A genome-wide association study on lodging resistant related traits in the Ethiopian germplasm collections of teff (*Eragrostis tef*)

(エチオピアのテフ（***Eragrostis tef***）遺伝資源における倒伏耐性関連形質に関するゲノムワイド関連解析**)**

Muluken Bayable Tadege

The United Graduate School of Agricultural Sciences
Tottori University, Japan

2021

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Muluken Bayable Tadege

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Major Supervisor

Nigussie Haregeweyn Ayehu (Prof.)

Co–supervisors

Atsushi Tsunekawa (Prof.)

Tsugiyaki Masunaga (Prof.)

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DEDICATION

I would like to dedicate this dissertation to:

My MOM, **Yigardush Terefe**, who shoulder a lot of burdens and pave my way to reach the current status.

The Ethiopian small scale farming community for their brave wisdom to explore the food values, domestication and conservation of the miraculous crop, teff (*Eragrostis tef*) which the world could not see its importance for centuries.

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CHAPTER 1

1. General Introduction

1.1. Background

Agriculture in Ethiopia is a cereal based mixed farming where crop production and livestock rearing is a common tradition. It is the main stay of about 85% of the population. Agriculture is still predominantly small scale and subsistence in which food production is the priority. Although, the climatic and agroecological situation in Ethiopia is suitable for several crop species to grow, teff is the top priority crop grown from drought prone to water logged Vertisol areas of the country. Teff is the stable food cereal and had high demand in the local market. As a result, farmers considered it as the main cash crop. Ethiopia is the home of the largest collections of teff germplasms also known as genetic resources mainly landraces. Landraces are known by their genetic heterogeneity, good adaptation to the local environmental conditions, but low in productivity. They are the main sources of desirable trait genes that can be used to increase the productivity of modern cultivars. Thus, characterization and documenting relevant information is mandatory for efficient utilization and conservation of the teff genetic resource.

1.1.1. Botanical descriptions of teff

Teff belongs to the grass family Poaceae, sub-family Chloridoidae, and the genus *Eragrostidae*. Although several binomial nomenclatures were given to this crop by different botanist through the past years, "*Eragrostis tef* (zucc.) Trotter" is the most accepted nomenclature of teff. Teff is a C_4 warm season annual plant with Kranz anatomy physiological pathways and classified intermediate between tropical and temperate grasses. Teff seed can be stored several years if direct contact to moisture is avoided (Ketema, 1997a), germination could occur within three days with ambient moisture condition. Teff is a short-day, completely self-pollinating species mainly a chasmogamous pollination behavior. Teff is a panicle bearing cereal with several panicle-lets and inflorescence. One single inflorescence

can produce up to 1000 seeds, and one plant up to 10,000. Teff has high tillering capacity. The teff accessions are known to differ in terms of their panicle form, lemma and caryopsis color.

1.1.2. Origin and distribution

Knowledge on the origin and distribution of crop species plays an important role in locating regions of germplasm collection and conservation which sources new genetic variations to the breeding program of the species. The genus *Eragrostis* is one of the largest genera in the grass family comprised of about 350 different species. The Russian botanist, Nikolas Vavilov, visited Ethiopia in 1927 and collected seed samples of several crops to characterize, conserve and utilize in the breeding program. He concluded from his investigation that Ethiopia as one of the places of crop domestication and center of origin for a number of crop species (Vavilov, 1951). Teff is one of them, though the exact place and time of domestication is not yet known. However, reports showed that teff domestication and cultivation is believed to started between 4,000 – 1,000 BC in the Northern highlands of Ethiopia (Simoons, 2009). In addition, Shaw (1976) speculated that the pre-Semitic people have started the domestication of teff in Ethiopia before wheat and barley were introduced; otherwise it would not have a chance to compete with and survived as a cultivated cereal. The distribution of Eragrostis species is largely tropical and sub-tropical and dominantly exist in the dry weedy habitats (Clayton and Renvoize, 1986). Reports indicate that about 43% are believed to 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). From the 54 Eragrostis species observed in Ethiopia, about 26% are particularly endemic (Cufodontis, 1974).

According to Burtt-Davy (1917), the distribution of teff to other countries was primary lead by the Royal Botanical Garden, Kew. This garden imported the seed of teff from Abyssinia, former name of Ethiopia, from the 1886 and distributed it to different botanical gardens and other institutions in India, Natal, and the colonies. The main aim of introducing teff to those countries was to get the advantage of its fast growing and drought resistant characteristics and relieve hunger in India and utilize as a high value fodder crop in other countries. The value of the grass as a fodder crop was surprisingly more than expected, while the attempt to expand as a food crop was not easy due to the people could not adopt the test of the bread (Bosman, 1927; Burtt-Davy, 1917). This might be due to lack of experience on dough preparation and baking of the teff flour. Even at this time bread making from teff flour particularly the Injera in most other countries, could not have equivalent quality as beaked in Ethiopia.

1.1.3. Production and Importance

Crop production in Ethiopia is mainly cereal based which accounts about 72% of the total crop land and 88% of total production. Grain yield of teff in Ethiopia shows an increasing trend during the past decades mainly due to the introduction of improved cultivars (Chanyalew et al., 2019). According to the Ethiopian Central Statistical Agency's (CSA) report, the productivity of teff was only 0.7 t/ha in 1995 where seed supply of the improved teff cultivars were at its infant stage and it reached 1.76 t/ha during the 2018 census report (**Figure 1.1**).

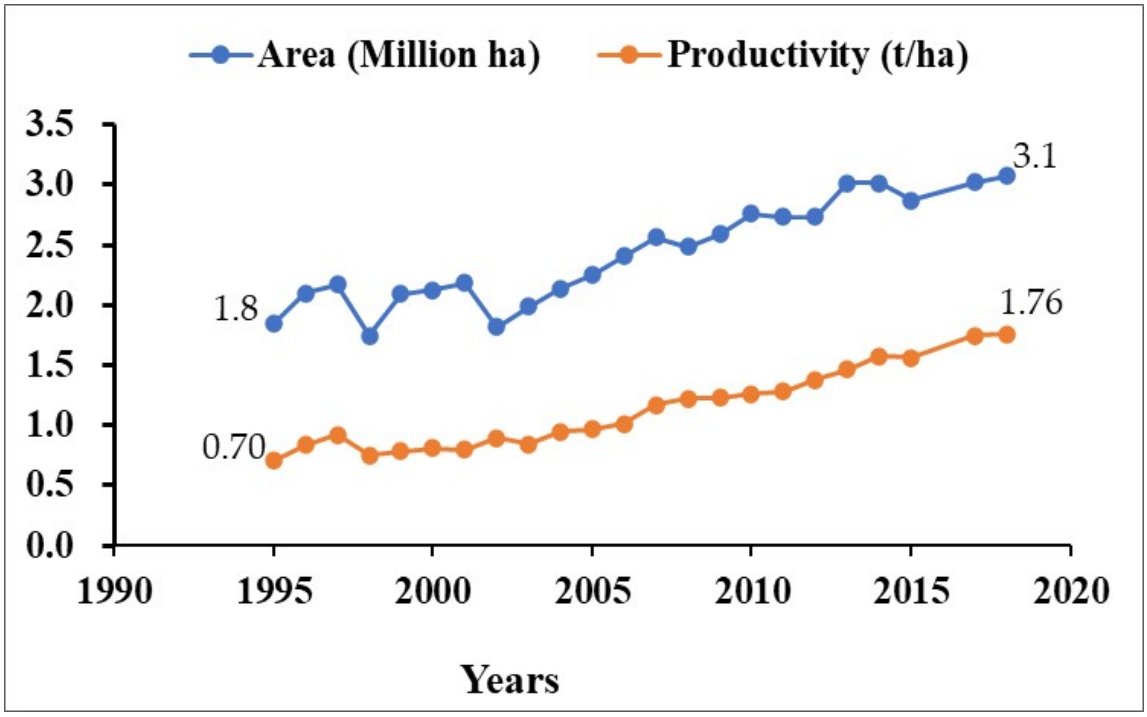

Figure 1-1: Nation-wide average trends of teff productivity (t/ha) and area coverage (million hectares) since 1995 (Source: Central statistics Agency of Ethiopia (CSA), available at: https://www.statsethiopia.gov.et/our-survey-reports/).

Teff production keeps its first rank in terms of area coverage among the other cereal crops which accounts about 30% of the land allotted to cereal production followed by maize, sorghum, and wheat (**Figure 1.2**). However, the lowest in its productivity which is only one third of the average wheat productivity of the nation (CSA, 2019a). Owing the low productivity with large share in area coverage, previous attempts of the country to oust teff production and replace with relatively higher productive cereal crops like wheat and maize was failed. This was mainly due to the fact that farmers were not convinced by the government plan mentioning the tremendous importance of the teff crop to their mixed farming system. Through that challenges, today nearly three million hectares of land have been covered by teff and more than six million small scale farmers are involving in teff cultivation in each year. In addition, teff started attracting consumers other than Ethiopia due to its health benefits like *amaranth* and *quinoa*.

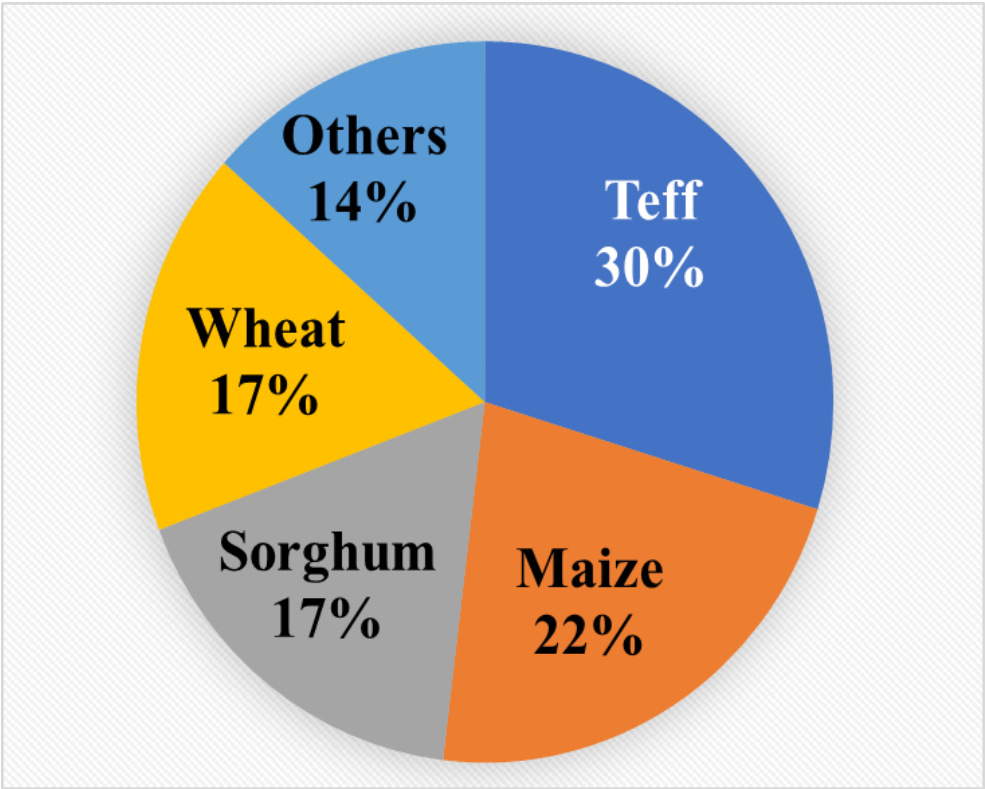

Figure 1-2: Pie chart showing the share of area under major cereal crops in Ethiopia.

Advantages of teff cultivation compared to other cereal crops includes:

- ✧ Tolerant to extreme growing conditions particularly to water lodging. Teff is unique in its ability to grow on poorly drained Vertisol of the cool environment where other cereals couldn't tolerate.
- ✧ The seeds are resistant to storage pests and can be stored longer without losing viability even under local storage conditions.
- ✧ Teff is also considered as a reliable and low risk catch crop where main crops failed due to intermittent drought
- ✧ Teff is gluten free and good in mineral and dietary fiber content and can be adopted as an alternative component of healthy food formulation.

- ✧ Higher flour production upon milling, an indicator of higher test weight, coupled with its gluten freeness makes the teff flour suitable for baking best quality traditional flat thin pancake with several honeycomb-like eyes on its upper part called Injera.
- ✧ The straw is also a valued livestock feed usually given to highly valued animals like lactating cows.

1.1.4. Nutritional values of teff

Among the 30,000 plant species grown in the world, only five cereal crops (wheat, rice, sorghum, millet, and maize) are currently providing more than 50% of the calory demand worldwide. Crop domestication through artificial selection for higher grain yield is believed responsible to cull out those valuable alleles good for nutrient uptake and use efficiencies of a species from the current cultivated crops gene pool. With current healthy food particularly gluten-free food demand increase, costing more than 1.6 billion USD (Cooper, 2015), there are an attempt to re-discover ancient crops as functional food sources. Dietary minerals are essential elements for human health and metabolism. The crude protein content is reported from 10.4 to 12.8% from samples in Ethiopia and the Netherlands respectively (Bultosa, 2007; Hager et al., 2012). The dietary fiber, crude fiber, and soluble fiber content of teff reported as 4.5, 3, and 0.9% respectively have been found much higher than maize, wheat, and rice (Baye, 2014).

1.1.5. Production constraints of teff

Despite all the aforementioned importance of teff in Ethiopia, its productivity is very low which is about 1.76 t/ha (CSA, 2019a). This largely because of susceptibility to lodging and accompanied challenges. Teff is completely self-pollinating species mainly a chasmogamous pollination behavior. Consequently, improved teff cultivar development strategy largely

depends on mass and pure line selection from landrace collections (Tefera et al., 2001). Therefore, low yielding cultivars can also be considered among the production constraints.

1.1.6. Lodging: Definitions and types

Lodging, defined as the irreversible displacement of the plant stem from its vertical is a complex mechanical plant stress (Berry et al., 2004b). The standing ability of the stem or the anchorage capacity of the root to hold up the shoot leverage is influenced by weather conditions. It is largely influenced by wind, rain, light, temperature, topography, soil type, soil fertility, plant density and diseases (Berry et al., 2004b). Lodging is also cultivar dependent and a tall, weak-stemmed cultivar is more susceptible to lodging than a semi-dwarf variety with stiffer stem. Thus, it is frequently associated with plant biomechanical and agro-morphological characteristics (Pinthus, 1974). It occurs in the form of stem lodging or failure of the anchorage system - root lodging (CROOK and Ennos, 1993; Pinthus, 1974; van Delden et al., 2010).

1.1.7. Effects of lodging on crop production

Lodging could cause both direct and indirect yield loss on most cereal crops. The direct loss has been related with complications of plant growth and development arising from falling-over or breaking of the plant culm (Berry et al., 2004a). Thus, it could limit efficient light interception, reduce translocation and assimilation, increase respiration and chlorosis, shattering, pre-mature sprouting, and creating conducive environment for mold to develop. The latter two could also deteriorate the quality of the harvested grain. The indirect loss is usually related with fear of the farmers in applying appropriate crop management options such as below optimum application of nitrogen fertilizer. Furthermore, lodging could also restrict mechanical seed harvesting which in turn increases the cost of production and limit the profitability.

Lodging susceptibility is the most significant production problem for teff. During average year, lodging is responsible for the estimated 17 - 25% grain yield loss nationally (Tefera and Ketema, 2000), whereas in some high rainfall areas particularly when rain comes during the grain filling period could routinely experience losses more than 50% (Bennetzen et al., 2009; Ketema, 1993). Lodging in tef is usually attributable to the bending and breaking of the thin culm rather than by uprooting of the plant ((Smith et al., 2012). On the other hand van Delden et al. (2010), reported root lodging is more prominent than stem bending and breaking in teff lodging phenomenon.

1.1.8. Lodging control options

Because of the significant role in the global food security, wheat and rice were the two main field crops in which a yield loss due to lodging of high yielding traditional cultivars has been substantially reduced. As a result, lodging resistant breeding has contributed significantly to the achieved yield increase in cereals since the Green Revolution following the discovery and introgression of dwarfing genes from the Japanese cultivar, Norin-10. This cultivar was derived from a cross between the native Japanese dwarf cultivar, Daruma, with American tall and high yielding wheat cultivar, Fultz, in 1917 (Milach and Federizzi, 2001). The Daruma – Fultz cross was then crossed with Turkey, a Russian wheat landrace, in 1925 resulted in the release of a new cultivar, Norin-10, in Japan during 1935 (Hanson et al., 1982). However, it was after the second world war that breeders realized Japanese farmers were growing short and stiff stemmed cultivars which invites the extensive exploitation and introgression of the sturdy short features of Norin-10 into high yielding lodging susceptible wheat cultivars in the USA and CIMMYT, Mexico. The introgression of these genes has been continued into modern cultivars in many parts of the world and more than 70% of the current wheat cultivars carry at least one of the dwarfing genes (Hedden, 2003).

Similarly, the lodging problem in rice was reduced through incorporation of a dwarfing gene from the Chinese cultivar called Dee-geo-woo-gen (DGWG) which led the release of IR 8 as the first semi-dwarf rice with good lodging resistance in 1966 (Milach and Federizzi, 2001). Therefore, reducing the plant height through introgression of reduced plant height genes (Rht-B1 and Rht-D1) in wheat and semi-dwarf genes (sd-1) in rice was the target trait of lodging resistance improvement in modern cultivars of wheat and rice.

The other option widely used for the management of crop lodging is the application of plant growth regulators which still used to reduce the plant height. Control of plant growth, such as culm length, has been achieved through the application of plant growth regulators. A number of plant promotors or retardants have been applied to control the vegetative growth of the plant traits that could aggravate lodging susceptibility. Plant growth regulators particularly gibberellin biosynthesis inhibitors such as chlormequate chloride and mepiquat-Cl are among the most widely used growth inhibitors. The application of chlormequate chloride controlled lodging in wheat by reducing the plant height, but also decreased the grain yield through decreasing the number of seeds per spike (Tripathi et al., 2004). Similarly, chlormequate chloride and Paclobutrazol reduced the culm length of teff, particularly the lower internodes with comparable grain yield but reduced panicle bearing tillers (Gebre et al., 2012). Paclobutrazol treated rice plants showed reduced plant height, higher bending resistance, and an increased grain yield compared to the control (Rani Sinniah et al., 2012).

1.1.9. Previous researches on teff lodging

Reports suggested that short, stiff-strawed genotypes as important sources of lodging resistance in teff (Berhe, 1982). Other reports showed a high correlation of lodging with stem diameter, plant height, and panicle length (Ketema, 1993; Mengesha et al., 1965). Plant height, culm length, panicle length, culm diameter, panicle weight, and shoot biomass

associated highly significantly and positively with lodging resistance (Hundera et al., 2000b). On the other hand, stem morphological related traits, such as total height, number of nodes, thickness and length of basal internodes were identified as important lodging components (Assefa et al., 1999). Similarly, Yu et al. (2007) showed a strong positive correlations between lodging, panicle type, culm thickness, and grain yield. Recent study outlined that the angle of the panicle contributed to lodging in teff and genotypes with compact panicle form and reduced height showed increased lodging resistance (Blosch et al., 2020). They also indicated that the landraces were lodged less than the improved cultivars.

1.1.10. Application of genome-wide association studies

The development of molecular markers, have played a significant role in the assessment of genetic diversity and in the dissection of the genetic basis of complex traits in several crop species. In human genetics, gene mining of important traits particularly those variants associated with diseases were difficult to study. To avert this challenge, genome-wide association mapping usually called genome-wide association study (GWAS) has developed and used to identify a host of susceptibility single nucleotide polymorphisms (SNPs) associated with important human diseases (Jeck et al., 2012). With the advent of high throughput genomic tools and quantitative genetics methods, analyzing the phenotype-genotype connection in plant species is getting more attention. Hence, GWAS has become a routine and powerful approach and applied in dissecting the genetic basis of trait variations in several crop species (Huang et al., 2010; Yang et al., 2014). Genes responsible for grain yield under stressed and non-stressed growing environments have been identified in wheat (El Gataa et al., 2021; Kulwal et al., 2012; Liu et al., 2017; Tadesse et al., 2019; Wang et al., 2019). Genomic regions responsible for wheat rust disease resistance were also identified and used for marker assisted selection (Letta et al., 2014). Similarly, in rice association study is extensively applied in dissecting the genetic basis of several important traits (Huang et al.,

2010; Huang et al., 2011). For example, a number of candidate genes controlling culm thickness from landrace japonica rice, which is lacking in modern cultivated cultivars, were identified (Chigira et al., 2020). Two other genomic regions and their candidate genes responsible for culm strength were also reported from indica rice (Ookawa et al., 2010). These are a clear indication that the landraces harbor superior alleles that could be exploited in the future breeding program.

1.2. Gaps and statement of the problem

Lodging is a key production constraint in teff production. In recent decades, similar attempts to introduce dwarfing genes to increase lodging resistance have been carried out in teff. These efforts have centered on mutation breeding, in particular the use of physical mutagens and the TILLING (Targeting Induced Local Lesions in Genomes) technique (Assefa et al., 2011; Tadele, 2019). This approach has been used mainly because teff is chasmogamous and completely self-pollinated with a microscopic size of the florets that makes emasculation and pollination very difficult and hinders the establishment of sufficient segregating populations. A reverse genetics approach to explore the presence and functioning of the Green Revolution genes has been also considered (Smith et al., 2012). Through the application of these breeding techniques, a number of dwarf and semi-dwarf teff accessions and mutant lines have been identified and evaluated, but no lodging-resistant cultivars have been developed. A comparison of genetic improvement related to trait changes in teff and wheat (Assefa et al., 2011) confirmed that the aforementioned efforts have not improved the harvest index of teff and reduced its susceptibility to lodging. This conventional approach of introducing a dwarfing gene through mutation breeding to increase lodging resistance seems unlikely to produce a semi-dwarf cultivar with sufficient lodging resistance in the near future. Overall, besides no lodging resistant teff cultivars developed, lack of consensus and knowledge on the traits to look for lodging resistance breeding continues one of the critical

gaps in the teff breeding program. Therefore, it will be highly important to strengthen the search of potential sources of lodging resistance, adopt other techniques such as targeted hybridization program to create additional genetic variabilities, a detailed analysis of the genetic control mechanisms and mode of inheritance of lodging resistant related traits, and most important lodging resistant related traits should be identified to increase the selection efficiency.

The GWAS have extensively applied in dissecting the genetic basis of important traits in several crop species as outlined in section 1.1.10. However, application of GWAS to dissect the genetic basis of important traits in teff is lacking.

Therefore, investigation and understanding of the diversity in germplasm resources and the genetic control mechanisms of lodging resistance related traits could play an important role and help the teff breeder (i) to analyze the genetic variability and identifying diverse parental combinations to create segregating populations with maximum genetic variability for further selection, (ii) introgression of target genes from diverse germplasm sources into the known genetic background, (iii) can facilitate reliable classification of germplasm collections, identification of subsets of core collections with possible utility for specific breeding purposes, and identify the gaps for further collection undertakings. On top of these, knowledge on the genetic basis of natural variations is a key in improving the breeding program through marker assisted selection and genomic manipulation.

In this study we characterized teff germplasm collections of Ethiopia for their biomechanical properties and agro-morphological trait variations aiming to investigate potential parental lines that could be used as sources of lodging resistance, determine the most lodging resistance related traits as a selection criterion and dissect the basis of genetic control using GWAS.

1.3. Objectives

1.3.1. General objective

To dissect key genomic regions that are essential for the control of lodging resistant related traits of teff through genome-wide association mapping using germplasm collections of Ethiopia.

1.3.1. Specific objectives

1. To evaluate the yield potential and determine the variabilities of teff genotypes under lodging controlled and non-controlled growing condition.
2. To examine the variability of stem mechanical properties and agro-morphological traits with their associations with lodging.
3. To explore the genetic diversity, population structure and trait-SNP associations using next generation sequencing (NGS) genotyping method.

1.4. Organization of the thesis

This PhD thesis is composed of five chapters (Figure 1.2). It starts from the general introduction as chapter-1 where I described briefly about the teff plant botany, origin, distribution, importance, production constraints, past research undertakings, the present research gaps that the research work under this thesis addressed. The general and specific objectives are also part this chapter.

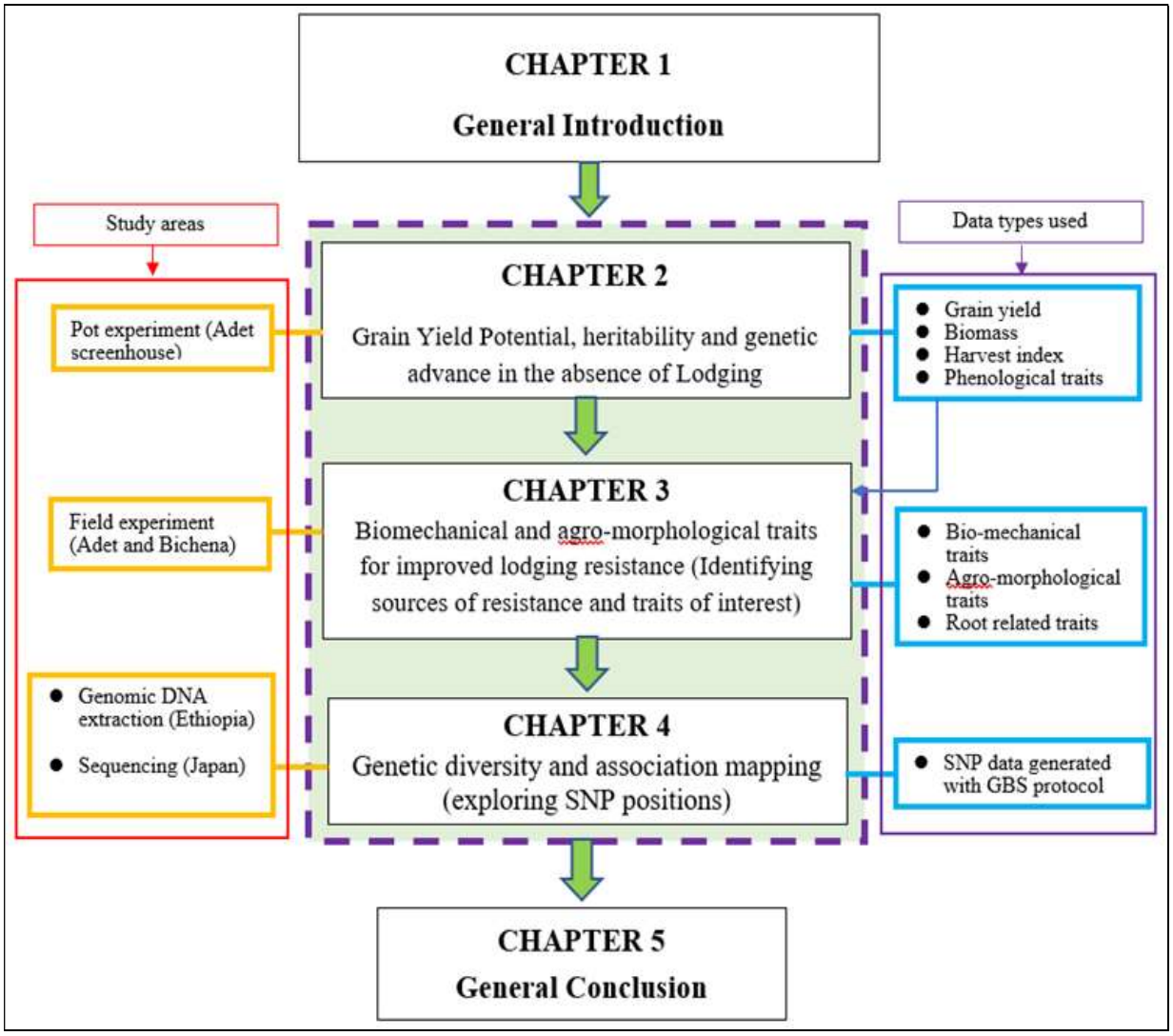

Figure 1-3: Flowchart showing the structure of the thesis.

