



**Agronomic and nutritional qualities of mixed genotypes of
Ethiopian tetraploid wheat (*Triticum durum/turgidum* and
related species) collections**

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**Agronomic and nutritional qualities of mixed genotypes of
Ethiopian tetraploid wheat (*Triticum durum/turgidum* and
related species) collections**

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Abstract

Agronomic and nutritional qualities of mixed genotypes of Ethiopian tetraploid wheat (*Triticum durum/turgidum* and related species) collections

Mulugeta Tilahun Melaku, MSc Thesis

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Ethiopian farmers have been traditionally growing several varietal mixtures in the same field that might have advantages of reducing the risks of pests and unusual environmental conditions in addition to adding variety to their diets. Replacement of farmers' varieties with genetically uniform varieties, results in changes to crop patterns and land uses largely affecting the magnitude of the genetic diversity in Ethiopian tetraploid wheats. Therefore, this study was conducted at two locations of each Amhara, Oromia and Tigray regions with the objective of evaluating the effects of mixtures of Ethiopian tetraploid wheat collections on agronomic and nutritional quality traits and maintaining the diversity of the crop. The experiment was laid out by using alpha lattice design with three replications. The experiment had fifteen genotypes with two categories (Category one: mixtures and pure stands of Ethiopian tetraploid wheat collections and Category two: mixtures and pure stands of improved varieties). Agronomic and proximate as well as selected minerals data were taken and subjected to analysis using SAS procedures. Since genotype by location interaction was significant for agronomic traits, analysis was conducted for each locations and GGE-biplot was used to select best fit genotypes for each location. The analysis of variance showed significant differences for both agronomic and nutritional quality traits. The range of grain yield was 0.2 to 2.7tha⁻¹ in Adet, 1.5 to 2.6tha⁻¹ in Geregera, 0.6 to 3.0tha⁻¹ in Debre Zeit, 2.4 to 3.4tha⁻¹ in Chefe Donsa, 1.4 to 2.6tha⁻¹ in Ayiba and 2.8 to 3.8tha⁻¹ in Hageresalam. Based on the analysis of individual locations and GGE-biplot mega environment results; mixtures of top ten Amhara Region, mixtures of ten improved varieties, Assasa and Mikuye are best genotypes for Adet and Geregera of Amhara Region. Mixtures of Oromia Region, GEBRE of Oromia Region, Assasa and Mikuye are best genotypes for Debrezeit and Chefe Donsa of Oromia Region. Whereas mixtures of top ten Tigray Region, GG2 of Tigray Region, GEBRE of Oromia Region and Mangudo are best genotypes for Ayiba and Hageresalam of Tigray Region. In regarding to nutritional quality, amounts ranged from 8.1 to 13.0% for protein, 73 to 78.9% for total carbohydrate, 1.4 to 2.9% for fat, 2.5 to 3.4% for fiber, 1.1 to 3.4gm/100gm for Fe and 1.9 to 2.7gm/100gm for Zn. Significantly higher protein contents (13.0 and 11.9%) were recorded from Mangudo, mixtures of Tigray Region and TIKUR SINDE of Amhara Region. Maximum total carbohydrate (78.9%) was obtained from GEBRE of Oromia Region. Higher fat contents (2.9, 2.6 and 2.5%) were obtained from TIKUR SINDE of Amhara Region, mixtures of top ten Tigray Region and Mangudo respectively. Maximum Fe 3.4 mg/100gm and Zn (2.7 mg/100gm) were obtained from mixtures of parental lines. Therefore, it can be concluded that mixtures as well as pure lines of farmers' varieties performed well comparatively with pure lines of high yielding improved varieties in this experiment and farmers can maintain the diversity of the crop and add supplement their diets by growing mixtures of Ethiopian tetraploid wheat genotypes.

Key words: Diversity maintenance, farmers' varieties, GGE-biplot, mega environment

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List of Acronyms

| | |
|----------|--|
| AAS | Atomic Absorption Spectrum |
| ANOVA | Analysis of Variance |
| CCP | Composite Cross Population |
| CSA | Central Statistical Authority |
| DAP | Di-ammonium Phosphate |
| EBI | Ethiopian Biodiversity Institute |
| EHNRI | Ethiopian Health and Nutrition Research Institute |
| FAO | Food and Agricultural Organization |
| GGE | Genotype plus Genotype by Environment |
| HCL | Hydro Chloric Acid |
| IBC | Institute of Biodiversity Conservation |
| IUCN | International Union for Conservation of Nature and Natural Resources |
| m.a.s.l. | Mean above sea level |
| NAM | Nested Association Mapping |
| r | Correlation coefficient |
| SNP | Single Nucleotide Polymorphism |

CHAPTER ONE

1. INTRODUCTION

1.1. Background

Wheat is among the main staple crops in the world and it has been producing for many years in the world which has a great role in human as well as animal consumption. Ethiopia is one of the largest producers of wheat in Sub-Saharan Africa with an area of 1,664,564.62 ha and total production of 4.22 million tons with an average yield of 2.5 tha^{-1} (CSA, 2016). While Amhara, Tigray and Oromia regions have an average yield of 2.2, 1.7 and 2.8 tha^{-1} respectively (CSA, 2016). Wheat is the third and fourth most important cereal in Ethiopia in terms of area and production respectively after teff (*Eragrostis tef*), maize (*Zea mays*) and sorghum (*Sorghum bicolor*) (CSA, 2016). It plays an appreciable role in supplying the population with protein, carbohydrates, and minerals (Schulthesis et al., 1997).

The total area and production of tetraploid wheat is respectively about 20 million hectares and 30 million metric tons globally (Kahrizi et al., 2010). It is cultivated on 10 to 11% of the world wheat areas and accounting for 8% of the total wheat production (Ganeva et al., 2011). In Ethiopia, tetraploid ($2n = 4x = 28$) wheat genotypes have been under cultivation since ancient times and the country is considered as the center of genetic diversity (Vavilov, 1997). The central Ethiopian highlands are the major tetraploid wheat producing areas and a wide range of diversity has been observed in terms of spike form, spike density, awn size, glume colour, glume pubescence, kernel size, and vitreousness (Tesfaye Tesemma, 1991). The majority (85%) of Ethiopian tetraploid wheat genotypes are farmers' varieties

comprising mixture of several agro types and subspecies (Tesfaye Tesemma, 1987; Firdissa Eticha et al., 2006). Ethiopian farmers traditionally grow several varietal mixtures in the same field that might have advantages to reduce the risk of pests and diseases or unusual environmental conditions and to add variety to their diets (Endeshaw Bekele, 1984). Jain (1961) also stated that traditionally farmers often continue to grow even less productive types and crop wild relatives, and, therefore, preserve cultivars and genetic diversity.

Replacement of farmers' varieties with genetically uniform varieties, changes in crop pattern and land uses have largely affected the magnitude of the genetic diversity of the indigenous crops. For instance, Ethiopian tetraploid wheat is giving way to TEF and new dwarf bread wheat varieties, particularly in areas where extensive wheat breeding activities have occurred since the sixties (IBC, 2007; Royo et al., 2009). As a result, approximately 80% of the wheat area in Ethiopia is planted to bread wheat, implying that 20% of the wheat area is planted to tetraploid wheat (Negassa et al., 2012 as cited by Dejene Kasahun et al., 2015).

The approach of creating uniform and genetically stable cultivars that are deployed over large areas in monocultures through conventional breeding system is inappropriate for dealing with the current and predicted threats to agriculture though they are well equipped to cope with one particular stress (Verboom et al., 2010; Döring et al., 2011). Suneson (1956) introduced a concept of evolutionary plant breeding to increase crop diversity by assembling seed stocks with diverse evolutionary origins, recombination by hybridization, the bulking of F1 progeny, and subsequent prolonged natural selection for mass sorting of

the progeny in successive and changing natural cropping environments as a supplement of conventional plant breeding.

Crop diversity through evolutionary plant breeding system can be created either by taking seeds from different genotypes and sowing a mixture with the same proportions of all genotypes every year or by developing composite cross populations from different parents through crossing (Ceccarelli, 2009; Döring et al., 2011). And this method can generate plant populations that are neither uniform nor stable. On the contrary, they are genetically highly diverse and changing in their genetic constitution over changing environment. Genotype mixtures can serve as on-farm conservation which has been defined as the continuous cultivation and management of a diverse set of populations by farmers in the agro-ecosystems where a crop has evolved or diversified (IUCN, 1992; Bellon et al., 1997).

On the other hand, the large increment in the percentage of people suffering from micronutrient malnutrition over the last four decades is associated with the global expansion of high yielding and input responsive cereal cultivars and high consumption of diets with little diversity consisting one or two staple foods (Welch, 2002; Welch and Graham, 2002; Cakmak et al., 2004). Among micronutrient deficiencies, Zn and Fe deficiencies are the most prevalent ones. Children are particularly susceptible to Zn deficiency and it has been shown to be responsible for 450,000 deaths of children under 5 years of age (Black et al., 2008). Iron deficiency is also the most widespread nutrient deficiency in the world, estimated to affect over 2 billion people (Stoltzfus and Dreyfuss, 1998). Deficiencies of Zn and Fe cause various serious health complications such as stunting, increased susceptibility to infectious diseases, impaired brain function and mental

development, poor birth outcomes and anemia (Kennedy et al., 2003; Hotz and Brown, 2004).

Genotype mixtures also have important effects on reduction of biotic stresses particularly diseases by different mechanisms (Browning and Frey, 1969, Chin and Wolfe, 1984). Furthermore, Sarandon & Sarandon (1995) and Finckh et al. (2000) reported that mixtures can give better yield and other yield related traits than their sole components. This increment in yield and yield related traits is achieved by different mechanisms like complementarity, compensation and facilitation among mixed genotypes (Khalifa and Qualset, 1974; Willey, 1979; Yachi and Loreau, 1999; Garcia-Barríos, 2002; Ceccarelli, 2009).

