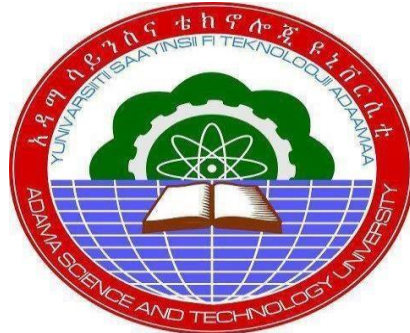


PHENOTYPIC AND SNPs BASED SCREENING OF SELECTED TEF (*ERAGROSTIS
TEF*) ACCESIONS FOR DROUGHT TOLERANCE AT VEGETATIVE STAGE.



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A THESIS SUBMITTED TO THE DEPARTMENT OF APPLIED BIOLOGY
SCHOOL OF APPLIED NATURAL SCIENCE

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE
DEGREE OF MASTER'S OF SCIENCE IN APPLIED BIOLOGY (BIOTECHNOLOGY)
OFFICE OF GRADUATE STUDIES

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

JUNE, 2023
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JUNE, 2023
ADAMA, ETHIOPIA

THESIS APPROVAL

We hereby certify that the recommendations and suggestions made by the board of examiners are appropriately incorporated into the final version of the dissertation entitled “Phenotypic and SNPs based Screening of Selected Tef (*Eragrostis tef*) Accessions for Drought Tolerance at Vegetative Stage” developed by Doni Hinsene.

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We, the undersigned, members of the Board of Examiners of the thesis by Doni Hinsene have read and evaluated the thesis entitled “Phenotypic and SNPs based Screening of Selected Tef (*Eragrostis tef*) Accessions for Drought Tolerance at Vegetative Stage” and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Biotechnology.

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DECLARATION

I hereby declare that this Master Thesis entitled “phenotypic and SNPs based Screening of Selected Tef (*Eragrostis tef*) Accessions for Drought Tolerance at Vegetative Stage” is my own original work and has not been submitted to any University or institute for the same purpose. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation

Name of the student

Signature

Date

RECOMMENDATION OF ADVISORS

We, the advisors of this thesis, hereby certify that we have read the revised version of the thesis entitled “phenotypic and SNPs based Screening of Selected Tef (*Eragrostis tef*) Accessions for Drought Tolerance at Vegetative Stage” prepared under our guidance by Doni Hinsene Wolabu, submitted in partial fulfillment of the requirements for the degree of Mater’s of Science in Biotechnology. Therefore, we recommend the submission of revised version of the thesis to the department following the applicable procedures.

Major Advisor

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ACKNOWLEDGEMENT

First of all, I would like to acknowledge and extend my sincere gratitude to my advisors Dr. Kero Jemal and Dr. Dejene Girma, who made this work feasible. Their consistent guidance was very valuable to realize this thesis. I am truly appreciative for their incredible mentorship and devotion.

Additionally, Mr. Abebaw Misganaw deserves my gratitude for his sincere assistance with the analysis of molecular data as well as for his insightful remarks and recommendations. I also want to extend my heartfelt thanks to all individuals who helped me during my study especially Dr. Kafiyalew Negisho and Mr. Sisay Kidane. I am also deeply grateful to Mr. Aleka Argachow who is always beside me in all aspects next to God, thank you for your generosity and encouragement in my work. I am also very thankful to the team and the greenhouse technicians at National Agricultural Biotechnology Research Center for their valuable support.

I would also like to thank National Agricultural Biotechnology Research Center for providing me the study opportunity and also financial support in this thesis research. Without the support of the NABRC this work would not have been possible. Thank you for your kind help.

My deepest gratitude and appreciation should also go to my beloved mother, Bushe Buta Dadhi, a courageous mom who raised me and my five brothers on her own. I thus, want to devote this thesis to my eternal love for her and to the memories of her that will always have a special place in my heart. I must also express my very profound gratitude to my wife Wasane Tesfaye and to Derartu Degafa, the daughter of my brother, for their support, motivation and excellent shouldering all responsibilities during the entire period of my study. My two cherished children Loko and Nedhi have consistently served as the cornerstones of my patience and the ribs of my bravery.

Last but not least, I would like to thank God for guiding me through every challenge. I have experienced your guidance day by day and it was you who allowed me to complete this work, I'll continue to put my future in your hands!

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LISTS OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of variance
CO ₂	Carbon dioxide
CRD	Completely Randomized Design
DArT	Diversity Arrays Technology
DNA	Deoxyribonucleic acid
DZARC	DebreZeit Agricultural Research Center
FDR	False Discovery Rate
GATK	Genome Analysis Toolkit
GVCF	Genomic variant call format
GWAS	Genome wide association study
K	Kinship matrix
LSD	Least Significant Difference
LTP	Leaf turgor pressure
MAB	Marker assisted breeding
MAS	Marker assisted selection
MLM	Mixed linear model
MTA	Marker trait association
PVC	Polyvinyl Chloride
Q	Population structure matrix
RAPD	Random amplified polymorphic DNA
RDWt	Root dry weight
RFWt	Root fresh weight
RL	Root length
SHDWt	Shoot dry weight
SHFWt	Shoot fresh weight
SHL	Shoot length
SNPs	Single nucleotide polymorphism
SSR	Single sequence repeat
TASSEL	Trait Analysis by aSSociation, Evolution and Linkage
TILLING	Targeting Induced Local Lesions IN Genomes
VCF	Variant call format

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ABSTRACT

Tef (Eragrostis tef (Zuccagni) Trotter) is a staple food supporting over 50 million people in Ethiopia. Ethiopia is the center of origin and diversity of tef. Tef production is low, specifically due to drought and lodging. Tef is more vulnerable to moisture stress during its early stages of development than it is at maturity. Moisture stress at pre-flowering stage can result in 69 - 77% grain yield reduction. Therefore, this study was aimed at morphological screening of 60 diverse tef accessions for moisture stress tolerance at their vegetative growth stage under water stress and non-stress greenhouse conditions and using SNPs marker. The experiment was laid out completely randomized design (CRD) with three replications. Phenotypic traits were recorded to evaluate the effect of water stress on tef. To complement the greenhouse experiment, 538,253 filtered SNPs generated from resequencing of the same accessions were used for Genome-wide association study (GWAS). GWAS based on mixed linear model (MLM) in TASSEL 5.2.9 identified SNPs with significant association ($FDR \leq 0.05$) to the studied traits. The two way ANOVA revealed existence of highly Significant ($P < 0.001$) differences among tef genotypes for root length, shoot length, root fresh weight, shoot fresh weight, root dry weight, and significant differences ($p < 0.05$) for shoot dry weight at both stressed and non-stressed conditions. The water treatment effect was also highly significant ($p < 0.001$) for all the studied traits except for root length. Accessions by Treatment interactions were highly significant ($p < 0.001$) for root fresh weight, shoot fresh weight and root dry weight, significant ($p < 0.05$) for shoot dry weight, and non-significant for root length and shoot length, indicating that thus water stress has detrimental effect on the majority of the evaluated phenotypic traits. Tef accessions found as tolerant to moisture stress; 243501, 225760, 243489, 243495, 243531 and 55183. GWAS identified a total of 215 loci significantly ($FDR < 0.05$) associated with the considered traits (shoot length, root dry weight, shoot fresh weight and root fresh weight) under water stress condition. The detected significant SNPs were distributed over the 20 tef chromosomes. Overall, in response to water stress, considerable genetic variability was found across the studied tef accessions, indicating promising possibility to develop water stress tolerant genotypes through integrated application of phenotypic and markers assisted breeding. Moreover, the detected significant markers could be used to screen more accessions in the tef gene pool and also to begin genome-assisted breeding of tef for water-stress tolerance.

Key words: Drought, Genome-assisted breeding, Genome-wide association study, Phenotype, SNPs, Tef.

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the study

Tef [*Eragrostis tef* (Zucc.) trotter] is an allotetraploid ($2n=4x=40$), small cereal grain crop that belongs to the family *Poacea*, sub-family *Eragrostoideae*, tribe *Eragrostidae* and genus *Eragrostis* (Ketema, 1997). Tef is a staple food supporting over 50 million people in Ethiopia (source). Ethiopia is the center of diversity and origin of tef (Vavilov, 1951). In Ethiopia, tef cultivation predates historical records from before wheat and barley were introduced to the country. Despite its low productivity, the Ethiopian farmers who engineered the domestication of this crop continued to cultivate it for millennia, with the area planted increasing over time (Assefa *et al.*, 2017)

Tef is a very nutritious grain. Its nutritional content is usually comparable to or better than that of the world's major grains such as wheat, rice, barley, and millet (Chanyalew *et al.*, 2019). It is superior in many aspects particularly in minerals such as calcium, iron, magnesium, phosphorus and potassium. Tef grains are also rich in essential amino acids, especially alanine, methionine, threonine, and tyrosine (USDA, 2013). In recent years, tef has become popular as a health and performance food in the global market. Spaenij-Dekking (2005) demonstrated that tef is useful as food for people suffering from gluten protein allergy diseases known as celiac disease, since the grains are gluten-free. Its low glycemic index, characterized by slow-release starch, makes it particularly suitable for diabetic patients (Baye, 2014). In addition, among people who consume tef as a staple food, its high iron content is related to the low prevalence of hookworm (Tafes *et al.*, 2020) and pregnancy-related anemia.

Tef has become world-famous and various products are available as health foods in Europe and North America, especially for gluten intolerant people (Saturni *et al.*, 2010). Straw is also a valuable source of animal feed. In South Africa, India, Pakistan, Uganda, Kenya and Mozambique, tef is mainly grown for fodder or forage (Assefa *et al.*, 2011). Tef grows under a wide range of ecological conditions from sea level to up to 3000 meter above sea level (m.a.s.l). However, its best performance occurs between 1800 and 2200 meter above sea level and mainly grown in the regions of Oromia, Amhara, Southern Nation and Nationalities and Tigray in Ethiopia (Tefera and Ketema, 2001).

Due to its diverse environmental adaptations, tef grows in high rainfall and drought-prone agro ecology. Yields in tef are relatively good during high rainfall seasons, but much limited during the drought period, without the complete crop failures that occur with other grains such as corn and sorghum (Abraha *et al.*,2015). Annual rainfall requirements for crops for optimal yields range from 950 to 1500 mm, but moderate yields are obtained even under low rainfall conditions ranging from 450 to 550 mm (Abraha *et al.*,2016). It also adapts to temperatures in the range of 10-27°C and flowers best under 12 hour day length. Although adapted to harsh environmental conditions, the productivity of tef is low in Ethiopia. The main factors limiting the productivity of tef are lodging and prolonged drought (Asefa *et al.*, 2011). Drought is so complex and destructive in plant biology which is comparable to cancers in mammalian biology. Its effects depend on the timing and intensity of stress on plant growth and development (Bhusal *et al.*, 2021). Plant responses to water stress are quite different and depend on the intensity and duration of stress, as well as the genotype and growth stage of the plant.

Understanding the response of plants to drought is important for developing drought-tolerant crops (Sohrawardy and Hossain, 2014). Drought tolerance is a complex quantitative trait controlled by many genes and influenced by the environment and genotype by environment interactions (Xoconostle-Cazares *et al.*, 2010). Breeding for drought tolerance depends on the accumulation of additive genes, a controlled stress screening environment and high-throughput selection methods to maximize the breeding advantage (Khan *et al.*,2016). Thus, the aim of this study was to screen selected tef accessions at vegetative stage through integrated application of morphological and SNPs marker to identify drought tolerant tef accessions.

1.2. Statement of the problem

Drought is a severe abiotic stress that has a considerable impact on agricultural productivity in the majority of African countries (Numan *et al.*, 2021). Tef production is low, specifically due to drought and lodging (Assefa, 2013). When compared to maize (4 tons ha⁻¹) and wheat (2.7 tons ha⁻¹) in Ethiopia, tef produced an average yield of only 1.7 tons per hectare (Cochrane and Bekele, 2018). Moisture stress is one of the major tef production constraint causing 26% to 51% decrease in grain yields (Shiferaw *et al.*, 2012). The impact of drought becomes severe and significant after planting specifically during flowering and grain filling stages. Tef is more vulnerable to moisture stress during its early stages of development than it is at maturity (Tefera *et al.*, 2000 cited in Abraha, 2016). Low moisture stress caused tef yield reductions up to 40% (Ayele, 1993). Ferede *et al.* (2018) reported that drought has reduced tef grain yields by 7.3% to 85% at the anthesis stage in greenhouses, and by 69% to 77% in the pre-flowering stage under field conditions. Therefore, identifying drought tolerance source materials is important for further tef breeding program. So far the integrated application of morphological and molecular approaches in tef breeding for drought tolerance is largely limited. Therefore, the present study was designed with the following general and specific objectives.

1.3. OBJECTIVES OF THE STUDY

1.3.1. General objective

To screen drought tolerance in selected tef accessions using morphological and molecular marker approaches.

1.3.2. Specific objectives

- ❖ To screen tef accessions for drought tolerance at vegetative stage under greenhouse moisture stress conditions based on morphological traits.
- ❖ To identify genomic regions underlying vegetative stage drought tolerance in tef.

CHAPTER TWO

2. LITRATURE REVIEW

2.1. Origin, distribution and diversity of Tef

Ethiopia is one of the richest agricultural origination centers in the world, producing various key crops such as *Eragrostis tef* and other related species (Reda, 2014). Ethiopia is the origin and diversification center of tef (Vavilov, 1951) and the crop species has coevolved with Ethiopians over the years. This is because Ethiopia not only has a wealth of crop species diversity, but it is also thought to be the site of genesis for its domestication, including the presence of putative wild progenitors. According to Reda (2014), crop variability does not exist everywhere in the globe except in Ethiopia, where the crop originated and was domesticated. The genus *Eragrostis* is one of the largest in the grass family, with over 350 species (Assefa *et al.*, 2017). About 43% of these species are thought to have originated in Africa, 18% in South America, 12% in Asia, 10% in Australia, 9% in Central America, 6% in North America, and 2% in Europe. Fourteen of the 54 tef species found in Ethiopia are indigenous to the country (Assefa *et al.*, 2017). Globally, there is no doubt that tef is originated in Ethiopia; however, the precise area where it was initially domesticated in Ethiopia is uncertain. Several studies (Melak Hail, 1965; Seyfu, 1993; Tiruneh *et al.*, 2000; Kebebew *et al.*, 2001 as cited in Tesema, 2013) confirmed that Tef is extremely diverse and variable in terms of morphological and agronomic aspects. The spread of the crop across various agro-ecological zones and the careful selection of farmers led to the emergence of several varieties with distinctive traits. Days to maturity (60 to 120 days), number of grains/plant (9,000–90,000 seeds), plant height (31–155 cm), number of tillers/plant (5–35), panicle type (from very loose open to very compact) figure1, flag leaf area (2-4 cm²), and culm diameter (1.2–5 mm) are among the parameters or characters with the greatest diversity. Tef has been distributed over the world by a variety of organizations and people. The date of tef's worldwide footmark, however, varies according to the sources. The Royal Botanical Gardens, Kew, London, United Kingdom, acquired tef seeds from Ethiopia between 1866 and 1886 and delivered them to certain British colonies, including India, Australia, the United States of America, South Africa, and British Guyana (Seyfu, 1997).Tadesse (1975) claims that Burt Davy brought tef to California (USA), Malawi, Zaire, India, Sri Lanka, Australia, New Zealand, and Argentina in 1916. It was

brought by Skyes to Zimbabwe, Mozambique, Kenya, Uganda, and Tanzania in 1911. Tef was introduced to Palestine by Horwitz in 1940.



Figure 1. Diversity in the form of tef panicles. (A) Very compact, (B) semi-compact, (C) fairly loose, (D) very loose. Source:(Assefa *et al.*, 2015)

2.2. Morphology and physiology of tef

2.2.1. Morphology of tef

Morphologically, tef is a fine stemmed tufted annual grass with a lag crown, many shoots and a shallow diverse root structure. It has a fine grain inflorescence that ranges in color from white and red to dark brown and its panicle can be loose or compact (Dijkstra and Polman, 2009) figure2. Under field circumstances, the root system of the tef plant is thin and fibrous (thread-like), rarely emerging from nodes above the base and growing 4-8 cm deep (Tadesse Ebba, 1969). The stems are typically upright (ascending), although in certain cultivars they geniculate (creep, bend or elbow) and are jointed with hollow internodes separated by nodes. One leaf with a sheath and a blade is present on each culm internode other than the most basal one. The Paniculate tef inflorescence ranges in form from very compact (whip-like) to very loose and open.

The panicles branch into primary, secondary, and tertiary bearing spikelets. Each spikelet bears a pair of unequal sized glumes at the base and a number of florets (3–17) above. A tri-nerved lemma, a two-nerved bow or boat-shaped palea, three stamens (arising from the ovary base and with extremely thin and slender filaments bearing length-wise opening anthers at the apex) and an ovary or a pistil are all present in each floret. The ovary has two or in rare and exceptional circumstances, three styles, each of which ends in a plumose (feathery), yellowish-white stigma. Tef is a highly self-fertilized species with just approximately 0.2-1% natural outcrossing (Seyifu, 1993). Basipetal (top-down) flowering, anthesis, maturity of florets and grains occur on a panicle basis and acropetal (bottom-up) on a spikelet basis.