Chapter two presents the heritability, expected genetic advance under selection, genetic potential, and analyze the yield gaps based on field and controlled experimentation with conventional and intensive management condition respectively. It also outlined trait relationships at the phenotypic and genotypic levels. Under the intensive management condition when lodging was controlled with support, higher harvest index was observed than the conventionally managed field experiments. On the other hand, high coefficient of variation, heritability and expected genetic advance for grain yield were observed in both experiments. The harvest index showed strong positive relationship with grain yield under the

intensive management but weak positive correlation with grain yield and a negative correlation with biomass under the field experiment, in which lodging was relatively higher. In addition, the yield gap analysis showed exploitable yield gap of about 67% and a potential yield gap of 150% compared to the current average farm yield of teff. All these suggests the presence of wide genetic diversity in the teff gene pool and its opportunity to enhance the productivity on one hand and the importance of lodging control options to exploit the potential.

The third chapter characterizes teff landrace accessions for lodging resistance in terms of the mechanical properties of the culm and lodging related agro-morphological traits using three hundred twenty teff genotypes under two contrasting locations for two consecutive growing seasons. This study revealed the presence of wide variability in both biomechanical and agro-morphological traits among the accessions. It is also noted that lodging index, failure moment, pushing resistance and lodging related traits such as culm diameter had a strong positive correlation with plant height. This suggests the shorter the plant height the lower the material strength of the teff culm. In contrast, tiller number showed a significant negative correlation with lodging index. The peduncle–panicle length, which generally accounted for 59% of the plant height, should be a target when breeding for semi-dwarfism. Root system development, which reached a depth of more than 1 m in tall and 57 cm in dwarf teff accessions, signifies the presence of genetic variabilities for future root lodging studies in teff, and it may also explain why teff performs well in drought-prone areas of Ethiopia. The observations indicated that stem failure account more likely than root failure for lodging in teff. However, our observations that the tillers initially grew mainly laterally and start to hold upright later in the growth stage further implies space competition at the crown, and teff has a relatively narrow root–shoot jointing point (crown). Thus, root failure could not be ruled out and needs detailed investigation in the future.

The fourth chapter outlines the genetic diversity, population structure and dissect Single Nucleotide polymorphisms (SNPs) that represent the putative Quantitative Trait Loci (QTL) associated with the target trait using double digest Restriction site Associated DNA sequencing (ddRAD-seq) derived SNPs. The admixture model bar chart of a STRUCUTURE program, neighbor-joining phylogenetic tree and the PCA scatter plot of TASSEL program consistently showed the presence of three sub-populations. The dendrogram based on biomechanical and agro-morphological traits contributing to lodging resistance also showed expected level of similarities among the members of each sub-population ranging from 15 to 33 %. The AMOVA showed only 1% of the variations was due to genetic variability among the sub-populations, whereas 99% of the variation was within populations. Further the low value of F_{ST} which is about 0.025, implies smaller population differentiation characterizes the teff population panel included under this study. Therefore, the information generated here will help in prioritizing breeding targets, and sensitizes to commence advanced genomic research such as genomic selection and marker development for efficient selection.

The fifth chapter is about the general conclusions and future directions. The high grain yield performance, wider range of harvest index under the intensive growing condition, and the associated moderate to high coefficient of genetic variation, heritability and genetic advance witnessed the available genetic potential for further improvement. On the other hand, our study on the lodging resistance variation of teff germplasms indicated a strong positive correlation of lodging index with plant height. Identified genotypes could be a good sources of culm strength for lodging resistance breeding in teff. The GWAS identified a significant marker-trait association for stem strength, stem diameter, and tiller number which could enhance the knowledge about molecular marker development and implementation of marker assisted selection in the teff breeding program. This could also enhance the opportunity to implement targeted genomic manipulation such as genome editing for lodging resistance.

CHAPTER 2

2. Yield potential and variability of teff (*Eragrostis tef* (Zucc.) Trotter) germplasms under intensive and conventional management conditions

Part of this chapter is published as:

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2.1. Introduction

Global climate trends such as recurrent drought and warming due to elevated carbon dioxide are the concerns of diminishing crop production (Cleland et al., 2007; He et al., 2020; Kurukulasuriya and Rosenthal, 2013). Consequently, the levels of poverty, food insecurity, and malnutrition are becoming a routine pain for human beings particularly in less developed countries (Tadele, 2019). On the other hand, food demand is expected to increase as the world population keeps increasing (Alexandratos and Bruinsma, 2012; Foulkes et al., 2011). The increased grain yield achieved in most common cereals during the Green Revolution largely resulted from improved partitioning of biomass to grain which is usually expressed as increased harvest index. However, such an improvement seems to have stagnated since the 1990s on major cereal crops (Foulkes et al., 2011; Hawkesford et al., 2013; Lin and Huybers, 2012; Ray et al., 2013; Rose and Kage, 2019) mainly due to the fact that attainable yield reached a plateau in favorable environments on the one hand and the challenging biotic and abiotic production factors are still threatening the agricultural system on the other hand. Investigations witnessed that modern wheat cultivars have reached a harvest index of greater than 0.50 which is close to the maximum hypothetical value of 0.62 (Austin et al., 1980; Hawkesford et al., 2013). In the world's leading rice producing countries, yield gain, which reached 36% during the 1980s has declined to 7% (Long, 2014). These facts suggest that to further increase productivity, the biological yield potential ceiling needs to be raised, or mechanisms to enable crops to cope with unfavorable growing conditions need to be improved. Clearly, without another breakthrough, the task of ending world hunger remains daunting.

In addition to exerting a substantial effort to increase grain yield in unfavorable environments through crop stress resistance and resource use efficiency in traditional cereal crops, it is also important to consider some niche crops that could contribute to food and

nutrient security with a potential for improvement. Several food crops play an important role in the food security and income of small-scale farmers in less developed countries (Lee, 2018; Tadele, 2019), yet despite their importance and significant area coverage in the center of diversity, the grain yield per unit area is very low due to less scientific research interventions, and they have remained under-utilized for centuries. Among those crops, the Ethiopian small cereal called teff is known for food and nutrition security and income for the local population. Currently, because teff is gluten free (Cannarozzi et al., 2014; Spaenij-Dekking et al., 2005; Zhu, 2018) and has high dietary fiber content (Zhu, 2018), it is attracting global attention from consumers, researchers, and food processing companies. This interest indicates that teff has the potential to be adapted as one component of the healthy food and beverage production industry. Consequently, adaptation and pilot level production has been underway in India, China, Australia, and Europe, while the United States has increased its teff production and supply to the world market (Lee, 2018).

Teff (Eragrostis tef (Zucc.) Trotter) is a C4 cereal and the major staple food crop grown in Ethiopia. Although the productivity is still regarded as low, grain yield of teff shows an increasing trend during the past decades mainly due to wide dissemination and adoption of improved cultivars (Assefa et al., 2015; Chanyalew et al., 2019). In Ethiopia, teff productivity, which was only 0.7 t/ha in 1995, when the seed supply of the improved cultivars was limited, reached 1.76 tone/ha in 2018 (CSA, 2019a). Teff production keeps its first rank in terms of area coverage among the other cereal crops which accounts about 30% of the land allotted to cereal production followed by maize (23%), sorghum (18%), and wheat (17%) (CSA, 2019a). However, the lowest in its productivity which is only one third of the average wheat productivity of the nation (CSA, 2019a). Today, nearly three million hectares of land are covered annually by teff and more than six million small scale farmers are involved in teff cultivation.

In addition to susceptibility to lodging, low grain yield potential has been supposed as one of the major production constraints of teff (Abraha et al., 2016a). Grain yield is a complex trait influenced by the growing environment, the genotype, and their interactions. The success of any crop management interventions to boost the productivity in a given environment is highly dependent on the genetic yield potential of the cultivars used. This is mainly because an increase in yield potential will uplift the actual farm yield. Yield potential is defined as the maximum yield that can be achieved by a cultivar when grown under nutrient and water non-limited environments to which it is adapted with stresses such as pests, diseases, weeds, and lodging effectively controlled (Evans and Fischer, 1999). Understanding the yield potential among the teff germplasm collections could play an important role in selecting best parental lines for desirable genetic recombination as it indicates how far breeders can attempt to increase the grain yield. However, how to quantify the yield potential continues debating. Although most yield potential studies used model-based prediction (Senapati and Semenov, 2020)[22], experimental quantification is also another option of estimating the yield potential with all the stresses being controlled as much as possible.

Genetic variability for yield and desirable traits is the backbone of any improvement program. The existence of considerable genetic variability has been reported in teff (Abraha et al., 2017; Assefa et al., 2001b; Ayalew, 2011). As the main yield determining factor, the variability, heritability, and genetic advance of harvest index in teff has been reported to be exceptionally low (Abraha et al., 2016a). Unlike the achievements observed during the Green Revolution on wheat and rice, reports in teff witnessed that the grain yield improvement achieved has been found due to an increase in plant height (Teklu and Tefera, 2005). Consequently, the harvest index of teff is low and significant improvement has not been made despite decades of breeding efforts (Ayalew, 2011; Jifar et al., 2015; Teklu and Tefera, 2005). On the other hand, due to differences in the nature of the genotypes used and the target

environments, reports showed highly variable results of the genetic parameters on grain yield and related traits. Thus, further investigation of the genetic parameters using large number of germplasm collections of teff is important to implement guided breeding strategies. Therefore, the objectives of this study were to evaluate the yield potential and determine the variabilities of teff genotypes under pot experiment with known stresses being controlled and field experiments with conventional management condition.

2.2. Materials and Methods

2.2.1. Description of experimental sites and materials

Two separate experiments were carried out. A pot experiment - hereafter, intensive growing condition - which was conducted at Adet Agricultural Research Center, Ethiopia in a screenhouse with full irrigation, high fertilizer rate application, and lodging controlled by providing a string support during 2017/18 off-season (December 2017 to May 2018). The second, field experiment with conventional management-hereafter, field growing condition - was conducted at two representative teff growing locations of Ethiopia. The same 317 teff genotypes were used for both experiments except three improved cultivars added in the field experiment. The locations were Adet (11° 28′ N, 37° 48′ E; 2216 m above sea level) and Bichena (10° 46′ N, 38° 19′ E; 2541 m above sea level) during the 2018 and 2019 main cropping seasons. Adet represents the Nitosol teff production areas whereas Bichena represents poorly drained Vertisol production areas (Figure 2.1).

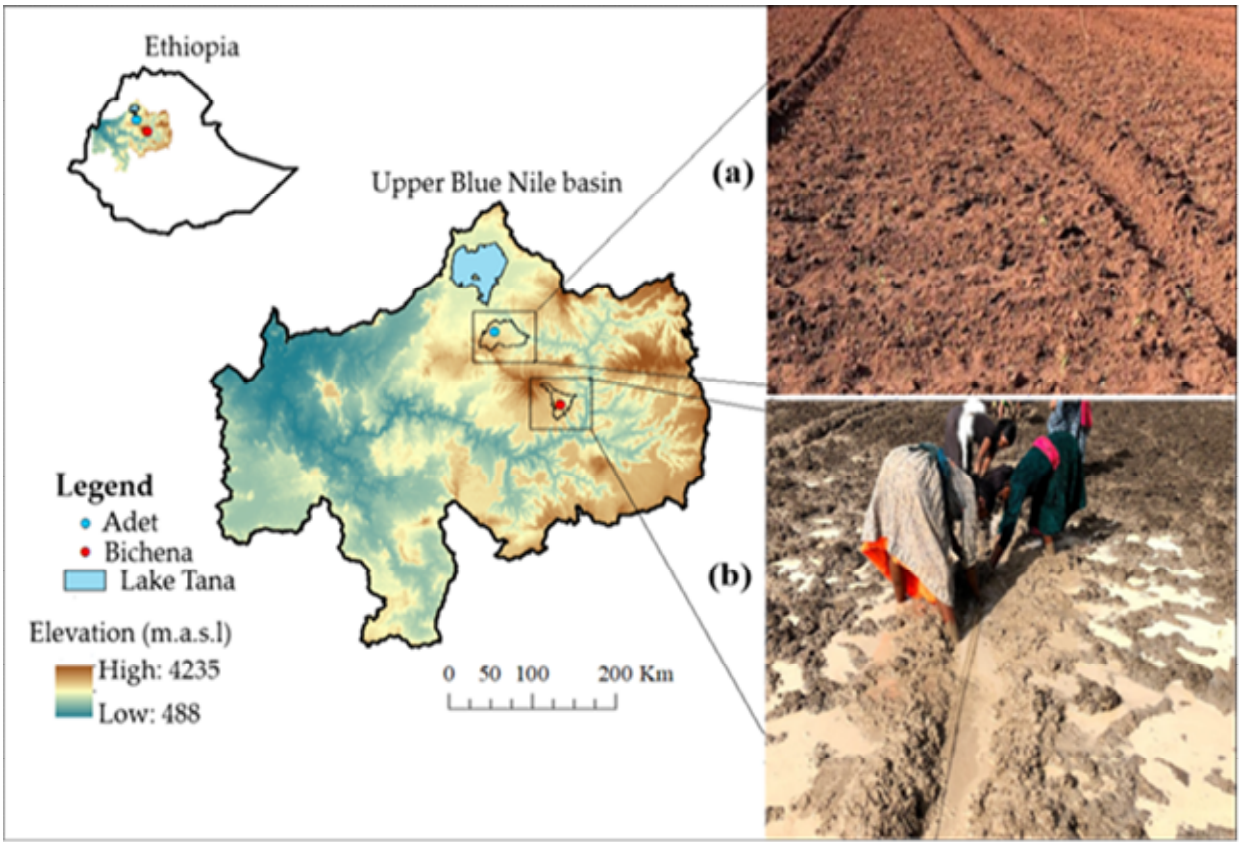

Figure 2-1: Map of the study areas and pictures (a) and (b) showing the field conditions and land preparation at the two stations Adet and Bichena respectively during teff planting time.

Adet received about 1512 mm annual rainfall, whereas Bichena received about 1156 mm with 75% comes from June to September. The average minimum temperature which is about 11 °C was similar at both locations. The maximum average temperature was 26 °C and 24 °C at Adet and Bichena, respectively. The germplasm panel used in this study was composed of 312 landrace collections from different teff growing regions of Ethiopia with an altitude range of 1200 to 3000 m above sea level, 2 breeding lines, and 6 improved cultivars (Kora, Quncho, Etsube, Abola, Dega-Tef, and DZ-Cr-37). The genotypes were accessed from Adet Agricultural Research Center, Ethiopian Biodiversity Institute, and Debre Zeit Agricultural Research Center. Seeds of all the landrace accessions were derived from individual panicle selections with a subsequent purification so that true to types were maintained. The seeds of

improved cultivars were taken from the breeder seed stocks of Adet Agricultural Research Center.

2.2.2. Experimental set up and management

2.2.2.1. Intensive growing condition

Non-transparent pots of 22 cm surface diameter and 18 cm depth were filled with soil leaving the top 3 cm empty for proper watering. The soil from the same paddock of Adet experimental station taken from the forest area within the top 10 cm depth was mixed with chicken manure in a 10:1 ratio. In total, 317 teff genotypes (312 landrace collections, 2 breeding lines, and 3 improved cultivars) were used for this particular study. The genotypes were assigned to each pot following the randomized complete block design with two replications. Folded blocks were created with 50 pots aligned in one row. Prior to planting, the pots were watered three days and planting was done on the fourth day at a seeding rate of 10 kg/ha. NPKS fertilizers were applied in the rate of 194, 120, 46, and 22 kg/ha respectively. The whole P and S, one-fifth of the N, and half of the K were applied at planting, while the remaining K and two-fifths of the N were applied at early tillering stage and the last two-fifths of the N was applied at early flowering stage. After two weeks from emergence, thinning was carried out to keep only 10 seedlings per pot. Each pot was watered on a daily basis with roughly equal volume of tap water to avoid moisture stress throughout the growing period. To reduce the effect of lodging, string supports at four different heights spanning 30 cm following the growth stage of the crop were made.

2.2.2.2. Field experiment under conventional condition

The same genotypes used in the intensive growing condition and three additional improved cultivars (Kora, DZ-Cr-37, and Dega-tef) - in total 320 teff genotypes - were planted in an 8

× 40 alpha lattice design with two replications. Each plot was 2 m long and 0.6 m wide and composed of three rows with 0.2 m spacing between rows. Following the local farming practice, teff sowing was done in mid-July at Adet station and in the first week of August at Bichena station in both experimental years. Teff seeds were sown in each row by hand drilling and three weeks after seedling emergence, thinning and transplanting were carried out to maintain 8 cm spacing between plants. N, P, and S fertilizers were applied in the form of urea (46% N) and NPS (19% N, 38% P, 7% S). N was applied at 64.5 kg/ha at Adet and 87.5 kg/ha at Bichena. Equal rates of P (60 kg/ha) and S (11 kg/ha) were applied at both stations. The whole P and S and half of the N were applied at planting, while the last half split of the N was applied at the tillering stage. All other field management practices were applied as per the recommendations of the crop.

2.2.2.3. Data collection and measurements

In both experiments, the heading date was taken when approximately 50% of the plants in the experimental units (plot/pot) had emerged heads, whereas the maturity date was determined when 90% of the plants in the plot reached physiological maturity or when the stem turns to golden yellow color. The plant height, panicle length, panicle weight, and peduncle length were measured on five representative plant samples. In both experiments, all plants in each experimental unit were harvested, threshed, and cleaned by hand. The grain yield per plot/pot was then converted into grain yield per plant according to the stand counts at harvest. The biomass yield was recorded after sufficient sun dying of the harvested samples.

2.2.2.4. Statistical data analysis

Single environment and combined over environments analysis of variance were computed following the Proc ANOVA (analysis of variance) model of the alpha lattice design for the field growing condition experiment data and Proc ANOVA model of the randomized

complete block design for the intensive growing condition experiment using the Statistical Analysis System software program (SAS, 2010). The genotypic, environmental, genotype by environment interaction, and residual variance components were determined using META-R software program of CIMMYT (CIMMYT, Mexico City, Mexico) using the residual maximum likelihood (REML) method (Alvarado et al., 2020). Broad sense heritability (H) as a ratio of genotypic variance (σ^2_G) to phenotypic variance (σ^2_P) was computed using the same META-R software program with the best linear unbiased predictors (BLUP) procedure. The phenotypic variance under the intensive growing condition ($\sigma^2_{P_i}$) and under the field growing conditions combined over environments ($\sigma^2_{P_{ij}}$) were estimated using the formula in Equations (2) and (3), respectively, as outlined in (Burton and Devane, 1953; Johnson et al., 1955). The genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), and genetic advance as percent of mean (GA) assuming 5% selection intensity were calculated with the formula described in (Singh and Chaudhary, 1985) as indicated in Equations (1), (4), and (5), respectively. Clustering of accessions based on grain yield and related traits was carried out using agglomerative hierarchical clustering procedure with square Euclidean distance as a measure of dissimilarity and incremental sum squares as a grouping strategy in the R software program.

$$\mathrm{GCV} = \frac{\sqrt{\sigma^2_G}}{\bar{x}} \times 100 \tag{1}$$

$$\sigma^2_{P_i} = \sigma^2_G + \frac{\sigma^2_e}{r} \tag{2}$$

$$\sigma^2_{P_{ij}} = \sigma^2_G + \frac{\sigma^2_{GE}}{l} + \frac{\sigma^2_e}{rl} \tag{3}$$

$$PCV = \frac{\sqrt{\sigma^2_P}}{\bar{x}} \times 100 \tag{4}$$

$$GA = \frac{K \times H \times \sqrt{\sigma^2_P}}{\bar{x}} \times 100 \tag{5}$$

where σ^2_e, σ^2_{GE} $\bar{x}$, r, l represents the residual variance, variance due to genotype by environment interaction, grand mean of the trait, number of replications, and number of environments, respectively, and K is the selection differential having a value of 2.06 at 5% selection intensity.

2.3. Results

2.3.1. Grain yield, Biomass and Harvest index

The analysis of variance results in the intensive growing condition revealed a highly significant difference ($p < 0.01$) among the test genotypes for grain yield, biomass, harvest index, and all other yield-related traits considered in this study. The performance of the top ten high yielding genotypes, cultivars, and the bottom three low yielding genotypes under the intensive growing condition are presented in Table 2.1. The grain yield performance of the test genotypes ranged from 4.2 to 8.8 g/plant with overall mean yield of 6.2 g/plant. Accession 242138-1, 236756-2, and 242200-1 were among the top yielding genotypes, whereas accession 55069-3, 239373-2, and 219850-1 were among the low yielding genotypes. Among the improved cultivars included in this study, Etsub recorded about 6.7 g/plant, while Quncho and Abola each gave about 6.4 g/plant grain yield which is equivalent to the overall mean grain yield. BLUP-adjusted mean grain yield also showed nearly 30% yield advantage of the high yielding genotypes over the best yielding cultivar. The above ground biomass yield ranged from 14.6 g/plant to 23.1 g/plant (Table A1). Accession 234431-1 and 229971-2 were the lowest in biomass yield while accession 204596-3 and 234430-1 were the highest in biomass. The harvest index ranged from 0.25 to 0.45 with a mean value of 0.34 (Table A1). Under the intensive growing condition, high yielding genotypes showed higher harvest index, tall plant stature, and relatively late maturing with high grain filling period.

In the field growing condition, single environment analysis of variance showed a highly significant variation ($p < 0.01$) among the genotypes for grain yield, biomass, and harvest index (data not presented). Similarly, the combined analysis of variance showed a highly significant difference among the genotypes for all the traits considered in this study. BLUP-adjusted mean genotype performance across the four environments indicated that accession

229971-3, and 236765-3 were the highest yielding genotypes with 10% yield advantage over the best yielding improved cultivar (Table 2.2). The grain yield performance of the genotypes combined over the four environments ranged from 1.8 g/plant to 4.3 g/plant with a mean value of 3.1 g/plant. Among the improved cultivars, the highest grain yield was recorded with the cultivar Estub about 3.9 g/plant with 12.8 g/plant biomass yield and 0.30 harvest index. Quncho, one of the most widely grown improved cultivar gave about 3.0 g/plant grain yield. The biomass yield was ranged from 7.4 g/plant to 13.5 g/plant with a mean value of 10.7 g/plant (Table A2). The harvest index ranged from 0.23 to 0.34 with an overall mean value of 0.29. Maximum grain yield about 5.7 g/plant was recorded at Adet during the 2018 season whereas the least, 1.4 g/plant, was at Bichena during 2019 season (Figure 2.2). Generally, the average grain yield of the two seasons at the Nitosol location (Adet) was higher (3.5 g/plant) than the Vertisol location (2.7 g/plant). However, the harvest index was higher in the Vertisol location (0.30) than the Nitosol location (0.27). The environmental and the genotype by environment interaction effect were also highly significant on the genotypes tested for grain yield, biomass, harvest index, and other yield related traits.

Table 2-1: Best linear unbiased predictor (BLUP)-adjusted mean grain yield and yield-related traits of the top ten high yielding, cultivars, and the bottom three low yielding teff genotypes under the intensive growing condition.

Genotypes	Accession number	GY	BM	HI	DH	DM	GFP	PH	PL	PW
Ten high yielding genotypes	242138-1	8.8	18.7	0.45	63	129	67	128.1	35.7	1.9
	236756-2	8.7	19.0	0.44	59	131	73	128.9	33.3	2.2
	242200-1	8.5	21.6	0.38	65	130	66	136.0	30.7	1.8
	235671-1	8.3	19.4	0.41	63	123	60	141.1	31.1	1.8
	227786-4	8.2	17.8	0.45	66	130	65	130.9	32.9	1.9
	229101-1	8.2	19.3	0.41	60	125	65	134.5	35.8	2.3
	Abishlemne	8.1	21.1	0.38	63	125	63	115.4	35.1	1.7
	229971-3	8.1	20.3	0.40	57	123	66	139.5	31.0	1.8
	244783-3	8.1	17.9	0.44	58	130	73	134.4	33.3	2.3
	234430-1	8.1	23.1	0.34	59	130	72	128.3	32.4	2.2
Cultivars	Etsub	6.7	22.4	0.30	60	130	71	138.5	36.8	2.1
	Quncho	6.4	17.8	0.36	60	123	63	128.5	32.7	1.8
	Abola	6.4	19.6	0.33	63	121	58	144.2	42.1	2.3
Low yielding genotypes	219850-1	4.3	16.7	0.27	62	108	46	102.9	34.5	1.7
	239373-2	4.3	18.0	0.25	63	111	48	100.7	28.0	1.4
	55069-3	4.2	16.1	0.28	60	109	49	115.3	21.6	1.6
Grand mean		6.2	18.4	0.34	60	121	60	126.9	32.2	1.8
LSD		0.9	4.0	0.1	6.7	11	12	15.0	6.1	0.4

GY = grain yield (g/plant), BM = above ground biomass (g/plant), HI = harvest index, DH = days to heading, DM = days to maturity, GFP = grain filling period, PH = plant height (cm), PL = panicle length (cm), PDL = peduncle length (cm), PW = panicle weight (g), LSD = least significant difference at 5% level of significance.

Table 2-2: BLUP-adjusted mean grain yield and yield related traits of the top ten high yielding, cultivars, and the bottom three low yielding teff genotypes under field growing condition with conventional management (combined over years and locations).

Genotypes	Accession number	GY	BM	HI	DH	DM	GFP	PH	PL	PW
Ten high yielding genotypes	229971-3	4.3	10.9	0.33	55	127	71	107.7	31.5	1.5
	236765-3	4.3	11.1	0.33	62	129	67	109.7	32.1	1.5
	234430-1	4.3	11.6	0.32	60	129	68	104.8	33.2	1.5
	236756-2	4.2	11.4	0.31	59	129	70	113.6	35.2	1.5
	DZ-01-3502	4.2	11.2	0.31	58	130	71	94.1	31.0	1.3
	RIL-260	4.1	12.6	0.30	61	131	69	128.6	42.2	1.8
	203010-4	4.1	13.5	0.27	61	131	69	125.0	38.7	1.5
	202978-2	4.1	11.0	0.32	62	129	67	108.7	33.8	1.3
	238223-2	4.0	11.9	0.29	58	128	69	104.1	30.1	1.0
	235659-3	4.0	12.1	0.30	59	127	67	110.1	32.3	1.2
Cultivars	Etsub	3.9	12.8	0.30	60	128	68	110.2	34.8	1.4
	Quncho	3.0	10.6	0.28	58	123	65	106.7	32.2	1.4
	Abola	3.4	12.8	0.27	64	130	66	113.4	37.4	1.5
Low yielding genotypes	229101-3	1.8	8.3	0.22	62	124	62	93.0	31.4	0.9
	234775-4	1.8	7.5	0.24	62	130	65	97.7	32.6	0.9
	219882-4	1.8	8.3	0.22	64	125	62	94.1	33.0	0.9
Grand mean		3.1	10.7	0.29	60	126	65	99.3	31.2	1.1
LSD		0.5	2.0	0.1	2.7	4.3	5.0	8.3	4.3	0.2

GY = grain yield (g/plant), BM = above ground biomass (g/plant), HI = harvest index, DH = days to heading, DM = days to maturity, GFP = grain filling period, PH = plant height (cm), PL = panicle length (cm), PDL = peduncle length (cm), PW = panicle weight (g), LSD = least significant difference at 5% level of significance.