1.2. Statement of problems, Research questions and Hypothesis

1.2.1. Statement of problems

Ethiopian farmers' varieties of tetraploid wheat have been largely replaced by monocultures of pure genotypes in their centre of diversity or the place where they evolved (Negash Geleta and Grausgruber, 2013b). This genetic erosion resulted in significant loss of valuable genetic diversity of the crop which is important for adaptation to changing environment and source of breeding material in the future (Mohammed Abinasa et al., 2011; Negash Geleta and Grausgruber, 2013a). The pure genotypes may not have the wide adaptation and the diverse genetic background which the farmers' varieties may have. The development of new varieties from populations of farmers' varieties is a viable strategy to improve the yield and yield stability of modern varieties, especially under stress and existing climate change conditions (Döring et al., 2011). Therefore, maintaining the

diversity of the crop and its source as future breeding material available to farmers is very vital. Most of the farmers in our country have fragile lands which cannot maintain all existing genotypes. Thus, growing mixtures of many genotypes in their small land is the best solution to reduce the genetic erosion of our endangered farmers' tetraploid wheat varieties.

Limited progress in undernourishment and child underweight is observed in developing countries especially in sub-Saharan Africa, but dietary imbalance is still a problem (FAO, 2015). Malnutrition has been mainly caused by deficiency in micronutrients like Fe and Zn, which result in underweight, wasting and stunting in children over many years (Stoltzfus and Dreyfuss, 1998; Black et al., 2008). It is believed that diversifying the diet to include micronutrient-rich traditional foods is the right solution to these challenges (Frison et al.; 2006). However, many of the world's poorest people who depend on major staple crops do not have access to a wide variety of nutritionally dense food crops (Graham et al., 2001). Increasing the diversity of staple crops through mixing is considered a better solution as the crops will have the ability to supply these nutrients sufficiently (Murphy et al., 2008).

Mixed cropping of different varieties of one crop has been commonly practiced by Ethiopian farmers for many years. Cultivating composite populations of wheat, barley, sorghum and other major crops had been the rule rather than the exception for Ethiopian farmers in the past. However, this traditionally framed practice is not well developed and studied as to its scientific basis and application to actively tackle and respond to the problems faced now. Therefore, this study was initiated to evaluate the performance of

mixed populations of different Ethiopian tetraploid wheat genotypes in selected areas of Amhara, Tigray and Oromia regions of Ethiopia.

1.2.2. Research questions

- How can the diversities of Ethiopian tetraploid wheat be possibly maintained by farmers while they keep on using mixtures of the crop?
- Can mixtures of Ethiopian tetraploid wheat collections give better yield and yield related traits?
- How would mixtures of Ethiopian tetraploid wheat collections reduce pressure from diseases?
- Can mixtures of Ethiopian tetraploid wheat collections enhance nutritional quality of the crop?
- Can mixtures of Ethiopian tetraploid wheat collections used as source of breeding materials for future crop improvement programmes?

1.2.3. Research hypotheses

- Farmers in Ethiopia cannot maintain the diversity of Ethiopian tetraploid wheat collections while regularly using the mixtures of the crop for cultivation
- Mixing seed collections of Ethiopian tetraploid wheat genotypes from different locations has no effects on agronomic traits, disease resistance, nutrient contents and diversity

1.3. Objectives of the study

1.3.1. General objective

- To evaluate the effects of mixtures of Ethiopian tetraploid wheat collections on agronomic and nutritional quality traits and maintenance of the diversity of the crop.

1.3.2. Specific objectives

- To determine the effect of mixtures of Ethiopian tetraploid wheat collections on the crops' phenology, yield and yield related traits
- To assess the effect of mixtures of Ethiopian tetraploid wheat collections on disease pressure
- To evaluate the effect of mixtures of Ethiopian tetraploid wheat collections on nutritional quality of the harvested grain on farmers' field at a sample location (Chefe Donsa)
- To increase the availability of Ethiopian tetraploid wheat genotypes for possible crop improvement researches
- To maintain the diversity of the crop at farmers level by making available mixtures and combinations of seeds with better yields, nutrition and diversity features.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Origin and evolution of wheat

The first cultivation of wheat is believed to have occurred about 10,000 years ago, as part of the 'Neolithic Revolution', which saw a transition from hunting and gathering of food to settled agriculture. The earliest cultivated forms of wheat were diploid (genome AA) (einkorn) and tetraploid (genome AABB) (emmer) wheats and their genetic relationships indicate that they originated from the south-eastern part of Turkey (Heun et al., 1997; Nesbitt, 1998; Dubcovsky and Dvorak, 2007). Cultivation spread to the Near East by about 9000 years ago when hexaploid bread wheat (*Triticum aestivum?*) made its first appearance (Feldman, 2001). The spread of wheat from its site of origin across the world has been described by Feldman (2001) and the main route into Europe was via Anatolia to Greece (8000 BP) and then both northwards through the Balkans to the Danube (7000 BP) and across to Italy, France and Spain (7000 BP), finally reaching the UK and Scandinavia by about 5000 BP. Similarly, wheat spread via Iran into central Asia reaching China by about 3000 BP and to Africa, initially via Egypt. It was introduced by the Spaniards to Mexico in 1529 and to Australia in 1788.

2.2. Botanical descriptions

Wheat is a tall, annual plant with a height ranging from two to six feet in early varieties. The plant is made up of leaves surrounding a slender stalk that terminates in spikes, or ears, of grain at the top of wheat (International Starch Institute). Each spike, ear, of grain is made

up of spikelets, which encloses the wheat grain in between the lemma and the palea. The wheat grain is in the shape of an oval and is what gives wheat its nutritional value. The grain may also vary in its length of brush hairs, either long or short. Cultivated wheat is most commonly grown with physical characteristics of uniform spikes, awned (bearded) and are easily threshed (McKevith, 2004). Domestic wheats are also bred for strong seed heads which will not shatter during processing (Katz, 2003). Cultivated forms of diploid, tetraploid, and hexaploid wheat all have a tough rachis apart from the spelt form of bread wheat. Similarly, the early domesticated forms of einkorn, emmer, and spelt are all hulled, whereas modern forms of tetraploid and hexaploid wheat are free-threshing (Shewry, 2009).

2.3. Nutritional values of wheat

The nutritional value of wheat is usually addressed through analyses of its macronutrient and micronutrient components. These groups consist of carbohydrate, proteins and lipids, for macronutrients, and vitamins, minerals and phytochemicals for micronutrients. It contains 78.10% carbohydrate, 8–15% protein, 2.10% fat, 2.10% minerals and considerable proportions of vitamins (thiamine and vitamin-B) and minerals (zinc, iron) (Kumar et al., 2011). Endosperm is about 83% of the kernel weight; it is the source of white flour. The endosperm contains the greatest share of the protein in the whole kernel, carbohydrates, iron as well as many B-complex vitamins, such as riboflavin, niacin, and thiamine. Bran is about 14.5% of the kernel weight. The bran, which consists of pericarp, testa and aleurone, is also a dietary source for fiber, potassium, phosphorus, magnesium, calcium, and niacin in small quantities. Wheat germ is the embryo of the wheat kernel. The germ or embryo of the wheat is relatively rich in protein, fat and several of the B-vitamins.

The high content of starch, about 60–70% of the whole grain and 65–75% of white flour, means that wheat is often considered to be little more than a source of calories. Despite its relatively low protein content (usually 8–15%), wheat provides considerable protein for human and livestock nutrition (Shewry, 2009).

Unlike macronutrients, micronutrients are not as concentrated within wheat and therefore cannot be compared within their percentages. However, they provide an extremely important purpose to the human body, specifically in their roles of promoting health and preventing disease (Basey, 79). Iron deficiency is the most widespread nutrient deficiency in the world, estimated to affect over 2 billion people (Stoltzfus and Dreyfuss, 1998). Although many of these people live in less developed countries, it is also a significant problem in the developed world. Zinc deficiency is also widespread, particularly in Sub-Saharan Africa and South Asia, and has been estimated to account for 800,000 child deaths a year (Micronutrient Initiative, 2006), in addition to non-lethal effects on children and adults. Therefore, the nutritional importance of wheat proteins should not be underestimated, particularly in less developed countries where bread, noodles and other products (e.g. Bulgar, couscous) may provide a substantial proportion of the diet.

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The average concentration of Zn in whole grain of wheat in various countries is between 20 to 35 mg kg⁻¹ (Cakmak et al., 2004) and it is 2.2 to 35 mg kg⁻¹ for Fe (Graham et al., 2007). Most of the seed Zn is located in the embryo and aleurone layer, whereas the endosperm is very low in Zn concentration (Ozturk et al., 2006).

According to Koehler and Wieser (2013), wheat whole grain ranks third in terms of average moisture content (12.6%) after rye (13.6%), oats (13.1%) and rice (13.0%). It ranks first in average protein content (11.3%) followed by rice (11.1%) and millet (10.5%). Available carbohydrate content of 59.4% puts wheat above oats (56.2%) and below rice (73.7%), millet (68.2%), maize (65.0%) and rye (60.3%). Millet is first in lipid content (3.9%) and wheat is among the least with 1.8%. In its' fiber content (13.2%), wheat is the first and followed by rye (13.1%), maize and barley (9.8%). While with its mineral contents (1.7%), wheat ranks third next to oats (2.9%) and barley (2.3%).