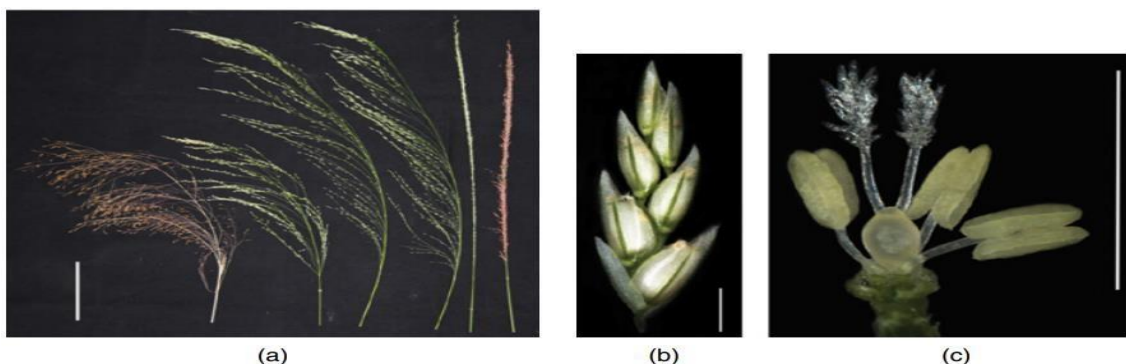


Figure 2. The inflorescence and flower of tef. a) Panicles of tef differ in color and size (scale bar = 10 cm); b) spike of tef showing individual spikelet (scale bar = 1mm); c) structure of tef flower indicating three stamens and a pair of hairy stigmas (scale bar = 1mm). Source: (Assefa *et al.*, 2017).

2.2.2. Physiology of tef

Tef is an annual herbaceous plant that matures physiologically between 60 and 140 days (Assefa *et al.*, 2011). It is a C4 plant that produces 4-carbon molecules (malic or aspartic acid) as the first by product of photosynthesis. It also demands a high optimal temperature for photosynthesis and whereas the typical carbon dioxide compensation points for C3 plants are in the range of 14-20 $\mu\text{l/l}$ and 5 $\mu\text{l/l}$ or less for C4 plants, the tef compensation point is $6 \pm 1 \mu\text{l/l}$ at 38°C. Furthermore, tef's leaf structure is Kranz type, with vascular bundles enclosed by bundle sheath cells in a circular pattern (Assefa *et al.*, 2011).

2.2.3. Taxonomy of tef

Tef belongs to the Poaceae family as do all economically important cereals. It is closely related to finger millet (*Eleusine coracana* Gaerth.) as both are in the subfamily Chloridoideae. The genus *Eragrostis* comprises about 350 species from which only tef is cultivated for human consumption. Although the crop species have had several synonyms previously used by several authors, presently its most accepted binomial nomenclature is [*Eragrostis tef* (Zucc.) Trotter] (Assefa *et al.*, 2011). Unlike wheat, barley and rice, which are all C3 plants, tef along with maize and sorghum is a C4 plant which efficiently utilizes carbon dioxide during photosynthesis. This can be seen by tef's Kranz-type leaf anatomy with vascular centers surrounded by bundle sheath cells containing a high number of chloroplasts and by the low CO₂ compensation point of the leaves, also typical of C4 as opposed to C3 species (Kebebew *et al.*, 2015). The genus *Eragrostis* is basically a complex taxon distinguished by the prevalence of polyploidy (approximately 69%) and common presence of cytological races. The species in the genus vary from diploids ($2n = 2x = 20$) to hexaploids ($2n = 6x = 60$). Tef is an allotetraploid ($2n = 4x = 40$) with disomic inheritance patterns that forms 20 bivalents in meiotic Metaphase I. (Berhe *et al.*, 2001). However, the probable diploid progenitors have not yet been found. *Eragrostis pilosa*, an allotetraploid, is thought to be the closest related and potential direct wild progenitor of tef, according to recent DNA-based investigations (Ayele *et al.*, 1999; Ayele and Nguyen, 2000; Ingram and Doyle, 2003). Assefa *et al.* (2011) stated that even by the standards of the genus, the chromosomes of tef are extremely tiny (0.8-2.9 μm); the largest tef chromosome is smaller than the smallest (1D) wheat chromosome (Gugsa *et al.*, 2001). Unlike several of its related *Eragrostis* species, neither chromosomal races nor aneuploidy have been found in this species. According to Kebebew Assefa *et al.* (2011), the average

nuclear genome size is 730 Mbp, which is nearly the same as the diploid sorghum genome (*Sorghum bicolor*, 735 Mb) and roughly 60% larger than the diploid rice genome (*Oryza sativa*, 430 Mb).

2.3. Economic importance of tef

Ethiopia is now the world's largest tef producer and the only country to have adopted tef as a staple crop, producing more than 90% of the world's tef (Tadele and Hibistu, 2022). The sole cereal crop in which the nation has a competitive trade advantage is tef. Tef is grown by 6.5 million smallholder farmers and many Ethiopians depend on it for their livelihoods (Tadele and Hibistu, 2022). More than 50 million people in the Horn of Africa rely on tef as a main diet today. Because it accounts for 72% of all cultivated land in Ethiopia, this sector is the most significant in the country's agricultural economy (Fikadu *et al.*, 2019). In Ethiopia, tef is mostly grown to harvest the economic portion of the grain for personal use. Once the grains have been processed, they are used to make Injera, Ethiopia's most well-known meal (Reda, 2014). Tef is one of the world's least well-known, most nutritious grains and Injera made from it has exceptional flavor, aroma, texture and durability (Tilahun, 2021). It is well known for its excellent nutritional value and 99% high return quality following milling as opposed to 60-80% from wheat (Reda, 2014). The grains may also be processed into flour, which is used to make porridge and alcoholic drinks such as tela and katicala. Additionally, Tef straw is used in the construction of traditional homes, granaries, and animal feed. Compared to other cereal straw, the straw is the most favored and appealing animal feed and it also has equivalent or even higher nutritional content than traditional forage crops (Miller, 2010). Because it is the primary choice of Ethiopians for food and animal feed, it fetches high market prices (Worede *et al.*, 2020). As a result, tef is Ethiopia's most significant commodity in terms of area planted and production value, and the second most important crop in terms of cash generation after coffee, producing over \$500 million each year for local farmers (Fikadu *et al.*, 2019). Studies indicate that the value of Injera exports in 2015 was over \$10 million (Hassen *et al.*, 2018; Fikadu *et al.*, 2019). Tef has a greater economic surplus than the nation's three other major cereals (sorghum, maize, and wheat) when taken as a whole. Tef is also a more profitable commodity in Ethiopian markets and it is a significant cash crop for farmers since it frequently fetches a market price two to three times more than maize and the grain with the biggest production volume in the nation (Assefa, 2015).

2.4. Nutritional value of Tef and its significance to human health

Tef's nutritional value, health benefits and quality value as a gluten-free grain have drawn interest globally (Sridhara *et al.*, 2022). Tef has highly appealing nutritional profile and gluten-free grain make it a suitable substitute for wheat, maize and other cereals in food applications, particularly for people with celiac disease. As a result, interest in tef has increased significantly (Girma *et al.*, 2017). Numerous gluten-free products might not provide enough fiber, vitamins, or minerals on a daily basis. As a result, they must be fortified to meet the daily intake requirements (Oliveira *et al.*, 2018). Compared to many other grains, tef naturally has a higher nutritional value, so it doesn't require fortification (Gebremariam *et al.*, 2014). The grain has high nutritional values, including a significant amount of protein, carbohydrate, fat, vitamin A and C, fiber, thiamin, riboflavin, and niacin, as well as important minerals like calcium, chloride, chromium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc (Girma *et al.*, 2017). Tef grains include all eight essential amino acids (isoleucine, leucine, methionine, lysine, phenylalanine, threonine, tryptophan and valine). Tef grains contain an average of 9 - 15% protein, 2% to 4% fat, 2% to 4% fiber, 68% to 74% carbohydrates, 10% to 13% moisture, and ash content between 2% and 3%, according to a study by the Ethiopian Institute Biodiversity Conservation laboratory using 114 tef variants collected from Ethiopia (Science, 2019). In addition, tef contains more of the amino acid lysine than other cereals despite having a protein content that is comparable to that of other common cereals like wheat. Tef is rich in phytochemicals like polyphenols and phytates, as well as fatty acids, minerals, fiber, and fiber-related nutrients (Baye, 2014). Tef's high fiber, calcium, and iron content, according to Dekking and Koning (2005), made it essential for preventing pregnancy anemia. In comparison to other cereal crops like wheat, sorghum, rice, barley, and maize, the crop has a longer shelf life and a slower rate of aging of its bread products. The grain is associated with a number of health advantages, including the prevention and treatment of diseases like celiac disease, diabetes and anemia, as stated by Gebru *et al.* (2020). Tef consumption also has a number of positive health effects, such as the growth of sturdy bones and teeth, a decrease in premenstrual syndrome, blood sugar regulation and sustained energy. The iron deficiency in the diet can be corrected with a daily dose of 100 g of tef (Sridhara *et al.*, 2022).

2.5. A significant turning point in Tef research history and improvement

In the late 1950s, scientific investigation into tef improvement started in Jimma at what was formerly known as Jimma Technical and Agricultural High School. The study was transferred in 1960 to the Central Experiment Station, which is now the DebreZeit Agricultural Research Center (Kebebew and Chanyalew, 2018). The following three related phases are identified as part of the overall history of tef Breeding: The first phase took place from 1956 to 1974, and was distinguished by germplasm enhancement (collection/acquisition, characterization and evaluation, systematics and conservation), genetic improvement solely based on mass and/or pure line selection directly from the existing germplasm and the beginning of induced mutation techniques.

The second phase was from 1975 to 1995 years and was marked by the discovery of the artificial crossing technique by Berhe (1975) and the subsequent incorporation of intraspecific hybridization in the genetic improvement program. Additionally the chasmogamous floral opening behavior of tef flowers (from approximately 6:45–7:30 am) was discovered in this period. The third phase was from 1995 to the present, and it is distinguished by the following activities: (1) initiation of molecular/genomic approaches, which involves the development of molecular markers, genetic linkage maps and analyses of molecular genetic diversity; (2) incorporating of *in vitro* culture techniques and interspecific hybridization; (3) re-evaluating induced mutagenesis, particularly for lodging and leaf rust disease resistance and (4) stepping up the use of participatory breeding techniques. As a result, over the past 20 years there has been advancement in the study of tef genetic architecture and genomics (Numan *et al.*, 2021) as illustrated in figure3.

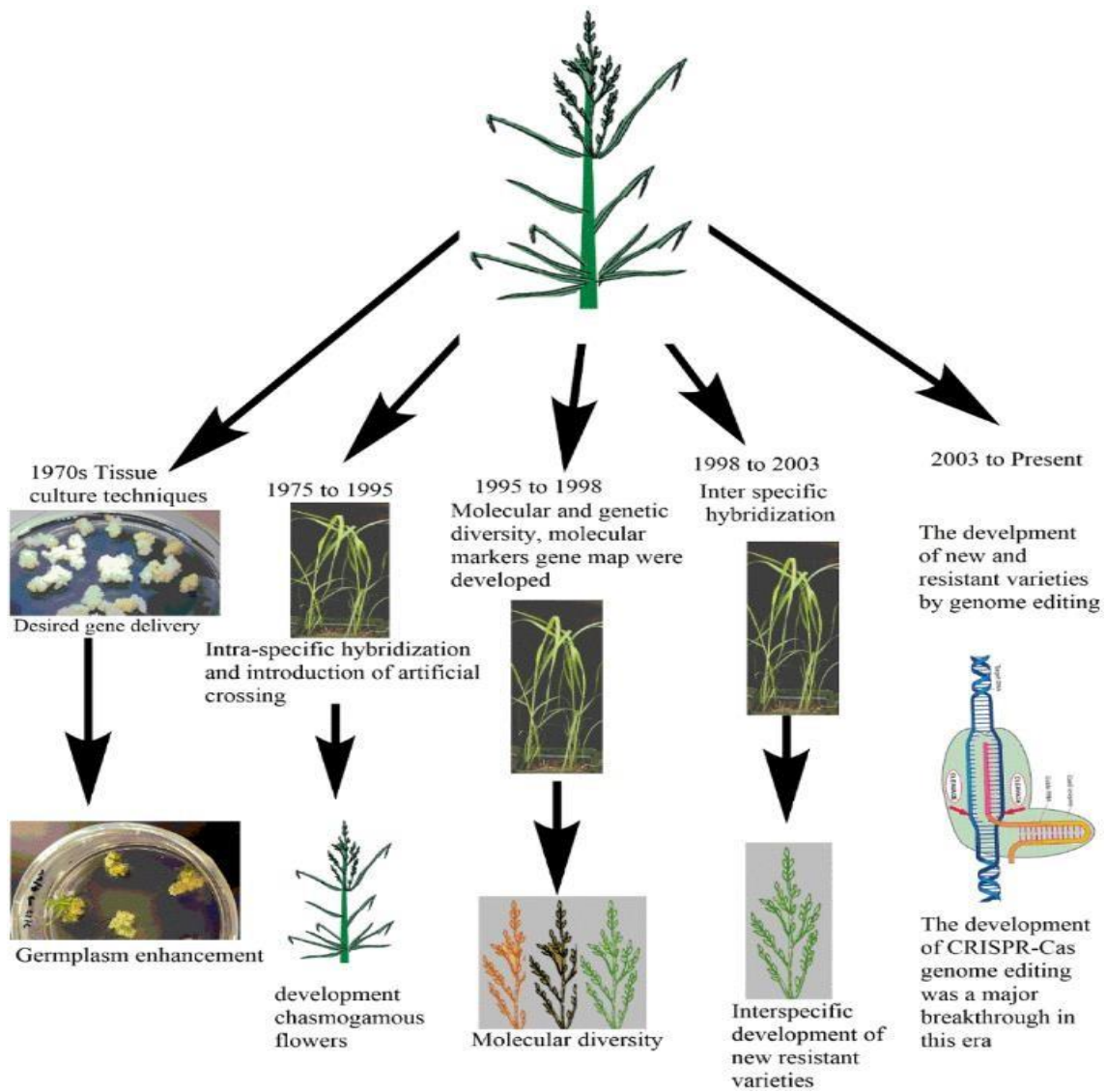


Figure 3. Improvement of tef varieties over the last 50 years. The improvement of tef started back in 1970s with tissue culture techniques, followed by hybridization, the study of molecular diversity, molecular marker analysis, the development of resistant varieties by interspecific hybridization and mutation and the recently emerged clustered regularly interspaced short palindromic repeats (CRISPR)-associated proteins (CRISPR-Cas) based genome editing technique. Source: (Numan *et al.*, 2021).

2.6. Mechanisms of drought tolerance in Tef

In order to develop alternative strategies for enhancing yield and quality, it is crucial to understand the degree of stress tolerance in crops. One of the main abiotic factors impacting plant growth and development is drought. In order to cope with drought, plants have developed a variety of strategies for drought tolerance (Sofia *et al.*, 2013; Mickelbart *et al.*, 2015). Modifications in stomatal conductance, osmotic adjustment, the establishment of a deep root system, and maintenance of cell membrane stability are some of the processes that have been reported in tef (Araya *et al.*, 2011). Many crops, especially tef have important drought stress tolerance mechanisms, including the development of a deep root system and osmotic adjustment. Previous research on tef found a correlation between plant height, root depth and thickness are related to tolerance to drought stress (Numan *et al.*, 2021).

Recent research on tef and other cereals established a correlation between plant height and drought tolerance, with semi-dwarf plants being proven to be drought-tolerant (Plaza *et al.*, 2016). It is also known that osmotic adjustment allows tef leaves to sustain leaf turgor pressure (LTP) during extreme drought circumstances by retrieving and absorbing water even from dry soils. Modification of root growth parameters in response to water scarcity is another strategy used to mitigate drought stress. Another technique for reducing drought stress is to modify root growth characteristics in response to water stress (Numan *et al.*, 2021). For example, the increase in root length of cowpea, peanut and soybean plants when exposed to drought enabled them to absorb deep soil water (Merrill *et al.*, 2002). Similarly, growing deep-rooted tef plants with a broad and extensive root system is a desired characteristic for drought tolerance.

2.7. Mechanisms of gene action for drought tolerance

Drought tolerance is a complex feature in which its expression is determined by the action and interaction of several genes and the environment, which influence morphological, physiological and biochemical characteristics. The success of any breeding effort for generating drought-tolerant cultivars is dependent on exact estimates of genetic variance components of the traits of interest, which primarily consist of additive, dominant and epistasis genetic influences (Abraha *et al.*, 2015). Additive gene action is connected with the presence of heterozygotes that perform intermediately between the two homozygotes in terms of a certain trait. Dominance is a sort of gene activity in which the heterozygote

performs the same as the dominant homozygote. Epistasis is a phenomenon in which two or more gene loci interact to influence a genotype's performance (Forneris *et al.*, 2017). To choose the best selection techniques, it is helpful to have knowledge about the gene action mode of the desired traits of a particular crop in a certain environment (Acquaah, 2015). If additive gene action predominates in self-pollinated species, the breeder can successfully select at different levels of inbreeding and produce a significant improvement of the trait of interest since additive effects are reversible and easily passed down from one generation to another. On the other side, sufficient non-additive gene activity may justify the generation of hybrids since it optimizes heterosis.