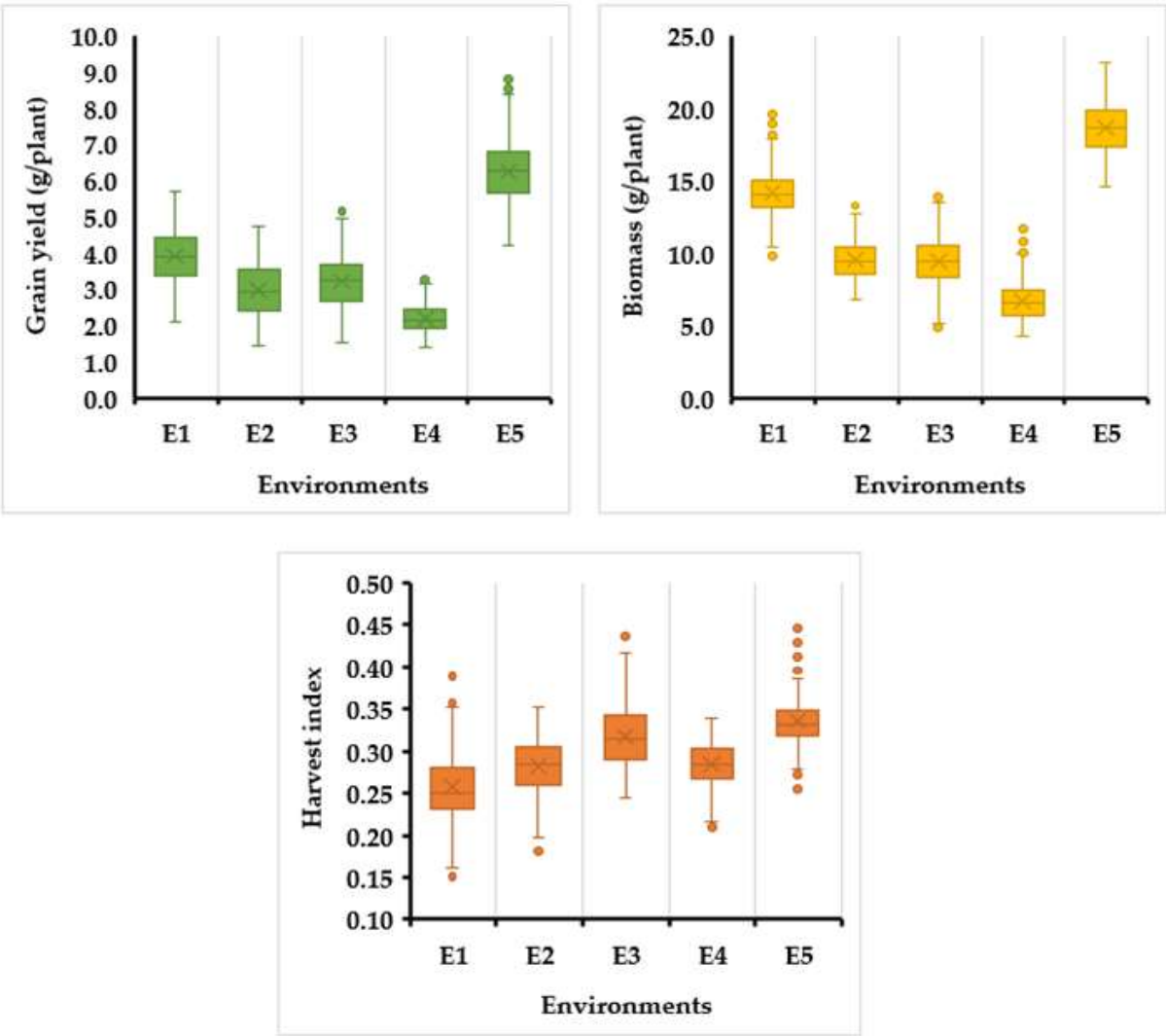

Figure 2-2: Genotype performance across the test environments for grain yield, biomass, and harvest index based on BLUP-adjusted mean values. E1 = Adet 2018, E2 = Adet 2019, E3 = Bichena 2018, and E4 = Bichena 2019. E5 refers the intensive growing condition and the data were analyzed separately.

The grain yield performance under the intensive management experiment and field experiment with conventional management showed a weak positive significant relationship ($r = 0.17$, $p = 0.05$) (Figure 2.3). Although the mean grain yield value is by far lower under the field experiment, the scatter plot also showed the presence of genotypes that performed consistently from the intensive management to the field growing condition.

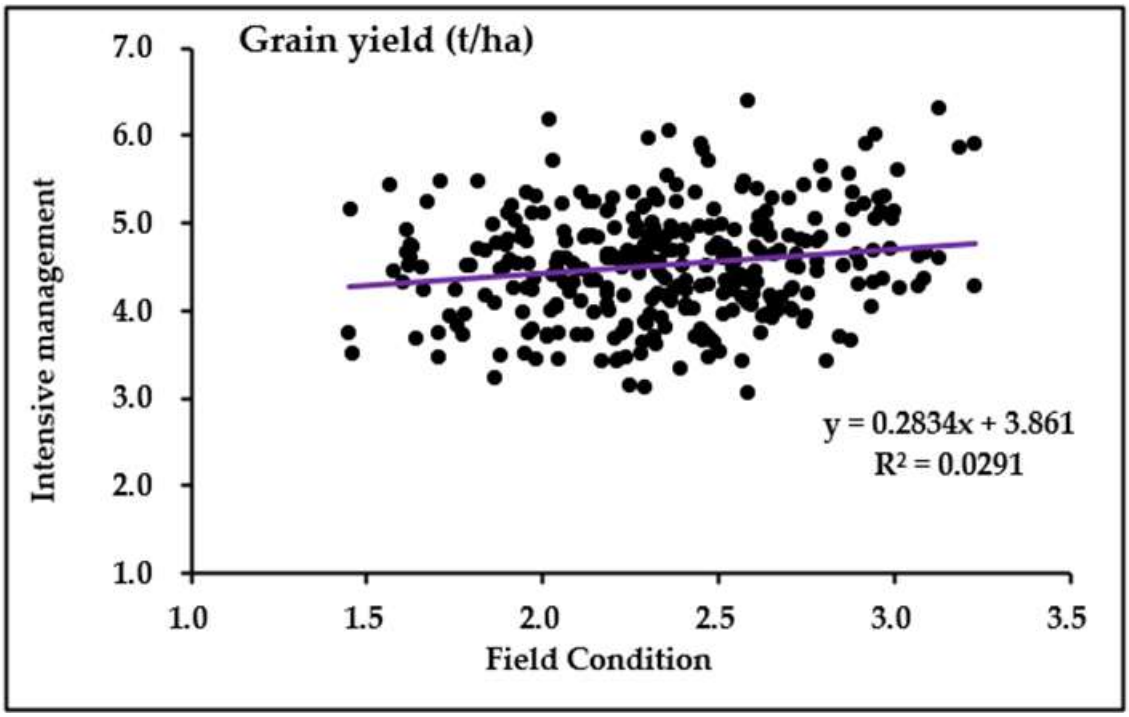

Figure 2-3: Scatter plot showing the relationship of teff grain yield under the field experimental condition with conventional management versus under the intensive management condition.

2.3.2. Phenology

The analysis of variance in both growing conditions showed a highly significant difference ($p < 0.01$) among the genotypes for days to heading, days to maturity, and grain filling period. Under the intensive growing condition, days to heading of the test genotypes ranged from 51 to 73 days with overall average of 60 days (Table A1). Accession 237709-2, 237572-1, and 234431-1 were among the early genotypes, whereas 212930-4, 212929-1, and 239373-2 were among the late heading genotypes. The maturity date ranged from 107 to 140 days with average value of 121 days. The grain filling period, the number of days from heading to maturity, ranged from 35 to 79 days (Table A1). Under the field growing condition, the early genotype took about 51 days to heading and 108 days to maturity with 53 days for grain filling (Table A2) whereas the late maturing genotypes took about 66 days to

heading and 131 days to maturity. Accession 234431-1 and 204569-1 were early and 203008-5, 229766-5, and 234782-2 were late maturing genotypes. Despite the rank differences, most of the early genotypes performed consistently across the two growing conditions while considerable rank difference was noted among the late genotypes across the two growing conditions. It was also noted that most early genotypes were associated with short plant stature.

2.3.3. Variance components, heritability and genetic advance

Estimates of genetic parameters and mean squares of the important traits in the intensive growing condition are summarized in Table 2.3. Among the traits, peduncle length and harvest index showed the lowest heritability, 0.59 and 0.67, respectively. Genetic advance generally ranged from moderate to high. The lowest genetic advance was observed for days to maturity and days to heading whereas the highest genetic advance was observed in grain filling period, grain yield, panicle length, and panicle weight. Grain filling period, grain yield, panicle length, and panicle weight also showed the highest coefficient of genetic variation, while the days to maturity and days to heading were the lowest in genetic coefficient of variation. The grain filling period which varied from 35 to 79 days showed the highest genetic coefficient of variation, and heritability with the highest genetic advance. On the other hand, the genetic coefficient of variation for the harvest index was small, 8.8% with a genetic advance of 15.2%.

Table 2-3: Mean squares, variance components, and estimates of variability for important traits of 317 teff genotypes under the intensive growing condition.

Traits	Mean Square		Means	Variance		GCV (%)	PCV (%)	H	GA (%)
	G	σ^2_e		σ^2_G	σ^2_P				
DH	40.46 **	6.69	60.39	16.88	20.23	6.80	7.45	0.83	12.73
DM	146.27 **	30.25	120.77	58.01	73.13	6.31	7.08	0.79	11.52
GFP	192.41 **	34.71	60.37	78.85	96.21	14.71	16.25	0.82	27.44
PH	307.11 **	59.43	126.90	123.84	153.56	8.77	9.77	0.81	16.29
PL	51.26 **	9.59	32.19	20.83	25.63	14.18	15.73	0.81	26.24
PDL	22.54 **	9.27	26.39	6.63	11.27	9.76	12.72	0.59	15.46
PW	0.12 **	0.02	1.77	0.05	0.06	12.88	14.01	0.84	24.24
GY	2.24 **	0.42	6.24	0.91	1.02	15.29	16.19	0.81	27.01
BM	10.54 **	2.40	18.62	4.07	4.70	10.83	11.64	0.77	18.47
HI	0.004 **	0.001	0.34	0.001	0.001	8.82	11.00	0.67	15.20

**, indicates significant difference at $p < 0.01$, G = genotype, σ^2e = residual variance, DH = days to heading, DM = days to maturity, GFP = grain filling period, PH = plant height (cm), PL = panicle length (cm), PDL = peduncle length (cm), PW = panicle weight (g), GY = grain yield (g/plant), BM = above ground biomass (g/plant), HI = harvest index, GCV = genotypic coefficient of variation, PCV = phenotypic coefficient of variation, H = broad sense heritability, GA = genetic advance as percent of mean, σ^2_G = genotypic variance, and σ^2_P = phenotypic variance.

Estimates of genetic parameters and mean squares of the combined analysis of variance in the field growing condition are summarized in Table 2.4. The variance component partitioning indicated that the environmental variance was consistently higher than the genotypic variance for all the traits considered (data not presented). Consequently, the phenotypic coefficient of variation surpassed the genotypic coefficient of variation. The phenological traits such as days to heading, days to maturity, and grain filling period showed the lowest genotypic coefficient of variation with higher heritability value. On the other hand, grain yield and panicle weight showed the highest coefficient of genetic variation. Therefore, the genetic advance under selection was higher for grain yield and panicle weight, but it was

the lowest for phenological traits. Harvest index showed the least heritability compared to other traits with 13.9% genetic advance.

Table 2-4: Mean squares, variance components, and estimates of variability for important traits of 320 teff genotypes under field growing condition with conventional management (combined over environments).

Traits	Mean Square			Means	Variance		GCV (%)	PCV (%)	H	GA (%)
	G	E	G x E		σ^2_G	σ^2_P				
DH	69.82 **	23941 **	9.70 **	60.27	7.40	8.58	4.51	4.86	0.86	8.61
DM	165.7 **	27092 **	27.42 **	125.61	17.60	20.84	3.34	3.63	0.84	6.29
GFP	123.0 **	9017 **	29.6 **	65.34	12.20	15.68	5.35	6.06	0.78	9.74
PH	815 **	58858 **	27.4 **	99.26	93.62	98.72	9.75	10.01	0.95	19.59
PL	146.2 **	35059 **	39.5 **	28.20	13.05	17.87	11.58	13.55	0.73	22.50
PDL	63.0 **	3004.8 **	8.85 **	24.52	6.70	7.79	10.56	11.38	0.86	20.17
PW	0.44 **	32.28 **	0.08 **	1.08	0.05	0.05	19.64	21.67	0.82	36.60
GY	3.07 **	231.0 **	0.45 **	3.10	0.33	0.38	20.74	22.36	0.86	35.20
BM	18.3 **	6087.1 **	6.08 **	10.70	1.53	2.25	12.46	15.10	0.68	19.64
HI	0.009 **	0.385 **	0.004 **	0.29	0.0007	0.001	8.79	11.82	0.57	13.90

**, indicates significant difference at $p < 0.01$. G = genotype, E = environment, G x E = genotype by environment interaction, DH = days to heading, DM = days to maturity, GFP = grain filling period, PH = plant height (cm), PL = panicle length (cm), PDL = peduncle length (cm), PW = panicle weight (g), GY = grain yield (g/plant), BM = above ground biomass (g/plant), HI = harvest index, GCV = genotypic coefficient of variation, PCV = phenotypic coefficient of variation, H = broad sense heritability, GA = genetic advance as percent of mean, σ^2G = genotypic variance, and σ^2P = phenotypic variance.

2.3.4. Phenotypic and genotypic correlations

The phenotypic and genotypic correlation coefficients of grain yield and related traits under the intensive growing condition are presented in Table 2.5. Days to maturity showed a highly significant positive phenotypic and genotypic correlation with grain yield, biomass, and harvest index. Except non-significant negative phenotypic correlation with biomass, days to heading generally showed a significant negative phenotypic and genotypic correlation with

grain yield, biomass, and harvest index. Similarly, the grain filling period, plant height, panicle length, and panicle weight showed a highly significant positive phenotypic and genotypic correlation with grain yield, biomass, and harvest index. However, the correlation of harvest index with panicle length and panicle weight was significant at 0.05 p-value. Grain filling period also showed a highly significant negative genotypic and phenotypic correlation with days to heading, whereas positive and highly significant correlation with days to maturity. The genotypic correlation of harvest index with grain yield and biomass was positive and significant but negligible phenotypic correlation with biomass. The peduncle length showed a negligible correlation with most of the traits considered in this study, except a positive significant correlation with days to heading.

Correlation coefficients based on the combined data under the field growing condition are presented in Table 2.6. Days to heading showed negative significant phenotypic and genotypic correlation with grain yield and harvest index, whereas its correlation with the biomass yield was non-significant. Days to maturity showed a non-significant correlation with grain yield and harvest index, but a positive significant correlation with biomass yield. Days to maturity, on the other hand, showed a highly significant positive phenotypic and genotypic correlation with grain filling period. Although the magnitude is small, the grain filling period correlated positively and significantly with grain yield, biomass, and harvest index. Both plant height and panicle length showed a highly significant positive correlation with biomass yield, significant correlation with grain yield, but negative significant correlation with harvest index. Panicle weight showed a positive significant correlation with biomass and grain yield, but non-significant with harvest index. The correlation between biomass yield and harvest index was positive and significant. A significant negative phenotypic and genotypic correlation was observed between peduncle length and panicle length. Correlation coefficients based on individual location data are presented in Table A3.

The phenotypic correlation between biomass and harvest index was significantly negative at Adet but significantly positive at Bichena. Plant height and panicle length showed a highly significant negative genotypic correlation with harvest index at Adet, but non-significant at Bichena. Similarly, the relationship between panicle weight and harvest index was highly significantly negative at Adet but significantly positive at Bichena.

Table 2-5: Genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficients between the important traits of 317 teff genotypes under the intensive growing condition.

	DH	DM	GFP	PH	PL	PDL	PW	GY	BM	HI
DH		−0.06	−0.52 **	−0.24 *	−0.13 ns	0.35 *	−0.10 ns	−0.22 *	−0.15 *	−0.22 *
DM	−0.04 ns		0.89 ***	0.61 **	0.49 **	−0.07 ns	0.51 **	0.77 ***	0.71 ***	0.54 **
GFP	−0.49 **	0.89 ***		0.64 **	0.48 **	−0.22 *	0.48 **	0.76 ***	0.68 ***	0.56 **
PH	−0.19 *	0.65 **	0.65 **		0.63 **	0.02 ns	0.50 **	0.69 ***	0.70 ***	0.44 **
PL	−0.09 ns	0.55 **	0.52 **	0.68 ***		−0.14 *	0.45 **	0.52 **	0.63 **	0.23 *
PDL	0.20 *	−0.02 ns	−0.11 ns	0.04 ns	−0.08 ns		0.001 ns	−0.03 ns	−0.03 ns	0.002 ns
PW	−0.08 ns	0.55 **	0.51 **	0.56 **	0.51 **	0.02 ns		0.53 **	0.55 **	0.33 *
GY	−0.17 *	0.78 ***	0.76 ***	0.74 ***	0.60 **	0.002 ns	0.58 **		0.81 ***	0.82 ***
BM	−0.11 ns	0.59 **	0.57 **	0.58 **	0.53 **	−0.01 ns	0.47 **	0.67 ***		0.33 *
HI	−0.14 *	0.51 **	0.51 **	0.48 **	0.34 *	0.02 ns	0.37 *	0.74 ***	−0.004 ns	

*, **, ***, indicates significant correlation coefficients at a p values of 0.05, 0.01, and 0.001 respectively, ns = non-significant at $p < 0.05$. DH = days to heading, DM = days to maturity, GFP = grain filling period, PH = plant height (cm), PL = panicle length (cm), PDL = peduncle length (cm), PW = panicle weight (g), GY = grain yield (g/plant), BM = above ground biomass (g/plant), HI = harvest index.

Table 2-6: Genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficients between the important traits of 320 teff genotypes under the field growing condition.

	DH	DM	GFP	PH	PL	PDL	PW	GY	BM	HI
DH		0.56 **	−0.10 ns	0.46 **	0.34 *	−0.18 *	0.38 *	−0.23 *	−0.05 ns	−0.45 **
DM	0.51 **		0.77 ***	0.42 *	0.31 *	0.09 ns	0.45 **	0.10 ns	0.15 *	−0.03 ns
GFP	−0.14 *	0.78 ***		0.16 *	0.12 *	0.25 *	0.25 *	0.30 *	0.21 *	0.31 *
PH	0.41 *	0.36 *	0.11 ns		0.96 ***	−0.01 ns	0.89 ***	0.26 *	0.60 **	−0.25 *
PL	0.30 *	0.24 *	0.06 ns	0.83 ***		−0.38 *	0.89 ***	0.36 *	0.67 ***	−0.14 *
PDL	−0.17 *	0.07 ns	0.21 *	−0.01 ns	−0.36 *		−0.06 ns	−0.10 ns	−0.16 *	0.06 ns
PW	0.34 *	0.39 *	0.20 *	0.80 ***	0.72 ***	−0.04 ns		0.46 **	0.69 ***	0.05 ns
GY	−0.22 *	0.09 ns	0.26 *	0.23 *	0.28 *	−0.07 ns	0.40 *		0.94 ***	0.88 ***
BM	−0.05 ns	0.13 *	0.18 *	0.49 **	0.49 **	−0.10 ns	0.55 **	0.84 ***		0.66 **
HI	−0.34 *	−0.03 ns	0.21 *	−0.22 *	−0.13 *	0.05 ns	0.02 ns	0.71 ***	0.24 *	

*, **, ***, indicates significant correlation coefficients at a *p* values of 0.05, 0.01, and 0.001 respectively, ns = non-significant at 0.05. DH = days to heading, DM = days to maturity, GFP = grain filling period, PH = plant height (cm), PL = panicle length (cm), PDL = peduncle length (cm), PW = panicle weight (g), GY = grain yield (g/plant), BM = above ground biomass (g/plant), HI = harvest index.

2.3.5. Cluster analysis

The hierarchical cluster analysis using Ward's method in the intensive growing condition revealed 7 different groupings of the 317 teff genotypes with 16 to 89 member genotypes (dendrogram not presented). The cluster mean values of the 10 important traits under the intensive growing condition are summarized in Table 2.7, and cluster members are reported in Table A4. Cluster-I particularly composed of teff genotypes with shorter peduncle length and moderate grain yield. Most members in cluster-II were characterized by high in biomass yield, longer grain filling period, and late to mature. Cluster-III consisted of higher grain

yielding, higher harvest index, and late maturing member genotypes. Cluster-VII consisted of genotypes with shorter grain filling period due to late heading date of the member genotypes, and lower harvest index and grain yield. Similarly, cluster-V consisted of low yielding genotypes with early heading date and shorter grain filling period. Cluster-VI consisted of genotypes with longer peduncle length, moderate grain yield, and average harvest index.

Table 2-7: Cluster means of grain yield and yield related traits of teff genotypes under the intensive growing condition.

Cluster	DH	DM	GFP	PH	PL	PDL	PW	GY	BM	HI
I	58.60	121.35	62.73	128.21	33.75	24.14	1.87	6.38	19.32	0.33
II	60.22	128.32	68.35	136.57	35.60	26.50	1.91	6.98	20.28	0.34
III	61.34	126.90	65.78	133.60	33.04	26.96	1.96	7.92	18.71	0.41
IV	63.78	115.62	51.73	127.87	31.39	27.09	1.70	6.01	18.53	0.33
V	58.84	114.32	55.24	117.26	28.48	26.16	1.60	5.26	17.07	0.32
VI	58.91	119.86	60.90	126.18	32.12	27.24	1.76	6.19	18.17	0.34
VII	70.11	114.56	44.42	109.89	28.12	27.14	1.59	5.31	16.77	0.30

DH = days to heading, DM = days to maturity, GFP = grain filling period, PH = plant height (cm), PL = panicle length (cm), PDL = peduncle length (cm), PW = panicle weight (g), GY = grain yield (g/plant), BM = above ground biomass (g/plant), HI = harvest index.

Under the field growing condition, six clusters were identified with 27–109 member genotypes (data not presented). Cluster-I and cluster-VI were both characterized by shorter plant height, lower panicle weight, and lower grain yield (Table 2.8). However, cluster-VI was different from cluster-I having member genotypes with shorter panicle and relatively better harvest index. Cluster-II composed of higher grain yielding, tall plant stature, higher in panicle weight, longer panicle, and late maturing genotypes. Cluster-V included late heading genotypes with intermediate plant height, longer panicle length and shorter peduncle length. Whereas cluster-III consisted of genotypes with longer peduncle length with intermediate

plant height. Among the improved cultivars, Etsub and Kora were grouped together with the high yielding genotypes under cluster-II. On the other hand, relatively older cultivars such as DZ-Cr-37 and Dega-tef were grouped under cluster-III where the second-high yielding and intermediate plant height genotypes included.

Table 2-8: Cluster means of grain yield and yield related traits of teff genotypes under the field growing condition.

Cluster	DH	DM	GFP	PH	PL	PDL	PW	GY	BM	HI
I	60.91	125.80	64.92	97.84	31.37	24.22	0.99	2.60	9.15	0.27
II	60.11	128.52	67.93	121.73	35.37	24.61	1.38	3.81	11.61	0.29
III	60.02	126.69	66.58	101.87	31.45	25.31	1.15	3.32	10.35	0.29
IV	58.74	118.18	59.92	95.67	30.79	23.34	1.01	2.98	9.97	0.29
V	63.13	128.61	65.65	106.26	34.06	21.60	1.21	2.89	10.11	0.27
VI	59.47	125.37	65.88	87.24	26.61	25.87	0.86	2.68	8.95	0.29

DH = days to heading, DM = days to maturity, GFP = grain filling period, PH = plant height (cm), PL = panicle length (cm), PDL = peduncle length (cm), PW = panicle weight (g), GY = grain yield (g/plant), BM = above ground biomass (g/plant), HI = harvest index.

2.4. Discussion

Understanding the existing genetic potential which is highly influenced by the yielding ability of the cultivar could play an important role in designing appropriate use of genetic variations in the breeding program. Thus, assessment of the genetic potential of teff genotypes could improve the breeding efficiency and increase the yield gains of the subsequent selection (Rajaram, 1999). For example, a strong positive correlation between grain yield in irrigated and non-irrigated growing conditions reported in (Hawkesford et al., 2013) suggests yield potential itself determines the farm-gate yield that farmers could harvest. Similarly, our results showed positive correlation between the grain yield under intensive and

field growing conditions ($r = 0.17$, $p < 0.05$). This positive correlation signifies the possibility of the presence of genotypes that perform consistently well under both growing conditions. The grain yield performance of genotypes was elevated from 3.1 g/plant under the field growing condition with conventional management to 6.2 g/plant in the intensive growing condition when lodging was artificially controlled. The mean grain yield performance of genotypes in the field growing condition of this study was higher than the previous reports (Abraha et al., 2016a; Assefa et al., 1999). Compared to the respective improved cultivars included, 30% yield advantage of the top yielding genotypes over the best yielding improved cultivar was observed in the intensive growing condition. However, this advantage has diminished to 10% in the field growing condition with conventional management. This clearly indicates that the actual yield is by far lower than the genetic potential due to poor management practices used and lodging susceptibility. Poor agronomic practices have been mentioned as the major factor that limits improved cultivars to express their maximum genetic ceiling and increased yield gaps in the developing countries (George, 2014). The observed high genetic coefficient of variation, heritability, and the corresponding genetic advance for grain yield and important related traits such as panicle length and weight in this study witnessed the immense genetic diversity and the potentials for further improvement of teff through desirable trait recombination and selection.

Harvest index was higher in the intensive growing condition compared to the field experiment which is in agreement with previous results that prevail harvest index is generally higher in favorable growing environments (Hay, 1995). Similarly, a relatively higher harvest index of teff has been reported when the supplemental irrigation frequency increased in the semi-arid areas of Ethiopia (Araya et al., 2010). Contrary to the present results under field condition, (Assefa et al., 2000) reported lower heritability for harvest index but equivalent genetic advance under selection as percent of the mean. On the other hand, (Ayalew, 2011)

reported lower heritability but higher genetic advance for harvest index. This discrepancy might arise due to differences in the genotypes used. This further emphasizes the need of combined use of both higher heritability and genetic advance during decision making for selection as it is also outlined by (Johnson et al., 1955). The wider range and relative increase of harvest index under the intensive growing condition when lodging being controlled (0.25–0.45) over that of field growing condition with conventional management (0.23–0.34) might indicate the existing genetic potential to improve the harvest index of teff. However, its positive correlation with plant height might complicate the improvement of harvest index in teff due to lodging. In addition, the observed significant positive genotypic and phenotypic correlations of both panicle length and weight with harvest index under the intensive growing condition, but negative significant correlation of the harvest index with panicle length and a negligible correlation with panicle weight under the field growing condition indicates how improved crop management and lodging control options could alter the harvest index.

In a crop–livestock mixed farming system, particularly in the drylands of Ethiopia, crop residues played an important role as the livestock feed (Duncan et al., 2016; Funte et al., 2009). The teff straw is repeatedly reported as a well-known feed resource for animals (Mengistu, 2003; Mottaleb and Rahut, 2018) mainly during the dry season when the availability of pasture is limited and as a supplement during the rainy season when pasture is available (Gebremariam and Belay, 2016). Therefore, with respect to the livelihood of small-scale farmers in Ethiopia, straw yield is as important as grain yield. Despite that fact, however, straw yield is rarely considered a target trait for teff improvement. Taking this into account, our results demonstrated a wide genotypic variability for dry above ground biomass production and straw yield of teff with high heritability and moderate genetic advance. This finding, coupled with the modest but significant positive correlation between aboveground biomass and grain yield under the intensive growing condition, suggests the opportunity to

improve both grain yield and straw yield to a certain extent. This is mainly due to the strong positive correlation of plant height with both grain yield and above ground biomass. Although (Abraha et al., 2016a; Bayable et al., 2020) identified relatively better lodging tolerance in the tall plant stature teff genotypes, this kind of correlation might not be desirable in the practical point of view mainly because an increase in plant height could aggravate lodging severity in most cereal crops (Jost et al., 2015; Madić et al., 2016). Previous reports (Bayable et al., 2020; Teklu and Tefera, 2005) indicated improved teff cultivars are tall in plant height. This might be due to selection for higher grain yield alone without considering the harvest index as an important component of the selection tool kit favored tall genotypes.