With regards to Ethiopian mixtures, mixtures of wheat whole grains have an average of 11.3 % moisture content which is below mixtures of millet (11.7%) and above mixtures of sorghum (9.7%) and mixtures of teff (10.7%). In terms its average protein content (10.0%), mixtures of wheat whole grains is above all mixtures of millet (7.55%), teff (8.4%) and sorghum (7.6%). Its carbohydrate content (75.3%) puts mixed whole grain wheat below

all sorghum (77.9%), millet (76.3%) and teff (75.7%). An amount of 1.8% fat content of mixed whole grain wheat puts it below that of sorghum (3.1%) and teff (2.55%), but similar with mixed whole grain of millet (1.8%). While its' 3.0% fiber content puts wheat below millet (5.8%) and teff (3.2%) but above sorghum (2.5%). Mixed wheat whole grain has 1.6% ash content which is below sorghum (1.7%), teff (2.7%) and millet (2.7%). In terms of its' 3.7 mg/100 Fe content, mixed whole grain is below all Sorghum (17.3), teff (35.3) and mixed millet (36.5) mg/100gm (EHNRI, 1997).

2.4. Importance of tetraploid Wheat

The major use of tetraploid wheat is for pasta products, particularly in the European and North American countries whereas in other areas (the Middle East and North Africa) it is also used for production of couscous and various types of bread. The quality of end-products is related to the quality of the tetraploid grain, which, in turn, is mainly determined by the genotype, but also by the environment (weather and nutrition) and crop management (Troccoli et al., 2000).

Tetraploid wheat is used predominantly for pasta and couscous but the use of tetraploid wheat in flat and specialty breads is also common in Mediterranean countries, the Middle East, and North Africa. Traditionally, pasta is manufactured solely from tetraploid wheat, which results in a product considered to be of superior quality to pasta made from cheaper common wheat or a blend of the two species (Bryan et al., 1998; Knödler et al., 2010). GENFO, ATMIT, KITA, ANEBABERO, CHUKO, CHECHEBSA, DABO-KOLLO, gulban, and MULMUL are some specialty dishes in Ethiopia. It is also used for preparing local drinks like TELA and AREKE (Bayush Tsegaye and Berg, 2007).

Grain protein in the range appropriate for the various end uses of wheat is critical for making acceptable quality products such as bread, various types of noodles and biscuits (Lemon, 2007). Protein content is a primary determinant of pasta cooking quality, while gluten strength plays an important part in both dough extrusion properties and in the quality of cooked pasta (Aalami et al., 2007).

2.5. Tetraploid wheat production and its diversity in Ethiopia

The central Ethiopian highlands are the major tetraploid wheat producing region and a wide range of diversity has been observed in terms of spike form, spike density, awn size, glume colour, glume pubescence, kernel size, and vitreousness (Tesfaye Tesemma, 1991). The crop is traditionally grown by farmers on heavy black soils (vertisols) under rain fed conditions at altitudes of 1800–3000 m.a.s.l. and sometimes even above 3000m even though 1900-2700 m.a.s.l. is the most suitable for the crop (Perrino and Porceddu, 1990). Most tetraploid wheat varieties in Ethiopia are landraces/farmers' varieties which are often species mixtures (Firdissa E ticha et al., 2006) and composites made of landraces maintained by farmers in the mixed form.

Genetic diversity is the extent of heritable variation in a population, or species, or across a group of species, e.g. heterozygosity, or number of alleles, or heritability (Frankham et al., 2002). Genetic diversity is required for populations to evolve in response to environmental changes and if there is no genetic diversity in a population or species, it is likely to go extinct in response to major environmental changes. Very high genetic diversity is found in Ethiopia in four of the world's widely grown food crops wheat, barley, sorghum and peas (Frankham et al., 2002).

Ethiopian farmers have been growing tetraploid wheat for centuries, and as a result it covered 60–70% of the arable land under wheat cultivation in Ethiopia until the 1980s, with bread wheat (*Triticum aestivum*) covering only the remaining 30–40%. However, the introduction of improved bread wheat from international breeding programs into Ethiopia and their wide adaptation with satisfactory yield potential has shifted the predominance to bread wheat and left tetraploid wheat landraces almost an orphan crop. Now, approximately 80% of the wheat area in Ethiopia is planted to bread wheat, implying that 20% of the wheat area is planted to tetraploid wheat (Negassa et al., 2012 as cited by Dejene Kasahun et al., 2015). Even though the area of land devoted to tetraploid wheat is diminishing in Ethiopia, there is high genetic variability in the country which is vital for tetraploid wheat improvement programs (Dejene Kasahun et al., 2016; Dejene Kasahun and Pè, 2016; Dejene Kasahun et al., 2015; Zewdie Bishaw et al., 2014). Jemanesh Haile et al. (2013) also found similar diversity for Ethiopian tetraploid wheat landraces. But these authors revealed that all the improved tetraploid wheat varieties of Ethiopia were clustered in the same group except the landrace varieties ‘DZ04’ which clustered with the landraces. These results point out that the diversity of Ethiopian tetraploid wheat varieties that are released over the last 43 years is relatively low. This research might show that only a small portion of genetic diversity of farmers’ varieties has been exploited for tetraploid wheat improvement.

In general, different level of diversity for tetraploid wheat which indicated Ethiopians’ richness in its diversity have been reported by different authors (Firdissa Eticha et al., 2005; Arega Gashaw et al., 2007; Yifru Teklu and Karl, 2008; Faris Hailu et al., 2010; Mohammed Abinasa et al., 2011; Negash Geleta and Grausgruber, 2013a). According to

these findings, the presence of high genetic diversity of Ethiopian tetraploid wheat genotypes reveals the presence of materials which are good opportunities for breeding programs.

2.6. Tetraploid wheat genetic erosion in Ethiopia

Genetic erosion is defined as the loss of variability from crop populations in diversity centers, i.e. areas of domestication and secondary diversification (Brush, 1999). Genetic erosion implies that the normal addition and disappearance of genetic variability in a population is altered so that the net change in diversity is negative. FAO (1997) estimates that since the beginning of the twentieth century, about 75% of the genetic diversity of agricultural crops has been lost.

Until the 1970's, the diversity in the landraces was unaffected significantly. However, due to repeated drought in some areas of high crop diversification in the country and diffusion of exotic seed varieties that have been displacing the landraces, the pace of genetic erosion tremendously increased after 1970's (IBC, 2007). Displacement of indigenous landraces by genetically uniform varieties, changes in crop pattern and land use have largely affected the magnitude of the genetic diversity of the indigenous crops. Tetraploid wheat is giving way to tef and new dwarf bread wheat varieties, particularly in areas where extensive wheat breeding activities have occurred since the sixties (IBC, 2007; Royo et al., 2009).

Many studies which are conducted in different locations of Ethiopia supported the above statement. For instance, Bayush Tsegaye and Berg (2007) reported genetic erosion (loss of diversity) in wheat landraces of East Shewa and the overall loss of the once popular tetraploid wheat landraces in the study area accounted for 77% of the formerly available

diversity. According to these authors, there was no single factor stand alone to account for loss of diversity. The combination of factors that contributed to the loss were displacement by bread wheat varieties, expansion of tef, absence of local seed supply for landrace wheat, decline in size of farmland holdings, changes in land use and cropping patterns, lack of policy support, and expansion of improved tetraploid wheat varieties. Another study which is conducted in Dandi and Ambo districts resulted in genetic erosion in tetraploid wheat reached greater than 60%. The loss of landraces was accounted to different causes. These included the expansion of improved bread wheat varieties, low soil fertility and poor yield of the landraces, and short rainy season period since most landraces are long maturing types (Negash Geleta and Grausgruber, 2013b).

2.7. Genetic resource conservation strategies

It is essential to conserve and utilize genetic resources in a sustainable manner to ensure the continuous improvement of the new wheat cultivars. There are two ways of conserving genetic resources: *in situ* and *ex situ*, which are basically complementary but not exclusive, to each other. *In-situ* conservation, including on-farm conservation, has been defined as the continuous cultivation and management of a diverse set of populations by farmers in the agro-ecosystems where a crop has evolved (IUCN, 1992; Bellon et al., 1997). *Ex-situ* conservation means the conservation of components of biological diversity outside their natural habitats (IUCN, 1992). Practically, most of the ex-situ conservations of wheat are in the form of seeds stored in gene banks. The underlying principle of successful seed storage is to maintain genetic integrity of accessions such as seeds with high viability for long periods.

2.8. Production constraints of Ethiopian tetraploid wheat

Although some tetraploid wheat cultivars are high yielding and improved varieties have been released and distributed to farmers, the area under improved varieties is very small (Tesfaye Tesemma, 1987). Furthermore, the national average yield is also low, less than 2.5 t/ha (CSA, 2016). Some of the major factors limiting yield of tetraploid wheat in Ethiopia are lack of widely adapted varieties, low soil fertility, heavy black soils (Vertisols) which are characterized by cracking when dry and waterlogging during the rainy season, (Hailu Beyene et al., 1990; Tesfaye Tesemma and Demissie Mitiku, 1992; Bekele Geleta et al., 1994).

Biotic stresses like diseases, insect pests and weeds are another important factors. Fungal diseases cause a considerable yield loss in tetraploid wheat, among these, leaf rust (*Puccinia recondita*), stem rust (*P. graminis tritici*), and stripe rust (*P. striiformis*), are the most important. Problematic diseases in some areas include septoria leaf blotch (*Septoria tritici*), glume blotch (*S. nodorum*), leaf spot (*Helminthosporium* spp.), bunt or stinking smut (*Tilletia foetida* or *T. caries*), scab or root rot (*Fusarium* spp.), powdery mildew (*Erysiphe graminis*), and bacterial stripe (*Xanthomonas translucens*) (Tesfaye Tesemma, 1991).