2.8. Development of molecular markers

Molecular markers are the most recent technology that friendly to examine gene localization and can be effective in plant breeding through desirable gene selections. The history of molecular markers development indicates that genetic positions of markers has improved in genomes over the last two decades that is providing fast and easy support for breeders and scientists. Molecular markers use the technology of genome analysis that helps to create information database for common use. The use of molecular markers in tef improvement began in 1995-1998 (Assefa *et al.*, 2011). Basically closely linked chromosomal regions are inherited together, and hence, marker-assisted breeding (MAB) or marker-assisted selection (MAS) uses molecular markers close to the target genes to track the introgression or presence of the target gene (Ibitoye and AkinIdowu, 2011). They enable for the efficient utilization of alleles during phenotypic selection. Microsatellites (simple sequence repeats; SSRs), amplified fragment length polymorphisms (AFLPs) and single nucleotide polymorphisms (SNPs) are the most often utilized markers. SSRs, expressed sequence tags, restriction fragment length polymorphisms and random amplified polymorphic DNA (RAPD) have all been developed for tef (Yu *et al.*, 2006). Abraha *et al.* (2016) used SSR markers to identify and enhance certain critical features in tef, such as grain yield, days to maturity, panicle length and plant height. Similarly, diversity in tef accessions was detected using AFLP markers, which may be employed in seed multiplication and breeding programs (Numan *et al.*, 2021). The use of these markers might have a significant impact on environmental stress tolerance in tef, resulting in increased productivity. Targeting induced local lesions in genomes (TILLING) was employed in tef to target and enhance beneficial agronomic features such as dwarfism, seed size and drought tolerance (Chanyalew *et al.*, 2019)

2.9. Single nucleotide polymorphism (SNPs) marker

Molecular markers are used to identify gene positions or genomic areas that regulate important traits in plants. SNPs are a widely used and popular DNA marker for identifying genomic regions for important traits that significantly speeds up plant breeding (Dwiningsih *et al.*, 2020). It refers to a change in one base pair at a particular locus involving two alleles, where the unusual allele frequency is more than 1 %.(Mokhena *et al.*, 2016). It is a single nucleotide base difference between two DNA sequences. Thus, it denotes the point in the DNA sequence where there is a one-base change. Based on nucleotide substitution, they are divided into two categories: transition, which is the exchange of pyrimidines (C/T) and purines (A/G), and transversion, which is the exchange of the purine base for the pyrimidine (G/C, A/T, A/C and G/T).

The SNP is present in both plants and animals and its frequency range in plants is between 100 and 300 bp (Edwards *et al.*, 2007; Xu, 2010 cited in Alsamdani, 2018). SNPs that are classified as insertions or deletions can be detected in a coding or non-coding regions of crop plants. Some individuals within a species may be heterozygous or ambiguous at SNP locus, which implies they have both nucleotides on the same position on that gene. As a result when screened phenotypically, such individuals will exhibit an intermediate phenotype. Due to the expansion of sequence information and the identification of gene function as a result of genomic research, single nucleotide polymorphism is now a widely preferred genetic marker. They are the ideal marker for plant germplasm screening or characterisation and identification of functional genes for traits of interest because of their abundance in the genome and the development of new SNP genotyping tools (Gray *et al.*, 2000; Chen *et al.*, 2003; Zhou *et al.*, 2016). SNPs are easily automated using high throughput techniques and are used to segregate large populations.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Plant materials

A total of 60 tef (*Eragrostis tef*) accessions used in this study, were obtained from Debre Zeit Agriculture Research Center (DZARC), which were collected from diverse agro-ecological regions of Ethiopia, ranging in altitude from 1100 to 2950 m.a.s.l. The detail information of accessions used for the screening experiment is shown in appendix 1.

3.2. Experimental site and its description

The greenhouse experiment was conducted at the National Agricultural Biotechnology Research Centers' Holeta located at a latitude and longitude of 9°3'N 38°30'E/ 9.050°N 38.500°E, respectively and an altitude of 2391 meters above sea level. Array based Sequencing of the tef accessions was carried out at Colorado State University, USA.

3.3. Phenotyping

The greenhouse experiment was conducted using PVC tubes (30cm length and 5cm diameter), each filled with 534g of pre-sieved mixed soil of sand and top forest soil (1:3). Before sowing, all PVC tubes were watered for seven days in order to check whether there may be other or wild tef in the soil and then weeded. Then, one day before sowing, all PVC tubes containing soil mix, were watered uniformly to field capacity. Eight seeds of each accession were sown in each tube and 100 ml Hoagland nutrient media of full strength were supplied according to Kittiwongwattana and Vuttipongchaikij (2013) ten days after seedlings were fully germinated to each experimental unit once per-week. The greenhouse day/night temperature was 26 /18 °C. Two weeks after sowing; the seedlings were thinned to three plants per-PVC tubes. Two treatment (moisture stressed and non-stressed) conditions were conducted with three replications arranged in completely randomized design (CRD). The accessions were phenotyped under water-stressed and non-stressed conditions. All accessions were allowed to grow under optimum water conditions from 25 days until water stress treatment was commenced. The PVC tubes were separated into two sets; one set of accessions was kept supplying nutrient media and watered in a regular manner (well-watered) to the end of the experiment , while the second set of treatment with similar accessions, withheld water and supplying of nutrient media for 14

days. Soil moisture in all PVC tube was maintained to field capacity one day before starting treatment. The experiment was terminated at 39 days after sowing. The effect of water stress was determined on every treatment by recording data on root length (cm) and shoot length (cm) using ruler, and root fresh weight (gm), shoot fresh weight (gm), root dry weight(gm) and shoot dry weight(gm) using a sensitive balance. In order to avoid breaking during data collection, soil in the PVC tubes was softened by watering, plants were carefully removed and rinsed gently with tap water and placed on tissue paper to absorb moisture. Then fresh weight (gm) of individual plants was measured. Plants were then carefully wrapped in aluminum foil and kept in an oven set at 65°C for 24 hours to get their dry weight. For all parameters in each replication under non-stressed and stressed conditions, a mean of three plants' measurements from each experimental unit, for all tef accessions were utilized for statistical analysis.

3.4. Statistical analysis of phenotypic data

For all parameters in each replication under non-stressed and stressed conditions, the mean of three plant measurements from each accession were utilized for statistical analysis. Collected data of seedling traits were analyzed using the two-way analysis of variance (ANOVA) in R (version 4.2.2). Data normality and homogeneity of variance was tested using Shapiro-Wilk test and Levene's tests. Significant means were separated using LSD at $p < 0.05$ significance level. Pearson's correlation coefficients were also performed based on the mean value of studied seedling traits under stress and non-stress conditions using METAN package in R Studio to clarify how different traits interact with one another and find qualities that might be effectively utilized to select for drought tolerance at this stage. The broad-sense heritability (H^2) of all traits was also calculated using VARIABILITY package in R.

3.5. Genomic DNA extraction and genotyping

Genomic DNA isolation was carried out from three weeks old leaves of individual plants from each accession grown in the greenhouse using the DArT plant DNA extraction protocol (<https://ordering.diversity arrays.com/file/DArT DNA isolation.pdf>). DNA quality and quantity was checked with 1.5% agarose gel and Nano drop spectrophotometer (Thermo Fisher Scientific), respectively. DNA concentrations were normalized to 50 ng/ μ l and sent to Colorado State University, USA for sequencing and library preparation. Genomic library of the 60 Tef's accessions were prepared and paired end whole genome

sequencing (2×150 bp at~10× coverage) was done using an Illumina NovaSeq 6000 platform.

Adapter sequences were trimmed from the raw paired-end 150nt reads using Fastp v0.19.6, as well as low-quality base pairs and reads shorter than 35 bp were removed. Trimmed reads were then aligned to most recent *tef* genome assembly [*Eragrostis tef* (*tef*) (vV3, id50954)] using BWA-MEM with default settings. The resulting Sequence Alignment Map files generated after read mapping were then sorted by chromosome, annotated for read group information, filtered for improperly paired reads and reads with a mapping quality less than 10, converted to Binary Alignment Map format, and subsequently indexed using SAM tools before proceeding to SNP calling using GATK. SNP calling was performed according to the best practice protocol of GATK. Haplotype Caller was used to genotype individual samples with default settings. All resulting Genomic Variant Call Format (GVCF) files were then combined into a single database using Genomic DBImport to perform joint genotyping with Genotype GVCF. The resulting Variant Call Format (VCF) files were filtered to contain only bi-allelic single nucleotide polymorphisms (SNPs) that had quality-by-depth ≥ 30 , Fisher strand bias ≥ 10 , minimum read mapping quality ≤ 42 , read depth within a 90% confidence interval by taxa, and passing genotype calls using the R package “vcfR” and GATK Select Variants and Variant Filtration tools. Finally, TASSEL was utilized to remove loci with a minor allele frequency $\leq 5\%$ and heterozygous genotype calls $\geq 50\%$; as well as taxa that possessed a genotyping rate $\leq 80\%$, and SNPs that missed % genotypes. The resulting filtered VCF comprised of 538,253 SNPs across 57 samples. The remaining 3 genotypes were excluded from genotyping due to poor DNA quality. Filtered data were received in VCF format and association analysis was done by converting the SNPs in VCF format into hapmap format using the TASSEL software.

3.6. Genome-wide association studies

TASSEL V5.0 was used to determine marker-trait associations using filtered SNP markers. The detection of association between SNP markers and the phenotypic traits was done using a mixed linear model (MLM). According to Mathew *et al.* (2019) population structure matrix (Q) was fitted as a fixed factor while the kinship matrix (K) was treated as a random factor. The MLM model with a default configuration (P3D for variance component analysis and no compression level) was taken into consideration throughout the study, and population structure was regarded as a covariate with five principal components (Begum *et al.*, 2020). The false discovery rate (FDR), which was estimated using the

qvalue package in R software, was set at 5% (FDR adjusted p-value) (Storey *et al.*, 2019) to determine statistically significant marker-trait association. To reduce the risk of false marker-trait associations, the markers were deemed significant for each trait separately at a critical p-value of 1% and a false discovery rate of 5 % (Gupta *et al.*, 2014). Using the CMplot package in R, rectangular Manhattan plots and Quantile-Quantile plots were plotted based on the TASSEL summary statistics output, which included the marker names, chromosomes, positions on the chromosomes, and p-values to categories the actual marker-trait association(Yin,2018).

3.7. Linkage disequilibrium

Based on trait-specific genome-wide markers whose locations were unique to the polymorphic SNP markers, Linkage Disequilibrium (LD) analysis was conducted. The significant markers were considered to plot the LD heatmap in order to accurately estimate coinheritance and historical linkage for each pair of loci (Begum *et al.*, 2020). Haploview 4.2 was used to create the LD that was visualised as a heat map. Map chart 2.32 is also used to illustrate the chromosomal location and QTL intervals of trait-associated SNPs on the A and B genomes for all evaluated traits.

CHAPTER FOUR

4. RESULTS

4.1. Phenotypic data

4.1.1. Root length, shoot length and their respective biomasses

ANOVA revealed that RL, SHL, RFWt, SHFWt and RDWt were highly significantly ($p < 0.001$) influenced by the main effects due to accessions at both stressed and non-stressed conditions (Table1). The genotypic effect on SHDWt was also significant ($p < 0.05$) at both growth conditions. Different accessions responded differently for all the traits investigated (Table3). The analyses confirmed the presence of genetic variability among tested tef accession for drought tolerance. The water treatment effect was also highly significant ($p < 0.001$) for all traits except for RL (Table1). ANOVA also confirmed that the interaction effect of genotype-by water treatment conditions were highly significant ($p < 0.001$) for RFWt, SHFWt and RDWt, significant ($p < 0.05$) for SHDWt, and non-significant for RL and SHL (Table1). Table 3 and figure4 displayed mean performances of all accessions in both non-stressed and stressed conditions. A lot of significant variations were observed among sixty tef accessions in response to water stress treatment when comparing with non-stressed condition. Those with the best phenotypic performance under stressed scenario were considered to be potential candidates for drought tolerance breeding.

Table 1. Analysis of variance for seedling traits of 60 tef accessions based on morphological traits evaluated under non-stressed and stressed conditions.

Source of variation	DF	MS					
		RL	SHL	RFWt	SHFWt	RDWt	SHDWt
Replication	2	63.56 ^{ns}	120.1*	0.0001 ^{ns}	0.00114 ^{ns}	0.0000047 ^{ns}	0.000008 ^{ns}
Treatment	1	43.06 ^{ns}	2426.8***	0.0018***	0.083 ***	0.0004***	0.0023***
Accessions	59	126.88***	64.1***	0.0004***	0.00761***	0.0000286***	0.00009*
Replication*Treatment	2	68.66 ^{ns}	3.1 ^{ns}	0.0002 ^{ns}	0.00023 ^{ns}	0.0000079	0.000007 ^{ns}
Treatment* Accessions	59	61.64 ^{ns}	41.2 ^{ns}	0.0004***	0.00317***	0.00004***	0.00009*

RL, root length; SHL, shoot length; RFWt, root fresh weight; SHFWt, shoot fresh weight; RDWt, root dry weight; SHDWt, shoot dry weight. *, **, *** significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, ns non-significant, DF, degrees of freedom, MS, mean square.

Root length ranged from 15cm to 42cm in the non-stressed and 12cm to 37cm in the stressed environment, showing the influence of drought stress on root length. Among tested tef accession, the highest root length was recorded in accession number 55062 followed by accession 243497 and 243531 under non-stressed, which highly decreased under stressed condition. Under water-stressed condition, accession number 243501 showed maximum root lengths followed by accession number 55068 and 243500. They were collected from Amhara region at altitudes of 2950, 2400 and 2230, respectively. These accessions also performed best under well-watered condition. However, due to its poor performance, accession 212834 was identified as being vulnerable to water stress conditions, as seen in table 3. According to earlier research; root length was a crucial characteristic for coping with drought stress (Ahmed *et al.*, 2019). The root system is therefore generally considered as the most important organ with respect to improving crop adaptation to water stress (Vadez, 2014).

Under non-stressed condition, shoot length ranged from 27 cm- 45.5cm. While it ranged from 13.5cm to 38.3cm under water stressed condition. The longest shoot length was recorded in accession number 225761 under non-stressed conditions which was followed by the accessions 225751 and 225760. On the other hand, in water-stressed condition, accession number 225760 showed the maximum shoot lengths, which was followed by accession 225759 and 243495. These accessions also showed very good performance under non-stressed and displayed tolerance to stressed condition.

With regard to root weight, the lowest (0.003g) and maximum (0.060g) root fresh weight were recorded under non-stressed condition. The tef accession 243494 showed the maximum root fresh weight followed by 55135 and 243497 under non-stressed environment. While the same accessions (243494 and 243497) which had recorded, the highest root fresh weight in non-stressed environment displayed the lowest root fresh weight under stressed condition and they are considered as susceptible to water stress. Under stressed condition, the roots fresh weight ranged from 0.001g to 0.048g. Under this stress condition, the tef accession 243489 possessed the largest root fresh weight followed by accession 243492 and 212616, and, they were collected from Amhara region at altitudes of 2920, 2935 and 1460, respectively. Similar accessions performed well under non-

stressed condition as well (Table 3). Shoot fresh weight ranged from 0.020g to 0.250g in the non-stressed and 0.009g to 0.126g in stressed conditions, revealing the impact of drought on shoots fresh weight. On Non-stressed environment, the accession 230773 showed the highest shoot fresh weight followed by the accessions 243495 and 225760 accessions, while under stressed condition accession number 243495 collected from Amhara region had maximum Shoot fresh weight. The study confirmed that the tef accessions 243495 and 225760 showed maximum shoot fresh weight under both conditions, and hence, they could be considered as tolerant to stress condition (Table 3). The tested genotypes showed considerable variation in root dry weight in both non-stressed (0.002g - 0.025g) to stress (0.001g to 0.009) condition, demonstrating the significant of water stress (drought) on tef root dry weight. Under non-stressed condition, accessions 243494 showed maximum root dry weight followed by 243497 and then by 243507, which radically decreased under stressed condition and these accessions were screened as drought susceptible. Under water stress condition, the highest root dry weight was recorded in accession 243531 and 212616 followed by accession 243533 which were collected from Amhara region at altitudes of 2605, 1460, 2390, respectively. The accession 212933 collected from SNNP also showed high root dry weight under stressed conditions. Likewise, considerable shoot dry weight difference was observed among tested in both non-stressed (0.035g to 0.012g) and stressed conditions (0.033g to 0.002). Accordingly, the tef accession 225760 sourced from Amhara region possessed highest shoot dry in non-stressed environment. In contrast these accessions reduced under stressed condition and screened as susceptible to water-stressed scenario. Whereas the accession 55183 obtained from Amhara and accession 230580 collected from Oromia showed maximum shoot dry weight under stressed condition (Table 3).

Broad sense heritability also done under moisture stress and non-stress condition using R software. The broad-sense heritability (H^2) under moisture stress condition ranged from -0.5 to 0.13 and under non-stressed condition, it ranged from -0.23 to 0.41 and also their phenotypic variance, genotypic variance, phenotypic coefficient of variance and genotypic coefficient of variance has shown in table2

Table 2. Phenotypic variance, genotypic variance, phenotypic coefficient of variance, genotypic coefficient of variance and broad sense heritability (H²)

Trait	Stressed					Non-stressed				
	Vp	Gv	Pcv	Gcv	H ²	Vp	Gv	Pcv	Gcv	H ²
RL	87.80	10.99	35.46	12.55	0.13	72.16	8.12	33.01	11.07	0.11
SHL	29.80	-14.87	17.92	12.66	-0.50	52.11	21.38	20.25	12.97	0.41
RFWt	1e-04	0	101.12	0	0	3e-04	0	120.24	0	0
SHFWt	8e-04	-1e-04	51.00	18.01	-0.125	0.0031	-7e-04	65.49	31.12	-0.23
RDWt	0	0	0	0	NaN	0	0	0	0	NaN
SHDWt	1e-04	0	56.1	0	0	1e-04	0	43.730	0	0

VP, Phenotypic variance; Gv, genotypic variance; Pcv, phenotypic coefficient of variance; Gcv, genotypic coefficient of variance; H², broad sense heritability.