Under the current and future unpredictable weather conditions combined with restricted adaptation range of cultivars, growing opportunities of the traditional cultivars of several crop species will be limited. Recently, (Woldeyohannes et al., 2020) pointed out that the suitability of teff cultivation in Ethiopia will decrease due to elevated temperature, causing production to decline in the future. In this regard, adjusting the phenology of the new cultivars would reduce the risk and maximize the yield gain. The genotypic coefficient of variation for days to heading and maturity was relatively low compared to other traits under both the intensive management and field growing conditions. Similarly, lower genetic coefficient of variation for phenological traits have been reported in teff and white lupin (Abraha et al., 2017; Assefa et al., 1999; Atnaf et al., 2017). However, the observed wide range and moderate expected genetic advance with high heritability of days to heading and maturity, and the highest genetic coefficient of variation and genetic advance in grain filling period under the intensive growing condition could suggest the presence of sufficient diversity to develop teff cultivars suitable to the prevailing weather variabilities of the target mega-environments. More specifically, this diversity in teff might enable the development of early maturing cultivars with better yield performance that could adapt better in the dryland areas where rain fall is

erratic and unpredictable. In the Mediterranean environments for example, yield improvement in wheat and barley has been achieved through early cultivar development (Francia et al., 2011; Giunta et al., 2007; Yang et al., 2019)[49–51]. Early maturing cultivars could therefore be used to intensify the agricultural system by adopting double and relay cropping systems. It could also play a significant role in reducing the risk associated with failure of the main crop due to intermittent drought or other environmental hazards as both practices are common in Ethiopia (Molla and Muhie, 2011). The negative correlation between days to heading and harvest index indicates that shortening the vegetative growth period in teff increases the grain filling period which in turn creates a good opportunity to enhance the harvest index provided lodging being controlled. The strong positive correlation of the grain filling period with both grain yield and harvest index in the intensive growing condition implies grain yield of teff could be maximized by selecting early heading but late maturing genotypes in suitable environments where rainfall is sufficient or irrigation water is available.

The important features of conventional breeding are the identification, creation, and exploitation of heritable genetic variations for desirable traits. The common way of doing this is assessing the diversity within the landrace collections of a species. Multivariate analysis, such as clustering and principal component analysis, of a large number of genotypes could provide ample information about which genotypes to select as a potential parental line for recombination. The cluster analysis in this study grouped the teff genotypes into seven and six clusters under the intensive and field growing conditions, respectively. In both growing conditions, high yielding genotypes were tall in plant stature, late maturing, and had relatively higher harvest index. Similarly, (Abraha et al., 2016a) reported high yielding teff genotypes were associated with tall plant height and late maturity. The current results demonstrated the presence of wide genetic diversity in the yielding potential of teff.

In conclusion, the coefficient of genetic variation, heritability, and expected genetic advance for grain yield were the highest in both experiments. Among the phenological traits, the grain filling period in the intensive growing condition exceptionally showed the highest genetic coefficient of variation, heritability, and genetic advance. This signifies the presence of immense genotypic variation for important traits. The high grain yield performance and wider range of the harvest index observed under the intensive growing condition further indicates the extent of genetic potential available in the teff germplasm collections. However, to exploit the genetic potential and narrow the yield gaps, improving the lodging resistance and thereby increasing the harvest index of teff should come to the front page.

CHAPTER 3

3. Biomechanical properties and agro-morphological traits for improved lodging resistance in Ethiopian teff (*Eragrostis tef* (Zucc.) Trotter) accessions

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3.1. Introduction

Teff [*Eragrostis tef* (Zucc.) Trotter] is a traditional, culturally important indigenous cereal food crop in Ethiopia, where it is eaten by half of the population at least once a day (Gebre et al., 2012; Jost et al., 2015; Mohammed et al., 2019). Besides its nutritional importance, teff is known for its ability to adapt to extreme conditions, including in areas prone to waterlogging or drought, where most cereal crops might fail. In Ethiopia, where 67% of the total area is regarded as dryland and climate variability affects crop production (Haregeweyn et al., 2015), teff continues to be the main economic crop of small-scale farmers. Teff has been cultivated in some parts of the world for livestock feed since the Royal Botanical Garden, Kew, distributed in the late 19th century from Ethiopia (Burtt-Davy, 1917; Costanza et al., 1979), its place of origin and the center of diversity. In Ethiopia, however, it has been cultivated for human food for centuries (D'Andrea, 2008; Ebba, 1975b). Today, as a result of the Ethiopian diaspora in different parts of the world and because it is both nutritious and gluten free, other consumers have become interested in teff. As a result, it has become an important agricultural commodity for export in Ethiopia (Lee, 2019). The increased export demand coupled with low crop productivity, however, has raised the market price of teff beyond the purchasing capacity of local consumers. Consequently, the Ethiopian government was forced to temporarily suspend the export of teff in 2006. This situation has led to the realization that unless there is a significant productivity boost, teff will cease to be the staple food of many Ethiopians.

The average nationwide teff yield in Ethiopia is only about 1.76 t/ha (CSA, 2019a), but it has been estimated that if lodging can be prevented, the yield might rise to 4.6 t/ha (Assefa et al., 2001a; Teklu and Tefera, 2005). Lodging, the irreversible displacement of a plant stem from its vertical due to a weak culm and/or to poor root system anchorage, which can be aggravated by winds, rain or hail (Pinthus, 1974), is a major factor limiting yield and quality

(Gebre et al., 2013; Jost et al., 2015; Tadesse, 1993). In Ethiopia, lodging occurs in teff fields every year regardless of the weather conditions and causes estimated yield losses of up to 25% (Tefera and Ketema, 2000) and can be greater than 50% in the worst cases (Bennetzen et al., 2009). Lodging not only lowers the yield directly but also leads to the deterioration of the quality of the crop. When rain falls during the crop's maturity growth stage, it can cause premature seed sprouting and mold growth. In addition, fear of lodging that hindered farmers in applying optimum fertilizers is a historic drawback on the efforts to maximize grain yield.

Lodging is not usually a one-time event during the growing season; rather, the crop is likely to suffer several cycles of oscillation associated with rainstorms or strong winds (Chauhan et al., 2020). It is of two types: lodging caused by anchorage failure or root lodging; and lodging caused by stem mechanical failure also known as buckling or stem lodging (Berry et al., 2006). Stem lodging can also be defined as breaking and bending type (Hirano et al., 2014). Lodging can occur at various crop growth stages, but it occurs most often between the panicle emergence and grain filling stages. Its economic effect also varies with the time of occurrence; the earlier it occurs, the higher the economic loss (Acreche and Slafer, 2011). The timing and severity of lodging depend on several factors, including plant population density, cultivar type, amount and timing of N fertilizer application, and overall crop management.

Higher N fertilizer application can lead to slender internodes, thin stem walls, and increased plant height, all of which decrease stem strength, thus increasing the lodging risk (Zhang et al., 2017). Similarly, (Wu et al., 2012; Wu et al., 2019) indicated a reduced lodging resistance with increasing N rates in spring wheat and rice. However, N optimization has been shown to reduce the total length of the lower internodes and to increase culm diameter, culm wall thickness, and breaking resistance in rice (Pan et al., 2019). Other studies in wheat

have reported greater lodging severity in early sown at a high rate with high N than in late sown at a low rate with low N (Griffin, 1998). A lower sowing rate has also been shown to reduce lodging susceptibility in teff (Ben-Zeev et al., 2020). Genotypic differences in stem mechanical strength and lodging-related morphological traits have been reported in wheat (Kelbert et al., 2004), rice (Islam et al., 2007), and barley (Chen et al., 2014). As these studies show, lodging is a complex phenomenon that is influenced by the environment, agronomic practices, genetic factors, and their interactions, and it is a major production constraint on cereal crops worldwide (Berry et al., 2004b; Chen et al., 2014; Miller et al., 2016). Moreover, there is no clear consensus among studies as to whether root or stem lodging predominates in cereal crops. For example, (Berry et al., 2003) investigated root lodging, whereas (Sterling et al., 2003) observed stem lodging as a dominant type. A study of lodging in two cultivars of teff grown in sandy soils found root lodging to be predominant, but suggested the need of improvements in both the shoot strength and root anchorage (van Delden et al., 2010).

Previous efforts to curb lodging in major cereal crops have focused on reducing the plant height by pyramiding semi-dwarfing genes into high-yielding cultivars and applying plant growth regulators. During the Green Revolution of the 1960s, the yield potential of wheat and rice was boosted significantly following the introgression of semi-dwarfing genes: reduced height-1 (*Rht-B1* and *Rht-D1*) in wheat and semi-dwarf-1 (*sd-1*) in rice (Spielmeyer et al., 2002). The mechanism was an increase in culm strength, which was associated with decreased plant height, along with a larger spike or panicle and more seeds per unit area (Fischer and Stockman, 1986). Modern wheat and rice cultivars having these semi-dwarf genes also have a higher response to inputs, including fertilizers, and more efficient assimilation of photosynthates into seeds instead of vegetative growth and, therefore, a higher harvest index (Evenson and Gollin, 2003).

In recent decades, similar attempts to introduce dwarfing genes to increase lodging resistance have been carried out in teff. These efforts have centered on mutation breeding, in particular the use of physical mutagens and the TILLING technique (Assefa et al., 2011; Tadele, 2019). This approach has been used mainly because teff is chasmogamous and completely self-pollinated with a microscopic size of the florets that makes emasculation and pollination very difficult and hinders the establishment of sufficient segregating populations. A reverse genetics approach to explore the presence and functioning of the Green Revolution genes has been also considered (Smith et al., 2012). Through the application of these breeding techniques, a number of dwarf and semi-dwarf teff accessions have been identified and evaluated, but no lodging-resistant cultivars have been identified. A comparison of genetic improvement related trait changes in teff and wheat (Assefa et al., 2011) confirmed that the aforementioned efforts have not improved the harvest index of teff and reduced its susceptibility to lodging. This conventional approach of introducing a dwarfing gene through mutation breeding to increase lodging resistance seem unlikely to produce a semi-dwarf cultivar with sufficient lodging resistance in the near future.

Another potential strategy is to breed accessions to increase the mechanical strength of the plant stems (Miller et al., 2016). Currently, breeding programs for various model crops are seeking to produce lodging resistance in plants with long culm stature. In rice, for example, a recombinant inbred line with long culm stature along with high grain yield and lodging resistance has been developed (Nomura et al., 2019). Two approaches to characterize the biomechanical properties of cereals have been suggested and widely utilized. The safety factor approach where the self-weight moment of the stem could be determined using the angle of inclination from the vertical, mass of the above ground plant part, height from center of gravity, and the acceleration due to gravity (Crook and Ennos, 1994; van Delden et al., 2010; Wu et al., 2020). This approach has not considered the main lodging factors such as the

rainfall, wind speed and the turbulence induced natural frequency of the environment. In contrast the modeling approach developed by (Baker et al., 1998) considered both the external environmental factors and the geometry of the plant culm particularly the section modulus. Although the former gets attention in recent studies, in reality it is not only the self-weight moment, but also the weather conditions are significantly important factors to lodging (Wu and Ma, 2018). In addition, from practical point of view we found the point of bending along the culm were variable in the teff accessions included in this study which makes measurement of the angle of inclination at similar position difficult.

The objectives of this study were therefore, to examine the variability of stem mechanical properties and agro-morphological traits, together with trait associations with lodging, in landrace accessions of teff, and then to infer possible selection criteria for developing lodging-resistant cultivars. We expect the findings of this study to be a useful resource for related biomechanical studies and for developing future strategies for improving lodging resistance in teff.

3.2. Materials and Methods

3.2.1. Description of the study sites

This study was carried out during the main cropping seasons in 2018 and 2019 at Adet (11°28′N, 37°48′E; 2216 m a.s.l) and Bichena (10°46′N, 38°19′E; 2541 m a.s.l) experimental stations, operated by the Adet Agricultural Research Center, northwestern Ethiopia. Adet and Bichena are located 42 and 222 km, respectively, southwest of Bahir Dar city. Average maximum daily temperatures during the growing period (July to December) of the study years were similar at the two stations, but average minimum daily temperatures differed between the stations, particularly during the early crop growth stages (July to September) (Figure 3.1). The rainfall distribution pattern which is a unimodal mostly occur

from June to September varied considerably between them. The sites also differed in soil type and soil physicochemical properties (Table 3.1). At Adet, the soil is a well-drained brown Nitosol, whereas at Bichena, the soil is a black Vertisol with a high water-holding capacity and poor drainage. The study locations are, therefore, distinct and represent the two mega-teff growing environments of the northwestern part of the country.

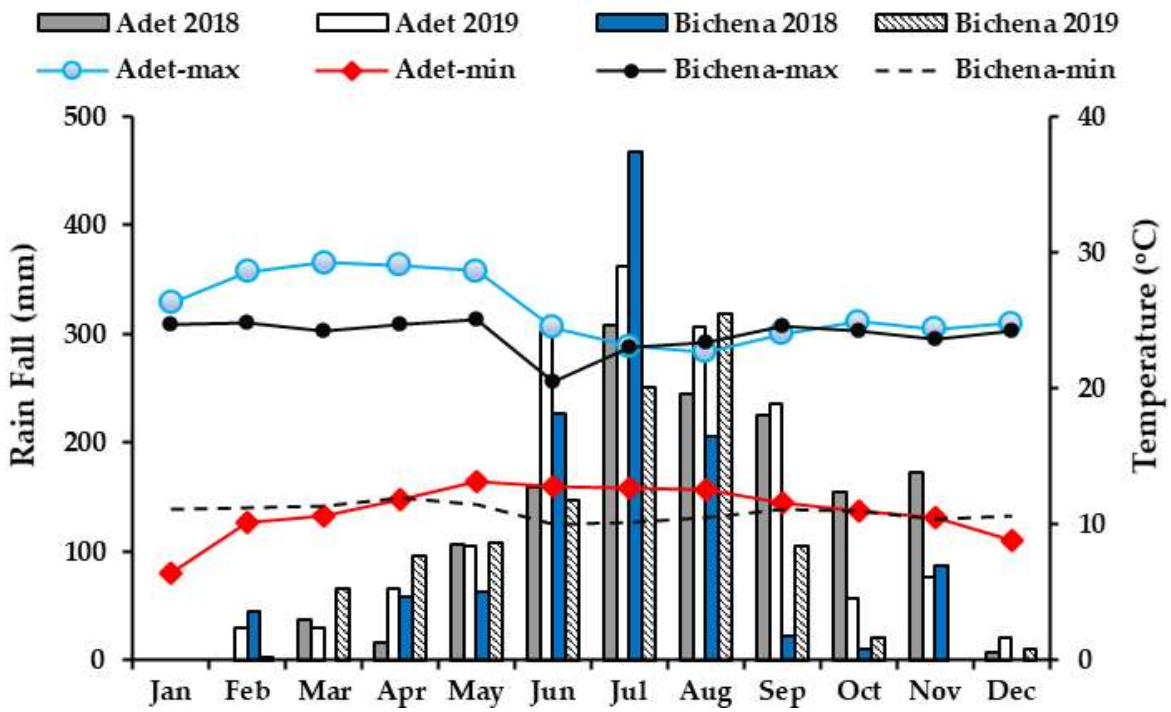

Figure 3-1: Monthly rainfall distribution (bars), maximum daily average temperature (max), and minimum daily average temperature (min) at the Adet and Bichena experimental stations during 2018 and 2019. The temperature data shown for each station are the averaged values of the two years. These data were provided by the Ethiopian Meteorology Agency, Bahir Dar region.

Table 3-1: Soil chemical properties of the test sites in the top 0 – 15 cm depth.

Study site	pH	EC* (dS/m)	Total N (g/kg)	Available P (mg/kg)	Available K (mg/kg)	Available Mn (mg/kg)	Available S (mg/kg)
Adet	5.17	0.07	1.57	27.69	0.88	78.12	48.09
Bichena	5.85	0.08	1.04	35.23	1.34	31.52	31.88

*Electrical conductivity of the soils

3.2.2. Population panels and design

The population panels included in this study were accessed from Adet and Debre-Zeit Agricultural Research Center teff breeding programs and from the Ethiopian Biodiversity Institute. Prior to the establishment of the field experiment, the true to types of each genotype were maintained in two consecutive cycles of head to row selection. A total of 320 teff genotypes (mostly landrace accessions, except for six improved cultivars: Tsedey, Dega-teff, Etsub, Quncho, Abola and Cora) were planted in an 8 × 40 alpha lattice design with two replications. Each plot was 2 m long and 0.6 m wide and composed of three rows with 0.2 m between rows. Seeds were sown in each row by hand drilling and were not covered over with soil. Three weeks after seedling emergence, thinning and transplanting were carried out to maintain an 8-cm spacing between plants, with two seedlings in each hill. Then the rows were covered with soil. Teff was planted in mid-July at Adet station and in the first week of August at Bichena station in both years, following the local farming practice. N, P, and S fertilizers were applied in the form of urea (46% N) and NPS (19% N, 38% P, 7% S). N was applied at 64.5 kg/ha at Adet and 87.5 kg/ha at Bichena. Equal rates of P (60 kg/ha) and S (11 kg/ha) were applied at the two stations. All of the P and S and half of the N were applied at planting, and the remaining half of the N was applied at the tillering stage. Harvesting was done in the third week of November and December for Adet and Bichena stations, respectively.

3.2.3. Measurements and data collection

Data of agro-morphological traits and biomechanical properties were collected timely during the growing seasons following standard methods and procedures. Data were collected on either a plot or a plant basis. Plant height, panicle length, peduncle length, tiller number per plant, and panicle weight were determined on a plant basis from five representative plant samples collected from each plot. Internode diameter, wall thickness and the biomechanical

properties (the internode breaking strength - the maximum force that the internode withstands before it failed) were measured using the same five samples. Pushing resistance, another biomechanical property, were measured at the heading stage of the crop. Grain yield was determined on a plot basis: each plot was harvested at physiological maturity, and then the crop was allowed to dry for a week in the sun before being threshed.

Visual lodging score and lodging index: The visual lodging score was determined on a plot basis by the method outlined by (Caldicott and NUTTALL, 1979). In this method, a score from 0 to 5 is assigned on the basis of the angle of lodging (leaning) from the vertical (0 = no lodging, with all plants upright; 5 = completely lodged, with all plants prostrate) and the percentage of plants in the plot exhibiting each degree of leaning. The index was then calculated as the $\sum$ (lodging score × % severity)/5.

Pushing resistance: Five hills were selected randomly from each plot when the crop was at the heading stage, and the pushing resistance of the whole plant, including tillers, was measured at a height of 20 cm above the ground with a prostrate tester (Daiki Rika Kogyo Co. Tokyo, Japan – model: DIK-7401). The device was positioned perpendicular to the plant stem at the height described above and pushed until the stem was bent 45° from the vertical position. The detailed procedures followed are outlined elsewhere (Hai et al., 2005; Kashiwagi and Ishimaru, 2004; Wu and Ma, 2016). The measurement was recorded in millimeters using the white spring (9.8 N/40 mm) and then converted to units of force by referring to the conversion chart provided by the company.

Culm breaking strength and bending moment: Samples of healthy culms were collected from each plot when the crop was near to physiological maturity. Each accession was sampled as it approached its maturity stage. Leaves and leaf sheaths were trimmed, and each internode was separated carefully. A flat smooth wooden table with two nails was used for a three-point

bending test. The two nails were hammered a short way into the table top, 8 cm apart test internodes that were ≥10 cm long and 6 cm apart for internodes that were <10 cm long. The internode was laid across the nail heads and then a digital force gauge was used to apply a downward force at its center. The maximum force (F_{max}) that the internode can withstand before it failed was recorded. Base on this value, the second basal internode breaking strength was calculated as $S_S = F_{max} \times L/4$, where L is the distance between the supporting points as described in (Crook and Ennos, 1996; Wu and Ma, 2016). The outer diameter and wall thickness of each internode were measured with a digital caliper of 0.01 mm accuracy. It is obvious that the plant culm deviates from a perfect hollow circular cross section, which is usually oval. The outer diameter was then measured both in the major and minor radius at the mid-point of the internode and the average was used for the downstream calculation. Since accessions with thin culm were included in the study, direct measurement of the inner diameter was not appropriate. Instead, the internodes were dissected vertically with razor blade and the wall thickness was measured along the groove. The difference between the average outer diameter and the wall thickness was considered as the inner diameter. The maximum second basal internode bending stress (σ) usually used to define the material strength was determined following equation 1 by simplifying the ratio of breaking strength to section modulus as describe elsewhere (Berry et al., 2006; Wu and Ma, 2016). The geometry of the internode: second moment of area (I), and section modulus (SM) was computed using the standard mechanical equations 2 and 3 respectively. Failure moment (BS) of the second basal internode were calculated as described by (Baker et al., 1998) and later used by (Berry et al., 2006; Miller et al., 2016) using equation 4 under the assumption that the teff stem had a hollow circular cross section:

$$\sigma = \frac{L * R * F_{max}}{(R^4 - r^4)} \quad \text{----------(1)}$$

$$I = \frac{\pi(R^4 - r^4)}{4} \text{----------}(2)$$

$$SM = \frac{\pi(R^4 - r^4)}{4R} \text{----------}(3)$$

$$BS = \frac{\sigma\pi R^3}{4}\left(1 - \left(\frac{R-t}{R}\right)^4\right) \text{----------}(4)$$

where F_{max} is the maximum force that the internode could withstand before it broke or failed, L is the distance between the supports (nails), I is the second moment of area, t is the internode wall thickness, and R and r are the outer and inner radius, respectively, of the internode.

3.2.4. Root characterization

To characterize the roots, fifteen selected accessions were planted based on the first-year field phenotyping results. Plant height, panicle weight and lodging response were the main selection criterion and contrasting accessions were included in the study. A 1 m long and 20 cm in diameter PVC tubes were prepared by dissecting vertically and fastened tightly with a wire rod at two positions. The tubes were then buried vertically in a 90 cm depth pit prepared under a screen house. Uniformly mixed soils of Nitosol and sand in a 3:1 Ratio were filled into each PVC and slight compaction with sticks was made. Seedlings were grown in small pots separately and two three-week old seedlings were then transplanted into each PVC tube. N, P, and S fertilizers were applied using the optimum recommendation rates of Adet experimental stations as described above. Irrigation was done every day early in the morning. During the maturity stage of the crop, the above ground biomass was harvested and roots was carefully washed with water using "GARDENA" Comfort Multi Sprayer.

3.2.5. Data analysis

Analysis of variance and mean comparisons were performed using the Proc ANOVA model of the alpha lattice design in the Statistical Analysis System software program (SAS, 2005). The statistical significance of the mean differences was computed by using the Tukey

test; $p < 0.05$ was considered significant. To identify traits that the future breeding program has to focus, multiple linear regression and principal component analysis (PCA) were performed. The PCA was determined using the Statistical Tools for Agricultural Research (STAR) (IRRI, 2014). Statistical Package for Social Sciences (SPSS v.16) was also used for multicollinearity test and multiple linear regression analysis. The Pearson correlation coefficients and its significant test between all the possible pair of the biomechanical properties and agro-morphological traits was determined following the Proc CORR procedure of SAS. All charts were computed using the excel spreadsheet of Microsoft Office v.16. It is worthy to mention that the crop performance during the 2019 season at Bichena station was generally stunted and measurements on the biomechanical properties was not done intending this might mislead the analysis and inference of the results. Data collected over 2 years at Adet and 1 year at Bichena stations were therefore used for the analyses.

3.3. Results

3.3.1. Agro-morphological trait variability

All traits showed significant differences among the tested genotypes (Table 3.2). Panicle + peduncle length accounted for 50%–67% of the total plant height, with a mean of 59%. The panicle length to culm ratio ranged from 0.31 to 0.70, with an average of 0.51. Thus, panicle length accounted for 51% of the culm length and 34% of the total plant height. Peduncle length accounted for nearly 38% of the culm length (range, 21%–54%) and for about 26% of the total plant height (Table 3.3). The internode diameter, which was largest at the base of the plant and decreased upward, was significantly different within and between environments. It was generally larger at Adet than at Bichena, and also found to be higher in 2018 than in 2019 (Figure 3.2). Plant height and grain yield were greater at Adet than at Bichena. The lodging index based on visual score was generally lower at Bichena than at Adet during both years.

Tiller number, one of the most important traits that influence both grain yield and lodging resistance exhibited a wider range among the accessions. It ranged from 9 to 28 with the highest values observed on dwarf cultivars. Peduncle length exhibited the highest genotypic variability among the traits. In most of the cases, the longer the peduncle the shorter the panicle length was observed. Panicle weight and panicle length also varied significantly among the accession in all the environments (Table A5). Although for some traits the interaction of genotype by environment was significant, its contribution to the total sum of squares was minimal (Table 3.2). However, genotypic ranks for plant height, panicle length, internode diameter, and panicle weight were consistent across the environments.

Table 3-2: Mean squares and statistical significance test of the traits based on combined analysis of variance.

Trait	Sources of Variation[1]					% Explained[2]	CV (%)
	G	E	G × E	R	B(R)		
Plant height	759.4**	85122.3**	13.3 ns	3731.9**	1512.9**	1.7 (49.4)	6.8
Panicle length	194.5**	24600.8**	15.1**	406.2**	236.3**	7.6 (49.2)	3.7
Culm length	304.0**	18103.6**	22.6**	6263.6**	654.7**	8.1 (54.4)	6.1
Peduncle length	78.7**	2421.1**	0.237 ns	587.5**	102.3**	0.43 (70.8)	7.4
Tiller number	42.8**	1390.2**	11.2**	122.7**	25.2**	23.4 (44.9)	18.1
Panicle weight	0.404**	47.8**	0.099**	0.139 ns	0.346**	18.5 (37.7)	20.8
Grain yield	1.99**	144.3**	0.026 ns	1.29**	2.64**	1.6 (62.3)	9.6
Lodging index	1352.5**	18091.2**	519.7**	4.31 ns	885.0**	34.4 (44.8)	18.4
Diameter	0.819**	29.7**	0.043 ns	17.2**	1.39**	6.5 (61.4)	8.4
Pushing resistance	92.25**	16066.3**	9.26ns	1838.5**	251.5**	5.0 (49.7)	18.7
2nd moment of area	4.5**	146.4**	0.27**	89.0**	9.8**	7.6 (63.7)	25.7
Section modulus	1.35**	50.5**	0.061**	27.8**	2.82**	5.6 (63.4)	19.9
Base failure moment	0.027**	1.94**	0.0006 ns	0.51**	0.055**	2.57 (58.4)	11.8

[1] G = genotype, E = environment, G × E = genotype by environment interaction, R = replication, B(R) = block within replications, CV = coefficient of variation., ns = non-significant, $^{**}P < 0.01$.

[2] Percent explained by genotype by environment interaction (values outside the parenthesis) and genotype (values in parenthesis) from the total sum of squares.

Table 3-3: Descriptive statistics of agro-morphological traits and biomechanical properties of tested teff accessions based on mean values of data from the three environments (location × year combination).

Variable	Minimum	Maximum	Mean	SD*
Plant height (cm)	74.00	135.00	100.17	11.22
Panicle length (cm)	19.40	51.57	33.87	5.69
Culm length (cm)	49.10	87.17	66.30	7.12
Peduncle length (cm)	14.10	35.23	25.26	3.62
Tiller number per plant	8.57	28.30	14.35	2.67
Panicle weight (g)	0.46	2.20	1.09	0.26
Grain yield (t/ha)	0.89	3.52	2.18	0.58
Lodging index (%)	22.00	100.00	65.54	16.09
2nd basal internode diameter (mm)	1.51	3.89	2.47	0.37
Pushing resistance at heading (N)	1.59	7.47	3.85	1.18
2nd moment of area (10^{-12} m^4)	0.15	6.31	1.44	0.87
Section modulus (10^{-9} m^3)	0.19	3.27	1.06	0.47
Base failure moment (Nm)	0.03	0.25	0.11	0.03
Culm length to plant height ratio	0.59	0.76	0.66	0.03
Panicle + peduncle length to height ratio	0.50	0.67	0.59	0.03
Peduncle length to plant height ratio	0.13	0.41	0.26	0.05
Panicle length to plant height ratio	0.24	0.41	0.34	0.03
Panicle length to culm length ratio	0.31	0.70	0.51	0.07
Peduncle length to culm length ratio	0.21	0.54	0.38	0.06

* SD: standard deviation, abbreviations in parenthesis are SI units (cm = centimeter, g = gram, t/ha = ton/hectare, mm = millimeter, N = Newton, m = meter, Nm = Newton meter).