The Russian wheat aphid (*Diuraphis noxia*) commonly attacks tetraploid wheat causing crop losses of up to 100% on late-planted local cultivars (Hailu Beyene et al., 1990; Tesfaye Tesemma, 1991). Grasshopper damage also occurs frequently in some parts of the country when no heavy rainfall occurs immediately after seed emergence. Weeds are also major problems in wheat producing areas and a general review of weed science research activities

on wheat and barley in Ethiopia reported that weed competition reduced wheat yields by up to 36.4% (Rezenne Fessehaie, 1985 as cited by Hailu Beyene et al., 1990).

2.9. Concept of evolutionary plant breeding

The approach of releasing uniform and genetically ‘stable’ cultivars that are deployed over large areas in monocultures is inappropriate for dealing with the current and predicted threats to agriculture under conventional breeding system. Although well equipped to cope with one particular stress, they may not be able to cope with other stresses in a changing or fluctuating environment. In view of such variability, wider adaptation is needed, and this can be achieved via extended intra-specific plant diversity (Döring et al., 2011). Simmonds (1993) and Allard & Hansche (1964) explained mass reservoirs of genetic variability as supplements to conventional breeding to help broaden the genetic base of crops and as a means for dynamic conservation of genes and genotypes.

An evolutionary plant breeding which was given its formal name by Suneson (1956) can generate plant populations that are neither uniform nor ‘stable’; on the contrary, they are genetically highly diverse and changing in their genetic constitution over time. This method can generate two crop populations which are Composite Cross Populations (CCPs) and genotype mixtures, determined by the way in which they were created, *i.e.*, by crossing in the case of CCPs, and by physical mixing seed of existing genotypes in the case of genotype mixtures (Döring et al., 2011).

Based on their literature review, Phillips and Wolfe (2005) indicated that composite cross populations which can be developed by evolutionary plant breeding might be an efficient method for providing useful heterogeneous crops and for selecting pure lines particularly

for low input systems. This breeding method depends upon the outcome of mass trials by artificial and natural selection acting upon a heterogeneous mixture of competing genotypes (Jain, 1961). The advantage of heterogeneous cereal populations increases as the environment becomes more stressful (Frey and Maldonado, 1967).

Evolutionary plant breeding represents an attempt to make the process of *in-situ* conservation more dynamic by combining participation and evolution in participatory-evolutionary breeding programs. One way of exploiting the progressive adaptation of the evolutionary populations is to consider them as evolving sources of new cultivars, progressively better adapted to the evolving agronomic and climatic conditions – in other words, a living gene bank in farmers' fields (CENESTA, 2013).

In evolutionary participatory breeding method, farmers' knowledge and expertise are utilized to improve crop varieties specifically adapted to their individual farming systems and local soil and climatic conditions instead of farmers' selection of homogeneous varieties bred under a different farming system and different environmental conditions. Whereas participatory plant breeding emphasizes collaboration between breeders and farmers to share knowledge and skills to identify and improve suitable cultivars previously unavailable to low-input farmers (Murphy et al., 2004).

To decide the number of parents for the establishment of crop population one can use only two different parent varieties (Steele et al., 2004). On the other hand, as many parents as physically possible can be crossed with each other and which strategy is better will depend on how genetically different the parents are (Kovács, 2008). The choice of parent material

greatly depends on end use (Finckh and Wolfe, 1997). For example, high yield potential for feed wheat, or high baking quality, or good drought resistance.

Once a population is created by natural selection, where no direct choice of genotypes by humans, there are two more options to select them namely: mass selection and manual selection (Döring et al., 2011). Mass selection is the simultaneous selection of a large number of plants or grains with similar but not entirely uniform properties. For example, by passing cereal grain through a sieve before sowing, smaller grains can be separated from larger ones. The success of mass selection depends on the presence of sufficient diversity, but at the same time, mass selection leads to a potentially large decrease in diversity if only a particular selected fraction is maintained.

Manual selection can be done either by singling out individual plants or ears in the population and manually collecting them for use as seed in the next season or by removing individual plants or ears of undesired habit from the population so that only the remaining plants contribute to the next year's seed. For example, very tall and very small plants could be taken from the growing crop, in order to narrow the height range of the population.

After having gone through several generations of natural or mass selection, diverse crop populations provide a valuable resource for both conventional and further population breeding. When single ears are selected and sown in 'ear rows', evaluation of individual genotypes can take place, eventually resulting in the selection of new breeding material or even ready-to-use lines (Murphy et al., 2005)

Using CCPs as a source for new material in conventional breeding programs has been especially suggested when the goal is to breed for low input systems (Danquah and Barrett,

2002). However, it is not correct to assume that a CCP will per se produce genotypes that have a high pure stand yielding ability in the prevailing environment. Ceccarelli (1996) also argues that because breeding is mostly conducted in the presence of high inputs, it has systematically missed the opportunity to exploit genetic differences at low levels of inputs.

2.10. Genotype mixtures

Monocultures are subjected to genetic uniformity which also provides disadvantages because of uniform vulnerability to pests. Vulnerability associated with uniformity can be mitigated by increasing the number of genotypes in fields to increase genotypic diversity. The resulting 'genotype mixtures' are defined as mixtures of genotypes (i.e. cultivars) 'that vary for many characters including disease resistance, but have sufficient similarity to be grown together' (Wolfe, 1985).

According to Murray and Milgroom (2012), there are three types of mixtures recognized, in general, depending on how they are developed. These are: landrace mixtures, varietal mixtures and multiline varietal mixtures. Landraces and their heterogeneity are developed in localized areas by the management of farmers. Varietal mixtures are resulted from intentional or unintentional mixing of seeds of existing varieties which might be developed by modern breeding programs, local selection or a combination of both. Multiline varietal mixtures are mixtures of multiple lines that are developed intentionally by the breeder to be grown together.

Several trends of planting genotype mixtures have been seen in different countries. For instance, close to 50% of wheat fields in Europe and tens of thousands of hectares of rice in China have been sown as cultivar mixtures (Zhu et al., 2000; Mundt, 2002 as it is cited

by Tooker and Frank, 2012). In the United States, 18% of soft winter wheat were also planted in Washington State in 2000 and 7% of Kansas wheat planted in 2001 were cultivar mixtures (Bowden et al., 2001; Mundt, 2002 as it is cited by Tooker and Frank, 2012). These mixtures are typically constructed as random mixtures of five cultivars that vary in susceptibility to important diseases (e.g. rusts, powdery mildew), and yield nearly 30% better than monocultures when disease is present while maintaining yield, or even slightly improving it when disease is absent (Wolfe, 1985; Brown and Wolfe, 1993; Mundt, 1994 and 2002 and Smithson and Lenne, 1996 as cited by Tooker and Frank, 2012; Garrett & Mundt, 1999)

2.10.1. Genotype mixtures for diversity maintenance

Diversity and heterogeneity serve to disperse or buffer the risk of total crop failure due to unpredictable environmental variation and this can also be achieved through mixtures of different crops, different cultivars of the same crop, and/or heterogeneous cultivars to retain and maximize adaptation (Ceccarelli, 2009).

Conservation of genetic resources requires very high attention at present and in the future since rapid genetic erosion is occurring within all crops in the face of environmental change and as most current varieties are uniform (Hammer, 2004; Dawson and Goldringer, 2012). Historically, crops have been growing as populations or landraces which can allow for diversification of crop varieties, adaptation to contrasting environments and uses as maintenance of genetic diversity. Now a days, mixtures which are created from a known component can be used as genetic diversity maintenance and in the mean time they can

also be allowed to evolve through seed saving and replanting over several years (Dawson and Goldringer, 2012).

2.10.2. Genotype mixtures for better quality and nutritional enhancement

The increase of grain yield together with grain protein content is an important goal in wheat production. However, this is not easy to achieve because of the inverse relationship that exists between grain yield and grain protein content (Kramer, 1979; Rauch and Busch, 1985 as cited by Sarandon & Sarandon, 1995). Higher grain yield has been achieved through new cultivars with a greater efficiency in dry matter partition (harvest index) without an increase in total biological productivity (Austin et al., 1980; Scholz, 1984; Perrv and D'Antuono, 1989; Slafer and Andrade, 1989 as cited in Sarandon & Sarandon, 1995). However, this achievement has been associated with a decrease in grain protein content (Austin et al. 1977; Kramer, 1979; Fossati and Cao, 1985 as cited in Sarandon & Sarandon, 1995)).

In baking tests, mixtures of different numbered cultivars performed better than the mean of the individual components (Osman, 2006; Vogt-Kaute, 2006).

The large increases in the percentage of people suffering from micronutrient malnutrition over the last four decades is associated with the global expansion of high-yielding, input-responsive cereal cultivars (Welch, 2002; Welch and Graham, 2002). Although diversification of diet to include micronutrient-rich traditional foods is the preferred solution to these challenges (Frison et al., 2006), many of the world's poorest people do not have access to a wide variety of nutritionally dense food crops (Graham et al., 2001). However, many researches revealed that farmers' varieties have the ability to supply these

nutrients sufficiently. For instance, Murphy et al. (2008) found in his research that out of eight genotypes, seven of the historical cultivars had significantly higher grain mineral concentrations than the modern cultivars. Only Ca showed no significant difference between the historical and modern cultivars. But negative and no correlations were observed in this study between yield and mineral concentrations.