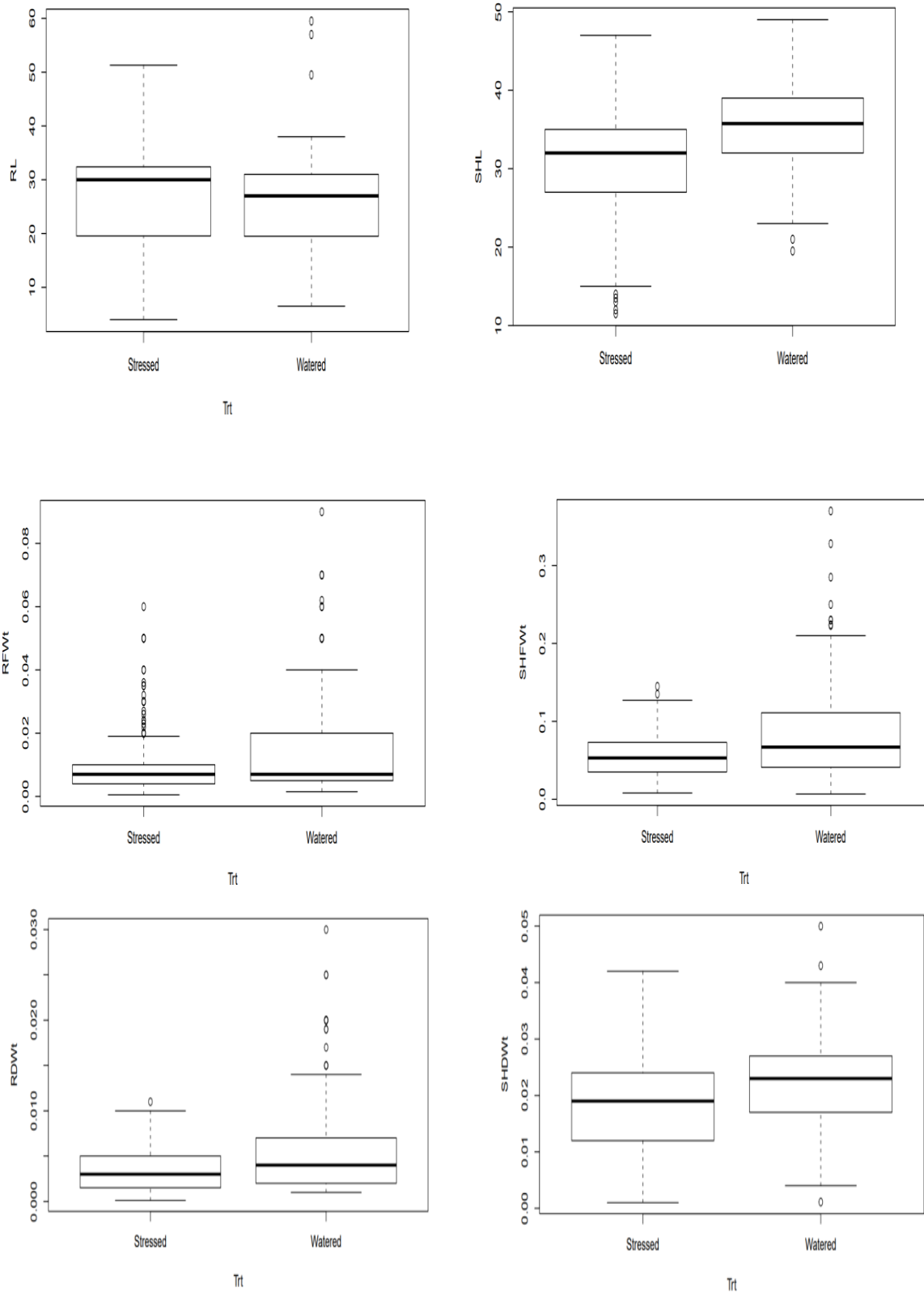


Figure 4. Box plots show mean value fluctuation of traits (RL, SHL, RFWt, SHFWt, RDWt and SHDWt) under stressed and non-stressed condition.

Table 3. Mean performance of seedling traits of 60 tef accessions under non-stress and water-stressed conditions.

Accessions	Root length(cm)		shoot length(cm)		Root fresh weight(g)		Shoot fresh weight(g)		Root dry weight(g)		shoot dry weight(g)	
	watered	stressed	watered	stressed	watered	stressed	watered	stressed	watered	stressed	watered	stressed
55183	18.900	26.833	37.667	31.167	0.004	0.008	0.041	0.054	0.003	0.002	0.024	0.033
212933	22.000	21.767	36.333	31.500	0.007	0.009	0.072	0.050	0.003	0.006	0.019	0.022
243522	17.667	17.533	34.433	34.267	0.038	0.012	0.029	0.039	0.006	0.002	0.016	0.021
243499	23.500	23.700	35.667	26.000	0.004	0.023	0.049	0.042	0.005	0.006	0.023	0.021
55186	28.400	21.533	34.667	24.833	0.006	0.015	0.070	0.024	0.005	0.003	0.025	0.007
230771	21.167	18.200	41.167	19.333	0.015	0.008	0.050	0.016	0.005	0.002	0.015	0.007
243494	32.000	28.833	41.000	34.500	0.060	0.006	0.135	0.037	0.025	0.003	0.029	0.010
243496	22.167	24.833	37.333	33.333	0.005	0.013	0.056	0.052	0.002	0.005	0.026	0.018
234726	18.500	31.333	28.500	31.667	0.005	0.016	0.040	0.041	0.002	0.003	0.014	0.009
243521	31.667	24.167	33.333	33.100	0.004	0.005	0.045	0.053	0.003	0.003	0.018	0.023
243506	31.333	29.000	36.333	29.667	0.006	0.007	0.049	0.060	0.004	0.002	0.023	0.019
243507	23.667	24.367	36.833	29.900	0.042	0.005	0.163	0.062	0.015	0.002	0.027	0.015
243533	26.500	12.933	34.667	13.500	0.006	0.022	0.105	0.070	0.002	0.007	0.019	0.019
225761	18.933	29.833	45.500	32.000	0.023	0.003	0.153	0.072	0.011	0.002	0.030	0.020
55102	26.167	20.000	39.200	29.133	0.008	0.004	0.120	0.084	0.004	0.003	0.028	0.026
243489	26.667	36.567	35.667	33.067	0.007	0.048	0.071	0.062	0.003	0.006	0.019	0.021
243495	29.833	25.733	37.800	36.533	0.023	0.005	0.213	0.126	0.009	0.002	0.030	0.028
230773	25.000	19.833	36.733	32.800	0.023	0.008	0.250	0.085	0.009	0.004	0.033	0.017
55184	16.667	31.000	34.167	32.500	0.004	0.007	0.047	0.045	0.003	0.002	0.024	0.017
243498	28.500	21.733	34.167	29.100	0.015	0.008	0.026	0.048	0.005	0.007	0.015	0.017
234435	24.867	19.500	36.833	32.833	0.027	0.004	0.094	0.055	0.013	0.002	0.023	0.008
234371	25.267	30.733	32.500	32.833	0.003	0.009	0.045	0.064	0.002	0.004	0.018	0.022
55334	20.333	22.867	33.333	29.333	0.005	0.007	0.027	0.029	0.005	0.003	0.017	0.016
55068	26.833	25.000	41.667	31.267	0.030	0.003	0.191	0.098	0.011	0.002	0.030	0.002
243531	35.200	31.000	37.367	28.833	0.007	0.012	0.151	0.064	0.003	0.009	0.025	0.019
243511	23.500	28.500	32.167	28.667	0.005	0.006	0.059	0.034	0.004	0.004	0.024	0.017
55100	24.167	26.333	29.333	29.833	0.004	0.005	0.031	0.045	0.002	0.004	0.018	0.021
55096	28.833	27.667	38.667	30.667	0.016	0.009	0.158	0.044	0.006	0.004	0.031	0.015
212834	17.333	11.833	40.100	33.333	0.056	0.021	0.157	0.097	0.003	0.004	0.027	0.011
225760	18.000	26.800	43.000	38.300	0.032	0.003	0.210	0.108	0.011	0.001	0.035	0.018
230580	26.000	32.900	38.833	34.167	0.013	0.008	0.025	0.100	0.005	0.005	0.026	0.027
55049	23.000	25.533	32.633	28.300	0.004	0.004	0.090	0.049	0.003	0.003	0.021	0.017
243500	25.667	34.667	37.167	26.800	0.015	0.006	0.052	0.034	0.004	0.003	0.025	0.015
55123	34.500	31.833	29.667	36.167	0.005	0.006	0.055	0.038	0.004	0.004	0.025	0.018
55034	15.000	22.000	34.833	21.000	0.003	0.004	0.084	0.041	0.002	0.002	0.028	0.018
225759	27.000	32.433	38.833	37.833	0.029	0.007	0.159	0.070	0.013	0.003	0.022	0.010
237205	21.567	33.167	31.067	33.667	0.006	0.012	0.050	0.084	0.004	0.005	0.015	0.021
243512	40.000	31.000	37.500	29.000	0.006	0.008	0.055	0.042	0.002	0.004	0.028	0.018
212835	24.800	31.167	33.400	30.333	0.018	0.006	0.088	0.058	0.004	0.004	0.012	0.022
243497	29.433	31.333	36.333	35.833	0.037	0.007	0.125	0.009	0.016	0.004	0.020	0.014
212616	24.033	30.100	31.000	25.967	0.005	0.035	0.060	0.037	0.003	0.009	0.021	0.013
243530	31.167	21.167	37.167	32.267	0.009	0.005	0.064	0.060	0.004	0.004	0.028	0.026
243539	31.667	28.000	33.000	29.167	0.008	0.006	0.042	0.046	0.005	0.002	0.019	0.019
243524	20.233	22.367	30.733	24.167	0.004	0.005	0.020	0.026	0.002	0.003	0.018	0.018
55188	24.500	33.333	29.500	22.500	0.006	0.005	0.051	0.040	0.004	0.002	0.016	0.015
243501	30.833	36.600	39.333	26.167	0.007	0.006	0.085	0.034	0.007	0.004	0.031	0.016
55187	23.333	30.667	27.333	28.833	0.004	0.006	0.075	0.060	0.003	0.004	0.021	0.014
243540	16.767	17.967	38.833	26.500	0.020	0.021	0.126	0.056	0.003	0.004	0.022	0.024
212931	25.333	29.333	33.667	31.000	0.006	0.008	0.066	0.057	0.004	0.003	0.026	0.019
243532	23.000	22.667	36.000	36.167	0.004	0.008	0.044	0.049	0.003	0.003	0.023	0.025
243523	26.100	19.900	34.500	32.667	0.004	0.001	0.040	0.049	0.003	0.004	0.022	0.025
243492	33.000	26.000	37.000	32.000	0.007	0.042	0.051	0.033	0.003	0.005	0.021	0.009
55125	27.667	29.800	35.600	30.833	0.035	0.004	0.059	0.046	0.014	0.002	0.014	0.009
243503	26.000	26.000	36.000	34.500	0.007	0.005	0.038	0.056	0.002	0.002	0.018	0.023
55031	30.333	21.333	33.100	24.167	0.007	0.005	0.068	0.035	0.005	0.002	0.028	0.014
243515	32.833	32.333	34.333	34.167	0.005	0.008	0.039	0.068	0.002	0.004	0.023	0.026
225751	28.000	26.500	42.333	35.333	0.033	0.016	0.198	0.062	0.014	0.002	0.031	0.017
55132	22.900	30.567	31.500	27.500	0.007	0.008	0.100	0.069	0.003	0.004	0.022	0.016
55062	41.667	34.333	34.667	33.500	0.008	0.008	0.059	0.050	0.003	0.005	0.021	0.024
55135	22.933	20.533	37.000	35.800	0.047	0.014	0.156	0.115	0.015	0.004	0.021	0.022

Grand mean	25.7	26.4	35.7	30.4	0.014	0.009	0.09	0.056	0.006	0.004	0.023	0.018
SD	7.995	8.8	5.59	6.7	0.016	0.01	0.062	0.03	0.005	0.002	0.008	0.497
CV	31%	33%	17%	22%	114%	102%	73%	53%	92%	67%	36%	48%

4.2. Relationship between traits under watered and stressed conditions

The correlation coefficient measures how closely two variables or factors are related. It also demonstrates the relationship between several traits. Under non-stressed condition, shoot dry weight was significantly ($p < 0.001$) and moderately positively associated with shoot fresh weight ($r = 0.63$), shoot length ($r = 0.59$) and root dry weight ($r = 0.31$), but negligible non-significant association with root length ($r = 0.12$) and root fresh weight ($r = 0.24$). Shoot fresh weight showed significant moderate positive association with root fresh weight ($r = 0.61$), root dry weight ($r = 0.58$) and shoot length ($r = 0.57$), but non-significant negligible association with root length ($r = -0.03$). Shoot length showed moderate positive association with root fresh weight ($r = 0.53$) and root dry weight ($r = 0.49$), but negative negligible association with root length ($r = 0.01$). Root dry weight showed significant strong positive ($r = 0.82$) association with root fresh weight, but its association with root length was negligible and non-significant ($p > 0.05$; $r = 0.07$). Moreover, root fresh weight and root length showed negative negligible ($r = -0.10$) association.

Root dry weight was significantly ($p < 0.001$) and moderately positively associated with root fresh weight ($r = 0.54$), but its association with shoot dry weight and root length was negligible and non-significant ($p > 0.05$; $r = 0.12$, $r = 0.09$) and also showed non-significant negligible negative association with shoot length and shoot fresh weight ($p > 0.05$, $r = -0.16$, $r = -0.03$). Root fresh weight showed non-significant negative negligible association with shoot dry weight ($r = -0.09$), shoot length ($r = -0.09$), shoot fresh weight ($r = -0.04$) and root length ($r = -0.01$). Root length showed negligible non-significant association with shoot dry weight ($r = 0.03$), shoot length ($r = 0.25$) and also showed negative negligible non-significant association with shoot fresh weight ($r = -0.1$). Shoot fresh weight showed significant moderate positive association with shoot dry weight ($r = 0.3$) and shoot length ($r = 0.4$). Moreover, shoot length and shoot dry weight showed negligible ($r = 0.22$) association. Figure 5 and 6 shows Pearson's correlation coefficients for selected morphological traits of tef accessions under non-stressed and stressed condition respectively.

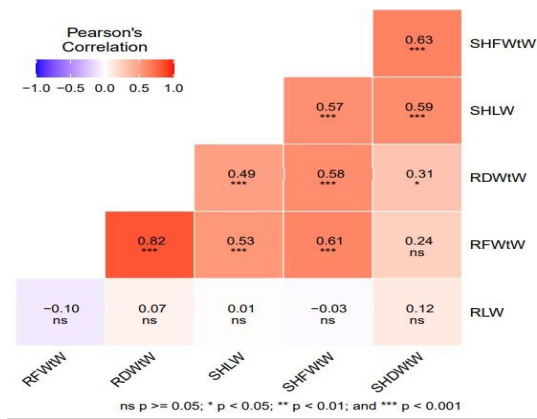


Figure 5. Phenotype correlation analysis among 60 tef seedling traits under non-stressed condition.

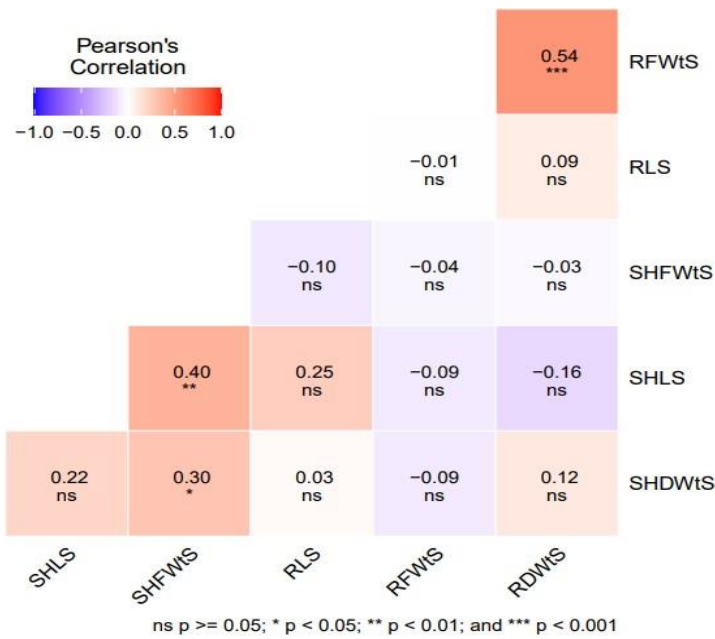


Figure 6. Phenotype correlation analysis among 60 tef seedling traits under stressed condition.

4.3. Marker trait association (MTAs) under water stressed condition

Marker-trait association (MTA) analysis was carried out for six phenotypic traits (RL, RFWt, RDWt, SHL, SHFWt and SHDWt) using 538,253 SNP markers to identify the key genomic regions responsible for water stress response at 5% significant threshold (FDR adjusted p-value) (Storey *et al.*, 2019). In four traits (SHL, SHFWt, RFWt, and RDWt) a total of 215 significant SNPs were identified at significance threshold of $p < 0.0001$ or $-\log_{10}(\text{p-values}) = 4.0$ (appendix 2). However, significant MTA was not detected for two traits (RL and SHDWt) at the used significance threshold. The highest number of MTA were identified for RFWt (204) followed by SHL (7) and then by RDWt (3), while the lowest MTA was detected for SHFWt (1) under water stress condition. Figure 10 depicts Manhattan plots constructed by plotting the negative log of the p-values i.e. $-\log_{10}(P\text{-value})$ of each SNPs on the y-axis against their corresponding genomic positions on chromosomes colored in alternating colors in the x-axis. The right side is the QQ plots indicating how well the used model controlled the confounding factors due to population structure (Q) and familial relatedness (k).

Linkage disequilibrium (LD) heat maps for MTA were generated using Haploview 4.2 to demonstrate coinheritance, marker-marker linkage, and non-random association patterns between SNPs figure 7. Using a window of physical distance in base pairs (bp) established by a pair-wise LD analysis of the genome-wide scanned SNPs. QTL under stress conditions were also discovered by merging the MTAs based on their genomic locations. Map chart 2.32 was used in order to visualize the chromosomal location and QTL intervals of the associated SNPs on the A and B genome for the evaluated traits. For some of the traits that were evaluated under stress condition, the chromosomal location and QTL intervals of the trait-associated SNPs on the A and B genome are shown in figure 9. Figure 8 depicts genome-wide linkage disequilibrium decay (LD-decay) in the total tef accessions utilized for this study.