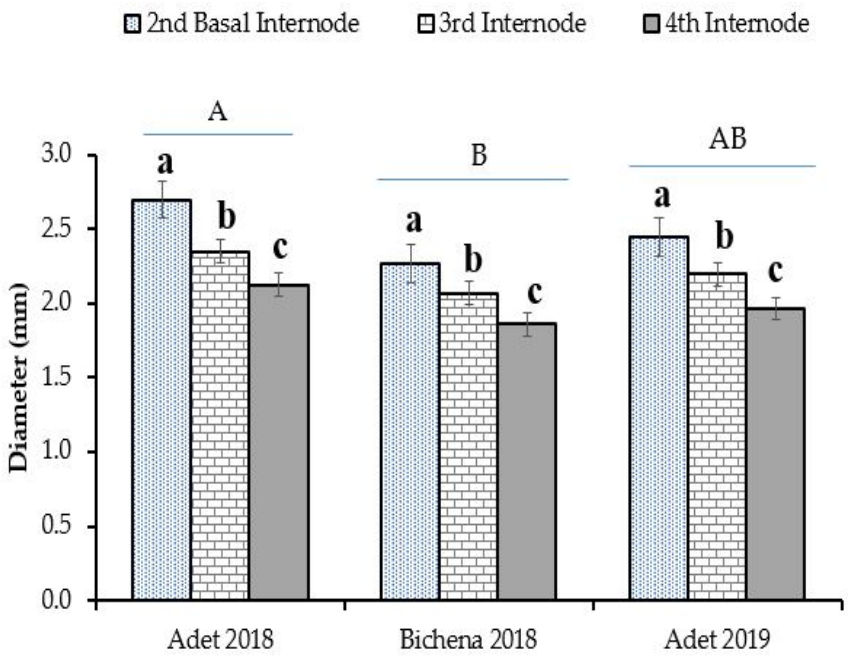

Figure 3-2: Internode and culm diameter trends across locations and years and along plants (internodes were counted from the ground upward). The same capital letters above bars indicates no significant differences ($p < 0.05$) among environments for the average culm outer diameter, and small letters indicates the significant difference between internode outer diameter within a culm.

3.3.2. Comparison of genotypes

We classified the population panels into four groups (dwarf, semi-dwarf, tall, and very tall) according to plant height, with the improved cultivars included as a fifth group (control), and then compared for important traits among these groups (**Figure 3.3**). The mean values of the biomechanical properties and agro-morphological traits of the improved cultivars were generally equivalent to those of the tall group, except that the grain yield of the control group was similar to that of the very tall group. In most traits, the mean values of the dwarf and semi-dwarf accession groups were lowest, except for tiller number.

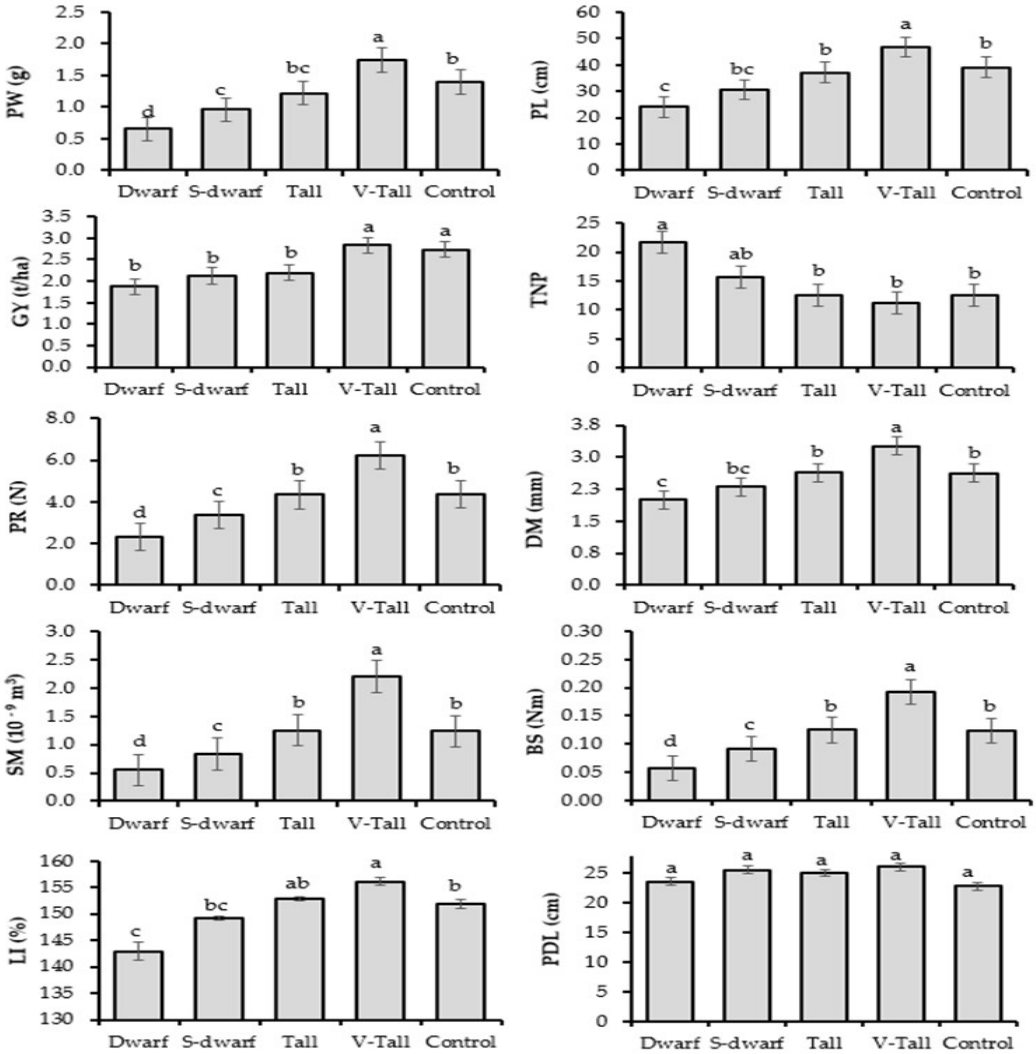

Figure 3-3: Comparison of genotypes by height group: Dwarf, ≤80 cm; Semi-dwarf (S-dwarf), 81–100 cm; Tall, 101–120 cm; Very Tall (V-Tall), ≥121 cm. The Control group comprises the averaged values of the six improved cultivars. Error bars indicate standard error. The same letter above bars indicates no significant differences between the groups ($p < 0.05$). The measured or calculated traits are panicle weight (PW), panicle length (PL), grain yield (GY), tiller number per plant (NTP), pushing resistance (PR), second basal internode diameter (DM), section modulus (SM), base failure moment (BS), Lodging index (LI), and Peduncle length (PDL).

Mean values of base failure moment, section modulus, and pushing resistance were highest in the very tall group (Figure 3.3). In general, tall genotypes were characterized by a larger internode diameter, longer panicle, heavier panicle weight, and higher grain yield than the dwarf genotypes. Even though some accessions in the very tall group were highly susceptible

to lodging, this group also included accessions identified as having both relatively high lodging resistance and good yield potential. In particular, accessions 123 (203010-4), and 149 (55114-4) in the very tall group and accession 7 (234430-1) in the tall group were characterized by high internode diameter and base failure moment values, a low tiller number, a smaller lodging score, and a good yield potential.

3.3.3. Mechanical properties and lodging index

We evaluated the plant base failure moment, section modulus, and pushing resistance of the stem (see section 2.3). The ANOVA results showed a significant difference among the genotypes in plant base failure moment, second moment of area, and the section modulus (Tables 3.2). Plant base failure moment ranged from 0.06 to 0.50 Nm with a mean value of 0.22 Nm. Pushing resistance ranged from 1.59 to 7.47 N (mean, 3.85 N), second moment of area from 0.15 to 6.3 × 10–12 m4 (mean, 1.4 × 10–12 m^4), and section modulus values from 0.19 to 3.27 × 10–9 m^3 (mean, 1.06 × 10–9 m^3).

The lodging index based on visual score was generally smaller at Bichena than at Adet during both years. It ranged from 22% to 100% at Adet and from 12.5% to 100% at Bichena. Accessions 123 and 149 had the lowest values. Accession 123 had a loose panicle form and a relatively high standing ability, but the lateral growth pattern of the tillers led to some bending (Figure 3.4D, E, F). In contrast, accession 149 had a compact panicle form and the lower stem had good standing ability, but breaking occurred at the neck or peduncle (Figure 3.4A, B, C). Both accessions had deep roots and a high root length density (data not shown). Generally, most accessions showed bending-type stem lodging, although some exhibited breaking-type stem lodging (Figure 3.4G, H, J).

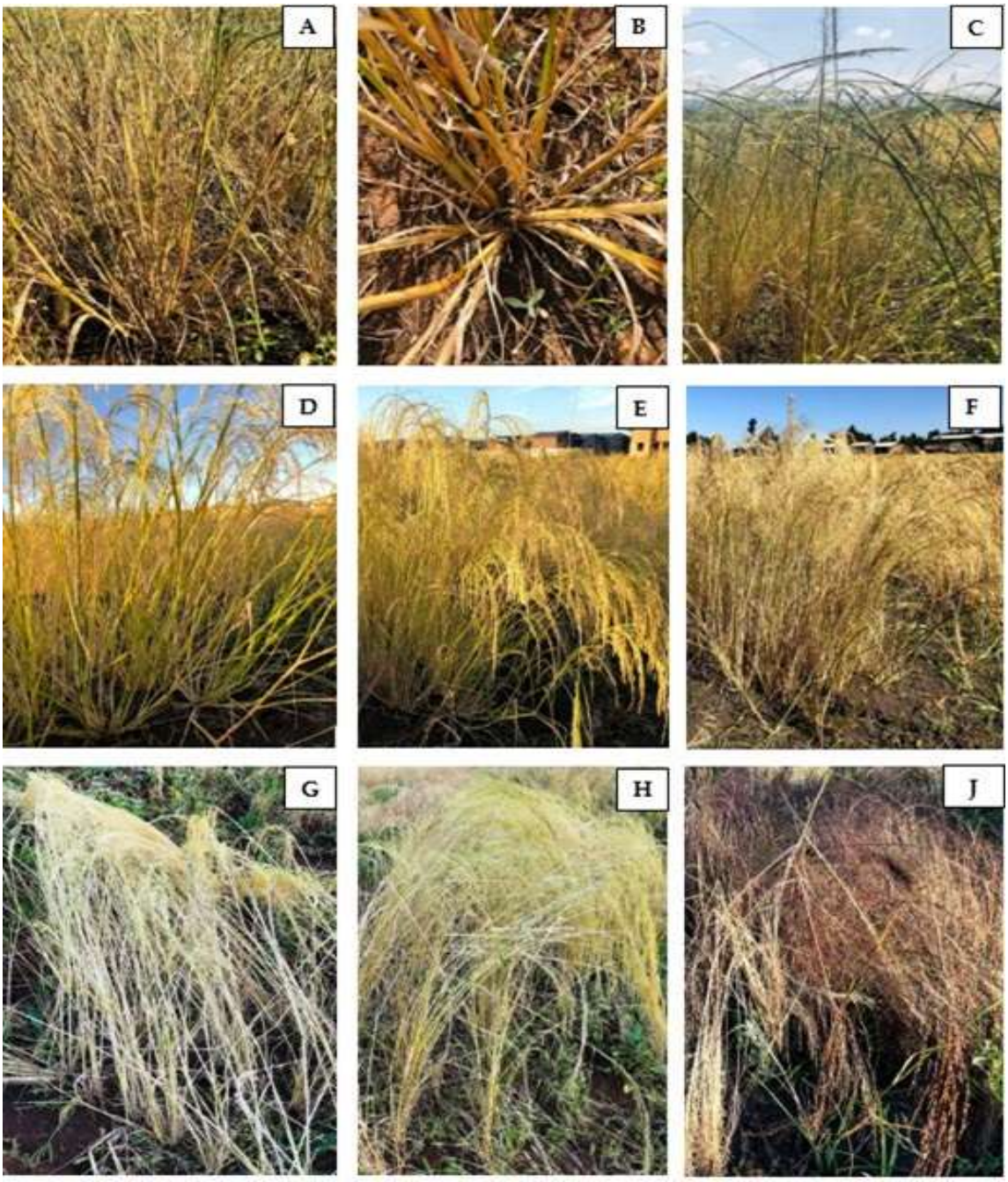

Figure 3-4: Lodging in selected teff accessions. (A–C) Accession 149 (55114-4). (E–G) Accession 123 (203010-4). This accession has relatively good lodging resistance but lateral tiller growth. Both (G) accession 199 (RILL-260) and (H) accession 201 (RIL-2) were susceptible to bending-type lodging. (J) Accession 63 (235368-1), with typical breaking-type stem lodging.

3.3.4. Trait associations

The relationships of plant height with pushing resistance during heading, plant base failure moment, the section modulus and Lodging index were linear and positive, with R^2 values of

0.39, 0.55, 0.44, and 0.46 respectively (Figure 3.5a). Similarly, the relationships of the section modulus with plant base failure moment, pushing resistance, and panicle weight were positive and significant (Figure 3.5b). The association of plant height and section modulus with lodging index is generally significantly positive. However, the association between section modulus and lodging index also shows a negative relationship for some sub-populations. The slope of the relationship between base failure moment and the section modulus (the maximum bending stress, an indication of the of material strength) differed significantly among the genotypes tested. Stem diameter, one of the traits used to characterize lodging, was positively related to plant height ($r = 0.66$, $p < 0.01$). Plant height, internode diameter, panicle weight, and panicle length correlated positively with each other and with the internode mechanical properties. The tiller number per plant, however, was negatively correlated with most of the traits, including the internode mechanical properties except the maximum stress of the internode (Figure 3.5c and Table A6).

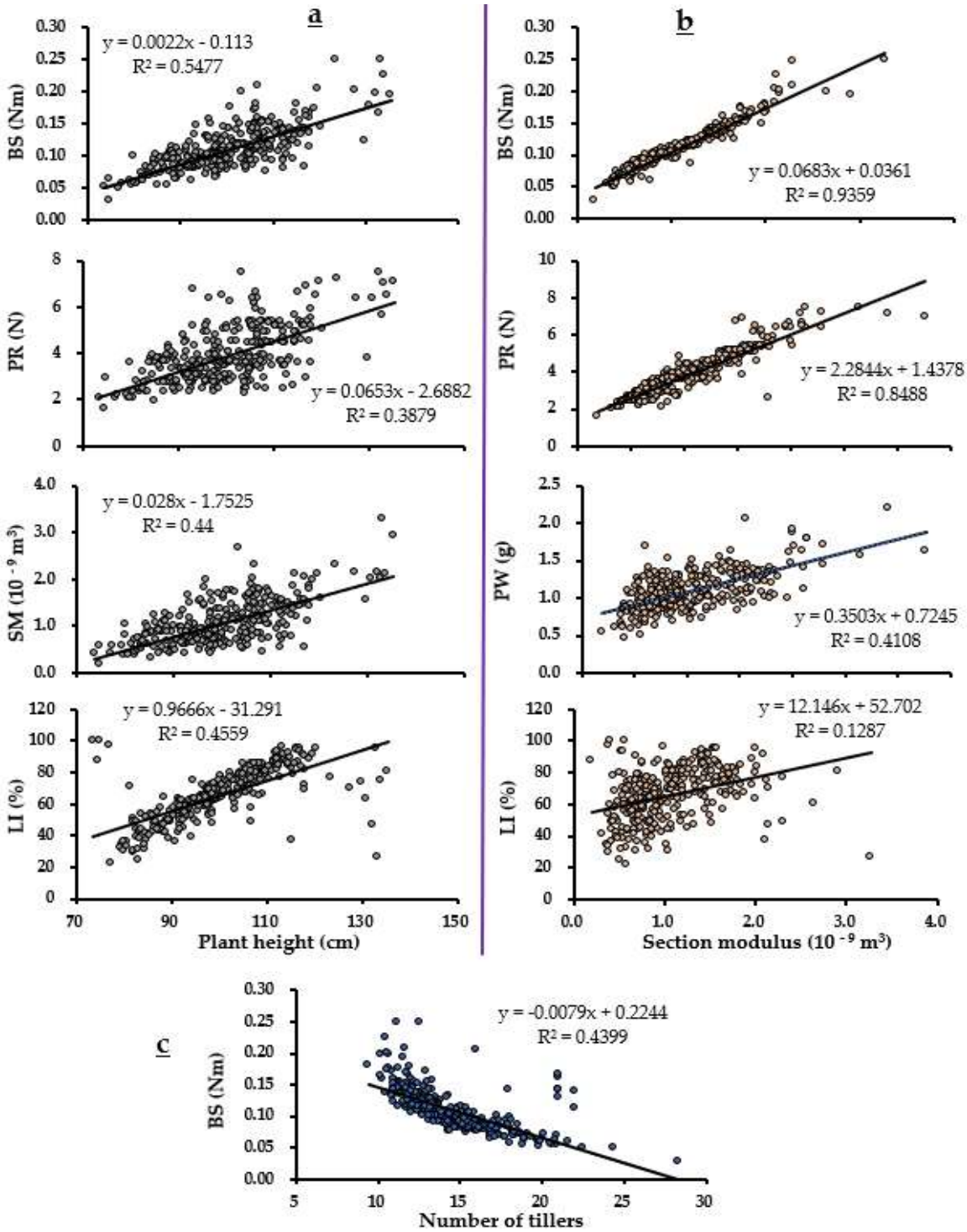

Figure 3-5: Bivariate analysis showing the relationship of the traits (320 accessions): a, the relationship of plant height with lodging index (LI), Section modulus (SM), pushing resistance (PR) and base failure moment (BS); b, the relationship of section modulus with lodging index (LI), panicle weight (PW), pushing resistance and base failure moment.

3.3.5. Root characteristics

The root characterization results showed significant differences in root system depth, root depth density, and the root to shoot ratio among the selected accessions. Despite the general consensus that teff has a shallow root system, root system depth and the root to shoot ratio were high in these accessions. Root system depth ranged from 56.7 to 105.3 cm, with a mean of 92.5 cm. The vertical root growth was proportional to the aboveground growth. Plant height ranged from 68 to 130 cm, with a mean of 106 cm which was comparable with the field observations. The root system depth and root depth density of the dwarf accessions were lower (Figure 3.6b2, b3) than those of the tall accessions and the standard cultivar Quncho (Figure 3.6b1, b4, b5). The thickness of individual roots was not measured, but they were very thin and abundant and distributed throughout the volume of the PVC tubes (Figure 3.6a).

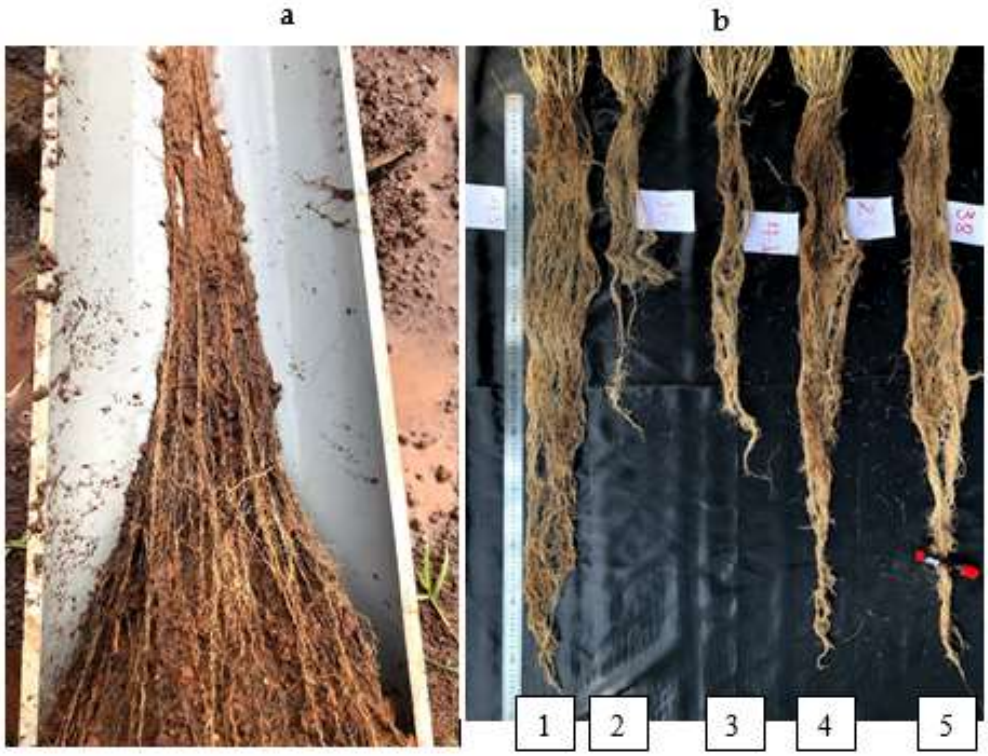

Figure 3- 6: Root systems of selected teff accessions after the PVC experiment. (a) Accession 149 during washing. (b) Roots of the selected accessions after drying: 1 = 149 (55114-4), 2 = 30 (234431-1), 3 = 116 (204596-2), 4 = 202 ('Quncho), 5 = 123 (203010-4).

3.3.6. Trait contributions to lodging

Plant base failure moment was among the parameters used to assess the mechanical properties of the plant stem. We carried out a multiple linear regression analysis with that as the dependent variable. Because of multicollinearity, most traits were excluded from the model. The results showed that pushing resistance at the heading stage, plant height, tiller number, and panicle weight were significantly contributed to the observed variation in base failure moment and together explained about 92% of the variance. Pushing resistance at the heading stage and plant height had the largest positive effect, and tiller number had a significant negative effect, on plant base failure moment (Table 3.4).

Table 3-4: Multiple linear regression results with base failure moment as the dependent variable.

Variables	**Coefficient**	**Std. Error**	***t*-statistic**	***P***
Constant	0.052	0.147	0.354	0.724
Plant height	0.143	0.001	4.592	0.000
Tiller number	–0.141	0.004	–4.615	0.000
Panicle weight	0.057	0.039	2.161	0.031
Pushing resistance	0.709	0.008	29.96	0.000
$\boldsymbol{R^2}$	0.922	Residual mean square		0.012
Adjusted R^2	0.921	*F*-statistic		930.1
Std. error of regression	0.108	Probability (*F*-statistic)		0.000

Similarly, the PCA (Figure 3.7) found pushing resistance, diameter, section modulus, base failure moment, panicle weight and plant height having the highest positive loading on the first component (PC1). The mechanical properties - pushing resistance, section modulus, and base failure moment - clustered with the second basal internode diameter which is indicated by the circle in the PCA plot. Accessions 201 (RIL-2), 123 (203010-4), 232 (237703-4), 20 (237707-

1), and 149 (55114-4) had high positive loadings on PC1. In contrast, tiller number (TNP) was the only trait with a strong negative loading on PC1. Accession 30 (234431-1) among the dwarf group showed the highest negative loading on PC1 due to the highest in tiller number per plant.

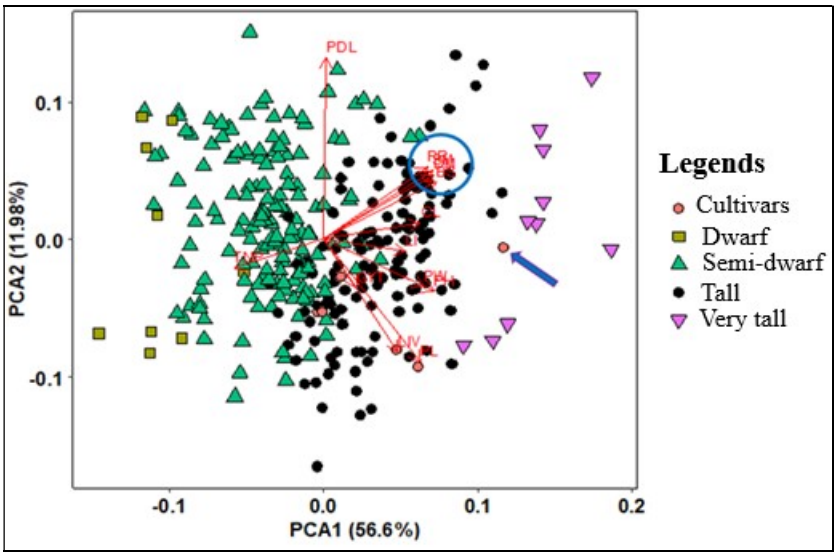

Figure 3-7: Principal component analysis results for the 12 agro-morphological and biomechanical traits of the 320 teff accessions. The length of vectors indicates the loading of each trait and the angle between vectors indicates the relationship among the traits: number of tillers per plant (TNP), peduncle length (PDL), panicle length (PL), lodging index (LI), plant height (PH), panicle weight (PW), culm length (CL), second basal internode diameter (DM), grain yield (GYT), pushing resistance (PR), section modulus (SM), second moment of area (MI), and base failure moment (BS).

3.4. Discussion

This study has investigated the variability and interrelationships of the culm biomechanical properties and agro-morphological traits of teff for lodging resistance. The grain yield performance of the improved cultivar group (Control) was comparable with the very tall accessions group. However, the improved cultivars were significantly lower in terms of internode diameter, pushing resistance and base failure moment. This clearly indicates the presence of untapped variations for important lodging related traits in teff accessions that can be exploited in the breeding program. Although not significant, the very tall group showed relatively lower lodging index which further indicated the presence of few accessions relatively

better in lodging resistance. It is important to note that the higher performance of the very tall group in its panicle weight and length which seems not contributed for grain yield likely diminish the visibility of lodging resistance in the group. The lower lodging index at Bichena was due to the low N content of the soil there. In addition, at Bichena, the midterm drought persisted throughout September during 2018 affected the growth and tillering capacity of the crop. Rainfall, particularly from the grain filling stage to maturity (October to December), was higher at Adet than at Bichena, indicating rainfall might account for the greater lodging observed at Adet station. Except a significant negative correlation with panicle length and positive relation with plant height, peduncle length showed no significant relationship with most of the traits. This implies the higher probability to reduce the plant height by reducing the peduncle length of teff through continues breeding. The significant negative relationship of tiller number per plant with all of the biomechanical properties considered in this study suggested suppressing the tillering capacity of the future cultivars might contribute for enhanced lodging resistance in teff. It will be worthy to note that the observed relatively higher genotype by environment interaction on lodging index and tiller number may signify the quantitative inheritance pattern of the traits which could slow the genetic gain under selection.

The observed root system depths, root to shoot ratios, and pushing resistance of the stubble are consistent with our observation that root lodging was minimal in the accessions tested. We carefully checked whether displacement of the root cone or uprooting was more likely in lodged plants but we did not observe any differences between lodged and unlodged plants. In addition, the stubble remaining after the crop was harvested tended to turn back to its upright position, whereas the stubble of root-lodged plants would be expected to remain bent even after the removal of most of the aboveground biomass. These observations indicate that stem failure rather than root failure accounts for lodging in teff. However, our observations that teff has a relatively narrow root-shoot jointing point (crown) (Figure 3.6),

compared with other cereal crops, are in agreement with previous findings (van Delden et al., 2010). Further, we observed that the tillers initially grew mainly laterally because of space competition at the narrow crown, and starts to hold upright later in the growth stage. Although root lodging could not be ruled out particularly the improvement of the root crown, we believe that shoot failure is a much more important factor in teff lodging. Root lodging may predominate in fields where inappropriate planting methods are used. Broadcast sowing, as practiced by most farmers in Ethiopia, leaves the small teff seeds on the ground surface, so the developed root crown remains above the soil surface, which may be mistaken for root lodging.

Despite the diversity among the accessions in panicle form and weight, we did not find any relationship between lodging and panicle form. The lower visual lodging scores of accessions with semi-compact panicle forms can be explained by their positive association with lower panicle weight and shorter panicle length, which reduced grain yield. This result implies that increased panicle weight and length increase lodging. Although lodging in wheat can be increased by heavier ear weight (Zuber et al., 1999), the effect is compensated by a heavier stem, which is strongly correlated with stem diameter and wall thickness. Our multiple linear regression analysis results show that greater panicle weight increased lodging. Similarly, in rice, a positive correlation of panicle weight with plant height has been reported (Ma et al., 2004), and heavier panicles have been shown to increase the bending moment of the basal internodes.