2.10.3. Genotype mixtures for reduction of biotic stress

Genotype mixtures have important effects on reduction of biotic stresses particularly diseases by different mechanisms. One mechanism is dilution effect by which disease is reduced in cultivar mixtures because of the increased distance between plants of the susceptible cultivar in the mixture (Browning and Frey, 1969, Chin and Wolfe, 1984). The presence of the resistant cultivar decreases the chance of the inoculum produced from the infected susceptible cultivar of landing on another susceptible cultivar. Most of the inoculum lands on the resistant cultivar, thus reducing the rate of disease spread in a population. The other mechanism is barrier effect through which the resistant cultivar provides a physical barrier that restricts the movement of the inoculum from the susceptible cultivar (Browning and Frey, 1969; Garrett and Mundt, 1999). For mixtures of differentially susceptible cultivars (i.e., both components are susceptible to different races of the pathogen), plants of cultivar A serve as a barrier for the race that attacks cultivar B, and vice versa. Induced resistance is also another mechanism which occurs when races that are non-virulent on a cultivar induce the plant's defense response mechanisms. As a consequence, any virulent race (genetically different isolate of the same pathogen that would normally infect the plant) invading exactly the same area of the plant cannot cause infection (Chin and Wolfe, 1984). For instance, some barley genotypes can produce

volatiles which induce other barley genotypes to become more resistant to pest attack (Ninkovic and Åman, 2009). The diversity of pathogen genotypes is expected to be higher in cultivar mixtures than in monoculture, thus increasing the chance of interactions and competition between pathogen races (Garrett and Mundt, 1999), this is called mechanism of Competition among pathogen races. Competition among different virulent races may prevent a certain race from dominating and overcoming host resistance in cultivar mixtures, thus reducing disease in the mixtures.

Genotype mixtures have also improved insect pest suppression in some crop studies (Cantelo and Sanford, 1984; Power, 1991). For example, in separate experiments, mixtures of two oat varieties or five corn varieties were found to harbour significantly fewer pests (aphids and leafhoppers, respectively) than monotypic stands, but this effect was only detected when pest populations were large (Power, 1991). Similarly, mixing a susceptible variety of potato with more resistant varieties lead to a reduction in numbers of potato leafhoppers (Cantelo and Sanford, 1984).

There is a tendency for a higher competitive effect on weeds of mixtures compared to pure stands of barley cultivars, but this depended on the combination of cultivars present in the mixture (Didon and Rodríguez, 2006). Contrasting characteristics between the cultivars as regards allelopathic activity and shoot development increased the competitive effect.

2.10.4. Genotype mixtures for increasing yield and yield related traits

One of the most important criteria for the success of mixtures in practice is their yielding ability. It has been argued that mixtures present greater yield advantages in low-yielding environments than in high yielding environments. Thomas et al. (1991) examined

composite crosses of wheat and reported yield improvements of a composite population of wheat of more than 15% over the mean of the parents in pure stands. The environments in which these populations grown were fluctuated from year to year in terms of temperature, rainfall and irrigation, and reflected standard regional barley-growing conditions. On the other hand Qualset (1968) reported F₂ generation of wheat outperforming the yield of parent stands by up to 26% under low yield conditions.

There are different mechanisms of genotype mixtures to increase yield and yield related traits. The first one is complementarity which occurs when genotypes are grown together in a diverse population which have different profiles of resource use (above and below ground), they complement each other in the exploitation of the limiting resource and therefore are subject to smaller between-plant competition. Such effects can be realized by mixing genotypes of different rooting patterns (shallow vs. deep), or different light interception strategies (Willey, 1979). Therefore, yield and yield related traits advantage occurs when cultivar components differ in their use of resources in space and time in such a way that overall use of resources is better than when components are grown separately.

Compensation is the second mechanism which usually happens between cultivars with different competitive abilities (Willey, 1979). It occurs when the yield of one component increases while the other decreases without affecting the overall yield of the mixture (Khalifa and Qualset, 1974). The risk of low yields mitigated by using the mix and this advantage is more pronounced under the higher variability conditions of the prevailing environment. In the mix, as one genotype fails, another one compensates the failure. In other words, the genotype diversity provides insurance against environmental fluctuations (Yachi and Loreau, 1999; Ceccarelli, 2009). For a genotype with a capacity to deal with

the specific environmental condition, crop diversity (in gene banks, in the field or via new crossings) is a central requirement to provide this capacity of specialist response.

There is also another mechanism which is called facilitation and it is happened when a component cultivar may benefit another component directly by improving microclimate, providing physical support or windbreaks, and ameliorating harsh environmental conditions, or indirectly by providing protection from other pests and diseases, and improving water holding capacity (Callaway, 1995; Garcia-Barrios, 2002).

CHAPTER THREE

3. MATERIAL AND METHODS

3.1. Experimental sites

The study was conducted at two locations of each Amhara, Tigray and Oromia regions of Ethiopia respectively in one year during the main growing period of 2016/2017. In Amhara Region, the study was conducted in Geregera and Adet farmers' field. In Oromia Region, the study was conducted in Debre Zeit research center and Chefe Donsa farmers' field. In Tigray Region, the study was conducted in Hagereslam and Ayiba farmers' field. These locations were distinct in soil type, altitude, maximum and minimum temperature as well as amount of average annual rain fall (Table 1) and their locations (Figure 1-3).

Table 1. Description of the experimental sites

| Regions | Locations | Soil types | Geographical coordinates | | | Average Temp.(°C) | | Average rainfall (mm) |
|---------|-------------|------------|--------------------------|-------------|--------------------|-------------------|------|-----------------------|
| | | | Latitude | Longitude | Altitude (m.a.s.l) | Max | Min | |
| Amhara | Geregera | Clay loam | 37° 36' 35" | 11° 10' 11" | 2650 | 27.7 | 11.5 | 672 |
| | Adet | Clay | 37° 29' 35" | 11° 16' 28" | 2240 | 26.7 | 13.2 | 1193 |
| Oromia | Ayiba | Clay loam | 39° 31' 35" | 12° 55' 38" | 2730 | NA | NA | 544 |
| | Hagereslam | Clay loam | 39° 10' 25" | 13° 38' 44" | 2583 | 22.2 | 11.2 | 656 |
| Tigray | Debre Zeit | Clay | 38° 57' 0" | 8° 43' 59" | 1900 | 27.1 | 11.5 | 864 |
| | Chefe Donsa | Clay | 39° 7' 23" | 8° 58' 12" | 2392 | 24.5 | 10.8 | 856 |

NA=Not available, Max=Maximum, Min=Minimum, m.a.s.l=Man above sea level

Source: Ethiopian National Meteorological Agency, Ethiopian Institute of Agricultural research and Amhara Region Agricultural Research Institute