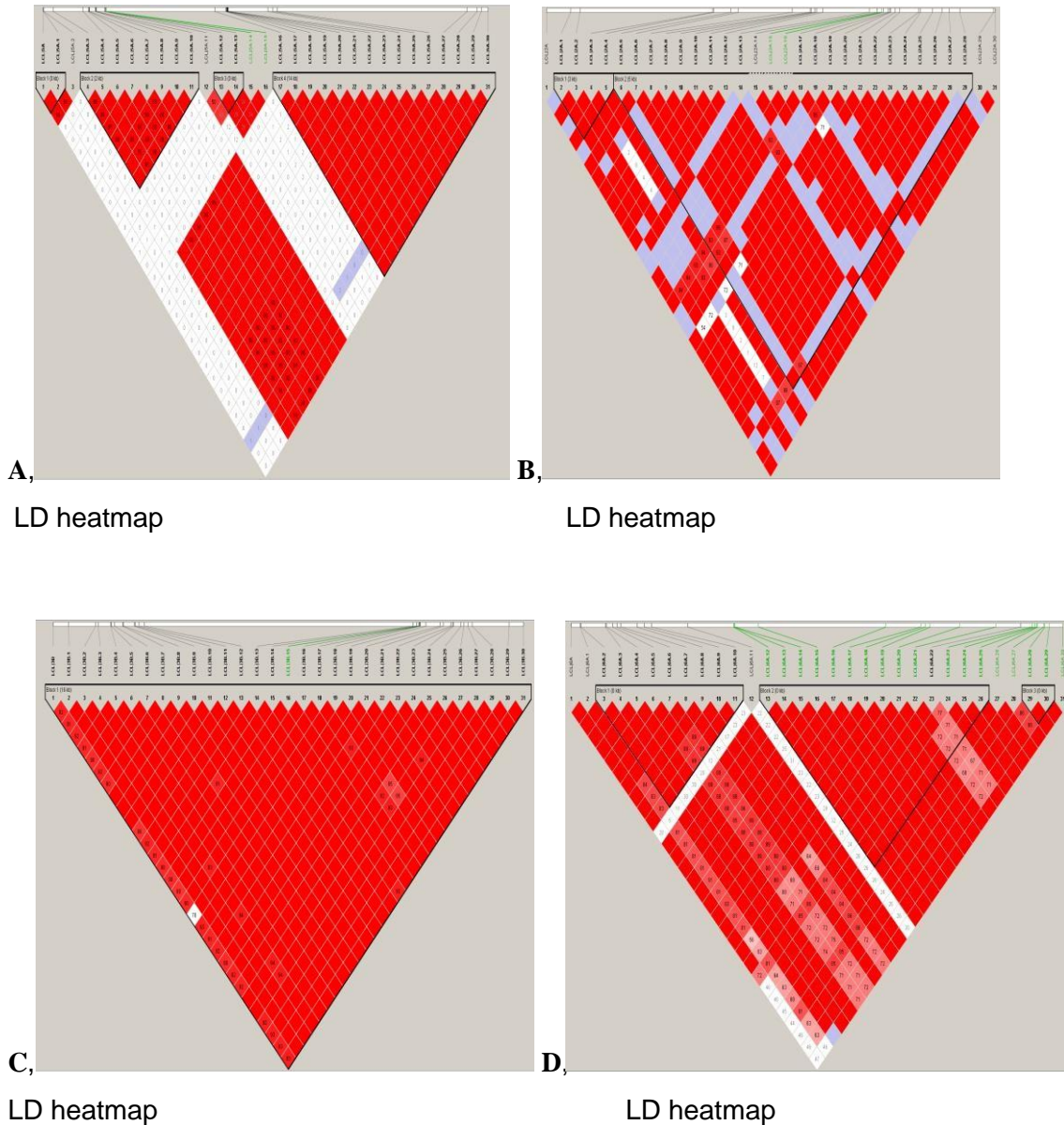


Figure 7. Linkage disequilibrium (LD) heat map generated by Haploview software for MTA on 5A; 2A, 3B and 6A respectively for shoot length (A), Root dry weight (B), shoot fresh weight (C) and Root fresh weight (D). Within each of the corresponding squares, the LD blocks/triangles are displayed as paired D' values that correspond to SNP pairings expressed as percentages (%) and LOD (log of the likelihood odds ratio), which measures the level of confidence in the value of D' . The amount and significance of pairwise LD between SNPs are represented by shading, with a red to white color gradient reflecting higher-to-lower LD values.

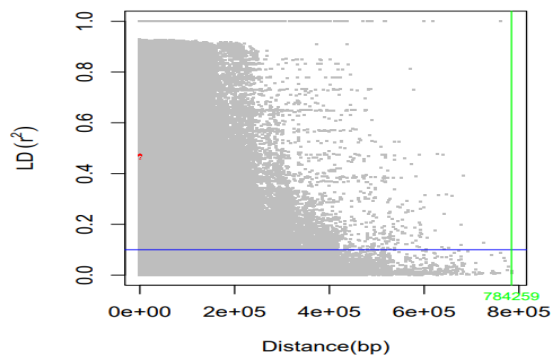


Figure 8.Scatter plot of genome-wide LD decay against total physical distance (bp) based on the r^2 values of the marker pairs.

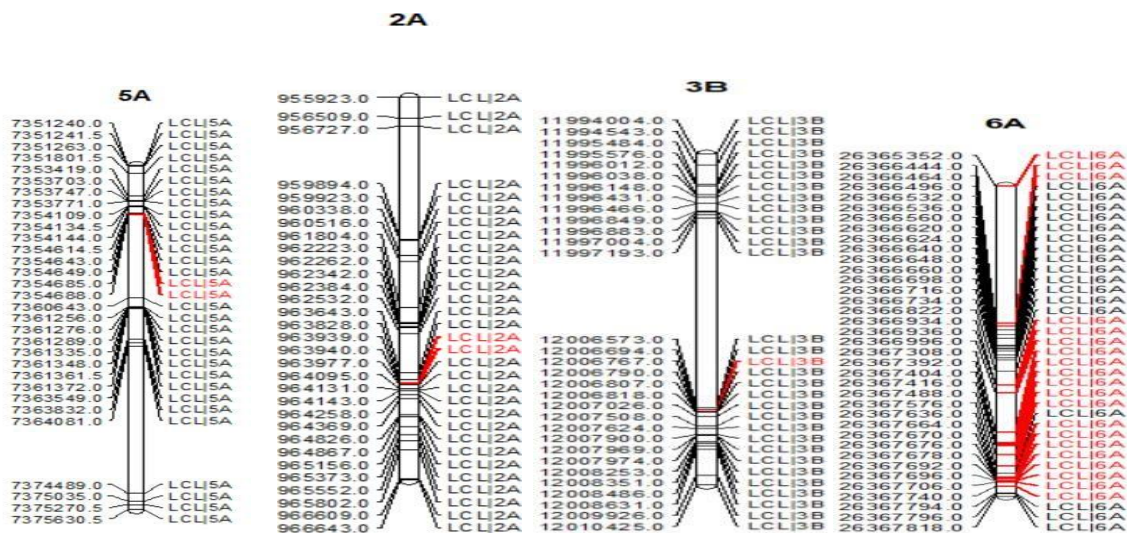


Figure 9.The related SNPs on the A and B genomes for MTA under water stress conditions were located on chromosomes by genome-wide analysis. SNPs are provided in millions of base pairs according to their physical positions on chromosomes. The vertical black lines in chromosomes denote the maker interval in LD where SNPs were located. The QTL are shown on the right (in red) the markers which are associated with shoot length(5A), Root dry weight(2A), shoot fresh weight(3B) and Root fresh weight(6A).

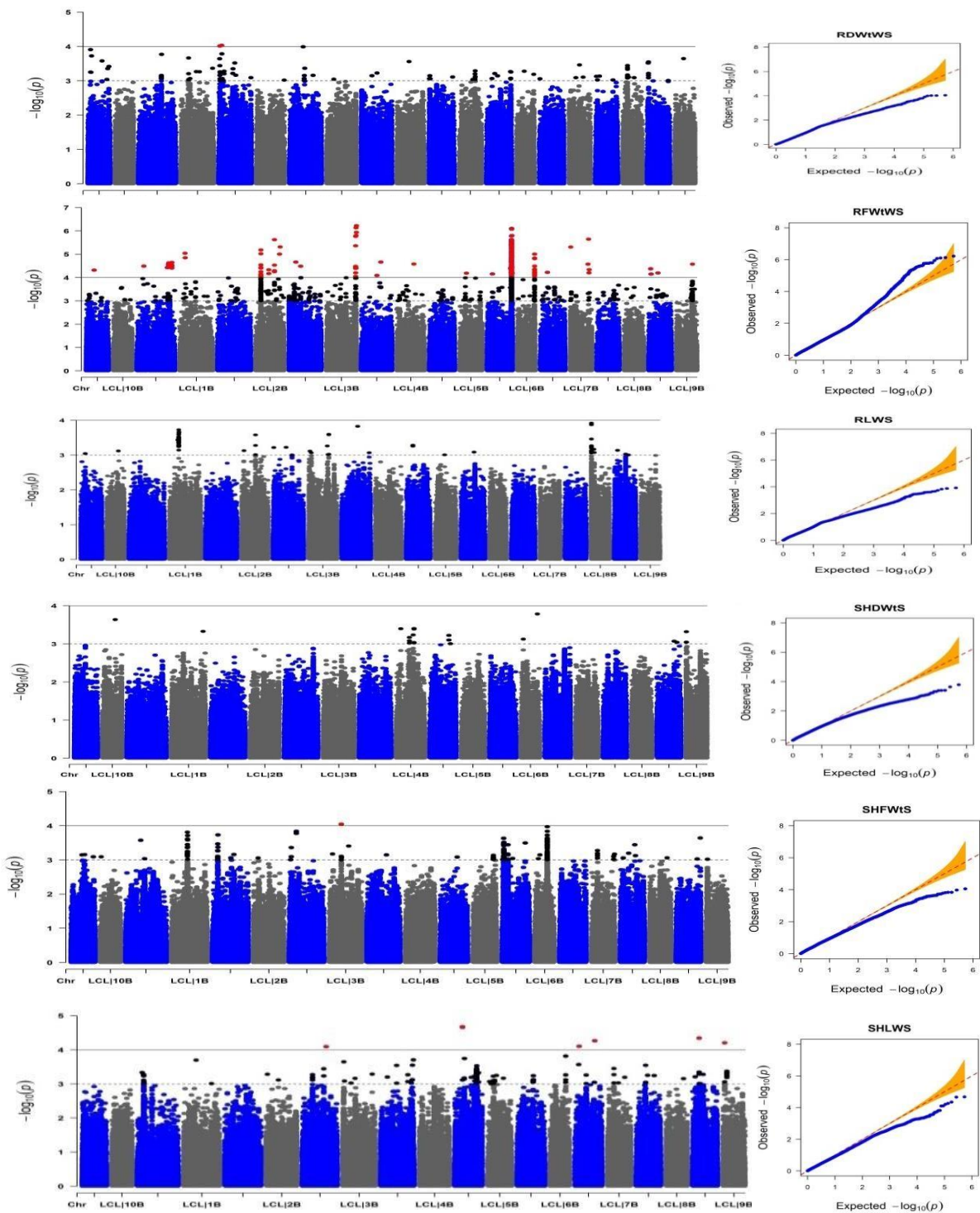


Figure 10.Manhattan and Quantile-Quantile (QQ) plots of genome-wide association study for six traits: RDWt, RFWt, RL, SHDWt, SHFWt and SHL. Each dot represents a SNP. On the X-axis is the genomic position of the SNPs on the corresponding chromosomes indicated in different colours.Y-axis is the $-\log_{10}$ of the P-value depicting the significance of the association test. Statistically significant MTAs are marked on the plots with red dot. Whereas for two traits (RL and SHDWt) there were no significant MTAs observed.

4.4. SNPs markers density

The physical distribution of targeted SNP markers across the 20 chromosomes was uneven, resulting in different densities on each chromosome. Figure 11 shows the chromosomal distribution of targeted SNP markers within a 1Mbp window size indicated by a color gradient; grey indicates no SNPs, green indicates low density, yellow indicates medium density and red indicates high density.

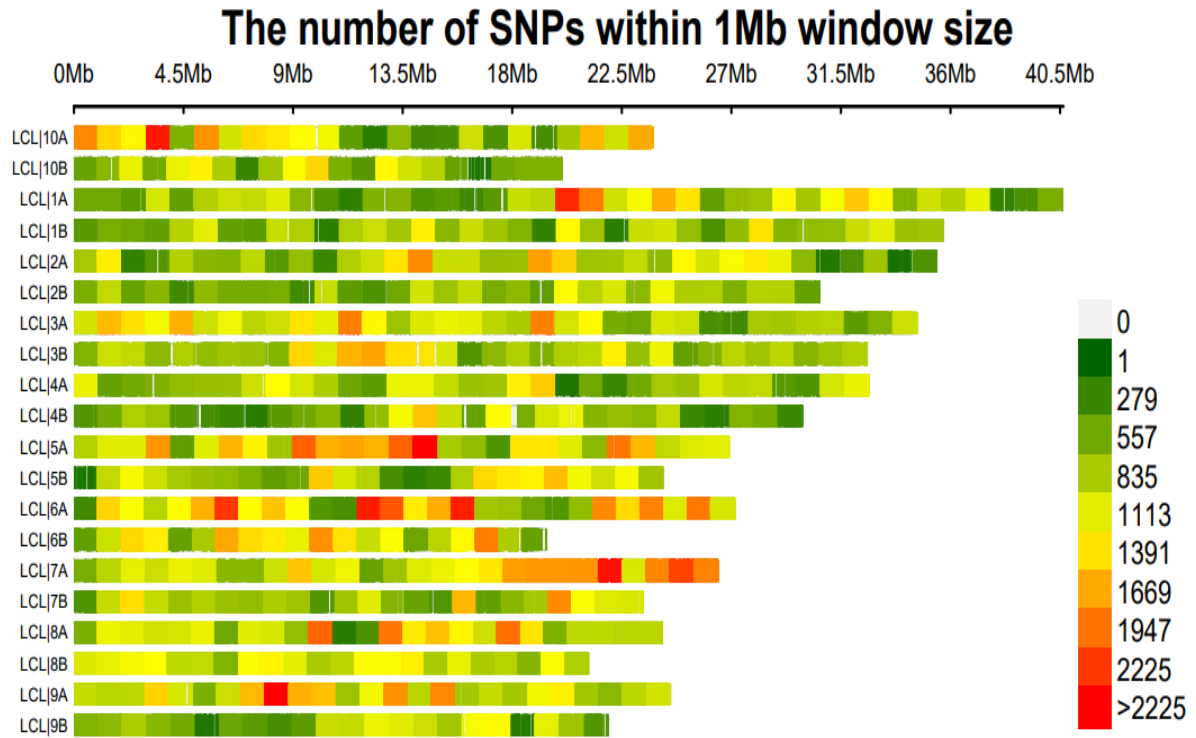


Figure 11. Distribution of chromosomal markers in terms of density. Green to red color coding represents low to high SNP densities.

5. DISCUSSION

Drought is one of the major abiotic factors that significantly influence and diminish the yield and productivity of food crop resulting in up to 70% yield loss globally (Lum *et al.*, 2014). It has a large influence on plant growth during germination, vegetative and reproductive stages. At each stage, it acts as a constraint to crop productivity. However, studies on drought tolerance have mostly neglected drought that occurs during the early stages of growth (Spontaneous *et al.*, 2016). There is limited information on drought stress tolerance of tef at vegetative growth stage. The current study was carried out to identify the best performing tef accessions under water stressed condition, and identify SNPs associated with drought tolerance to be used in further tef breeding program through MAS.

5.1. Effects of water stress on root length shoot length and their respective biomasses.

The results obtained from the present work clearly demonstrated the presence of significant variability among 60 tef accessions for the considered seedling traits at vegetative growth stage. The genetic variability offer opportunities for breeds to improve yield, yield related traits and resistance to both biotic and abiotic stresses through breeding. The analyses also confirmed that the effect of water treatment on genotypes performance was highly significant, indicating that germplasm greenhouse screening can be used to identify drought tolerant tef genotypes. Moreover, the very highly significance differences for the interaction effect of accession by water treatment indicated that the different tef accessions interacted or responded differentially with the different water treatment conditions. These findings are consistent with (Meo, 2000; Bibi *et al.*, 2010; Ali *et al.*, 2011a cited in Bibi *et al.*, 2012). Similarly, maize plants are found to be more susceptible to drought stress at the seedling stage (Badr *et al.*, 2020). The study confirmed that although both shoot and root growth was inhibited under water stressed condition, shoot growth was more sensitive than root growth. This finding is in line with the reports by Salih *et al.*(999) Younis *et al.* (2000) Okçu *et al.* (2005), Bibi *et al.* (2010,) and Ajithkumar and Panneerselvam (2014) that drought stress suppressed shoot growth more than root growth, and in certain cases root growth increased in sorghum at seedling stage. Similarly, Ahmad *et al.* (2014) stated that limited supply of water and nutrients under water stress condition often results in growth inhibition of shoot than roots.