A negative relationship between lodging resistance and plant height has been reported in wheat, rice, and barley (Navabi et al., 2006; Pinthus, 1974; Stanca et al., 1979; Zhang et al., 2016; Zuber et al., 1999), and this observation has led to selection for shorter plants. In teff plants, the strong positive relationship between panicle length and panicle weight might be

the main factor affecting the drag force and center of gravity of the plant, because in this study, genotypes with shorter panicles had lower lodging scores. However, the dwarf phenotypes, which tended to have very thin culms and high tiller numbers, were extremely susceptible to lodging. In rice, plant height is directly correlated with the plant's center of gravity, so in shorter plants the self-weight moment of the shoot is reduced. As a result, dwarf cultivars have higher lodging resistance than taller cultivars (Okuno et al., 2014). Thus, our results are contrary to findings in model crops such as wheat and rice, in which dwarf genotypes have superior lodging resistance because of their stiff, thick stems. A significant positive correlation between plant height and lodging score has been reported also in wheat (Zuber et al., 1999). However, an attempt to reduce the risk of lodging in wheat and oat through the introgression of extreme dwarfing genes such as *Rht3* and *DW-6*, respectively, failed because these genes are linked with deleterious traits. Extreme dwarf wheat cultivars accumulate less biomass and produce smaller seeds, which significantly decreases grain yield (Flintham et al., 1997). Similarly, poor extrusion of panicles from the flag leaf and lower seed numbers and size have been observed in extreme dwarf oat cultivars (Milach and Federizzi, 2001). It is possible, therefore, that previous attempts to develop lodging-resistant teff cultivars by the introgression of dwarfing genes might have failed because of linkages between the dwarfing genes and thin, low-weight panicles, reducing grain yields. Unlike in other cereal crops, the panicle-peduncle length in teff is much larger in high-yielding genotypes. In addition, selection for a vertical distribution of the internodes such that they form a single straight axis has resulted in improved lodging resistance in barley and other cereal crops (Madić et al., 2016). However, in our observations, the internodes interphase of most of the accessions showed deflection from the vertical axis, implying that the interphase point might be too weak to support the plant parts above it.

Stem diameter and internode wall thickness, particularly in the basal internodes, have been used as indicators of lodging resistance in major cereal crops (Tripathi et al., 2003). Selection for increased stem diameter and wall thickness along with semi-dwarf phenotype have significantly increased the lodging resistance of modern wheat and rice cultivars. In our study, genotypes showing better lodging resistance were associated not only with large stem diameter and wall thickness, but also with fewer tillers per plant. Stem diameter had a high positive association with plant height, visual lodging score, and base failure moment. This result implies that increasing the stem diameter alone would not necessarily increase lodging resistance in teff. Although significant differences in stem diameter were found among the genotypes in this study, a previous study of five teff genotypes found no significant difference in stem diameter among them (Blosch et al., 2020). This discrepancy is attributable mainly to the differences in the number of genotypes studied. The positive association between internode diameter and lodging resistance reported in previous studies of cereal crops was not seen in our study. The positive association between internode diameter and the visual lodging score in this study can be attributed mainly to the longer and heavier panicles in most of the accessions with wider internodes. Thus, the larger stem diameter in the tall teff accessions could not counterbalance the impact of a longer and heavier panicle. Contrary to our results, previous studies in wheat have reported a negative correlation of stem diameter and wall thickness of different internodes with lodging scores (Tripathi et al., 2003; Zuber et al., 1999).

A modeling study of teff panicle bending when force was applied to the upper section of the plant (Blosch et al., 2020) suggested that the bottom two internodes experience the highest stress. Similarly, other studies have shown that plant base strength, that is, the bending moment of the stem at the base of the plant, is closely related to lodging resistance (Baker et al., 1998). These findings support our inference that base failure moment is a key

parameter for lodging resistance in teff. We found significant differences in base failure moment among the accessions, and the positive association of base failure moment with plant height implies that the dwarf accessions had lower material strength than the tall accessions. Consistent with our result, the bending moment of most internodes in rice cultivars was significantly decreased in gibberellin-deficient and insensitive (dwarf) mutants, but increased in gibberellin-overproducing (tall) mutants (Okuno et al., 2014). In wheat and barley, however, greater base failure moment values in dwarf phenotypes than in tall phenotypes have been reported. These contradictory results might be due to morphological differences between wheat/barley and rice, because basal internode diameter and wall thickness are negatively correlated with plant height in wheat and barley. In addition, geometric factors such as the stem's dimensions and cross-sectional shape (i.e., the section modulus) can affect how stress affects a stem and, consequently, its behavior and properties.

This study demonstrated the presence of genotypic variation for agro-morphological traits and stem biomechanical properties. Accessions in the extreme tall group with good standing ability due to its higher base failure moment and pushing resistance with a reasonable yield potential were observed. These accessions can be used as a potential parental material in the breeding programs for increased lodging resistance. Besides, the ongoing attempt to reduce the plant height of teff for lodging resistance, selection for enhanced stem strength could be an alternative solution.

CHAPTER 4

4. Genetic diversity, population structure, and association mapping in teff (*Eragrostis tef* (Zucc.) Trotter) germplasm collections

4.1. Introduction

Teff, like most common cereal crops, belongs to the grass family called Poaceae under the Chloridoideae subfamily. Ethiopia is the origin and center of diversity of teff (Vavilov, 1951), holding more than five thousand accessions collected from different parts of the country in its ex situ seed-bank (Assefa et al., 2015). As pointed out in (Ketema, 1997b), it is also known that the country is the niche of a number of wild relatives, where nearly 14 of them are endemic to Ethiopia (Cufodontis, 1974). The archaeological study that (D'Andrea, 2008) commenced revealed the northern Ethiopian highlands was likely the place where teff domestication started. The genus *Eragrostis* consists of about 350 different species (Clayton and Renvoize, 1986; Zappacosta et al., 2011), but it is only teff and finger millet that have been cultivated for centuries as food crop in Ethiopia. The genus is therefore, known to grow wild as volunteer plant and considered as orphan or minor crop for centuries due to its non-economic value in the rest of the world (Girma et al., 2018; Ingram and Doyle, 2007) except *Eragrostis Pilosa and curvula* being cultivated for livestock feed in some countries (Ingram and Doyle, 2007; Somaratne et al., 2019). Consequently, teff benefited less from scientific discoveries particularly in terms of genetic improvement (Assefa et al., 2015) inflicting on the current low productivity which is about 1.76 t/ha (CSA, 2019b). Among the major production constraints that still needs breakthrough to boost its productivity is lodging (Assefa et al., 2011; Bayable et al., 2020).

Cytological analysis reported by (Jones et al., 1978; Tavassoli, 1986) revealed teff is a tetraploid with 2n = 4x = 40 chromosomes. The disomic inheritance of qualitative traits such as panicle form reported in (Berhe et al., 1989) indicated that teff is either an allotetraploid, containing two sets of chromosomes derived by genome duplication of the different diploid progenitors which are still unidentified or might be an effectively diploidized autotetraploid. Very recently (VanBuren et al., 2020) outlined that the teff genome is made up of two

complete sets of homoeologous chromosomes in which most of the genes maintain synteny among the gene pairs. However, the nuclear genome size is relatively small, 724 Mbp, which is only 50% larger than the rice genome and only 1/4th of the maize genome (Ayele et al., 1996; Hundera et al., 2000a).

The genetic variability study based on agro-morphological traits (Mengesha et al., 1965), the discovery of breeding techniques particularly the time when the flower opens (Berhe, 1975) and the first characterization of teff based on phenology and other agro-morphological characteristics by (Ebba, 1975a) shades light for the subsequent research commencements. Since then, a number of agro-morphological based characterization and diversity studies indicated the presence of wide phenotypic diversity among the teff germplasms for plant height, panicle length, maturity, seed yield, lodging, and panicle form (Assefa et al., 1999; Bayable et al., 2020; Tadesse, 1993; Tefera et al., 2003; Tefera et al., 1990). These studies could highlight the major milestones of teff morphological research and can provided an important information about the diversity of teff accessions. However, phenotypic based characterization is usually influenced by environmental factors. Therefore, Genetic diversity, population structure, and association mapping studies based on molecular markers are very important for characterizing the natural selection history and genetic relationships among genotypes (Begun et al., 2010). This increases the efficiency of characterization, conservation, and wise exploitation of the available germplasm resources to combat the existing and newly emerging production constraints (Assefa et al., 2015; Hedrick, 2005; Jost, 2008; Olsen et al., 2017).

Although, the application of high throughput molecular biology tools has dated several years back on some commercial and food cereals, most crops like teff have benefited less from the applications of those tools. However, few molecular studies have been documented in teff. Besides the determination of teff nuclear genome size with flow cytometry analysis

(Ayele et al., 1996), the genetic diversity studies within and among three Eragrostis species by (Ayele and Nguyen, 2000); and (Bai et al., 2000) were the pioneer in the molecular teff research using Amplified Fragment Length Polymorphisms (AFLP) and Random Amplified Polymorphic DNA (RAPD) markers. Results however, showed low level of polymorphism in teff compared to other cereal crops like wheat, barley, rice, sorghum and maize. Simple Sequence Repeat (SSR) based genetic diversity studies on the other hand revealed a broad genetic diversity in teff (Zeid et al., 2012; Zeid et al., 2011). These might be due to either the low level of diversity among the teff gene pool or the efficiency of the markers used. Following the first draft genome sequence of teff reported in (Cannarozzi et al., 2014), the diversity, population structure, and phylogeny of the germplasm panel composed of cultivated teff genotypes and wild Eragrostis species showed clear separation between the wild and cultivated species whereas low differentiation among the cultivated teff genotypes (Girma et al., 2018).

Dissecting the genomic regions that govern the inheritance of adaptive traits usually called quantitative trait loci (QTL) is important in evolutionary biology (Brachi et al., 2010). Two know methods have been employed in the detection of candidate genes responsible for the variation in trait phenotypes. Conventional linkage mapping used to dissect genes underlying variations is limited to the segregating genes in the bi-parental crossing populations. In contrast, the genome wide association mapping has been used in detecting common variants in natural accessions. Thus, genetic markers associated with important traits in the existing land race collections of a species has practical implications in the efficiency of plant breeding programs and progressively applied in several crop species. GWAS is particularly important in a species with complex flower biology that limits the capacity to generate sufficient bi-parental populations due to difficulty in emasculation like what has been seen in teff, and could be the main way to discover molecular markers and harness the

conventional breeding programs with marker assisted selection. Conventional linkage mapping using few SSR markers has shown a non-significant association of the markers with all of the traits except one marker associated with seed weight per plant (Zeid et al., 2012), and visual lodging index (Zeid et al., 2011). Although, association mapping is widely applied in several crops, no published report found in teff particularly using next generation sequencing protocols. This is mainly due to lack of chromosome level assembled reference genome. A new chromosome level assembled reference genome sequence has now been published (VanBuren et al., 2020). Since then, our work might be the first or among the few to assess the genetic diversity, population structure, and GWAS of teff.

The Genomic DNA sequencing of this study was done following double digest Restriction-site Associated DNA sequencing protocol (ddRAD-seq). It is one of the next generation sequencing method which allow genome wide polymorphism assessment based on restriction enzyme mediated genome complexity reduction approach (Davey and Blaxter, 2011; Miller et al., 2007). Although, it requires the conservation of enzyme recognition sites to isolated homologous fragments for sequencing to reduce the missing data (Eaton et al., 2017), the technique gets recent attention in a number of studies on organisms with and without reference genome (Basak et al., 2019; Nagano et al., 2018; Peterson et al., 2012; Yamashita et al., 2019). The objectives of this study were, therefore, to characterize the population structure, level of genetic diversity, and association mapping of 179 teff genotypes using ddRAD-seq derived SNPs with particular emphasis on lodging resistance related traits.

4.2. Materials and Methods

4.2.1. Plant Materials and Phenotyping

The population panel composed of 310 land race collections, 4 breeding lines, and 6 improved cultivars totally 320 teff genotypes were used for the field phenotyping activity.

Seeds of the panel were collected from Adet Agricultural Research Center and Debre-Zeit Agricultural Research Center, Ethiopia. All the land race collections were originally accessed from the Ethiopian Biodiversity Institute. Phenotypic evaluation of the population panel was carried out under field condition in two contrasting teff growing locations, Adet and Bichena, which are located 42 Km and 222 Km Southwest of Bahir Dar city, Northwestern Ethiopia. Adet represents a drained brown soil whereas Bichena represents a Vertisol with high water holding capacity and so that poor in drainage. The germplasms were planted following an alpha lattice design (8 x 40) with two replications in both locations during the 2018 and 2019 growing seasons.

The following traits were measured: Pushing resistance, Section modulus, Failure moment, Internode diameter, Plant height, Panicle length, Tiller number per plant, Panicle weight, Grain yield, and lodging index. Pushing resistance of a plant including the tillers was measured at a height of 20 cm above the ground with a prostrate tester (Daiki Rika Kogyo Co. Tokyo, Japan, model: DIK-7401) following the procedures outlined in (Hai et al., 2005; Kashiwagi and Ishimaru, 2004; Wu and Ma, 2016). The maximum force (F_{max}) that the internode can withstand before it failed was recorded using digital force gauge from which the culm breaking strength was computed as $S_S = F_{max} \times L/4$, where L is the distance between the supporting points as described in (Crook and Ennos, 1996; Wu and Ma, 2016). The outer diameter of the second basal internode was measured with a digital caliper of 0.01 mm accuracy. Failure moment of the second basal internode was then calculated as described in (Baker et al., 1998). Similarly, the section modulus of the second basal internode was computed using the standard mechanical Equations. Methodological details of the phenotyping including equations of this chapter is outlined in chapter three. Best Linear Unbiased Predictors, BLUP, adjusted mean values of nine important traits of 179 teff genotypes were then used for the current genome-wide association mapping analysis.

4.2.2. DNA Extraction

Prior to DNA isolation, the genotypes were planted in the field in a single row fashion and individual panicles was harvested from each genotype. These further purified and multiplied in a pot and the true to types were maintained. For genomic DNA extraction, seeds of 200 teff genotypes were then planted in small pots under the greenhouse of Holetta National Biotechnology Research Center, Ethiopia. One-month old leaves were harvested in a 2ml Eppendorf tube, immediately frozen with liquid nitrogen and crushed with stainless steel beads using a mixer mill-200 grinder. The standard CTAB protocol developed by DArT company with a slight modification in which SDS (sodium dodecyl sulphate) was used in place of sarkosyle for the DNA extraction buffer solution preparation. The quantity and quality of the DNA was checked by Nanodrop and running in a 1% agarose gel. After quality checking, the DNA quantity of each genotype was normalized approximately to 100 ng/ul and sent to Ryukoku University, Japan for library preparation and sequencing.

4.2.3. Genotyping and Raw Read Generation

A double digest RAD-seq library was prepared as described elsewhere (Sakaguchi et al., 2015) which is a modification of the original double digest RAD-seq library preparation protocol (Peterson et al., 2012). Genomic DNAs were digested with *Bgl*II and *Eco*RI, ligated with Y-shaped adaptors, amplified by PCR with KAPA HiFi HS ReadyMix (KAPA BIOSYSTEMS) and size-selected with the E-Gel size select (Life technologies, CA, USA) in Information Biology, Faculty of Agriculture, Ryukoku University, Japan. Approximately 450 bp library fragments were retrieved. Raw images and base calling were then generated with the Illumina HiSeqX (Macrogen, Japan) through an integrated primary analysis software called RTA 2 (Real Time Analysis 2). The BCL (base calls) binary was converted into FASTQ using Illumina package bcl2fastq2-v2.20.0.

4.2.4. Raw Read Processing and Analysis

Our ddRAD sequencing generated an average of 1.57 billion reads, with an average of 7.1 million reads per accession. The GC content ranged from 41.8 to 45.9 % with average value of 43.1%. To assemble loci and generate vcf file for downstream analysis, raw sequence data were processed with Genome Analysis Tool Kit (GATK). Reads with a Phred score less than 30 out of 40 and containing adapters were identified using FASTQC software program and trimmed with the cutadapt pipeline of the GATK. After quality filtering for Q30 and get adaptors removed, about 5.3 million reads per accession were recovered. The quality trimmed reads were further checked for its quality and PCR duplicates were removed. The quality filtered reads were then aligned to the newly chromosome level assembled teff reference genome available at CoGe under id50954 (VanBuren et al., 2020) using Bowtie2 pipeline under the GATK environment.

4.2.5. Analysis of Population Structure

The population structure was determined using admixture model with correlated allele frequencies based Bayesian clustering approach implemented in TRUCTURE v2.3.4 software program (Pritchard et al., 2000). Five independent runs were performed for each number of sub-populations (K) by setting the value of K from 2 to 10. A burn-in period of 10,000 followed by a Markov Chain Monte Carlo (MCMC) replication of 10,000 for each run was implemented. The most probable sub-populations (K-value) was determined using the online software program, Structure Harvester v0.6.94, (available at http://taylor0.biology.ucla.edu/structureHarvester/) (Earl and vonHoldt, 2012). The log probability of the data [LnP(D)] and delta K (ΔK) based on the rate of change in [LnP(D)] between successive K values were used to determine the best number of sub-populations as described elsewhere (Coleman et al., 2018; Earl and vonHoldt, 2012; Evanno et al., 2005).

Membership coefficient matrix from the STRUCTURE for the determined optimum sub-populations (K-values) were used in CLUMPP (Jakobsson and Rosenberg, 2007) to generate an individual Q-matrix and a population Q matrix which was then used to create a bar plot using DISTRUCT software (Rosenberg, 2004). The analysis of molecular variance (AMOVA) and the sub-population pair wise F statistic (F_{ST}) were computed in the GenAlex 6.5 software program (Peakall and Smouse, 2012).

4.2.6. Association mapping

Biomechanical and agro-morphological traits related to lodging resistance of 179 teff genotypes and the corresponding ddRADseq derived SNP data were used to compute the genome-wide association analysis under the TASSEL ver 5.0 software program. The general linear model (GLM) by considering the population structure Q-matrix generated from the STRUCTURE software as a covariate was used. False discovery rate (FDR) values were computed at 0.001 P-value following the standard procedures (Benjamini and Hochberg, 1995). The Manhattan plots were then visualized in the R software program following the *qqman* package (Turner, 2014) using the GWAS analysis result as an input data. The markers are declared significantly associated with the trait of interest when the negative log(P) values fall above the FDR as a cutoff point.

4.3. **Results**

4.3.1. Population Structure

In this study, SRUCTURE software with admixture model were used to compute the population structure of 179 teff genotypes using 2087 ddRAD-seq derived SNP markers. The analysis was carried out without providing a priori information about the population structure. The change rate of the log likelihood value which was the highest as the K value changed from 2 to 3 (Figure 4.1a) and the Delta K value which reached to the maximum at K = 3 and

rapidly decreased onwards (Figure 4.1b) indicates 3 is the optimum sub-population number of the genotypes under this study. The admixture model bar graph of posterior membership probabilities showed well separated three sub-populations with 57.9, 12.5 and 29.6 percent overall membership proportion of the genotypes in each of the three clusters (Figure 4.2). The individual genotype membership was decided using the membership coefficient matric (Q matrix) (Table A7). According to the cutoff criterion for inferred ancestry estimates, majority of the genotypes were clearly assigned to specific sub-populations when the Q value was set ≥ 0.6. Accessions with Q values less than 0.6 were regarded as admixtures of two or more sub-populations, that share common ancestry, and comprised of 30 genotypes which is about 16.8% of the population panel.

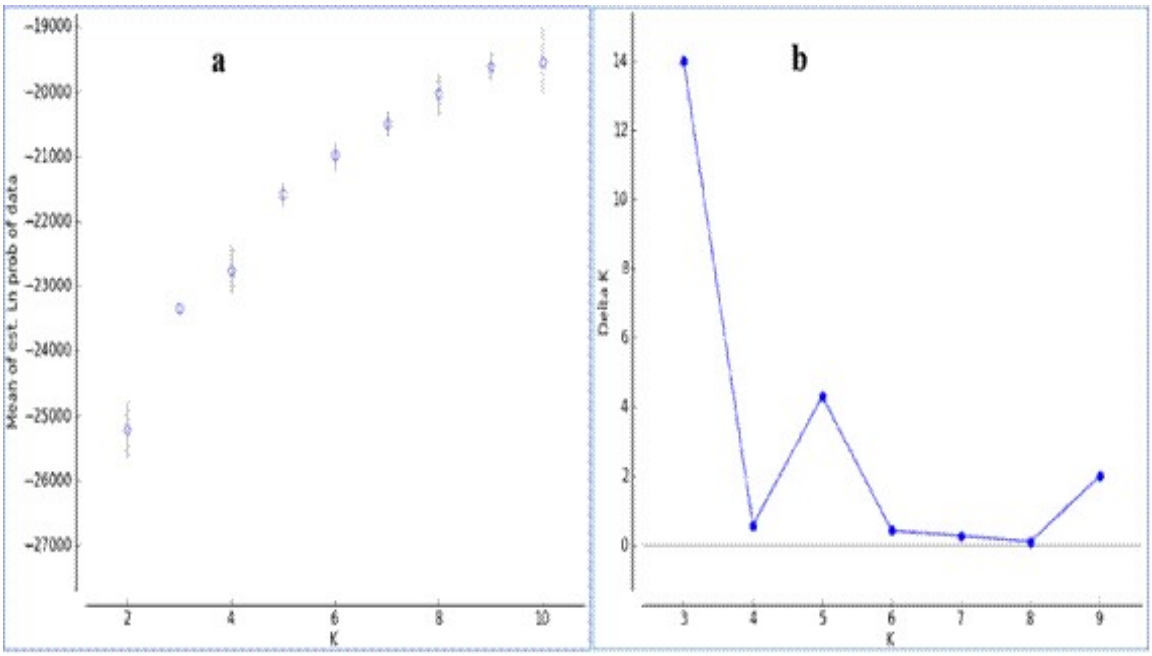

Figure 4-1: STRUCTURE Harvester estimation of the optimum number of sub-populations by setting the K values from 2 to 10. (a) plot of mean of estimated Ln probability of the data against the K and (b) delta K (mean(|L(K)|/SD(L(K)) values.

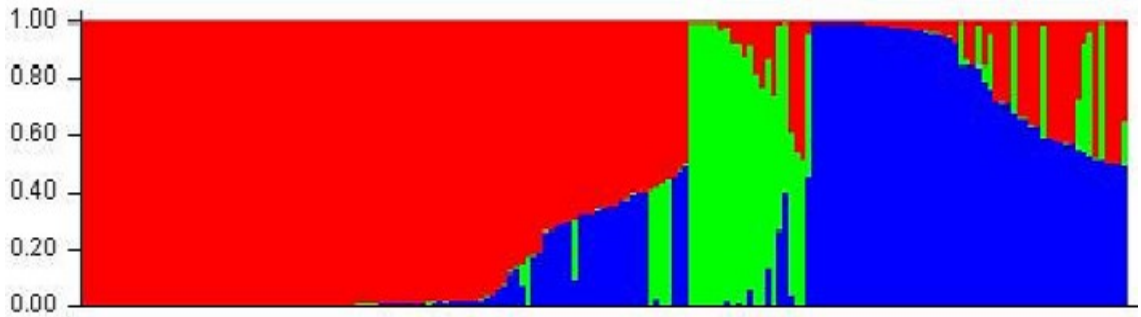

Figure 4-2: Population structure of the 179 teff genotypes based on ADMIXTURE model generated using STRUCTURE software. For each genotype, columns fully shaded with one color represents genetic homogeneity, while columns with mixed colors indicated admixtures whose genetic makeup is drawn from more than one of the identified K sub-populations.

A phylogenetic tree consisting of 179 teff genotypes, constructed based on unrooted neighbor-joining phylogenetic tree in TASSEL software program, also indicated the existent of three different sub-populations - red, blue, and green with 103, 49, and 27 member individuals respectively (Figure 4.3). To further assess the population subdivision, the principal component analysis (PCA) was carried out using TASSEL software program which indicated the clear division of the population panel into three sub-populations as identified by STRUCTURE program. PC1 explained 5% of the observed genetic variability, whereas PC2 explain 3.6% of the variation (Figure 4.4).

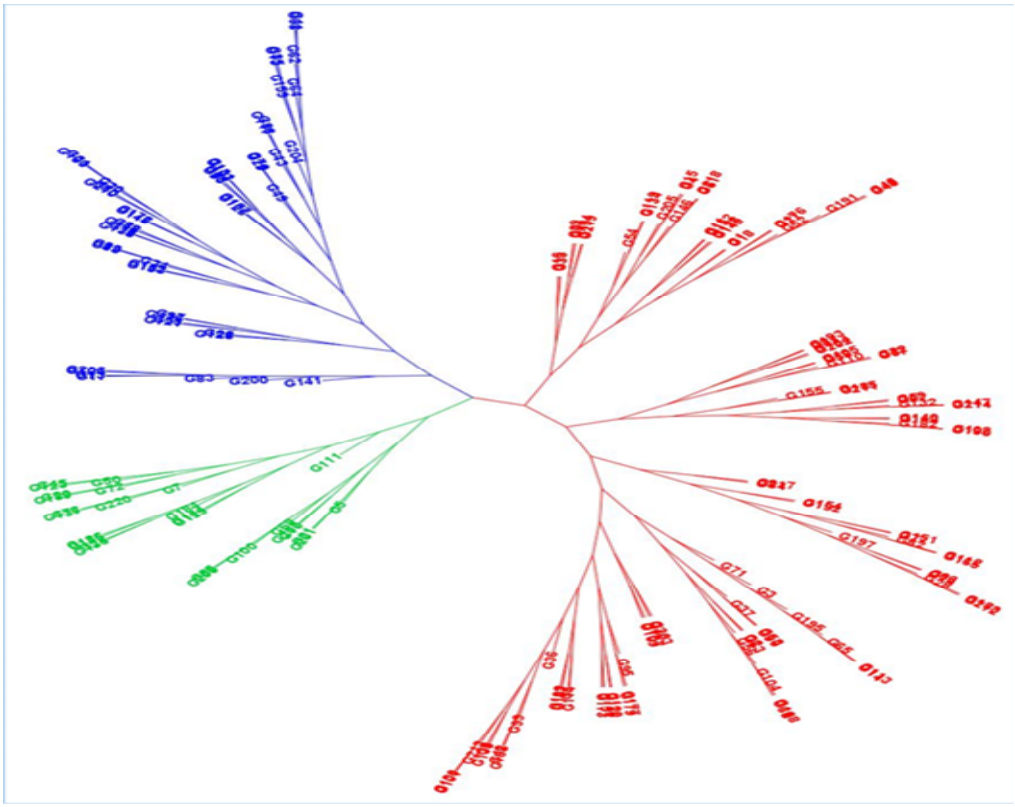

Figure 4-3: Unrooted phylogenetic tree generated using 2087 SNP markers and 179 teff genotypes based on neighbor-joining tree in TASSEL software program confirms the three different sub-populations.

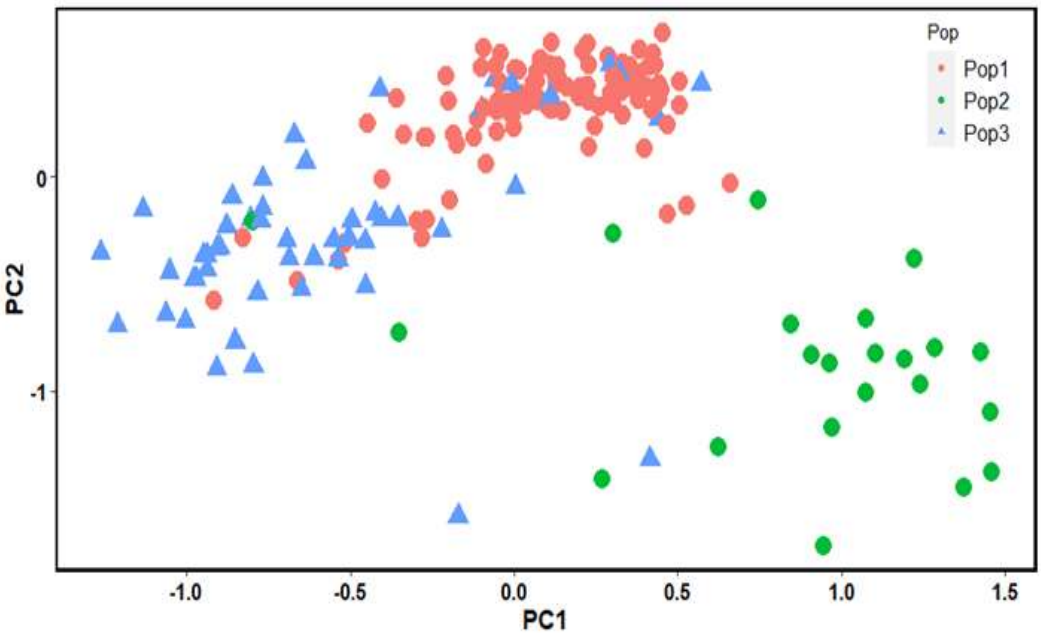

Figure 4-4: Principal component analysis-based scatter plot of 179 teff genotypes of the first two components (PC1 vs PC2) based on 2087 SNP markers.