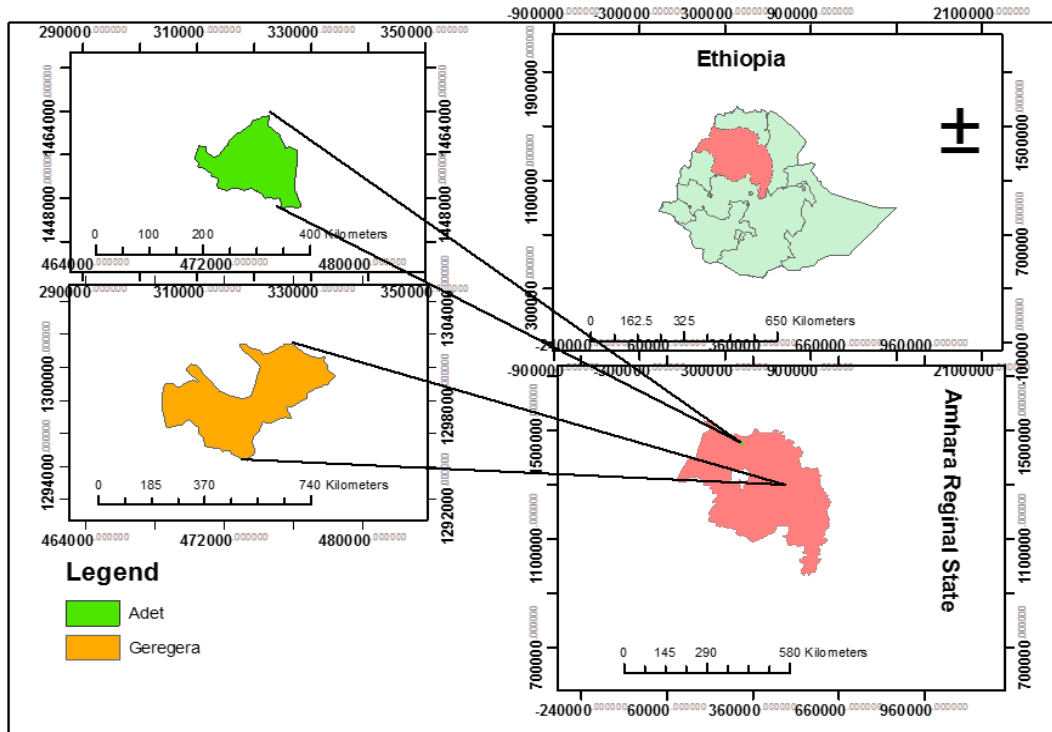


Figure 1. Map of Ethiopia and experimental sites in Amhara Region

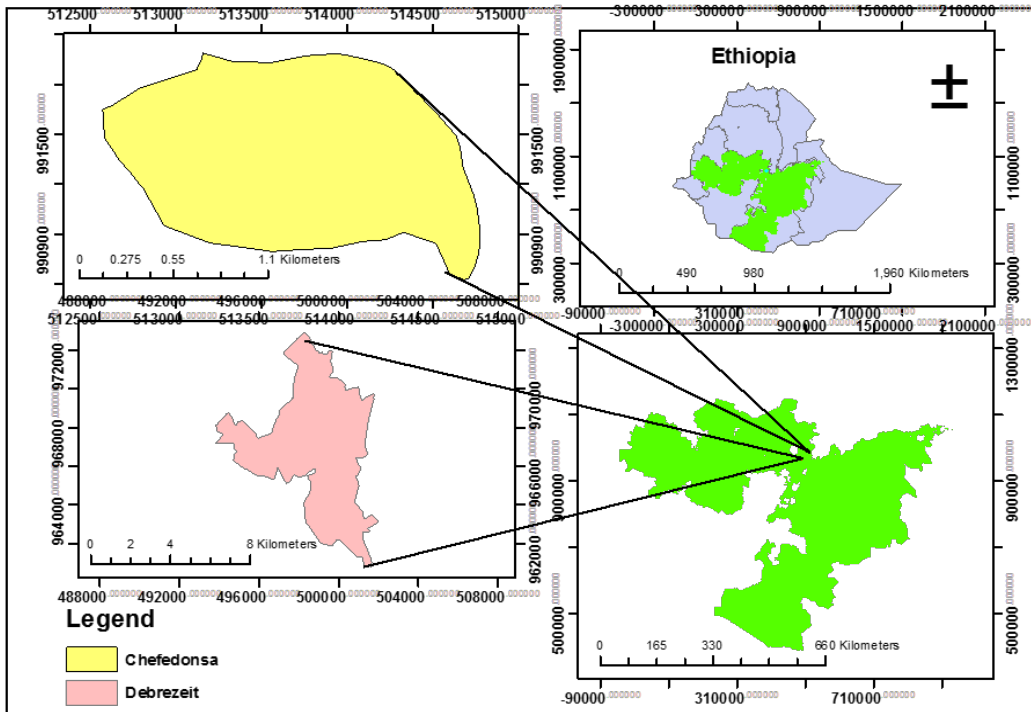


Figure 2. Map of Ethiopia and experimental sites in Oromia Region

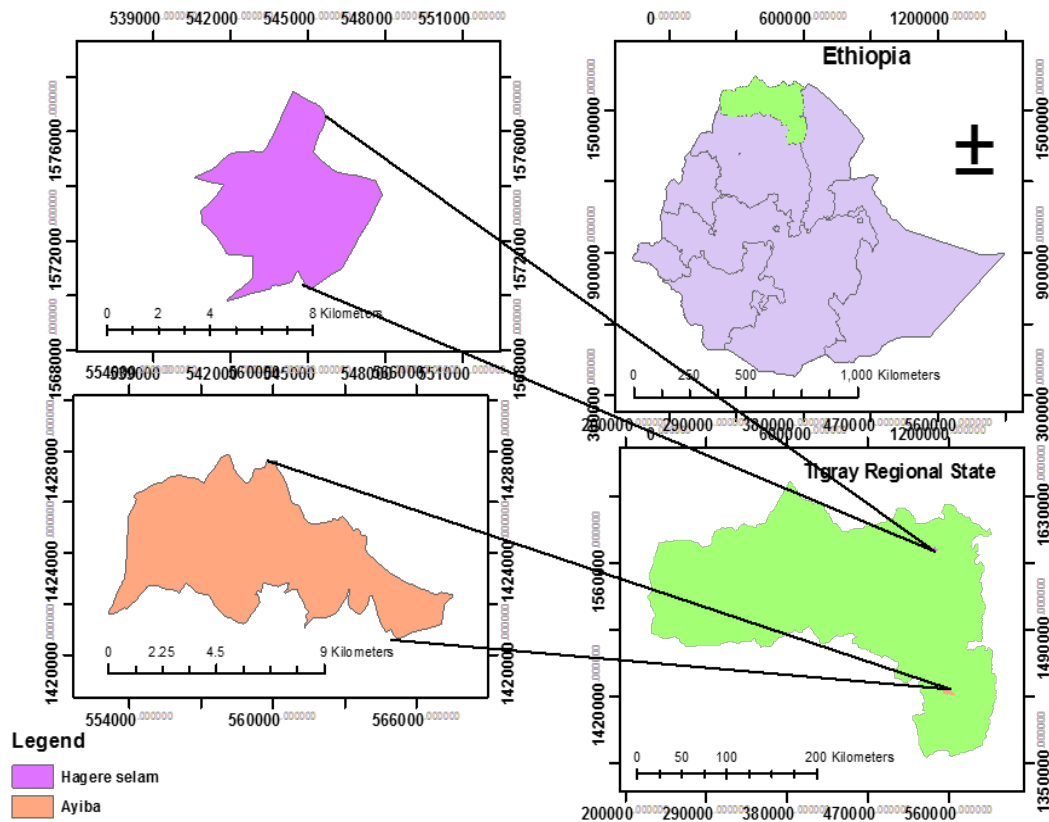


Figure 3. Map of Ethiopia and experimental sites in Tigray Region

3.2. Experimental materials and treatments

The experiment was done using two categories of tetraploid wheat genotypes as described below: Category one: mixtures and pure stands of Ethiopian tetraploid wheat collections which contain mixtures of 219 tetraploid wheat collections as mixtures of the country, mixtures of 29 collections as parental lines for NAM (Nested Association Mapping) population, mixtures of collections from each regions and pure stand of three farmers' varieties grown widely in each three regions. Totally we used 248 Ethiopian tetraploid wheat collections for mixtures which were characterized and selected by Dejene Kasahun et al. (2016) where they brought 373 accessions from EBI (Ethiopian biodiversity institute) and collected from different regions of Ethiopia (Appendix 6-10).

Category two: mixtures and pure stands of improved varieties which contain mixtures of hybrid lines (F7 generation) which were obtained from NAM experiment of Bioversity International, mixtures of ten improved varieties and three pure stands were used (Appendix 6).

Mixtures of the country are collections from Amhara, Oromia, Tigray and other different regions which were not specified well by the authors. All mixtures of Amhara, Oromia and Tigray Regions were collections from each regions and exclusive to each other during treatment set up. Mixtures of top ten Amhara and top ten Tigray Region were selected by their high yield performance and farmers' selection criteria since all collections were planted in these two regions (Dejene Kasahun et al., 2016). Accessions in mixtures of top ten Amhara were from Amhara Region only and were not included in all treatment arrangements, but accessions in mixtures of top ten in Tigray were not collections only from Tigray Region, but also from Amhara and Oromia Regions (Accessions 222541, 238579, 208192 and 208215A were from Amhara, 208316 from Oromia, 238137A from Tigray Regions) (Appendix 6) and included in each regions mixture. In the meantime, all pure stands of farmers' as well as improved varieties were not included in any treatment arrangements. All these were arranged in fifteen treatments and described below (Table 2).

Mixtures of NAM (Nested Association Mapping) population are mixtures of inbred lines which are at F7 generation and obtained by crossing 29 parental lines with Assasa improved variety (Dejene Kasahun et al., 2016). TIKURE SINDE of Amhara Region, GEBRE of Oromia region and GG2 of Tigray Region are farmers' varieties which are being grown widely in their respective regions. Whereas Assasa, Mikuye and Mangudo are improved varieties which are currently under cultivation in the country.

Table 2. Descriptions of experimental genotypes and their treatment arrangements

| Treatment number | Genotypes Code | Genotypes' descriptions | Number of collections | Genotypes status |
|------------------|----------------|-----------------------------------|-----------------------|--------------------|
| 1 | Mix 1 | Mixtures of the country | 219 | Accessions |
| 2 | Mix 2 | Mixtures of parental lines | 29 | Accessions |
| 3 | Mix 3 | Mixtures of Tigray Region | 10 | Accessions |
| 4 | Mix 4 | Mixtures of Amhara Region | 76 | Accessions |
| 5 | Mix 5 | Mixtures of Oromia Region | 92 | Accessions |
| 6 | Mix 6 | Mixtures of top ten Amhara Region | 10 | Accessions |
| 7 | Mix 7 | Mixtures of top ten Tigray Region | 10 | Accessions |
| 8 | Mix 8 | Mixtures of NAM population | 12 | Recombinants |
| 9 | Mix 9 | Mixtures of ten varieties | 10 | Improved varieties |
| 10 | Sole 1 | TIKURE SINDE of Amhara Region | 1 | Farmers' variety |
| 11 | Sole 2 | GEBRE of Oromia region | 1 | Farmers' variety |
| 12 | Sole 3 | GG2 of Tigray Region | 1 | Farmers' variety |
| 13 | Sole 4 | Assasa | 1 | Improved variety |
| 14 | Sole 5 | Mikuye | 1 | Improved variety |
| 15 | Sole 6 | Mangudo | 1 | Improved variety |

3.3. Experimental design

Planting was done in rows by using alpha lattice design with three replications (Figure 4a and b). The experiment was laid out with a plot size of 2.5 m x 2 m with 20 cm row spacing. There was 0.5 m and 1m spacing between plots and replication respectively. 100 kg ha⁻¹ seed rate and full dose of P (100 kg ha⁻¹ DAP) and half of N (50 kg ha⁻¹ urea) fertilizer were applied during sowing. Additional 50 kg ha⁻¹ of urea was applied at the beginning of tillering stage. Weeding practices were done as per recommendations.