Shoot length of tef accessions were decreased under water stressed condition in comparison to non-stressed condition that corresponds to the study of drought tolerance in maize (Zea *et al.*, 2018) and wheat (Aldesuquy *et al.*, 2012). Water stress reduced fresh as well as dry weight of root and shoot in tef. This result is in line with the results reported by Bibi *et al.*(2012) in some cultivars of sorghum, wheat, maize and sunflower. Farooq *et al.* (2008) also displayed that water stress on crop plants generally cause significant reduction in fresh and dry biomass production. Genotypes allocate biomass differently between roots and shoots (Weiner, 2004), and there are indications that drought tolerance can be improved via traits, such as root length, shoot and root biomass accumulation (Paustian *et al.*, 2016; Griffiths and Paul, 2017). Mwangoe *et al.* (2022) reported that accumulation of root and shoot biomass has been used as an indicator of drought tolerance. The results are useful for screening large number of tef accessions for water stress tolerance during its vegetative growth stage.

Pearson correlation among morphological traits was also assessed for both non-stressed and stressed circumstances. Results from the study demonstrated that strongest positive correlation was observed between shoot fresh weight and shoot length under both environmental conditions. Root dry weight and root fresh weight had significant positive correlation under both conditions. The present result is in line with the report of Ahmed *et al.* (2022) in wheat. Shoot fresh weight had also significant and positive association with shoot dry weight under both stressed and non-stressed condition. Traits that showed strong positive association can be improved simultaneously through indirect selection. Correlations are useful criteria for choosing genotypes that are drought tolerant (Darvishzadeh *et al.* (2010). Characterization of tef accessions with better stress tolerance traits and screening for drought tolerant tef are essential to the success of breeding programs. The broad-sense heritability (H²) under moisture stress condition ranged from -0.5 to 0.13 and under non-stressed condition, it is ranged from -0.23 to 0.41. This low heritability implies that the genetic variance is low compared to the phenotypic variance which means most of the differences are not due to genetic.

5.2. Marker trait association (MTAs) under water stressed condition

In agricultural plants, GWAS is becoming into a potent and cutting-edge method for identifying the genes, alleles or haplotypes that are strongly associated with agronomically important traits (Alqudah *et al.*, 2020). Furthermore, the advent of molecular breeding tools like as genome sequencing and the rapid increase of high-density SNP arrays pave the door for crucial trait association mapping/QTL mapping. In the current study, at significant $p < 0.001$ threshold, MLM model based GWAS identified a total of 215 SNPs significantly associated with various drought stress traits of tef such as shoot length, shoot fresh weight, root dry weight and root fresh weight at vegetative growth stage under water stressed condition. However, no marker-trait association was observed for root length and shoot dry weight ($-\log_{10}p\text{-value} \geq 4.0$); hence, the considerable variations in these two traits were not genetic. For RFWt trait the largest number of associated SNPs mapped was 204, for SHL (7), for RDWt (3) and for SHFWt (1) was associated (appendix2). The marker-trait association revealed here are particular to water stress conditions and to the best of my knowledge relevant information on these associations in *Eragrostis tef* is not currently accessible in the literature and can be considered as potential candidates for exploring SNP trait associations further in Tef accessions panels. It is known that Linkage disequilibrium is a popular and ideal method to explain the continuation of non-random association among alleles at two or more loci which can exist after the historical linkage and recombination events and mutation (Abeba M.A., 2020). LD decay can, however, be influenced by a variety of genetic and environmental factors such as mating system, selection, rate of mutation, rate of genetic recombination, genetic drift, and population structure (Gupta *et al.*, 2014). Therefore, linkage disequilibrium (LD) analysis was carried out to identify the potential candidate genes that may be responsible for the assessed parameters under water stress condition for each trait having significant SNPs across each chromosome in order to demonstrate the existence of non-random association between alleles at two or more loci and to define the candidate genomic region. The locus thought to be relevant for a given trait is the LD block that contains significant SNP markers for that trait (Figure 7). Figure 8 also shows the chromosomal location and QTL intervals of trait-associated SNPs on the A and B genomes for all evaluated traits. To the best of my knowledge, none of the known QTL had been documented in the literature on tef during its vegetative development stage under moisture stress conditions; hence, these QTLs may be regarded as novel.

6. CONCLUSIONS

Diverse tef accessions used in this study showed significant variations for different traits to drought stress recorded at the vegetative growth stage under water stress condition in the greenhouse. This implies that drought tolerance can be improved by exploiting the existing genetic variability through breeding. The study confirmed that water stress has a detrimental effect on the majority of evaluated phenotypic traits (RL, SHL, RFWt, SHFWt RDWt and SHDWt). This shows that it is important to consider these traits in selecting genotypes for drought tolerance. Correlation analysis revealed that some agronomic traits like shoot fresh weight and shoot dry weight showed strong positive associations, indicating that these traits can be improved simultaneously through indirect selection breeding. GWAS also identified a total of 215 significant MTAs from the scanned population of tef accessions collected from different regions of Ethiopia for shoot length, shoot fresh weight, root dry weight and root fresh weight under water stress condition. The physical distribution of significant SNP markers found across the 20 chromosomes was uneven; resulting in different densities on each chromosome. Current study's significant MTAs may be included into genomic prediction models to assess their selection potential under drought stress circumstances. We suggest the effect of detected QTLs needs to be validated before being deployed in genome assisted breeding of tef for drought tolerance. Overall, the identified drought tolerant tef accessions and the identified QTLs could be deployed in the tef breeding program for the development of drought stress tolerant tef varieties.

7. RECOMMENDATIONS

Up on the present results, the following recommendations have been forwarded.

1. Significant markers identified in the present study shall be validated for use in future tef breeding program.
2. The markers developed in this work could be highly beneficial for marker assisted breeding of tef under water-stressed environments.
3. More study should be conducted with large sample size across locations to identify large effect and stable QTLs useful in tef breeding for drought tolerance.

8. REFERANCE

- Abraha, M. T. (2016). Breeding tef [*Eragrostis tef* (Zucc.) trotter] for drought tolerance in Northern Ethiopia (Doctoral dissertation).
- Abraha, M. T., Hussein, S., Laing, M., and Assefa, K. (2015). Genetic management of drought in tef: Current status and future research directions, 3(3), 156–161.
- Abraha, M. T., Shimelis, H., Laing, M., and Assefa, K. (2016). Performance of tef [*Eragrostis tef* (Zucc.) Trotter] genotypes for yield and yield components under Drought-Stressed and non-stressed conditions. *Crop Science*, 56(4), 1799–1806.
- Abraham, R. (2015). Achieving food security in Ethiopia by promoting productivity of future world food tef: A review. *Adv Plants Agric Res*, 2(2), 00045.
- Acquaah, G. (2015). Conventional plant breeding principles and techniques. *Advances in plant breeding strategies: Breeding, biotechnology and molecular tools*, 115-158.
- Adanech, T. N. (2019). Morphological and Molecular Characterization of selected tef (*Eragrostis tef* (Zucc.) Trotter) Accessions from the Gene Bank of Ethiopia, 9(2), 31–40.
- Admas, S., and Belay, G. (2011). Drought-resistance traits variability in *Eragrostis tef* X *Eragrostis pilosa* recombinant inbred lines. *African Journal of Agricultural Research*.
- Ahmad, I., Khaliq, I., Khan, A. S., and Farooq, M. (2014). Screening of spring wheat (*Triticum aestivum* L.) genotypes for drought tolerance on the basis of seedling traits, 51(2), 367– 372.
- Ahmed, H. G. M. D., Sajjad, M., Li, M., Azmat, M. A., Rizwan, M., Maqsood, R. H., and Khan, S. H. (2019). Selection criteria for drought-tolerant bread wheat genotypes at seedling stage. *Sustainability (Switzerland)*, 11(9), 1–17.
- Ahmed, H. G. M., Zeng, Y., Shah, A. N., Yar, M. M., Ullah, A., and Ali, M. (2022). Conferring of drought tolerance in wheat (*Triticum aestivum* L.) genotypes using seedling indices.
- Aldesuquy, H. S., Hamed, S. A. A., Abbas, M. A., Elhakem, A. H., Aldesuquy, H. S., Hamed, S. A. A. and Abbas, M. A. (2012). Role of glycine betaine and salicylic acid in improving growth vigor and physiological aspects of drought wheat cultivars. *Journal of Stress Physiology and Biochemistry*, 8(1), 149–171.
- Alqudah, A. M., Sallam, A., Stephen Banziger, P., and Börner, A. (2020). GWAS: Fast-forwarding gene identification and characterization in temperate Cereals: lessons from Barley a review. *Journal of Advanced Research*, 22, 119–135.
- Alsamdani, N. M. (2018). Screening for Drought Tolerance Using Polymorphic SNP Markers in Barley (*Hordeum Vulgare* L).
- Araya, A., Stroosnijder, L., Girmay, G., and Kesstra, S. D. (2011). Crop coefficient, yield response to water stress and water productivity of tef (*Eragrostis tef* (Zucc.). *Agricultural Water Management*, 98(5), 775–783.

- Assefa, B., M. Demeke, and B. Lanos. "Analysis of Price Incentives for Tef in Ethiopia Technical Notes Series, MAFAP." Food and Agriculture Organization of the United Nations, Rome (2015).
- Assefa, K. (2013). Achievements and prospects of tef improvement. Proceedings of the Second International Workshop on Tef Improvement, Debre Zeit, Ethiopia. Nov. 7-9, 2011.
- Assefa, K., Chanyalew, S. and Tadele, Z. (2017). Tef, *Eragrostis tef* (Zucc.) trotter. Millets and Sorghum: Biology and Genetic Improvement, 226-266.
- Assefa, K., Yu, J. K., Zeid, M., Belay, G., Tefera, H., and Sorrells, M. E. (2011). Breeding tef [*Eragrostis tef* (Zucc.) trotter]: Conventional and molecular approaches. *Plant Breeding*, 130(1), 1–9.
- Assefa, Kebebew, and Chanyalew, S. (2018). Agronomics of Teff. *The Economics of Tef: Exploring Ethiopia's Biggest Cash Crop*, 39–70.
- Assefa, Kebebew, Cannarozzi, G., Girma, D., Kamies, R., Chanyalew, S., Plaza-Wüthrich, S., ... Tadele, Z. (2015). Genetic diversity in tef [*Eragrostis tef* (Zucc.) Trotter]. *Frontiers in Plant Science*, 6(MAR) 1–14.
- Ayele, M., and Nguyen, H. T. (2000). Evaluation of amplified fragment length polymorphism markers in tef, *Eragrostis tef* (Zucc.) Trotter and related species. *Plant Breeding*, 119(5), 403-409.
- Ayele, M., Tefera, H., Assefa, K., and Nguyen, H. T. (1999). Genetic characterization of two *Eragrostis* species using AFLP and morphological traits. *Hereditas*, 130(1), 33–40.
- Badr, A., El-shazly, H. H., Tarawneh, R. A., and Börner, A. (2020). Screening for Drought Tolerance in Maize (*Zea mays* L.) .Germplasm Using Germination and Seedling Traits under Simulated Drought Conditions. *Plants*, (9), 1–23.
- Baye, K. (2014). Teff: Nutrient composition and health benefits (ESSP working paper 67).
- Begum, H., Alam, M. S., Feng, Y., Koua, P., Ashrafuzzaman, M., Shrestha, A ... Frei, M. (2020). Genetic dissection of bread wheat diversity and identification of adaptive loci in response to elevated tropospheric ozone. *Plant Cell and Environment*, 43(11), 2650–2665.
- Benjamin, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal statistical society: series B (Methodological)*, 57(1), 289-300.
- Berhe, T., Gebretsadik, Z., Edwards, S., and Araya, H. (2011, November). Boosting tef productivity using improved agronomic practices and appropriate fertilizer. In *Achievements and prospects of Tef improvement*.
- Bhusal, B., Ram Poudel, M., Pandit, R., Regmi, R., Neupane, P., Bhattarai, K., ... Acharya, S. (2021). A Review on Abiotic Stress Resistance in Maize (*Zea mays* L.): Effects, Resistance Mechanisms and Management Corresponding Author. *Journal of Biology and Today's World*, 10(2), 1–003.
- Bibi, A.Sadaqat, H. A., Tahir, M. H. N., and Akram, H. M. (2012). Screening of sorghum (

Sorghum bicolor var *moench*) for drought tolerance at seedling stage in polyethylene glycol, 22(3).

- Blum, A. (2015). Towards a conceptual ABA ideotype in plant breeding for water limited environments. *Functional Plant Biology*, 42(6), 502-513.
- Chanyalew, S., Ferede, S., Damte, T., Fikre, T., Genet, Y., Kebede, W., Assefa, K. (2019). Significance and prospects of an orphan crop tef. *Planta*, 250(3), 753–767.
- Chen, X., and Sullivan, P. F. (2003). Single nucleotide polymorphism genotyping: biochemistry, protocol, cost and throughput. *The pharmacogenomics journal*, 3(2), 77-96.
- Cochrane, L., and Bekele, Y. W. (2018). Average crop yield (2001–2017) in Ethiopia: Trends at national, regional and zonal levels. *Data in Brief*, 16, 1025–1033.
- Darvishzadeh, R., Pirzad, A., Hatami-Maleki, H., Poormohammad-Kiani, S., and Sarrafi, A. (2010). Evaluation of the reaction of sunflower inbred lines and their F1 hybrids to drought conditions using various stress tolerance indices. *Spanish Journal of Agricultural Research*, 8(4), 1037.
- Dashora, A., Mehta, R., Singh, D., Urmila, and Singh, S. K. (2022). Genetic variability, association and diversity studies in wheat (*Triticum* spp. L.). *Journal of Environmental Biology*, 43(3), 390–400.
- Dijkstra, A., and Polman, J. (2009). Survey on the nutritional and health aspects of tef (*Eragrostis Tef*). *Educon.Javeriana.Edu.Co*, 319–382.
- Dwiningsih, Y., Rahmaningsih, M., and Alkahtani, J. (2020). Development of Single Nucleotide Polymorphism (SNP) Markers in Tropical Crops. *Advance Sustainable Science, Engineering and Technology*, 2(2), 1–13.
- Edwards, D., and Batley, J. (2010). Plant genome sequencing: applications for crop improvement. *Plant Biotechnology Journal*, 8(1), 2–9.
- Farooq, A. (2008). Analysis of some physio-genetic parameters related to drought tolerance in maize (Doctoral dissertation, M Sc thesis Department of Plant Breeding and genetics University of Agriculture, Faisalabad).
- Ferede, B., Mekbib, F., Assefa, K., Chanyalew, S., Abraha, E., and Tadele, Z. (2018). Evaluation of Tef (*Eragrostis tef* (Zucc.) Trotter) Somaclones for Drought Tolerance. *Advances in Crop Science and Technology*, 06(04), 4–11.
- Fikadu, A. A., Wedu, T. D., and Derseh, E. A. (2019). Review on Economics of Teff in Ethiopia. *Open Acc Biostatistics and Bioinformatics*, 2(3), 1–8.
- Forneris, N. S., Vitezica, Z. G., Legarra, A., & Pérez-Enciso, M. (2017). Influence of epistasis on response to genomic selection using complete sequence data. *Genetics Selection Evolution*, 49(1), 1–14.
- Gebremariam, M. M., Zarnkow, M., and Becker, T. (2014). Teff (*Eragrostis tef*) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages: a review. *Journal of Food Science and Technology*, 51(11), 2881–2895.
- Gebru, Y. A., Sbhatu, D. B., & Kim, K. P. (2020). Nutritional Composition and Health Benefits

- of Teff (*Eragrostis tef* (Zucc.) Trotter). *Journal of Food Quality*, 2020.
- Ghafoor, G. (2013). Correlation Analysis for Different Parameters of F2 Bread Wheat Population. *Pure and Applied Biology*, 2(1), 28–31.
- Gray, I. C., Campbell, D. A., and Spurr, N. K. (2000). Single nucleotide polymorphisms as tools in human genetics. *Human Molecular Genetics*, 9(16 REV. ISS.), 2403–2408.
- Griffiths, C. A., and Paul, M. J. (2017). Targeting carbon for crop yield and drought resilience. *Journal of the Science of Food and Agriculture*, 97(14), 4663–4671.
- Gupta, S., Kumari, K., Muthamilarasan, M., Parida, S. K., and Prasad, M. (2014). Population structure and association mapping of yield contributing agronomic traits in foxtail millet. *Plant Cell Reports*, 33(6), 881–893.
- Hameed, A., Goher, M., and Iqbal, N. (2010). Evaluation of seedling survivability and growth response as selection criteria for breeding drought tolerance in wheat. *Cereal Research Communications*, 38(2), 193–202.
- Hassen, I. W., Regassa, M. D., Berhane, G., Minten, B., and Taffesse, A. S. (2018). Teff and its role in the agricultural and food economy. *The economics of Tef, exploring Ethiopia's Biggest Cash Crop*. International Food Policy Research Institute (IFPRI), Washington DC, 11-37.
- Ibitoye, D. O., and Akin-Idowu, P. E. (2011). Marker-assisted-selection (MAS): A fast track to increase genetic gain in horticultural crop breeding. *African Journal of Biotechnology*, 10(55), 11333–11339.
- Ketema, S. (1997). Promoting the conservation and use of underutilized and neglected crops. *Frontiers in Plant Science* (Vol. 8).
- Khan, A., Sovero, V., and Gemenet, D. (2016). Genome-assisted Breeding For Drought Resistance. *Current Genomics*, 17(4), 330–342.
- Kibatu, G., Chacha, R., and Kiende, R. (2017). Determination of Major, Minor and Trace Elements in Tef Using Portable Total X-Ray Fluorescence (TXRF) Spectrometer. *EC Nutrition*, 1(May), 51–59.
- Kittiwongwattana, C. and Vuttipongchaikij, S. (2013). Effects of nutrient media on vegetative growth of *Lemna minor* and *Landoltia punctata* during in vitro and ex vitro cultivation, 60-69.
- Lum, M. S., Hanafi, M. M., Rafii, Y. M., and Akmar, A. S. N. (2014). Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. *Journal of Animal and Plant Sciences*, 24(5), 1487–1493.
- Ma, F., Xu, Y., Ma, Z., and Li, L. (2018). Genome-wide association and validation of key loci for yield-related traits in wheat founder parent Xiaoyan 6.
- Mathew, I., Shimelis, H., Shayanowako, A. I. T., Laing, M., and Chaplot, V. (2019). Genome-wide association study of drought tolerance and biomass allocation in wheat. *PLoS ONE*, 14(12), 1–21.