4.3.2. Genetic diversity

Nucleotide diversity and heterozygosity are among the commonly adopted genetic indices to examine genome wide patterns of genetic diversity. Nucleotide diversity within the sup-populations ranged from 0.06 to 0.08, whereas the observed heterozygosity between member genotypes of same sub-population ranged from 0.39 to 0.48 (Table 4.1) with the overall heterozygosity value of 0.14. The level of differentiation between the identified sub-populations (F_{ST}) was relatively higher in Pop-2 and the lowest for Pop-1. However, the pair wise differentiation between the sub-populations were generally low ranging from 0.013 between Pop-1 and Pop-3 to 0.04 between Pop-2 and Pop-3. The AMOVA result showed that only 1% of the total genetic variation observed was among the sub-populations. Whereas a large amount of the genetic variation, about 99%, was among individual genotypes within the sub-population (Table 4.2). The genetic distance among the teff genotypes calculated with the distance matrix option of TASSEL V5.2.52 varied from 0.06 to 0.32. The highest genetic distance was observed between genotype G38 and G183, whereas the lowest distance was found between G114 and G194.

Table 4-1: Genetic parameter estimates among the populations identified based on 2087 SNPs and 179 teff genotypes.

Populations	No. Genotypes	Inferred clusters	Ho	He	F_{ST}	
					Pop-2	Pop-3
Pop-1	94	0.579	0.39	0.06	0.025	0.013
Pop-2	16	0.125	0.48	0.08	–	0.037
Pop-3	39	0.296	0.43	0.07	–	–

H_e = gene diversity (expected heterozygosity), Ho = observed heterozygosity between individuals in same cluster, F_{ST} = pair wise genetic differentiation.

Table 4-2: Analysis of molecular variance (AMOVA) among and within sub-populations of the teff population panel.

Source	df	SS	MS	Estimated Variance	Variation explained (%)
Among Pops	2	775.94	387.97	2.70	1%
Within Pops	137	38225.60	279.02	279.02	99%
Total	139	39001.54		281.72	100%

4.3.3. Phenotypic variations

Wide range of phenotypic performance among the test genotypes were observed (Figure 4.5). For instance, among the most important lodging resistance related biomechanical traits, failure moment of the second basal internode ranged from 0.05 Nm to 0.25 Nm with mean value of 0.11 Nm. The pushing resistance varied from 2.0 N to 7.5 N (Mean = 4.0 N). The outer diameter of the second basal internode was also varied significantly from 1.7 mm to 3.9 mm. The multivariate clustering analysis based on four biomechanical (Pushing resistance, Failure moment of the second basal internode, Internode breaking strength and lodging index) and five agro-morphological traits related to lodging resistance (Plant height, Panicle length, Panicle weight, Internode diameter and Tiller number per plant) was computed following the Ward.D_2 hierarchical clustering algorism in R software program with *hclust* function of the *factoextra* package. The result showed three diverse clades outlined as blue (Cluster-I), green (Cluster-II) and red (Cluster-III) colors with 63, 19 and 97 member genotypes respectively (Figure 4.6). Although cluster-II is the least in terms of the number of member genotypes, the performance was the highest for the phenotypic trait mean values. For example, its average plant height was 120.1cm. Shorter plant height has characterized cluster-III which is about 89.6cm and cluster-I was intermediate in plant height with 104.5 cm. The failure moment of the second basal internode, a measure of material strength, was the highest

for cluster-II and the lowest for cluster-III. However, the number of tillers per plant was the highest for cluster-III and the lowest was recorded for cluster-I and II, both scored similar mean value.

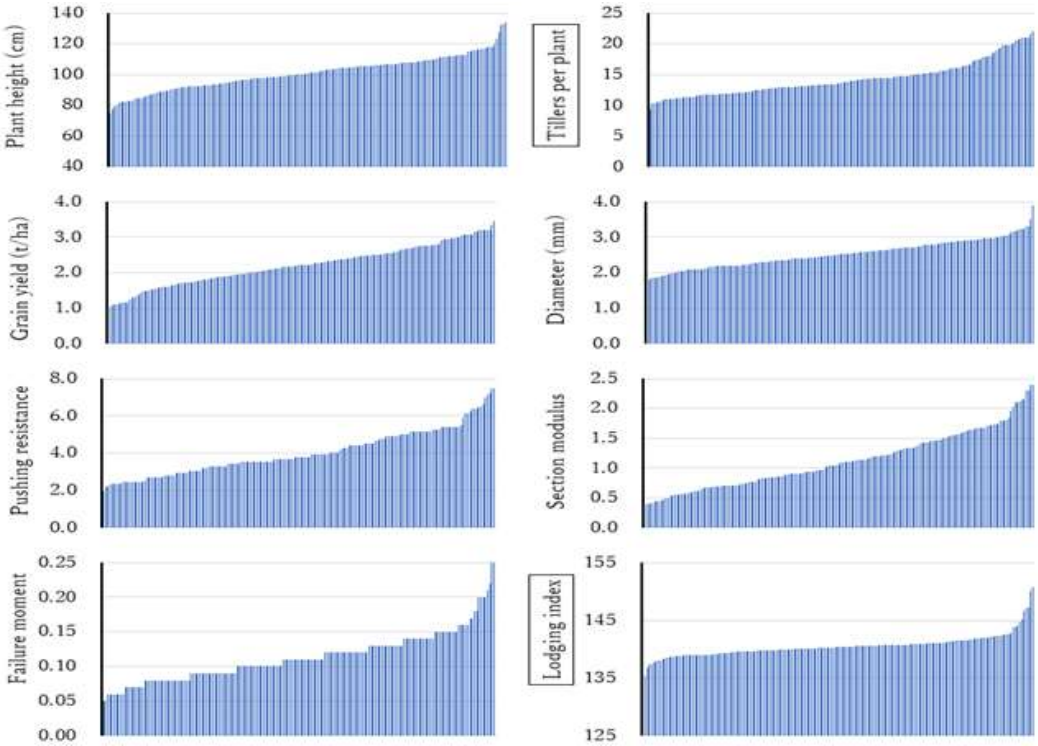

Figure 4-5: Phenotypic variability of biomechanical and agro-morphological traits of 179 teff genotypes.

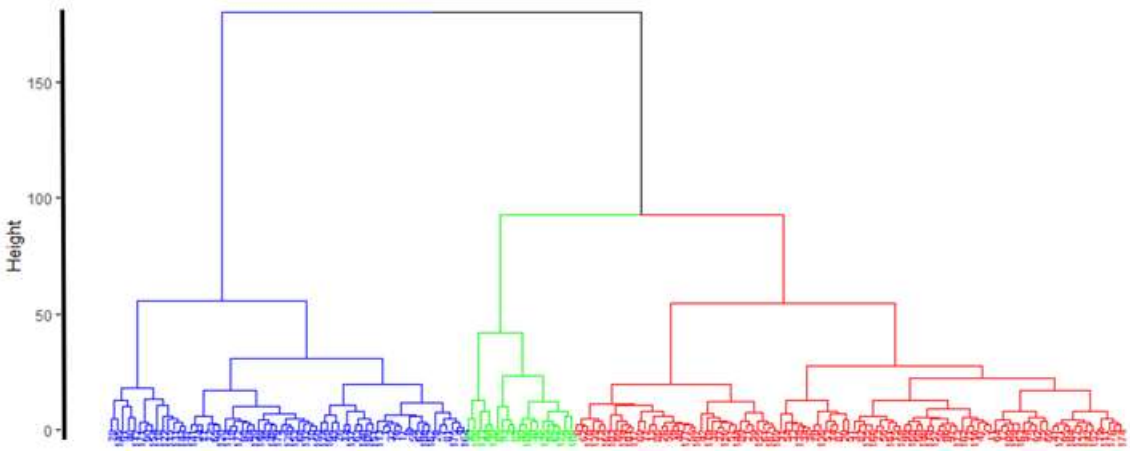

Figure 4-6: Dendrogram based on nine biomechanical and agro-morphological traits related to lodging resistance.

4.3.4. Marker-trait association analysis

Regarding to the biomechanical properties of the teff stem, 9 SNPs, which showed a significant association ($P < 0.001$) with the failure moment of the 2nd basal internode of the teff accessions were identified (Table 4.3). The individual SNPs explained ranged from 7% to 12% of the total phenotypic variations observed in failure moment. These SNPs together explained 83% of the total phenotypic variation observed due to failure moment. However, only six markers that showed a significant association with failure moment have a P-value of above the cutoff point. Chromosome 1A, 4A, and 8A were found to harbor the associated SNPs for failure moment at 24646607, 7366573, 4384808 positions (Figure 4.7a). These three SNPs explained 30% of the phenotypic variation observed due to failure moment. The Manhattan plot also showed five different SNPs having a significant association above the cutoff point with lodging index in chromosome 1B, 2A, 8B, and 9A at 26232557, 20596748, 408274, and 6096608 positions respectively (Figure 4.7b). Similarly, one SNP that is significantly associated with section modulus was found in chromosome 1A at 24646607 position. No significantly associated SNPs above the cutting point was found for pushing resistance in the teff accessions included in this study.

Among the agro-morphological traits, the second basal internode diameter and tiller number per plant found one SNP above the cutting point having a significant association which is located at chromosome 1A at 24646607, and 29560088 positions respectively (Figure 4.8). The identified SNP for the second basal internode diameter and tiller number per plant accounted for 11%, and 12% respectively of the observed phenotypic variation of each trait (Table 4.4). Although the genome wide association analysis result revealed a number of significantly associated SNPs for plant height and panicle length, the Manhattan plot showed all of them were below the cutoff point. Similarly, no significantly associated

SNPs above the cutoff point was found for both panicle weight and grain yield. On the other hand, two significantly associated SNPs were identified for days to maturity in chromosome 4A, and 6B at 5565758, and 8948060 positions respectively. None of the significantly associated SNPs for days to heading were found to be above the cutoff point.

Table 4-3: The chromosome position of most significant SNPs for failure moment of the lower 2nd basal internode and lodging index.

Base failure moment				**Lodging index**			
CHR	**SNP Position**	**P-value**	**R^2**	**CHR**	**SNP Position**	**P-value**	**R^2**
8A	4384808	4.22×10^{-5}	0.12	1B	26232557	1.80×10^{-5}	0.15
1A	24646607	9.87×10^{-5}	0.09	8B	408274	1.93×10^{-5}	0.19
8A	4360993	2.48×10^{-4}	0.09	9A	6096608	5.13×10^{-5}	0.09
4A	7366573	4.17×10^{-4}	0.10	2A	20596748	7.14×10^{-5}	0.09
8A	4384830	5.00×10^{-4}	0.09	9A	6096364	8.97×10^{-5}	0.09
8A	4384845	5.00×10^{-4}	0.09	9A	6096436	8.97×10^{-5}	0.09
8A	4385079	5.90×10^{-4}	0.09	6B	7027320	1.55×10^{-4}	0.12
7A	5544907	1.12×10^{-3}	0.07	1A	24646607	5.62×10^{-4}	0.07
8A	3503447	1.16×10^{-3}	0.07	6B	8200062	8.74×10^{-4}	0.07
				9A	6204950	8.86×10^{-4}	0.07
				2A	20596787	1.02×10^{-3}	0.07

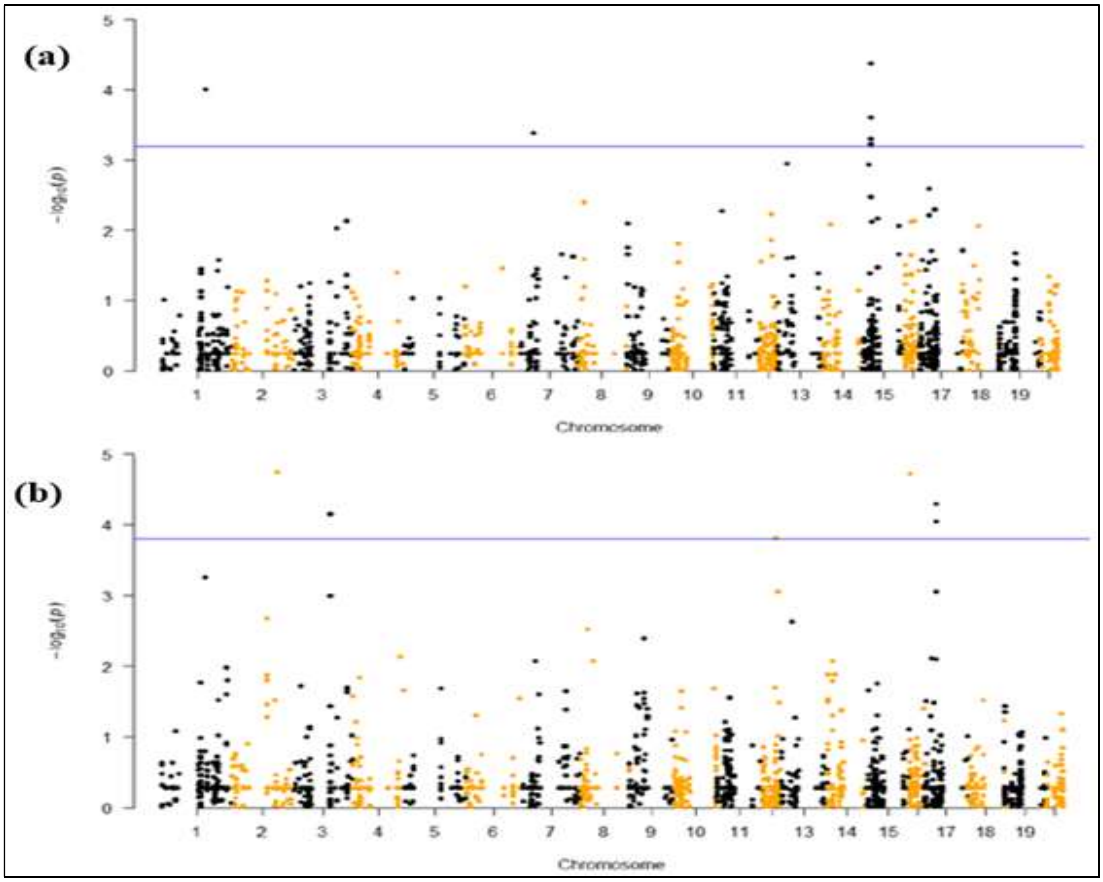

Figure 4-7: Manhattan plots of 179 teff accessions based on 2088 single nucleotide polymorphisms (SNP)for lodging related biomechanical properties: (a) base failure moment of the lower 2nd internode, and (b) lodging index.

Table 4-4: The chromosome position of most significant SNPs for stem diameter of the lower 2nd basal internode, and tiller number per plant.

Stem diameter				Tiller number per plant			
CHR	**SNP Position**	**P-value**	**R^2**	**CHR**	**SNP Position**	**P-value**	**R^2**
1A	24646607	8.31×10^{-6}	0.11	1A	29560088	2.10×10^{-4}	0.12
7A	5544907	8.20×10^{-4}	0.07	9A	6096364	1.57×10^{-3}	0.06
8A	3503447	1.08×10^{-3}	0.08	9A	6096436	1.57×10^{-3}	0.06
9B	9255300	2.43×10^{-3}	0.08	5B	4032592	1.82×10^{-3}	0.05
5B	4030086	5.29×10^{-3}	0.04	7B	2662840	2.10×10^{-3}	0.06
				1B	2725533	3.22×10^{-3}	0.07
				1B	2725494	3.57×10^{-3}	0.07
				1B	2725502	4.05×10^{-3}	0.07
				6A	20258592	5.04×10^{-3}	0.04

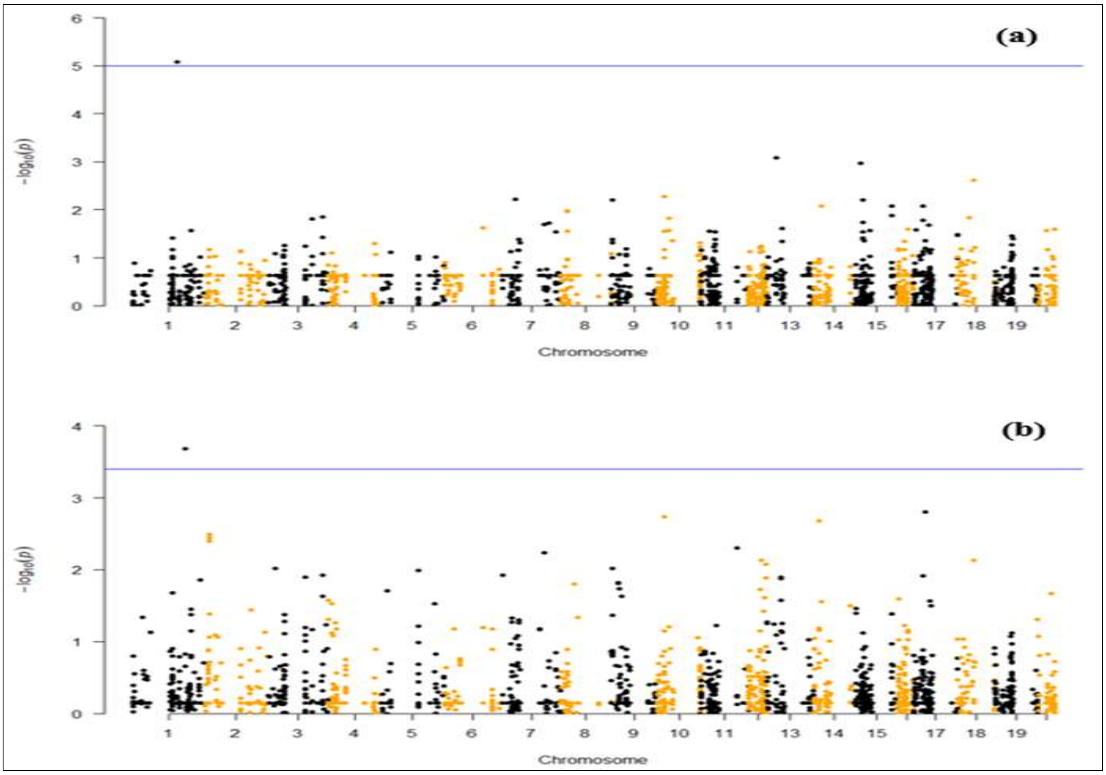

Figure 4-8: Manhattan plots of 179 teff accessions based on 2087 single nucleotide polymorphisms (SNP)for lodging related agro-morphological traits: (a) Stem diameter of the lower 2nd basal internode, and (b) Tiller number per plant.

4.4. Discussion

The genetic diversity and population structure studies documented previously were based on SSR and ISSR molecular markers except a phylogeny analysis by (Girma et al., 2018) who outlined clear separation between wild and cultivated species of teff using genotyping by sequencing (GBS) SNP markers. Regarding the genome-wide association mapping study, this is might be the first report on teff and compared to previous studies we relatively used large number of genotypes. To describe the properties of a certain populations, it would be important to classify each individual member into sets of sub-populations (Pritchard et al., 2000). One way of defining the population structure is using the various descriptors of individuals such as phenology, agro-morphological traits, and the geographic locations where the individual samples collected. With this approach, however, it is difficult to segregate

whether the classification is due to heritable genetic terms or simply due to the environmental stimuli. Since the heritable genetic term variation is crucial for any genetic improvement in the target traits, it would be wise to ascertain the variability is also consistent with the genetic information. Knowledge of DNA sequence based population structure and genetic diversity is therefore plaid an important role for any genetic improvement for a target trait (Laido et al., 2013). In addition, the classification and characterization of the population is a pre-requisite and shades light to commence high throughput genetic studies such as association mapping, genomic selection and marker development. This is mainly because the higher the genetic diversity existed in a working population panel, the higher the probability of increasing the selection efficiency of the breeding programs.

The admixture model bar chart of a STRUCUTURE program, neighbor-joining phylogenetic tree and the PCA scatter plot of TASSEL program consistently showed the presence of three sub-populations. The population stratification, a dendrogram based on biomechanical and agro-morphological traits contributing to lodging resistance (Figure 1) showed a fairly good membership correspondence with the molecular data based derived sub-populations. The similarity which ranges from 15 to 33% indicated the environmental effect on the phenotypic data was considerable. This result is in line with the reports of many genotype by environment interaction studies in cereal crops on which about 70% of the total observed phenotypic variance has been accounted for the environment (Jifar et al., 2019; Mohammadi et al., 2010). This implies the genetic and phenotypic variabilities are consistent except considerable discrepancies mainly due to expected environmental effects on the phenotype data. Previous studies using ISSR markers on 92 selected teff accessions from eight geographical origin, (Assefa et al., 2003) outlined six sub-populations at 60% Jaccard similarity level. Whereas (Abraha et al., 2016b), found only three distinct sub-population using 10 SSR markers on 60 teff germplasms.

Although teff is originated and domesticated entirely in Ethiopia and some of the teff accession collections lack georeferenced information at the time of collection, the relationship of genetic diversity with its geographic location of collection were evaluated in this study. The clustering of accessions based on the geographical place of collection did not show any clear pattern to classify accessions derived from same origin together. Lack of clear pattern in the population structuring and genetic diversity of several crop species with its place of origin and collection has been reported in Sesame (Chiang et al., 2019) and common bean (Tigist et al., 2020). Similarly, (Assefa et al., 2003), and (Abraha et al., 2016b) reported absence of clear association pattern between the population structure and geographic places of collection of the teff accessions using ISSR and SSR molecular markers. This implies the migration or gene flow between regions within Ethiopia through informal seed exchange and trade. This is expected in a country where up to 90% of the seed demand is satisfied with the informal seed system through exchange between farmers (Sisay et al., 2017). Thus, the ecological and geographical factors contributed less in the evolution of teff or absent of a certain population that evolve in an isolated geographical location.

The AMOVA result which indicates only 1% genetic variability among the sub-populations of the teff population panel considered in this study suggested the absence of complicated population stratification. This further supported by a lower value of pair wise population differentiation (F_{ST} = 0.025) implying very low population structuring characterizes the teff population panel. Similarly, a lower population stratification among the sub-populations of teff has been reported (Abraha et al., 2016b; Assefa et al., 2003; Girma et al., 2018). Such phenomenon is also common in other crop species, for instance (Cui et al., 2017) and (Basak et al., 2019) reported lower level of population differentiation among the sesame populations. The higher level of gene diversity within each sub-population between

individual genotypes indicated a considerable genetic variation in the population panel under this study.

The overall proportion of heterozygosity of this teff panel was 0.14 indicating high level of genome homozygosity. This is mainly because, teff is a chasmogamous self-pollinating plant and it is highly expected that all the accessions have passed with several generations of self-pollination. However, the observed 16.8% admixed individuals identified in the STRUCTURE program might be due to its very small seed size that increases the chance of mechanical seed mixtures or they might inherit portions of genomic regions from common ancestors during the polyploidization. The seriousness of the mechanical seed mixture in teff is also outlined by (Zeid et al., 2012) as a common phenomenon like in most other small grain cereals.

The efficiency of genome wide association studies has been influenced by a number of factors related to the nature of the population under investigation. Thus, genetically diverse, but not interrelated or not highly differentiated population panels are required (Flint-Garcia et al., 2003; Knowler et al., 1988). The population structure information generated with this study could provide valuable information to commence association mapping from which the future teff breeding program could benefit. In addition, the population panels included in this study have useful characteristics in terms of agro-morphological and lodging resistance related traits. The plant height, tillering capacity and the lower internode mechanical properties varied significantly among the genotypes and showed strong relationship with lodging index (Bayable et al., 2020).

As one of the potential approaches in dissecting genomic regions controlling traits of interest, association mapping has been applied widely in most cereal crops. For instance, quantitative trait loci (QTL) associated with culm strength have been identified and reported

in rice (Chigira et al., 2020). However, association mapping studies are lacking in teff due to the limitation of chromosome level assembled reference genome sequence. We conducted GWAS for important lodging related traits based on BLUP adjusted mean data combined over three environments. Three significantly associated SNPs with the failure moment of the second basal internode, a measure of stem strength, has identified on chromosome, 1A, 4A, and 8A. In addition, four significantly associated SNPs with lodging index were identified on chromosome 1B, 2A, 8B, and 9A. The identified SNP on chromosome 1A for failure moment was also significantly associated with internode diameter and section modulus. This might indicate the pleiotropic effect of this gene that simultaneously control these three traits or the strong correlation between them. Similar phenomenon has been reported in several crops. Four QTLs that showed significant association with visual lodging index was identified in a biparental linkage mapping study from a cross between *Eragrostis tef* and its wild relative *Eragrostis Pilosa* (Zeid et al., 2011). In the association mapping study using teff genotypes including wild relatives, (Zeid et al., 2012) reported no significantly associated markers found with lodging. However, one of the markers associated significantly with lodging index in (Zeid et al., 2011) was found to be associated with seed weight per plant rather than lodging. This discrepancy further indicates the association mapping result has to be validated with biparental linkage mapping.

CHAPTER 5

5. General Conclusion

Extensively cultivated in East Africa, particularly in Ethiopia, *Eragrostis tef* (Zucc.) Trotter continues a reliable crop cultivated from high rainfall to marginal low input agricultural systems. Despite its resilience to extreme growing conditions, gluten freeness, and good sources of minerals and dietary fiber, the crop suffers low productivity due to lodging and accompanied production constraints. Like other crop species, the usefulness of teff germplasms conserved in ex-situ gene banks depends on the extent and quality of information associated with its heritable variability on the important traits. The germplasm collections found in Ethiopia, more than five thousand accessions, is the largest if not the only source of the genetic material of teff in the world. These resources need to be characterized and documented for wise use and conservation.

The present study revealed higher coefficient of genetic variation, heritability and expected genetic advance for grain yield under both the intensive (higher fertilize application, fully irrigated, lodging artificially controlled) and field growing conditions with conventional management. Among the phenological traits, the grain filling period in the intensive growing condition exceptionally showed the highest genetic coefficient of variation, heritability, and genetic advance. Harvest index was also improved under the intensive growing condition indicating the importance of lodging control and presence of immense genotypic variation for yield potential. Therefore, to exploit the genetic potential and narrow the yield gaps,

improving the lodging resistance and thereby enhance the harvest index of teff should come to the front page.

The analysis for biomechanical and agro-morphological traits revealed a significant variation among the genotypes. It is also noted that the panicle-peduncle length of teff is large accounting about 59% of the total height. Wider stem diameter, higher stem strength, and reduced tiller number found important lodging related traits. Our result suggests the need to pyramid genes of those important traits, all of which are features of tall plant stature, into dwarf and semi-dwarf cultivars.

The molecular study provided the first insight into the genetic basis of lodging resistant related traits of teff using genome-wide association study (GWAS). Significantly associated SNPs for stem strength, stem diameter, and tiller numbed were identified. This in turn provides an opportunity to develop molecular markers for efficient selection, to implement targeted genomic manipulation such as genome editing for lodging resistance, and to emplace knowledge based targeted teff breeding programs.

The strong positive correlation of both grain yield and harvest index with plant height could limit the selection efficiency of the conventional breeding approach. Thus, strong hybridization program focusing on desirable trait recombination, study the mode of inheritance of the important traits, and selection based on lodging resistant related traits could benefit the future teff breeding program. In addition, more GWAS with a subsequent validation using the biparental QTL mapping is required to complement the breeding program with marker assisted selection.