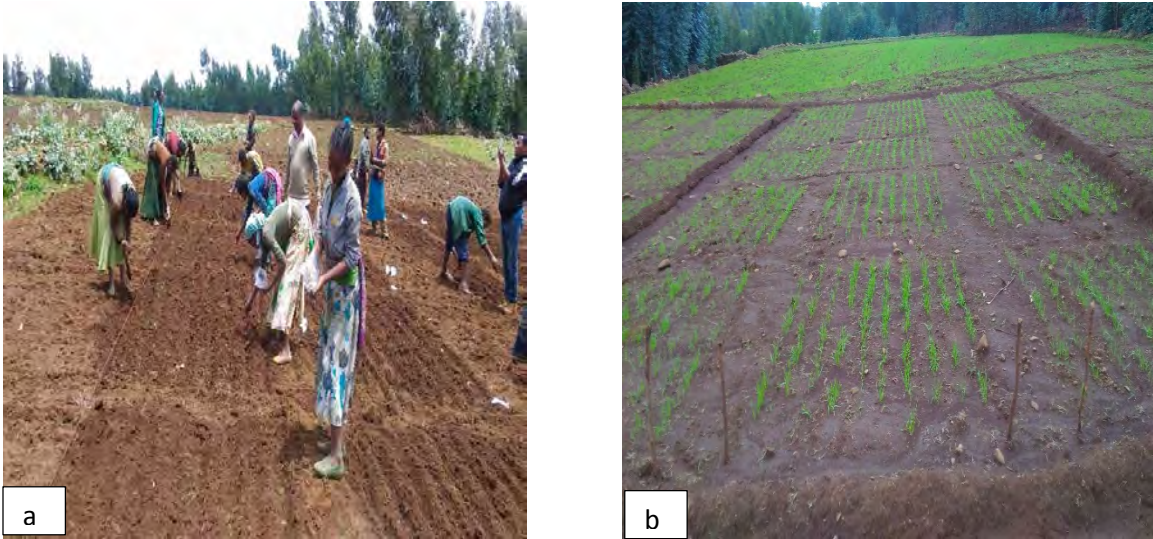


Figure 4. Pictures showing planting in rows and plants after emergence with their plot arrangements

3.4. Methods of data collection for agronomic traits

3.4.1. Phenological traits

Booting date: It was done by taking the number of days from date of planting to when 50% of plants in a plot had booted.

Heading date: It was done by taking the number days from date of planting to when 50% of plants in a plot had headed.

Flowering date: It was done by taking the number of days from date of planting to when 50% of plants in a plot had get flowered.

Maturity date: It was done by taking the number of days from date of planting to when 75% of physiological maturity.

Plant height: It was recorded by taking measurements from the ground to the tip of the spike of the crop from 25 randomly selected plants from the harvestable rows for all genotypes and an average was taken to have well represented values for mixtures.

3.4.2. Yield and yield related traits

Grain yield (tha^{-1}): The crop was harvested at full maturity with appropriate moisture content. The yield per plot was weighed and converted into ton per hectare at 12.5% moisture content.

Thousand kernel weight (gm): Randomly taken 1000 g rains were counted for each treatment and weighed with a sensitive balance.

Biomass yield (tha^{-1}): The weight of total aboveground parts per plot was measured from the harvestable plot area (4m^2) and converted in to ton per hectare.

Spike length (cm): Average length of spikes per plot was taken from twenty five randomly selected plants in harvestable rows to have well represented values for mixtures.

Number of Seeds per spike: Average number of seeds per spikes in each plots was counted and taken from twenty five randomly selected plants in harvestable rows.

3. 5. Methods of data collection for disease traits

Disease recording was done by estimating the whole plot from the harvestable rows for all treatments to have well represented values by reducing exclusion of individual genotypes during scoring of diseases in mixed treatments (Figure 5).

3.5.1. Rust diseases

According to the modified Cobb scale (Zadoxs, 1961), rust diseases were recorded by taking severity (percentage of rust infection on the plants) and plant responses (type of disease reaction). This recording process was depending upon visual observations and using intervals: Trace-100 percent rust infections on plant. Plant responses were recorded using the following letters:

- 0= No visible infection on plants.
- R= Resistant; visible chlorosis or necrosis and no uredia are present.
- MR= Moderately Resistant; small uredia are present and surrounded by either chlorotic or necrotic areas.
- M= Intermediate; variable sized uredia are present and some with chlorosis, necrosis, or both.
- MS= Moderately Susceptible; medium sized uredia are present and possibly surrounded by chlorotic areas.
- S= Susceptible; large uredia are present. Generally with little or no chlorosis and no necrosis.

Severity and plant response readings were usually combined. For example:

- tR = Trace severity with a resistant plant response,
- 5MR= 5% severity with a moderately resistant plant response and
- 60S= 60% severity with a susceptible plant response.

According to Stubbs et al. (1986), the final disease severity data for the stripe rust was converted into a coefficient of infection (CI) by multiplying severity with a constant value

for field response ($R=0.2$, $MR=0.4$, $MS=0.8$ and $S=1$) which it is used for ranking or rating varieties. Therefore,

$$CI = Severity \times CIF$$

3.5.2. Septoria disease

Septoria was recorded visually by taking disease severity using a double-digit (00 to 99), modified version of Saari and Prescott's scale (Saari and Prescott, 1975; Eyal et al., 1987; Sharma and Duveiller, 2007). The first digit (D1) indicates disease progress in plant height and the second digit (D2) refers to severity measured as the diseased leaf area.

$$Severity = (D1/9) * (D2/9) * 100$$

Where, D1= disease progress in plant height and D2= severity measured as the diseased leaf area

For instance, if we take 23 as on record, we mean that the disease reached 20% of the plant height and the plant has 30% diseased leaf area.



Figure 5. Picture showing disease data assessment and recording

3.6. Proximate and selected minerals determination

Nutritional quality traits of the crop like moisture, protein, carbohydrate, fat, ash and fiber content and iron and zinc minerals were determined in Addis Ababa University Food and Nutrition Center laboratory except crude fiber content which was determined in Ethiopian Public Health Institution laboratory following the standard procedures. Proximate contents of the raw wheat grain flour was determined according to AOAC (2000). Whereas iron and zinc mineral contents of the tetraploid wheat flour samples from each treatments were determined according to Osborne and Vooget (1978) by using Buck Atomic Absorption Spectrophotometer (AAS).

3.6.1. Moisture content

Moisture content of the raw wheat grain flour was determined according to AOAC (2000) using the official method of 979.09 which was used to remove water from the sample, and the loss of weight is used to calculate the moisture content of the sample.

The cylinders used for the moisture determination were dried at 105⁰c for 1 hr in drying oven and placed in desiccators for about 30 m inute to cool. The mass of cylinders was measured after cooling (M₁) and about 5 gm tetraploid wheat flour for each samples was taken and weighed together with dried cylinders (M₂) to dry at 105⁰C for 3 hours. After drying was completed and cooling it in a desiccator at room temperature, the mass was measured (M₃). The moisture content was calculated from the equation:

$$\text{Moisture (\%)} = \frac{M_2 - M_3 \times 100}{M_2 - M_1}$$

Where M_1 = mass of cylinder, M_2 = mass of the cylinder and the sample before drying and M_3 = mass of the cylinder and the sample after drying

3.6.2. Crude protein content

Protein content of the raw wheat grain flour was determined according to AOAC (2000) using the official method 979.09. The protein content was determined from the nitrogen content by the following procedure.

Addition of reagents. About 0.5 gm of tetraploid wheat flour for each samples was weighed by adventurer analytical balance, added to the Tecator tube and placed in the Tecator rack. Six ml of concentrated sulfuric acid was added to each samples and mixed immediately. A 3.5 ml of 30% hydrogen per oxide solution was added to each Tectator tubes step by step. The tubes were shacked until the violet reaction was disappeared. About 3 gm of catalyst mixture made from 10 g of copper sulfate and 150 gm of potassium sulfate was added in to each Tectator tubes and let for 5-15 minutes before digestion.

Digestion. The s olution for eac h samples w as digested at 370°c for about 1 hr. using Tecator digester until a clear solution was obtained. After digestion is completed, the rack was transferred in to the fume hood f or cooling and 30ml o f water was added for each solutions for dilution.

Distillation. After digestion and dilution, 40ml of 40% sodium hydroxide was added to each samples to neutralize the acid and to make the solution slightly alkaline. A 250ml conical flask containing 25ml of boric acid, 25ml of distilled water and indicator solution was placed under the condenser of the kejeltic distiller with its tip immersed in to the

solution. The distillation was continued until a total volume become between 200 and 250 ml.

Titration. Titration of each sample solutions with 0.1 N Hydrochloric acid to a reddish color was done by using kjeltic analyzer unit. Then finally the nitrogen and protein contents were calculated from the equation:

$$\text{Nitrogen (\%)} = \frac{(\text{V}_{\text{HCL}} - 0.2) \text{L} \times \text{NHCL}(\text{ca. } 0.1) \times 14.00 \times 100}{\text{W}_0}$$

Where V_{HCL} = volume of HCL consumed to the end point titration, 0.2 = volume of blank consumed, N = the normality of HCL (used often is 0.1 N), 14 = the molecular weight of nitrogen and W_0 = sample weight on dry matter basis.

$$\text{Protein content (\%)} = 6.25 \times \%N$$

3.6.3. Total ash content

Total ash content of the raw wheat grain flour was determined according to AOAC (2000) using official method of 999.10.

The crucibles used for the analysis were cleaned and dried for 30 min. at 550⁰c in a Muffle furnace. The crucibles were let to cool in a desiccator (with granular silica gel) for about 30 min. at room temperature and weighted (M_1). A 2.5 g of tetraploid wheat flour samples were weighed in to the crucibles (M_2). The samples were let to char on a hot plate under a fume-hood and the temperature was slowly increased until smoking ceased. The samples were let to ash in the Muffle furnace at 550⁰C for 5 hrs. The ash was clean and white in appearance when it was taken out from furnace. Finally the crucibles with ash were cooled

at room temperature and reweighted (M3) with placing them in a desiccator. Then the total ash was calculated from the equation:

$$\text{Ash (\%)} = \frac{M3 - M1 \times 100}{M2 - M1}$$

Where M_1 = mass of the dried crucible, M_2 = mass of the crucible and the fresh sample, M_3 = mass of the crucible and the ash.