- McCouch, S. R., Zhao, K., Wright, M., Tung, C. W., Ebana, K., Thomson, M. Bustamante, C. (2010). Development of genome-wide SNP assays for rice. *Breeding Science*, 60(5), 524–535.
- Merrill, S. D., Tanaka, D. L., and Hanson, J. D. (2002). Root length growth of eight crop species in Haplustoll soils. *Soil Science Society of America Journal*, 66(3), 913-923.
- Mickelbart, M. V., Hasegawa, P. M., and Bailey-Serres, J. (2015). Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. *Nature Reviews Genetics*.
- Miller, D. (2010). Teff grass: crop overview and forage production guide. Cal/West Seed Company. Woodland, CA, 95695.
- Mokhena, T., Mochane, M., Tshwafo, M., Linganiso, L., Thekiso, O., and Songca, S. (2016). We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists TOP 1%. Intech, 225–240.
- Moshelion, M., and Altman, A. (2015). Current challenges and future perspectives of plant and agricultural biotechnology. *Trends in Biotechnology*, 33(6), 337–342.
- Mulabachew, T. F. (2016). Genetic Diversity of Ethiopian Tef (*Eragrostis tef* (Zucc.) Trotter) Varieties as Revealed by Morphological and Microsatellite Markers, (October).
- Mwangoe, J., Kimurto, P. K., and Ojwang, P. P. O. (2022). Identification of drought tolerant finger millet (*Eleusine coracana*) lines based on morpho-physiological characteristics and grain yield, 16(April), 47–60.
- Naik, L., Muniraja, M., and Vijayalakshmi, M. (2020). Morpho-physiological and biochemical changes in finger millet [*Eleusine coracana* (L.) Gaertn.] under drought stress. *Physiology and Molecular Biology of Plants*, 26(11), 2151–2171.
- Numan, M., Khan, A. L., Asaf, S., Salehin, M., Beyene, G., Tadele, Z., and Ligaba-Osena, A. (2021). From traditional breeding to genome editing for boosting productivity of the ancient grain tef [*eragrostis tef* (Zucc.) trotter]. *Plants*, 10(4).
- Pang, Y., Liu, C., Wang, D., Amand, P. S., and Bernardo, A. (2020). High-Resolution Genome-Wide Association Study Identifies Genomic Regions and Candidate Genes for Important Agronomic Traits in Wheat.
- Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., and Smith, P. (2016). Climate-smart soils. *Nature*, 532(7597), 49-57.
- Plaza-Wüthrich, S., Blösch, R., Rindisbacher, A., Cannarozzi, G., and Tadele, Z. (2016). Gibberellin deficiency confers both lodging and drought tolerance in small cereals. *Frontiers in Plant Science*, 7(MAY2016), 1–14.
- Reda, A. (2014). Achieving Food Security in Ethiopia by Promoting Productivity of Future World Food Tef: A Review. *Advances in Plants and Agriculture Research*, 2(2), 86–95.
- Saturni, L., Ferretti, G., and Bacchetti, T. (2010). The gluten-free diet: Safety and nutritional quality. *Nutrients*, 2(1), 16–34.
- Science, F. (2019). Review to the Level of minerals in Teff [*Eragrostis tef* (Zuccagni) Trotter], grain samples Melaku Tafese Awulachew Department of food science and nutrition

research process, Ethiopian Institute of Agricultural Research, Kulumsa Agricultural Research Center, (11), 14–27.

Seyfu Ketema (1997). Tef (*Eragrostis tef* (Zucc.)Trotter) Promoting the Conservation and Use of Underutilized and Neglected Crops. 12. Inst. of Plant Genetics and Crop Plant Research, Gatersleben/Int. Plant Genet. Resour. Inst., Rome, Italy.

Shiferaw, W., Balcha, A., and Mohammed, H. (2012). Genetic Variation for Grain Yield and Yield Related Traits in Tef [*Eragrostis tef* (Zucc.)Trotter] under Moisture Stress and Non-Stress Environments. *American Journal of Plant Sciences*, 03(08), 1041–1046.

Sofia, A., de Almeida, A. M., da Silva, A. B., da Silva, J. M., Paula, A., Santos, D.Sousa Araujo, S. de. (2013). Abiotic Stress Responses in Plants: Unraveling the Complexity of Genes and Networks to Survive. *Abiotic Stress - Plant Responses and Applications in Agriculture*.

Sohrwardy, H., and Hossain, M. L. (2014). Response of Short Duration Tropical Legumes and Maize to Water Stress: A Glasshouse Study. *Advances in Agriculture*, 2014.

Spaenij-Dekking, L., Kooy-Winkelaar, Y., and Koning, F. (2005). The Ethiopian Cereal Tef in Celiac Disease. *New England Journal of Medicine*, 353(16), 1748–1749.

Spontaneum, L. S. S. P., Hordeum, B., Ssp, V. L., Tyagi, K., Park, M. R., Lee, H. Y. O. J., ... Steffenson, B. (2016). Fertile crescent region as sources of drought tolerance at early stage of plant growth of wild, (February 2011).

Sridhara, S., N, P. G. H., Manoj, K. N., and Gopakkali, P. (2022). Nutritional importance of Teff (*Eragrostis tef* (Zucc .) Trotter) and human health : A critical review, (May).

Storey, J. D., Base, A. J., Dabney, A., and Robinson, D. (2019). qvalue: Q-value estimation for false discovery rate control. R package version 2.10. 0. 2015.

Tadele, E., and Hibistu, T. (2022). Cogent Economics and Finance Spatial production distribution economic viability and value chain features of tef in Ethiopia.

Tadele, Z. (2019). Orphan crops: their importance and the urgency of improvement. *Planta*, 250(3), 677-694.

Tafes Desta, B., Meseret Gezahegn, A., and Eshetu Tesema, S. (2020). Planting Time Effects on the Productivity of Tef [*Eragrostis tef* (Zucc.)] Varieties in Ethiopia. *American Journal of Life Sciences*, 8(3), 34.

Tefera, H., and Ketema, S. (2001). Production and importance of tef in Ethiopian agriculture. Narrowing the Rift: Tef research and development. In *Proceedings of the International Workshop on Tef Genetics and Improvement*, Oct (pp. 16-19).

Tesema, A. (2013). Genetic Resources of Tef in Ethiopia. *Achievements and Prospects of Tef Improvement*.

Tilahun, A. (2021). Culturable microbial biology of fermenting teff (*Eragrostis tef* (Zucc)) dough and developing starter culture for injera production (Doctoral dissertation, Addis Ababa University).

- USDA, U. (2013). National nutrient database for standard reference, release 28. US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory.
- Vadez, V. (2014). Root hydraulics: The forgotten side of roots in drought adaptation. *Field Crops Research*, 165, 15–24.
- Vavilov, N. I. (1951). The origin, variation, immunity and breeding of cultivated plants (Vol. 72, No. 6, p. 482).
- Weiner, J. (2004). Allocation, plasticity and allometry in plants. *Perspectives in Plant Ecology, Evolution and Systematics*, 6(4), 207–215.
- Worede, F., Mehadi, T., and Wondimu, S. (2020). Stability analyses of tef (*Eragrostis tef* [Zucc.] Trotter) grain yield in dryland areas of Northeast Ethiopia. *Cogent Food and Agriculture*, 6(1).
- Xoconostle-Cazares, B., Ramirez-Ortega, F. A., Flores-Elenes, L., and Ruiz-Medrano, R. (2010). Drought tolerance in crop plants. *American journal of plant physiology*, 5(5), 241-256.
- Yin, L. (2018). CMplot: Circle Manhattan Plot; GitHub. Inc. San Francisco, CA, USA.
- Yu, J. K., Sun, Q., La Rota, M., Edwards, H., Tefera, H., and Sorrells, M. E. (2006). Expressed sequence tag analysis in tef (*Eragrostis tef* (Zucc) Trotter). *Genome*, 49(4).
- Zea, L., Rashid, M., Sajid, M. A., Noreen, S., Mahmood, S., and Shah, K. H. (2018). Study of adverse effects of drought stress on two different hybrids of maize, 7(4), 1316–1325.
- Zhou, L., Vega, F. E., Tan, H., Lluch, A. E. R., Meinhardt, L. W., Fang, W., ... Zhang, D. (2016). Developing Single Nucleotide Polymorphism (SNP) Markers for the Identification of Coffee Germplasm. *Tropical Plant Biology*, 9(2), 82–95.

9. APPENDICES

Appendix1. List of Eragrostis tef accessions, Regions, Zone, District, Latitude, longitude and altitude.

Serial no	Accession	Region/state/province	Zone	Woreda/district	Latitude	Longitude	Altitude
1	55183	Amhara	Mirab gojam	Burewemberma	10-47-00N	37-0300E	2550
2	212933	SNNP	Semen omo	Gofa zuria	36-54-00-N	06-19-00-E	1360
3	243522	Tigray	mehakelegnaw	Adwa	14-10-00N	38-51-00E	2050
4	243499	Amhara	Debub wallo	Tenta	11-20-00N	39-15-00E	2550
5	55186	Amhara	Agew Awi	Banja	11-06-00N	36-53-00E	2420
6	230771	Oromia	Borena	Moyale	05-03-00N	39-28-00E	1200
7	243494	Amhara	Debub Wallo	Tenta	11-20-00N	39-15-00E	2400
8	243496	Amhara	Debub Wallo	Tenta	11-20-00N	39-15-00E	2400
9	234726	Benishangul andGumuz	Metekel	Dibate	10-41-00N	36-23-00N	1450
10	243521	Tigray	Mehakelegnaw	Kola Temben	13-37-00N	39-59-00E	2010
11	243506	Amhara	Semen Wallo	Guba Lafto	11-51-00N	39-32-00E	2600
12	243507	Amhara	Semen Wallo	Habru	11-45-00N	39-41-00E	2020
13	243533	Amhara	Semen Gondor	Dabat	13-38-00N	37-55-00E	2390
14	225761	SNNP	Semen omo	Kucha	06-28-00N	37-30-00E	1290
15	55102	Amhara	Debub Wallo	Kutaber	11-18-00N	39-29-00E	2100
16	243489	Amhara	Debub Wallo	Dessie Zuria	10-57-00N	39-34-00E	2920
17	243495	Amhara	Debub Wallo	Tenta	11-20-00N	39-15-00E	2350
18	230773	Oromia	Borena	Moyale	05-04-00N	39-29-00E	1220
19	55184	Amhara	Mirab Gojam	Burewamberma	10-52-00N	36-57-00E	2590
20	243498	Amhara	Debub Wallo	Tenta	11-20-00N	39-15-00E	2400
21	234435	Tigray	Mirabawi	Tahtay koraro	14-33-00N	38-04-00E	1500
22	234371	Tigray	Mehakelegnaw	Adwa	14-12-00N	38-50-00E	2120
23	55334	Amhara	Agew awi	Dangela	11-07-00N	36-54-00E	2570
24	55068	Amhara	Misrak Gojam	Enarjenawga	10-35-00N	38-16-00E	2400
25	243531	Amhara	Semen Gonder	Dabat	13-59-00N	37-47-00E	2605
26	243511	Tigray	Debubawi	Endmahoni	12-45-00N	39-27-00E	2320
27	55100	Oromia	Mirab hararge	ciro	09-04-00N	40-46-00E	2030
28	55096	Amhara	Semen gonder	Gonder zuria	12-40-00N	37-30-00E	2350
29	212834	Oromia	Bale	Buluk	06-15-00N	39-47-00E	1250
30	225760	SNNP	Semen omo	Kucha	06-28-00N	37-30-00E	1290
31	230580	Oromia	Bale	Buluk	06-25-00N	39-52-00N	1150
32	55049	Amhara	Agew awi	Banja	10-58-00N	36-46-00E	2250
33	243500	Amhara	Semen wallo	DawuntnaDela nt	11-32-00N	39-15-00E	2230
34	55123	Oromia	Mirab hararge	ciro	09-09-00N	41-02-00E	2190
35	55034	Amhara	Debub gonder	este	11-38-00N	38-04-00E	2620
36	225759	SNNP	Semen omo	Kucha	06-28-00N	37-30-00E	1290
37	237205	Tigray	Mahakelegnaw	Meherab lehe	14-19-00N	38-49-00E	1350
38	243512	Tigray	Debubawi	endamehoni	12-45-00N	39-27-00E	2320
39	212835	Oromia	Bale	buluk	06-24-00N	39-50-00E	1340
40	243497	Amhara	Debu wallo	tenta	11-20-00N	39-15-00E	2400
41	212616	Amhara	Debub wallo	ambasel	11-25-00N	39-37-00E	1460
42	243530	Amhara	Semen gondor	dabat	13-59-00N	37-47-00E	2605
43	243539	Amhara	Semen gondor	gonder zuria	12-19-00N	37-33-00E	2050

44	243524	Tigray	mahakelegnaw	adw	14-13-00N	38-54-00E	2030
45	55188	Amhara	Agaw awi	dangela	11-21-00N	36-58-00E	2040
46	243501	Amhara	Semen wallo	Dawuntna Delant	11-34-00N	39-14-00E	2950
47	55187	Amhara	Agew awi	dangela	11-17-00N	36-54-00E	2160
48	243540	Amhara	Semen gondar	Gondar zuria	12-19-00N	37-33-00E	2050
49	212931	SNNP	Semen omo	Gofa zuria	36-59-00N	06-21-00E	1400
50	243532	Amhara	Semen godar	dabat	13-38-00N	37-55-00E	2390
51	243523	Tigray	mehakelegnaw	adwa	14-1000-N	38-51-00-E	2075
52	243492	Amhara	Dehub wallo	tenta	11-14-00N	39-15-00-E	2935
53	55125	Oromia	Misirak harerge	deder	09-18-00-N	41-26-00-E	2320
54	243503	Amhara	Dehub wallo	ambasel	11-28-00-N	39-00-00-E	2825
55	55031	Amhara	Agew awi	dangela	11-21-00-N	36-53-00-E	2150
56	243515	Tigray	mehakelegnaw	Degua temben	13-38-00-N	39-07-00-E	2580
57	225751	SNNP	Semen omo	Arbaminch zuria	05-45-00-N	37-22-00-E	1100
58	55132	Oromia	Misirak wallaga	Guto wayu	09-01-00-N	36-30-00-E	2400
59	55062	Amhara	Misirak gojam	enemay	10-29-00-N	33-11-00-E	2560
60	55135	Oromia	Misirak shewa	Ada'a chukala	08-44-00-N	39-00-00-E	2100

Appendix2. Significant marker trait associations at FDR corrected p value for four traits.

Trait	Marker	Chr	Pos	-Log10pvalue	pvalue	R ²
SHL	SLCL 5A_7354688	LCL 5A	7354688	4.676	2.11E-05	47.58%
	SLCL 5A_7354685	LCL 5A	7354685	4.658	2.20E-05	47.58%
	SLCL 9A_6324708	LCL 9A	6324708	4.344	4.53E-05	45%
	SLCL 7A_19475156	LCL 7A	19475156	4.264	5.44E-05	43%
	SLCL 9B_1756788	LCL 9B	1756788	4.203	6.27E-05	38.7%
	SLCL 7A_3857099	LCL 7A	3857099	4.102	7.90E-05	40%
	SLCL 3A_23582412	LCL 3A	23582412	4.093	8.06E-05	45%
RDWt	SLCL 2A_3132115	LCL 2A	3132115	4.033	9.271E-05	33%
	SLCL 2A_963939	LCL 2A	963939	4.011	9.74E-05	35%
	SLCL 2A_963940	LCL 2A	963940	4.011	9.74E-05	35%
SHFWt	SLCL 3B_12006767	LCL 3B	12006767	4.048	8.96E-05	34.9%
RFWt	SLCL 3B_32561289	LCL 3B	32561289	6.223	5.981E-07	62%
	SLCL 3B_32162476	LCL 3B	32162476	6.131	7.396E-07	56.58%
	SLCL 6A_26366995	LCL 6A	26366995	6.108	7.795E-07	57.4%
	SLCL 6A_26367664	LCL 6A	26367664	6.076	8.404E-07	57.59%
	SLCL 3B_32561392	LCL 3B	32561392	5.941	1.145E-06	54.36%
	SLCL 6A_26681681	LCL 6A	26681681	5.805	1.568E-06	50.54%
	SLCL 3B_32162717	LCL 3B	32162717	5.803	1.573E-06	50.55%
	SLCL 6A_26222292	LCL 6A	26222292	5.775	1.68E-06	55.53%
	SLCL 6A_26222296	LCL 6A	26222296	5.775	1.68E-06	0.55532
	SLCL 6A_26223268	LCL 6A	26223268	5.774	1.682E-06	58.69%
	SLCL 3B_31740835	LCL 3B	31740835	5.763	1.726E-06	50%
	SLCL 3B_31741200	LCL 3B	31741200	5.762	1.73E-06	53.75%
	SLCL 7B_20080883	LCL 7B	20080883	5.646	2.262E-06	47.3%
	SLCL 6A_26351564	LCL 6A	26351564	5.622	2.387E-06	51.1%
	SLCL 2B_20312365	LCL 2B	20312365	5.621	2.391E-06	64.37%
	SLCL 6A_26223193	LCL 6A	26223193	5.607	2.469E-06	49.46%
	SLCL 6A_26373787	LCL 6A	26373787	5.604	2.489E-06	49.47%
	SLCL 6A_26222471	LCL 6A	26222471	5.574	2.667E-06	50.74%
	SLCL 6A_26222473	LCL 6A	26222473	5.574	2.667E-06	50.74%
	SLCL 6A_26367487	LCL 6A	26367487	5.555	2.788E-06	51.88%
	SLCL 6A_26252205	LCL 6A	26252205	5.544	2.859E-06	52.64%
	SLCL 6A_26252237	LCL 6A	26252237	5.536	2.912E-06	49.52%
	SLCL 6A_26473259	LCL 6A	26473259	5.522	3.009E-06	51.46%
	SLCL 6A_26400693	LCL 6A	26400693	5.485	3.271E-06	62.94%
	SLCL 6A_26252212	LCL 6A	26252212	5.476	3.345E-06	47.75%
	SLCL 6A_26252219	LCL 6A	26252219	5.433	3.689E-06	48.48%
	SLCL 6A_26252210	LCL 6A	26252210	5.422	3.786E-06	47.44%
	SLCL 6A_26400927	LCL 6A	26400927	5.412	3.876E-06	61.75%
	SLCL 6A_26114037	LCL 6A	26114037	5.397	4.005E-06	49.71%
	SLCL 6A_26373811	LCL 6A	26373811	5.372	4.248E-06	48.3%