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SUMMARY

Teff (*Eragrostis tef*) plays an important role in the food and nutrition security of about half of the population in Ethiopia. The crop is rarely known in the other world as a food crop for long time. Due to its gluten freeness and high in minerals and dietary fiber, however, it is attracting other consumers in the world and seen as a potential component of healthy food and beverage production in the future. In addition to the nutritional importance, teff has an ecological advantage over other cereal crops. Adaptiveness to extreme growing conditions particularly tolerant to water logging Vertisol areas where other cereals might fail is consistently mentioned as one of the top merits of the crop. On the other hand, teff is tolerant to storage pests such as weevils and has higher seed longevity even under traditional storage facilities. In Ethiopia where drought and climate variability affect crop production, teff continues one of the main cash crops as both the grain and straw fetch high price. Despite the merits, the productivity of teff is very low which is about 1.76 t/ha. Lodging is the major yield and quality limiting factor of teff production directly and it also hinders farmers from applying optimum fertilizer rate fearing it could aggravate lodging.

Efforts have been carried out to search and utilize dwarfing genes in teff lodging resistance cultivar development. The attempt however, was not successful mainly due to strong association of reduced plant height with weak culm and lower grain yield potential. On the other hand, the smaller floret size limits the efficiency of hybridization and trait inheritance studies. Consequently, mutation more recently TILLING (Targeting Induced Local Lesions in Genomes) becoming an option to source new variabilities for lodging resistance. Lack of scientific knowledge is the key problem in the breeding program of teff particularly sources of culm mechanical strength, the genetic diversity and responsible genomic regions that govern lodging resistant related traits is either limited or yet not

explored. Phenotyping and genotyping studies were carried out using three hundred twenty teff genotypes under the field and screenhouse conditions with the objectives of (i) to evaluate the yield potential and determine the variabilities of teff genotypes under lodging controlled and non-controlled growing conditions, (ii) to examine the variability of stem mechanical properties and agro-morphological traits with their associations with lodging, and (iii) to explore the genetic diversity, population structure and trait-marker associations using next generation sequencing (NGS) method.

The first chapter outlines the general introduction of the thesis. It presents the botanical description, its origin and distribution, the importance, production constraints, the lodging effect, previous research undertakings and the statement of the problem and research gaps. The general and specific objectives and the overall structure of the thesis are also included.

Chapter two presents the grain yield potential, heritability, and genetic advance under selection of diverse teff genotypes under intensive and field growing condition. It also outlined trait relationships at the phenotypic and genotypic levels. Under the intensive growing condition when lodging was artificially controlled with support, wider range of harvest index was observed than the field growing condition. On the other hand, high coefficient of variation, heritability and expected genetic advance for grain yield were observed in both experiments. The harvest index showed strong positive relationship with grain yield under the intensive management but weak positive correlation with grain yield and a negative correlation with biomass under the field experiment, in which lodging was relatively higher. These suggests the presence of wide genetic potential in the teff gene pool and its opportunity to enhance the productivity.

The third chapter characterizes teff germplasm collections for lodging resistance in terms of the biomechanical properties of the culm and lodging related agro-morphological traits

using three hundred twenty teff genotypes. This study revealed the presence of wide variability in both biomechanical and agro-morphological traits among the accessions. It is also noted that lodging index, failure moment, pushing resistance and lodging related traits such as culm diameter had a strong positive correlation with plant height. This suggests the shorter the plant height the lower the material strength of the teff culm. In contrast, tiller number showed a significant negative correlation with lodging index. The peduncle–panicle length, which generally accounted for 59% of the plant height, should be a target when breeding for semi-dwarfism. Root system development, which reached a depth of more than 1 m in tall and 57 cm in dwarf teff accessions, signifies the presence of genetic variabilities for future root lodging studies in teff, and it may also explain why teff performs well in drought-prone areas of Ethiopia. The observations indicated that stem failure account more likely than root failure for lodging in teff. However, our observations that the tillers initially grew mainly laterally and start to hold upright later in the growth stage further implies space competition at the crown, and teff has a relatively narrow root–shoot jointing point (crown). Thus, root failure could not be ruled out and needs detailed investigation in the future.

The fourth chapter outlines the genetic diversity, population structure and dissect the genetic basis controlling lodging resistant related traits of 179 teff genotypes through genome-wide association study (GWAS) using double digest Restriction site Associate DNA sequencing (ddRADseq) derived single nucleotide polymorphisms (SNPs). The admixture model bar chart of a STRUCUTURE program, neighbor-joining phylogenetic tree and the PCA scatter plot of TASSEL program consistently showed the presence of three sub-populations. The dendrogram based on biomechanical and agro-morphological traits contributing to lodging resistance also showed reasonable level of similarities among the members of each sub-population. The analysis of molecular variance showed only 1% of the variations was due to genetic variability among the sub-populations, whereas 99% of the

variation was within populations. Further the low value of F_{ST} which is about 0.025, implies smaller population differentiation in the teff population panel. Significant SNP-trait associations were identified for stem strength, lodging index, stem diameter and tiller number. Therefore, the information generated here will help to prioritize breeding targets, and to commence advanced molecular research such as genomic selection and marker development for efficient selection.

The fifth chapter is about the general conclusions and future directions. The observed high grain yield performance, wider range of harvest index in the intensive management condition coupled with the moderate to high coefficient of genetic variation, heritability and genetic advance witnessed the genetic potential of teff for further improvement. The analysis for biomechanical and agro-morphological traits revealed significant variation among the genotypes. Higher stem strength, wider stem diameter, and reduced tiller number found important lodging related traits, all of which were found in the tall plant stature. The GWAS also identified significantly associated SNPs for stem strength, stem diameter, and tiller number. Thus, strong hybridization program focusing desirable trait recombination and selection based on the lodging resistant related traits could benefit the future teff breeding.

学位論文概要

テフ（*Eragrostis tef*）は、エチオピアの人口の約半数の食糧、栄養源として、重要な役割を果たしている。この作物は、他国では長い間食用作物として認知されてこなかった。しかし、テフはグルテンを含まず、ミネラルや食物繊維が豊富なため、健康食品としての潜在的可能性が認知され、世界中から注目され始めている。栄養上の重要性に加え、テフは他の穀物よりも生態学的に有利な特性を持っている。例えば、他の穀物生産が困難な湿潤なバーティソル地域への適応性などがあげられる。一方、テフはゾウムシなどの貯蔵害虫に耐性があり、整備されていない貯蔵施設でも長期間の貯蔵が可能である。干ばつと気候変動が作物生産に影響を与えるエチオピアでは、安定的に生産できるテフの穀粒と藁は、主要な換金作物でもある。テフはエチオピアの最重要作物であるが、生産性は約1.76トン/ haと非常に低い。テフ生産の主要な収量と品質の制限要因は倒伏であり、収量の低下は農家が倒伏を避けるために最適な肥料量を適用していないことに起因する。

テフ倒伏抵抗性品種の開発において矮性遺伝子を利用するための努力がなされてきた。しかし、この試みは、草丈の低下、稈の弱さと穀物収量の低下との強い関連性のために成功していない。一方、テフの小花は非常に小さく、人口交配を用いた遺伝研究が制限されている。その結果、突然変異体集団（TILLING集団）は、倒伏抵抗性の新しい遺伝資源と期待されている。科学的知識の欠如は、テフの育種プログラムにおける重要な問題であり、特に稈の機械的強度、遺伝的多様性、耐倒伏性に関連する形質を支配するゲノム領域の特定は未開拓である。本研究はエチオピアの320系統のテフの表現型および遺伝子型の研究を行った。（i）系統間の収量と、集中栽培条件（ポット試験）と圃場栽培条件における系統間の倒伏、収量の違い。（ii）茎の機械的特性と農業形態学的形質と倒伏との関連性。（iii）次世代シーケンサーによるジェノタイピング（ddRADseq）法を使用し、遺伝的多様性、集団構造、および形質とマーカーの関連性調査。

最初の章では、本博士論文を概説した。 テフの起源と分布、重要性、生産の制限因子、倒

伏、以前の研究活動、および現状の問題と研究との溝について記した。 本博士論文の目的、全体構造も含まれる。

第2章では、圃場栽培条件下での多様なテフ遺伝子型における収量、遺伝率、および遺伝的進歩について説明する。 また、表現型および遺伝子型レベルでの形質の関係についても概説した。人工的に倒伏を制御した集中栽培条件（ポット試験）では、圃場栽培条件よりも広い範囲の収穫指数が観察された。 一方、両方の実験で、高い変動係数、遺伝率、および穀物収量の予想される遺伝的進歩が観察された。 収穫指数は、集中栽培条件での穀物収量と強い正の相関を示したが、圃場栽培条件下では穀物収量と弱い正の相関、および倒伏が比較的高かった圃場栽培条件下では乾物重と負の相関を示した。 これらは、テフ遺伝資源に幅広い遺伝的多様性が存在し、生産性を高める可能性があることを示唆した。

第3章では、320のテフ系統を使用して、稈の機械的特性と倒伏に関連する農業形態学的特性の観点から、倒伏抵抗性とテフ遺伝資源とを特徴づけた。調査した系統間に生体力学的および農業形態学的特性の多様性を明らかにした。倒伏指数、折れる瞬間、押し付け抵抗、および稈の直径などの倒伏に関連する特性は、草丈と強い正の相関があった。これは、草丈が短いほど、テフ稈の材料強度が低いことを示唆した。対照的に、分げつ数は倒伏指数と有意な負の相関を示した。一般に草丈の59%を占める花柄-穂の長さは、半矮性育種の目標となる。地際部からの倒伏と根の関係を調べた、高性系統では1m以上、矮性系統で57cm以上の深さに根は達しており、根域の多様性を明らかにした。茎からの倒伏が地際部からの倒伏よりもテフの倒伏特性に関連が高いことを示した。しかし、テフの初期成長では分げつが主に横方向に成長し、成長段階の後半に直立し始めるという我々の観察は、根と茎の接合点（クラウン）での空間競争を意味した。したがって、地際部からの倒伏を除外することはできず、将来的にさらなる詳細な調査が必要である。

第4章では、179系統のテフの一塩基多型（SNPs）情報を次世代DNAシークエンサーを用いた（ddRADseq法）で取得し、遺伝的多様性、集団構造、ゲノムワイド関連解析（GWAS）を行い、テフの倒伏耐性関連形質を制御する遺伝的要因を分析した。 STRUCUTUREプログラムのグ

ラフ、系統樹、およびTASSELプログラムのPCA散布図は、一貫して3つの集団の存在を示しました。耐倒伏性に寄与する生体力学的および農業形態学的特徴に基づく樹状図も、集団の構成と類似していた。多様性の1%のみが集団間の遺伝的変異によるものであったのに対し、多様性の99%は集団内にあったことを明らかにした。さらに、0.025であるF_{ST}の低い値は、テフの集団内での遺伝的分化が小さいことを明らかにした。茎の強度、倒伏指数、茎の直径、分げつ数について、SNPと形質の有意な関連性がを明らかにした。したがって、育種目標の優先順位付けを提供するだけでなく、効率的な育種のためのゲノム選抜やマーカー開発などの高度なゲノム研究を可能にした。

第5章は、本博士論文の結論と今後の方向性について記述した。集中栽培条件（ポット試験）により、テフの多様な収穫指数が観察された。中程度から高い遺伝的変異係数、遺伝率、および遺伝的進歩は、テフ改良に利用可能な材料を明らかにした。テフには生体力学的および農業形態学的特性が多様であることを明らかにした。背の高いテフの倒伏耐性に重要な形質は茎の強度、茎の直径、少ない分げつ数であった。これらの形質を支配するゲノム領域をGWAS解析により明らかにした。よって、本博士研究で明らかにした倒伏耐性関連形質を制御するゲノム領域を交雑によってテフ品種に導入し選抜する道を切り開いた。

LIST OF PUBLICATIONS

1. Bayable, M., Tsunekawa, A., Haregeweyn, N., Ishii, T., Alemayehu, G., Tsubo, M., Adgo, E., Tassew, A., Tsuji, W., Asaregew, F. and Masunaga, T., 2020. Biomechanical properties and agro-morphological traits for improved lodging resistance in Ethiopian teff (*Eragrostis tef* (Zucc.) Trotter) accessions. *Agronomy*, 10(7): 1012. (doi:10.3390/agronomy10071012; online)

   [This publication has covered chapter 3 of the thesis]

2. Bayable, M., Tsunekawa, A., Haregeweyn, N., Alemayehu, G., Tsuji, W., Tsubo, M., Adgo, E., Tassew, A., Ishii, T., Asaregew, F. and Masunaga, T., 2021. Yield potential and variability of teff (*Eragrostis tef* (Zucc.) Trotter) germplasms under intensive and conventional management conditions. Agronomy, 11(2): 220 (doi:10.3390/agronomy 11020220; online)

   [This publication has covered chapter 2 of this thesis]

APPENDIXES

Table A1: Descriptive statistics of grain yield and related traits of 317 teff genotypes under the intensive growing condition.

Variable	Min		Mean	Max		SD
	Value	Genotype		Value	Genotype	
Days to heading	51	237709-2	60	73	212930-4	3.75
Days to maturity	107	230802-4	121	140	230774-5	6.78
Grain filling period	35	212930-4	60	79	230774-5	8.04
Plant height	94.7	234431-1	126.9	156.6	RIL-2	9.99
Panicle length	21.6	55069-3	32.2	46.2	204535-1	4.11
Peduncle length	20.8	234431-1	26.4	31.1	222122-3	1.98
Panicle weight	0.87	234431-1	1.77	2.6	204535-1	0.21
Grain yield	4.2	55069-3	6.24	8.8	242138-1	0.86
Biomass weight	14.6	234431-1	18.4	23.1	204596-3	1.77
Harvest index	0.25	239373-2	0.34	0.45	242138-1	0.03

SD = standard deviation.

Table A2: Descriptive statistics of grain yield and related traits of 320 teff genotypes under the field growing condition (Combined across locations and years).

Variable	Min		Mean	Max		SD
	Value	Genotype		Value	Genotype	
Days to heading	51	204596-2	60	66	214215-4	2.57
Days to maturity	108	234431-1	126	131	203008-5	3.89
Grain filling period	53	229123-2	65	71	249333-2	3.10
Plant height	73.9	234431-1	99.3	129.7	RIL-260	10.58
Panicle length	19.3	235676-4	28.2	39.2	204535-1	3.08
Peduncle length	16.4	234742-3	24.5	31.0	234778-4	2.40
Panicle weight	0.58	237709-2	1.08	1.84	204535-1	0.19
Grain yield	1.8	219882-4	3.1	4.3	229971-3	0.54
Biomass yield	7.4	244784-3	10.7	13.5	203010-4	1.02
Harvest index	0.23	219882-4	0.29	0.34	236261-4	0.02

SD = Standard deviation.

Table A3: Individual location based genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficients of important traits of 320 teff genotypes under the field growing condition.

a) Adet (Nitosol area) combined over two years

	DH	DM	GFP	PH	PL	PDL	PW	GY	BM	HI
DH		0.50**	-0.34*	0.47**	0.46**	-0.21*	0.46**	-0.30*	-0.08^{ns}	-0.43*
DM	0.48**		0.64**	0.30*	0.31*	-0.01^{ns}	0.31*	0.11^{ns}	0.21*	-0.17*
GFP	-0.07^{ns}	0.84***		-0.08^{ns}	-0.06^{ns}	0.18*	-0.06^{ns}	0.38*	0.32*	0.20*
PH	0.39*	0.27*	0.07^{ns}		1.00***	-0.06^{ns}	1.00***	0.21*	0.72***	-0.71***
PL	0.20*	0.15*	0.05^{ns}	0.67**		-0.05^{ns}	1.00***	0.21*	0.63**	-0.59**
PDL	-0.16*	0.04^{ns}	0.14*	0.02^{ns}	0.09^{ns}		-0.05^{ns}	-0.05^{ns}	-0.03^{ns}	0.01^{ns}
PW	0.40*	0.30*	0.10^{ns}	0.92***	0.63**	-0.03^{ns}		0.20*	0.63**	-0.62**
GY	-0.18*	0.06^{ns}	0.18*	0.27*	0.27*	-0.11^{ns}	0.30*		0.84***	0.45**
BM	-0.01^{ns}	0.11^{ns}	0.13*	0.53**	0.39*	-0.07^{ns}	0.51**	0.80***		-0.08^{ns}
HI	-0.25*	-0.09^{ns}	0.05^{ns}	-0.38*	-0.17*	-0.04^{ns}	-0.32*	0.30*	-0.31*	

b) Bichena (Vertisol area) combined over two years

	DH	DM	GFP	PH	PL	PDL	PW	GY	BM	HI
DH		0.89***	-0.45**	0.55**	0.37*	-0.16*	0.37*	-0.25*	-0.10^{ns}	-0.40*
DM	0.53**		0.04^{ns}	0.98***	0.59**	0.27*	0.93***	0.21*	0.17*	0.12*
GFP	-0.24*	0.70***		0.71***	0.37*	0.85***	0.91***	0.90***	0.50**	0.99***
PH	0.40*	0.42*	0.15*		0.77***	-0.04^{ns}	0.82**	0.20*	0.50**	-0.02^{ns}
PL	0.28*	0.26*	0.07^{ns}	0.74***		-0.55**	0.70***	0.21*	0.47**	0.01^{ns}
PDL	-0.16*	0.08^{ns}	0.23*	-0.03^{ns}	-0.52**		-0.02^{ns}	-0.04^{ns}	-0.06^{ns}	0.10^{ns}
PW	0.28*	0.43*	0.26*	0.63**	0.56**	-0.05^{ns}		0.54**	0.69***	0.34*
GY	-0.22*	0.08^{ns}	0.28*	0.19*	0.20*	-0.03^{sns}	0.43*		1.00***	0.98***
BM	-0.08^{ns}	0.13*	0.21*	0.35*	0.33*	-0.08^{ns}	0.49**	0.80***		0.86***
HI	-0.29*	0.01^{ns}	0.25*	-0.04^{ns}	-0.01^{ns}	0.07^{ns}	0.23*	0.83***	0.34*	

*, **, ***, indicates significant correlation coefficients at a p values of 0.05, 0.01, and 0.001 respectively, ns = non-significant at $p < 0.05$. DH = days to heading, DM = days to maturity, GFP = grain filling period, PH = plant height (cm), PL = panicle length (cm), PDL = peduncle length (cm), PW = panicle weight (g), GY = grain yield (g/plant), BM = above ground biomass (g/plant), HI = harvest index.

Table A4: Cluster members of the 317 teff genotypes under the intensive growing condition.

Cluster	Size	Cluster members
I	36	225819-3, 229985-2, 234435-3, 235758-3, 234746-3, 236763-1, 237695-4, 234391-1, 237574-2, 238223-1, 202975-2, 204596-4, 203010-4, 242186-1, 55104-1, 55096-,1 55096-2, 55246-2, Dz-01-3772, 229766-1, 219878-1, 229767-4, 237158-1, 229767-1, 234776-1, 234776-3, 234777-1, 234778-3, 244882-1, 219852-2, 229766-5, 234357-2, 249333-2, 236765-3, 237697-1, 236261-3.
II	89	229101-3, 244792-4, 244792-5, 234430-1, 219852-4, 234430-3, 229985-3, 229971-1, 237143-2, 237143-4, 238684-1, 237707-1, 237143-1, 224102-1, 229759-1, 229759-2, 225926-2, 235659-1, 219850-2, 55189-3, 55151-3, 55144-4, 55150-3, 202385-2, 238219-3, 235362-2, 237695-3, 238224-2, 237577-1, 235676-3, 244885-2, 237578-2, 237691-1, Abola, 237691-3, 237573-1, 237573-2, 237577-3, 244885-1, 212487-5, 212929-2, 212929-4, 206081-1, 212490-5, 212489-1, 204596-3, 212489-2, 202978-1, 204535-1, 203007-2, 55111-6, 55114-3, 55125-1, 55140-1, 55114-4, 55140-2, 235667-5, 235659-3, Dz-01-115, RIL-260, 242140-1, 234774-1, 239375-1, 229767-3, 237703-4, 235675-3, 202931-3, 214215-1, 212930-2, 239373-5, 244882-3, 230774-5, 244793-2, 230802-2, 230772-3, 55017-3, 238606-4, 236364-2, 234742-3, 236364-4, 434734-1, 236261-1, 230774-3, 230774-4, 234761-2, 234701-2, 244773-1, 237575-1, Etsub.
III	17	242200-1, 229101-1, 227786-4, 234462-1, 236766-1, 236756-2, 237788-1, 237574-3, 205151-1, 235659-2, 235671-1, Abishlemne, 236261-4, 229971-3, 229986-4, 244783-3, 242138-1
IV	31	229123-2, 244792-3, 244792-2, 236088-1, 55177-2, 55148-1, 55271-3, 55144-3, 235676-4, 239352-3, 237728-2, 234782-2, 212929-1, 202978-2, 202978-6, 55130-4, 235671-2, 235667-1, RIL-2, 235753-2, 239375-2, 242138-4, 222062-1, 212930-1, 214215-4, 229766-3, 244793-7, 230802-4, 230774-1, 230774-2, 242138-2
V	76	236331-2, 237729-2, 237698-1, 234431-1, 228970-1, 228970-2, 55151-2, 237573-3, 237572-1, 237576-2, 244887-1, 234746-2, 204596-1, 212695-2, 204596-2, 238609-2, 55052-2, 203008-5, 55091-1, 55091-2, 55052-1, 55130-3, 55096-3, 55102-2, 55126-3, 55126-4, 55150-1, 55150-2, 238223-2, 235474-3, 236745-2, 55002-2, 202375-2, 55192-2, 238618-1, 55034-1, 55001-2, 235667-4 , 235667-6, 236261-2, 236745-1, IBC-31, IBC-29, IBC-26, IBC-37, IBC-45, 234775-4, 244773-2, 219878-3, 229767-5, 219882-4, 237709-2, 222062-4, 244792-1, 222062-3, 222122-3, 225898-2, 225907-3, 225898-1, 229971-2, 244783-1, 214214-1, 236361-1, 236535-2, 234778-2, 215198-3, 202931-6, 219858-2, 229766-2, 244793-5, 230802-3, 229986-2, 244793-8, 55017-1, 234742-2, 237146-3
VI	52	237143-3, 234462-2, 239762-3, 225819-5, 239098-1, 229759-4, 229097-2, 228668-2, 225825-3, 225819-1, 55148-3, 55144-1, 235368-1, 236761-3, 238224-3, 238221-3, 237691-2, 206081-4, 237214-3, 212487-4, 202975-1, 212695-1, 237690-1, 204535-2, 55125-3, 55130-1, 55140-3, 202390-2, 202378-2, 55194-3, 55251-2, 202377-4, IBC-30, IBC-10, Dz-01-3502, Dz-01-3597, Quncho, 244784-3, 239373-4, 237700-3, 225906-1, 222124-2, 234776-2, 214214-2, 215198-2, 234778-4, 219852-1, 229766-4, 229987-1, 244793-9, 234357-1, 238606-1
VII	16	55225-4, 212930-4, 55069-3, 55002-1, 219850-1, 55192-4, 55002-4, 235667-3, 242149-3, 239373-2, 229767-2, 228668-3, 244784-5, 202931-5, 212489-4, 214215-3

Table A5. Means squares of single environment ANOVA for agro-morphological traits and biomechanical properties of 320 teff germplasms.

Sources of variation	PH	PL	CL	PDL	TNP	PW	GYT	LIV	DM	SM	BS	LI
Adet station 2018												
Replication	2297.5**	41.1**	2728.3**	223.7**	143.3**	1.95**	0.35**	17.2^{ns}	5.28**	11.3**	0.056**	119.9**
Block (Replication)	380.8**	82.1**	253.1**	36.6**	23.7**	0.16^{ns}	1.02**	405.6**	0.36**	0.85**	0.005**	32.9**
Genotype	198.1**	68.8**	95.1**	28.3**	15.4**	0.21**	0.76**	607.5**	0.29**	0.57**	0.003**	37.1**
Error	47.3	0.32	30.6	3.85	0.5	0.1	0.05	149.5	0.04	0.07	0.0003	7.33
CV%	6.69	1.58	8.22	7.4	5.2	24.7	7.9	16.9	7.7	19.1	11.5	2.0
Grand mean	102.7	35.4	67.3	26.5	13.6	1.3	2.7	72.3	2.7	1.4	0.14	137.8
Bichena station 2018												
Replication	928.8**	460.4**	1611**	187.6**	589.1**	1.36**	0.32**	9954**	6.13**	7.17**	0.034**	85.56*
Block (Replication)	603.8**	65.59**	225.6**	31.13**	40.28**	0.17**	0.63**	746.9**	0.51**	0.78**	0.0038**	29.91^{ns}
Genotype	319.9**	44.62**	131.3**	23.50**	33.39**	0.11**	0.51**	1011**	0.31**	0.39**	0.0024**	34.69**
Error	64.9	1.78	11.81	3.53	8.22	0.007	0.04	131.1	0.06	0.04	0.00014	19.12
CV%	9.2	4.9	5.7	8.2	17.9	10.7	10.9	18.3	10.4	23.5	12.8	3.2
Grand mean	87.6	27	60.6	23	16	0.79	1.81	62.7	2.27	0.86	0.09	137.4
Adet station 2019												
Replication	750.8**	49.58**	1999**	177.7**	290.8**	0.771**	0.655**	9844**	5.81**	9.61**	0.038**	584.7**
Block (Replication)	645.0**	128.1**	241.6**	35.02**	61.23**	0.304**	1.052**	620.5**	0.57**	1.29**	0.005**	79.29**
Genotype	267.8**	111.2**	123**	27.3**	16.28**	0.279**	0.777**	773.2**	0.301**	0.52**	0.002**	32.96**
Error	26.9	0.496	4.71	3.51	4.64	0.024	0.048	110.1	0.032	0.022	0.00008	6.48
CV%	4.7	1.8	3.1	7.1	16.1	12.9	10.9	14.7	7.3	15.9	9.9	1.7
Grand mean	110.2	39.1	71.1	26.2	13.4	1.2	2.0	71.4	2.4	0.93	0.09	149.5

Table A6: Pearson correlation coefficient between eight agro-morphological traits, five biomechanical properties and lodging index of 320 teff germplasms.

	PL	CL	PDL	TNP	PW	GYT	LIV	DM	PR	MS	SM	BS	SS	LI
PH	0.84***	0.903***	-0.02	-0.61**	0.79***	0.214	0.68**	0.66**	0.62**	-0.38*	0.66**	0.73***	0.73***	0.59**
PL		0.533**	-0.41**	-0.53**	0.73***	0.23*	0.65**	0.58**	0.52**	-0.36*	0.57**	0.62**	0.62**	0.51**
CL			0.30*	-0.54**	0.66**	0.16	0.54**	0.58**	0.56**	-0.31*	0.59**	0.66**	0.67**	0.53**
PDL				-0.05	-0.03	-0.03	-0.16	0.08	0.09	-0.06	0.09	0.09	0.10	-0.01
TNP					-0.54**	-0.03	-0.35*	-0.66**	-0.58**	0.53**	-0.61**	-0.66**	-0.655**	-0.53**
PW						0.39*	0.50**	0.64**	0.58**	-0.43**	0.64**	0.67**	0.66**	0.49**
GYT							0.16	0.20	0.17	-0.15	0.21*	0.20*	0.20	0.08
LIV								0.38*	0.36*	-0.25*	0.36*	0.41**	0.41**	0.39**
DM									0.91***	-0.87***	0.99***	0.96***	0.94***	0.55**
PR										-0.70***	0.92***	0.93***	0.93***	0.50**
MS											-0.81***	-0.72***	-0.70**	-0.46**
SM												0.97***	0.95***	0.50**
BS													0.99***	0.54**
SS														0.55**

*, **, *** significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$. Plant height (PH), Panicle length (PL), Culm length (CL), Peduncle length (PDL), Tiller number per plant (TNP), Panicle weight (PW), Grain yield (GYT), Lodging score (LIV), 2nd basal internode diameter (DM), Pushing resistance (PR), Maximum stress (MS), Section modulus (SM), Base failure moment (BS), Breaking strength (SS) and Lodging index (LI).