SLCL 3B_31617404	LCL 3B	31617404	5.363	4.331E-06	56.67%
SLCL 3B_31617405	LCL 3B	31617405	5.363	4.331E-06	56.67%
SLCL 6A_26367695	LCL 6A	26367695	5.363	4.333E-06	47.65%
SLCL 6A_26363799	LCL 6A	26363799	5.348	4.485E-06	58.12%
SLCL 6A_26351698	LCL 6A	26351698	5.336	4.616E-06	58.12%
SLCL 6A_26400942	LCL 6A	26400942	5.314	4.855E-06	60.26%
SLCL 2B_26373086	LCL 2B	26373086	5.313	4.863E-06	59.08%
SLCL 7B_746835	LCL 7B	746835	5.309	4.904E-06	55.99%
SLCL 6A_26363801	LCL 6A	26363801	5.268	5.394E-06	57.51%
SLCL 6A_26205849	LCL 6A	26205849	5.254	5.57E-06	58.54%
SLCL 6A_26367675	LCL 6A	26367675	5.200	6.305E-06	43.85%
SLCL 2B_5445420	LCL 2B	5445420	5.185	6.528E-06	52.86%
SLCL 6A_26367670	LCL 6A	26367670	5.177	6.649E-06	45.96%
SLCL 6A_26351937	LCL 6A	26351937	5.115	7.674E-06	46.05%
SLCL 6A_26365347	LCL 6A	26365347	5.087	8.184E-06	55.27%
SLCL 6A_26223601	LCL 6A	26223601	5.083	8.256E-06	54.73%
SLCL 6A_26363564	LCL 6A	26363564	5.082	8.283E-06	50.08%
SLCL 6A_26363565	LCL 6A	26363565	5.082	8.283E-06	50.08%
SLCL 6A_26365302	LCL 6A	26365302	5.069	8.522E-06	53.33%
SLCL 6A_26367706	LCL 6A	26367706	5.061	8.686E-06	42.59%
SLCL 1B_5743960	LCL 1B	5743960	5.043	9.048E-06	44.12%
SLCL 6A_26223587	LCL 6A	26223587	5.034	9.239E-06	55.77%
SLCL 2B_5445454	LCL 2B	5445454	5.022	9.514E-06	56.51%
SLCL 2B_25701012	LCL 2B	25701012	5.005	9.886E-06	44.08%
SLCL 6B_18553573	LCL 6B	18553573	5.002	9.945E-06	41.21%
SLCL 6A_26365351	LCL 6A	26365351	4.981	1.045E-05	53.76%
SLCL 6A_26363541	LCL 6A	26363541	4.976	1.058E-05	50.5%
SLCL 6A_26363542	LCL 6A	26363542	4.976	1.058E-05	50.5%
SLCL 6A_26363543	LCL 6A	26363543	4.976	1.058E-05	50.5%
SLCL 6A_26167041	LCL 6A	26167041	4.953	1.114E-05	50.5%
SLCL 6A_26363486	LCL 6A	26363486	4.927	1.183E-05	49.1%
SLCL 6A_26419578	LCL 6A	26419578	4.893	1.278E-05	52.78%
SLCL 6A_26425332	LCL 6A	26425332	4.883	1.311E-05	55.58%
SLCL 6A_26357954	LCL 6A	26357954	4.874	1.336E-05	52.29%
SLCL 6A_26161278	LCL 6A	26161278	4.869	1.353E-05	53.72%
SLCL 1B_5830583	LCL 1B	5830583	4.849	1.416E-05	40.06%
SLCL 6A_26363603	LCL 6A	26363603	4.844	1.433E-05	47.69%
SLCL 6B_18553614	LCL 6B	18553614	4.832	1.472E-05	39.42%
SLCL 6A_26357942	LCL 6A	26357942	4.817	1.523E-05	51.30%
SLCL 6B_18553594	LCL 6B	18553594	4.811	1.545E-05	39.60%
SLCL 6A_26166898	LCL 6A	26166898	4.792	1.616E-05	50.39%
SLCL 6A_26357860	LCL 6A	26357860	4.741	1.817E-05	48.39%
SLCL 6A_26346643	LCL 6A	26346643	4.736	1.835E-05	50.25%
SLCL 6A_26114624	LCL 6A	26114624	4.691	2.039E-05	46.33%
SLCL 6A_26114626	LCL 6A	26114626	4.691	2.039E-05	46.33%
SLCL 6A_26166916	LCL 6A	26166916	4.682	2.082E-05	48%

SLCL 6A_26357918	LCL 6A	26357918	4.680	0.0000209	49.5%
SLCL 4A_20739616	LCL 4A	20739616	4.665	2.161E-05	63.51%
SLCL 3A_6991749	LCL 3A	6991749	4.663	2.174E-05	47.86%
SLCL 6A_26223559	LCL 6A	26223559	4.658	2.2E-05	51.98%
SLCL 6A_26223560	LCL 6A	26223560	4.658	2.2E-05	51.98%
SLCL 6A_26223563	LCL 6A	26223563	4.658	2.2E-05	51.98%
SLCL 6A_26367307	LCL 6A	26367307	4.648	2.25E-05	39.83%
SLCL 1A_37457788	LCL 1A	37457788	4.646	2.259E-05	38.27%
SLCL 6A_26234902	LCL 6A	26234902	4.645	2.264E-05	37.39%
SLCL 6A_26367575	LCL 6A	26367575	4.644	2.268E-05	40.75%
SLCL 1A_34153852	LCL 1A	34153852	4.624	2.374E-05	39.15%
SLCL 4B_18368841	LCL 4B	18368841	4.581	2.625E-05	38.16%
SLCL 7B_19418910	LCL 7B	19418910	4.576	2.656E-05	39.43%
SLCL 9B_17129765	LCL 9B	17129765	4.575	2.663E-05	40.65%
SLCL 6A_26363762	LCL 6A	26363762	4.565	2.724E-05	45.55%
SLCL 1A_34021621	LCL 1A	34021621	4.562	2.74E-05	37.32%
SLCL 6A_26367678	LCL 6A	26367678	4.550	2.821E-05	44.72%
SLCL 6A_26205526	LCL 6A	26205526	4.547	2.839E-05	44.36%
SLCL 2B_5432473	LCL 2B	5432473	4.542	2.87E-05	38.18
SLCL 1A_34101912	LCL 1A	34101912	4.538	2.898E-05	35.76%
SLCL 2B_20312336	LCL 2B	20312336	4.534	2.927E-05	39.52%
SLCL 2B_20312337	LCL 2B	20312337	4.534	2.927E-05	39.52%
SLCL 1A_37425661	LCL 1A	37425661	4.533	2.929E-05	37.32%
SLCL 6A_26362923	LCL 6A	26362923	4.521	3.015E-05	36.79%
SLCL 1A_37566926	LCL 1A	37566926	4.497	3.182E-05	36.51%
SLCL 6A_26366445	LCL 6A	26366445	4.496	3.192E-05	38.16%
SLCL 6B_18654716	LCL 6B	18654716	4.491	3.229E-05	35.93%
SLCL 1A_7292023	LCL 1A	7292023	4.489	3.242E-05	43.48%
SLCL 3A_12394580	LCL 3A	12394580	4.486	3.267E-05	39.67%
SLCL 3B_31748526	LCL 3B	31748526	4.480	3.308E-05	36.86%
SLCL 6A_26166942	LCL 6A	26166942	4.480	3.309E-05	45.44%
SLCL 6A_26251870	LCL 6A	26251870	4.460	3.471E-05	38.9%
SLCL 6A_26682381	LCL 6A	26682381	4.445	3.593E-05	36.55%
SLCL 6A_26373794	LCL 6A	26373794	4.443	3.603E-05	46.71%
SLCL 1A_33980358	LCL 1A	33980358	4.441	3.625E-05	37.96%
SLCL 1A_32747669	LCL 1A	32747669	4.428	3.736E-05	34.63%
SLCL 6A_26219125	LCL 6A	26219125	4.425	3.756E-05	36.27%
SLCL 2B_5432446	LCL 2B	5432446	4.425	3.761E-05	37.98%
SLCL 3B_31743195	LCL 3B	31743195	4.420	3.799E-05	36.31%
SLCL 6A_26362855	LCL 6A	26362855	4.409	3.896E-05	36.08%
SLCL 1A_37445691	LCL 1A	37445691	4.407	3.915E-05	38.06%
SLCL 2B_5439319	LCL 2B	5439319	4.406	3.925E-05	36.35%
SLCL 6A_25382694	LCL 6A	25382694	4.405	3.932E-05	38.14%
SLCL 6A_26234967	LCL 6A	26234967	4.402	3.966E-05	35.02%
SLCL 3B_32160921	LCL 3B	32160921	4.398	3.999E-05	37.24%
SLCL 3B_31743205	LCL 3B	31743205	4.389	4.086E-05	36.32%

SLCL 6A_26366464	LCL 6A	26366464	4.388	4.092E-05	37.43%
SLCL 6A_25433046	LCL 6A	25433046	4.387	4.099E-05	37.69%
SLCL 6A_26358434	LCL 6A	26358434	4.384	4.131E-05	44.46%
SLCL 9A_1848686	LCL 9A	1848686	4.373	4.236E-05	0.9%
SLCL 6B_18654759	LCL 6B	18654759	4.355	4.419E-05	34.4%
SLCL 7B_20653376	LCL 7B	20653376	4.338	4.592E-05	50.93%
SLCL 2B_13971760	LCL 2B	13971760	4.331	4.67E-05	44.42%
SLCL 6A_26252272	LCL 6A	26252272	4.325	4.737E-05	48.69%
SLCL 6A_26234957	LCL 6A	26234957	4.323	4.748E-05	33.9%
SLCL 6A_26365097	LCL 6A	26365097	4.323	4.751E-05	33.71%
SLCL 6B_18651946	LCL 6B	18651946	4.322	4.769E-05	44.6%
SLCL 6A_26407818	LCL 6A	26407818	4.320	4.785E-05	32.81%
SLCL 6A_26114606	LCL 6A	26114606	4.320	4.788E-05	41.78%
SLCL 10A_8324689	LCL 10A	8324689	4.317	4.82E-05	34.17%
SLCL 6A_26252034	LCL 6A	26252034	4.306	4.946E-05	36.28%
SLCL 6A_26367403	LCL 6A	26367403	4.305	4.958E-05	34.57%
SLCL 6A_26167352	LCL 6A	26167352	4.300	5.01E-05	32.3%
SLCL 6A_26167284	LCL 6A	26167284	4.300	5.015E-05	35%
SLCL 2B_20312305	LCL 2B	20312305	4.293	5.098E-05	34.83%
SLCL 6A_26367415	LCL 6A	26367415	4.286	5.177E-05	34.85%
SLCL 6A_26399887	LCL 6A	26399887	4.252	5.594E-05	35.59%
SLCL 6A_26167552	LCL 6A	26167552	4.251	5.613E-05	34.9%
SLCL 2B_20312316	LCL 2B	20312316	4.250	5.623E-05	35.5%
SLCL 2B_5432427	LCL 2B	5432427	4.247	5.666E-05	33.46%
SLCL 6A_26367392	LCL 6A	26367392	4.246	5.681E-05	33.8%
SLCL 6B_18554372	LCL 6B	18554372	4.233	5.846E-05	38.18%
SLCL 6A_26114814	LCL 6A	26114814	4.226	5.949E-05	34.86
SLCL 7A_7459770	LCL 7A	7459770	4.223	5.984E-05	32.28%
SLCL 6B_18652386	LCL 6B	18652386	4.212	6.137E-05	32.82%
SLCL 6B_18652390	LCL 6B	18652390	4.212	6.137E-05	32.82%
SLCL 6A_26151834	LCL 6A	26151834	4.208	6.193E-05	34.53%
SLCL 7B_20530393	LCL 7B	20530393	4.203	6.271E-05	41.74%
SLCL 3B_31678920	LCL 3B	31678920	4.198	6.333E-05	49.4%
SLCL 6A_26374711	LCL 6A	26374711	4.195	6.38E-05	32.91%
SLCL 9A_9839695	LCL 9A	9839695	4.194	6.392E-05	33.31%
SLCL 6A_26219510	LCL 6A	26219510	4.191	6.434E-05	33.52%
SLCL 6A_26219511	LCL 6A	26219511	4.191	6.434E-05	33.52%
SLCL 6A_26878846	LCL 6A	26878846	4.191	6.443E-05	35.12%
SLCL 6A_26219582	LCL 6A	26219582	4.187	6.496E-05	33.82%
SLCL 5B_7338275	LCL 5B	7338275	4.187	6.504E-05	35.4%
SLCL 6A_26234970	LCL 6A	26234970	4.186	6.512E-05	33.83%
SLCL 6A_26409334	LCL 6A	26409334	4.184	6.55E-05	32.37%
SLCL 6A_26219535	LCL 6A	26219535	4.176	6.662E-05	34.53%
SLCL 6A_26113865	LCL 6A	26113865	4.174	6.696E-05	35.42%
SLCL 6A_26113868	LCL 6A	26113868	4.174	6.696E-05	35.42%
SLCL 6A_26366934	LCL 6A	26366934	4.169	6.779E-05	31.97%

SLCL 2B_13971537	LCL 2B	13971537	4.167	6.806E-05	41.72%
SLCL 6A_5589048	LCL 6A	5589048	4.155	7.002E-05	44.7%
SLCL 6A_26410540	LCL 6A	26410540	4.153	7.025E-05	33.04%
SLCL 6A_26438939	LCL 6A	26438939	4.152	7.042E-05	33.27%
SLCL 6A_26366936	LCL 6A	26366936	4.148	7.109E-05	32.14%
SLCL 6A_26366937	LCL 6A	26366937	4.148	7.109E-05	32.14%
SLCL 9A_2225591	LCL 9A	2225591	4.147	7.126E-05	36.21%
SLCL 2B_5521509	LCL 2B	5521509	4.144	7.173E-05	31.52%
SLCL 6B_18654756	LCL 6B	18654756	4.144	7.182E-05	32.34%
SLCL 2B_5427234	LCL 2B	5427234	4.137	7.288E-05	34.34%
SLCL 6A_26358485	LCL 6A	26358485	4.133	7.36E-05	32.66%
SLCL 2B_5495348	LCL 2B	5495348	4.118	7.624E-05	32.56%
SLCL 6A_26358503	LCL 6A	26358503	4.113	7.716E-05	32.62%
SLCL 6A_26219446	LCL 6A	26219446	4.112	7.729E-05	33%
SLCL 6A_26370572	LCL 6A	26370572	4.108	7.805E-05	32.82%
SLCL 6A_26424694	LCL 6A	26424694	4.106	7.841E-05	32.88%
SLCL 6A_26167271	LCL 6A	26167271	4.091	8.114E-05	33.24%
SLCL 4A_16672589	LCL 4A	16672589	4.090	8.124E-05	31.27%
SLCL 6A_26410483	LCL 6A	26410483	4.082	8.284E-05	32.46%
SLCL 6A_26357038	LCL 6A	26357038	4.078	8.357E-05	31.31%
SLCL 2B_5494649	LCL 2B	5494649	4.076	8.401E-05	34.32%
SLCL 6B_18562520	LCL 6B	18562520	4.067	8.579E-05	30.91%
SLCL 6A_26370785	LCL 6A	26370785	4.059	8.737E-05	31.25%
SLCL 3B_31742648	LCL 3B	31742648	4.046	8.988E-05	46.4%
SLCL 6A_26367739	LCL 6A	26367739	4.038	9.161E-05	50.82%
SLCL 6A_26365174	LCL 6A	26365174	4.036	9.196E-05	48.18%
SLCL 2B_5486670	LCL 2B	5486670	4.036	9.205E-05	30.08%
SLCL 6A_26161269	LCL 6A	26161269	4.035	9.223E-05	40.43%
SLCL 6A_26367692	LCL 6A	26367692	4.034	9.244E-05	40.31%
SLCL 6A_26167773	LCL 6A	26167773	4.029	9.361E-05	31.38%
SLCL 6A_26363755	LCL 6A	26363755	4.028	9.366E-05	45.9%
SLCL 6A_26161255	LCL 6A	26161255	4.026	9.409E-05	39.46%
SLCL 2B_5432363	LCL 2B	5432363	4.024	9.458E-05	33.54%
SLCL 2B_5432366	LCL 2B	5432366	4.024	9.458E-05	33.54%
SLCL 2B_5522094	LCL 2B	5522094	4.011	9.753E-05	32.77%
SLCL 6A_26167556	LCL 6A	26167556	4.007	9.84E-05	32.97%

Chr=chromosome; pos=position; SHL=shoot length; RDWt=root dry weight;
SHFWt=shoot fresh weight and RFWt=root fresh weight.