3.6.4. Crude fat content

Crude fat content of the raw wheat grain flour was determined according to AOAC (2000) using the official method 4.5.01.

The extraction cylinders were cleaned with hot water, removed any impurities and placed into an oven for 1 hr. at 105°C for drying. The cylinders were then taken out, placed into desiccator and measured for mass (M_1) by putting them back in a desiccator again. Cleaned and numbered extraction thimbles were taken by covering their layers with fat free cotton. Two gm of each samples were measured (M) and poured in tumblers by covering their top again with cotton which were then put in the extraction chamber. The extraction cylinders were taken out from the desiccator and put into the bracket by matching their numbers with extraction tumblers. A 50ml petroleum ether was added to each extraction cylinder and moved into heating plank where extraction left for 4 hrs. After 4 hours, extraction cylinders were disconnected and placed into an oven for 30 min. at 70°C . After drying was completed, extraction cylinders were placed into desiccator for 30 min. Mass of extraction cylinders having fat content (M_2) was taken immediately after they were taken out from desiccators. The crude fat content was calculated from the equation:

$$\text{Crude fat (\%)} = \frac{M_2 - M_1 \times 100}{M}$$

Where M₁= mass of extraction cylinder (gm.), M₂= mass of extraction cylinder and lipid extracted (gm.) and M=sample mass (gm.).

3.6.5. Crude fiber content

Crude fiber content of the raw wheat grain flour was determined according to AOAC (2000) using the official method of 962.09.

A mass of 1.6 g for each samples was measured in each of crucibles which were cleaned and dried in an oven for 1 hr. at 105⁰C (M). A 200 ml of 1.25% sulfuric acid solution was added to each crucibles and allowed to boil at 6-8⁰C for 30 min by rotating and steering periodically. During boiling the level was kept constant by addition of hot distilled water. After 30 min, 20 ml of 28% potassium hydroxide was added in to the crucibles and again allowed to boil for another 30 min. Then after 30 min the solution found in the crucible was filtered through crucibles containing sand by placing on Buchner funnel. During filtration the samples were washed with hot distilled water. The crucibles with their contents were dried for 5 hr. at 105⁰c and cooled in desiccators and measured for their mass (M₁). Then again the samples were ashed for 3 hr. at 525⁰c in furnace and cooled in desiccators. Finally the mass of each crucible was taken (M₂). Then crude fiber was calculated from the equation:

$$\text{Crude fiber (\%)} = \frac{M_2 - M_1 \times 100}{M}$$

Where M_1 = mass of the crucible, the sand and wet residue after drying, M_2 = mass of the crucible and the sand after ashing and W =sample weighed.

3.6.6. Carbohydrate content

Total and available carbohydrate contents of each sample were calculated by difference with the inclusion and exclusion of crude fiber.

$$\text{Total carbohydrate (\%)} = 100 - (\text{Moisture} + \text{Crude protein} + \text{Total ash} + \text{Crude fat})$$

$$\text{Available carbohydrate (\%)} = \text{total carbohydrate} - \text{Crude fiber}$$

3.6.7. Fe and Zn mineral contents

Iron and zinc mineral contents of the tetraploid wheat flour samples from each treatments were determined according to Osborne and Vooght (1978) by using Buck Atomic Absorption Spectrophotometer (AAS).

Nitric acid was added to each of the ash obtained previously and let it in finance at 550oC for 2 hrs to facilitate the digestion. Seven ml of 6N hydrochloric acid was added and allowed to digest on a Wegtech hot plate for 2 hr (Figure 6a). Then 5 ml of 3N hydrochloric acid was added again to each of the ashes and allowed to boil on a hot plate. The digests was cooled and filtered in to 50 ml long neck round bottom flask using the Whitman filter paper (Figure 6b). The Fe and Zn contents in the samples were determined using Buck atomic absorption spectrometer (AAS) at 248.3 and 213.9nm using air acetylene flame. The concentration of the elements in the samples was calculated as mg/100g.

$$\frac{mg}{100g} = \frac{(c_2 - c_1) \times 50}{w \times 10}$$

Where C1= concentration of the mineral in the blank solution, C2 =Concentration of the mineral in the sample solution and W= sample weight.

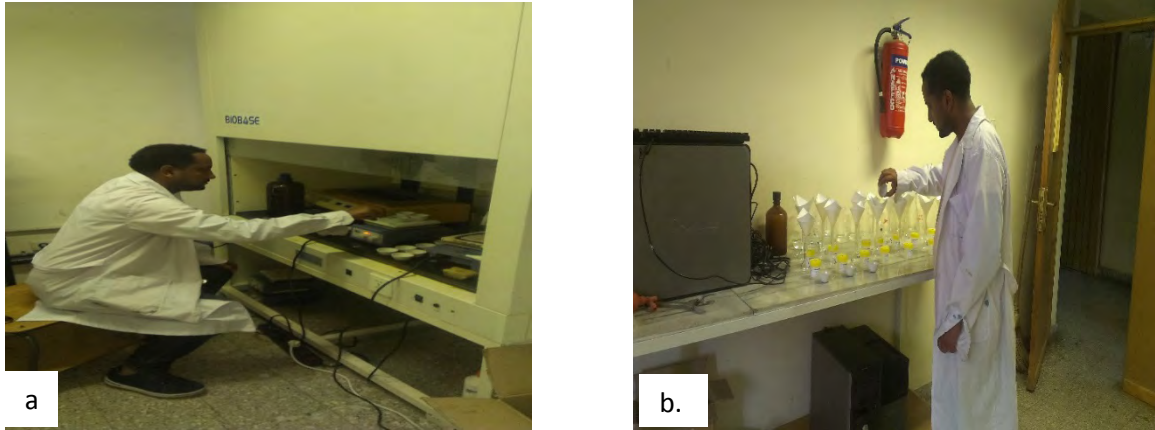


Figure 6. Pictures showing boiling and filtering organic compounds for minerals determination

3.7. Statistical analysis

Agronomic, disease and nutritional quality data were subjected to the analysis of variance (ANOVA) using SAS version 9.1.3 with a procedure of PROC GLM (SAS, 2004). The yield data were subjected to combined analysis of variance to partition yield variation into environments, genotypes, and GE interaction effects and it was done after homogeneity test using PROC VARCOMP. Mean separation was also carried out using Duncan's Multiple Range Test. The analysis for individual locations was based on alpha lattice design linear model:

$$Y_{ijk} = \mu + R_i + B_{ij} + G_k + e_{ijk}$$

Where, μ = the grand mean of trait Y, R_i =is the effect of Replicate i, B_{ij} = effect of Block j within Replicate I and G_k = effect of genotype k and e_{ijk} = random error

The analysis for combined locations was based on alpha lattice design mixed model:

$$Y_{ijk} = \mu + R_i + B_{ij} + G_k + E_h + G_k * E_h + e_{ijk}$$

Where, μ = the grand mean of trait Y, R_i =the effect of Replicate i, B_{ij} = effect of Block j within Replicate I and G_k = effect of genotype k, E_h = the effect of environment h, $G * E$ = the effect of genotype k by environment h interaction, and e_{ijk} = random error

Correlation among yield and nutritional quality traits were analyzed using PROC CORR procedure of SAS (SAS, 2004).

GGE-biplot Analysis

GGE-biplot analysis was conducted using GenStat 13th edition software. The GGE-biplot method (Yan et al ., 2000) was employed to analyze the genotype by environment interaction of yield. This analysis was based on the model:

$$Y_{ij} - \bar{Y}_j = \lambda_1 \xi_{i1} \eta_{j1} - \lambda_2 \xi_{i2} \eta_{j2} - \varepsilon_{ij}$$

where Y_{ij} is the mean yield of genotype i in environment j ; \bar{Y}_j is the grand mean yield over all genotypes in environment j ; $\lambda_1 \xi_{i1} \eta_{j1} - \lambda_2 \xi_{i2} \eta_{j2}$ are collectively called the first principal component (PC1) and the second principal component (PC2); $\lambda_1 + \lambda_2$ are the singular values for the first and second principal components, PC1 and PC2, respectively; $\xi_{i1} + \xi_{i2}$ are the PC1 and PC2 scores, respectively, for genotype i; $\eta_{j1} + \eta_{j2}$ are the PC1 and PC2 scores, respectively, for environment j ; and ε_{ij} is the residual of the model associated with the genotype i in environment j. Thus a GGE-biplot is constructed by plotting the PC1 scores against the PC2 scores for each genotype and each environment.

CHAPTER FOUR

4. RESULTS

4.1. Effects of mixtures of Ethiopian tetraploid wheat genotype collections on agronomic traits

Analysis of variance for each individual and combined location conducted for yield and yield related traits showed significant difference ($P < 0.001$) for days to booting, days to maturity, plant height (cm), spike length (cm), biomass yield, 1000 seed weight (gm) and grain yield (Table 3). Significant interaction between genotypes and locations indicated that genotypes performed differently in each specific locations. Thus it was better observe the analysis of variance for each location to select genotypes which performed well for specific locations.

Table 3. Combined mean squares for agronomic traits of fifteen genotypes over locations ($P < 0.001$)

| Traits | Sources of variations and their level of significance | | | |
|-----------------------------|---|----------|-----------|--------|
| | D.F. | Location | Treatment | L*G |
| Days to booting | 5 | 6278.20 | 233.56 | 50.07 |
| Days to maturity | 5 | 18809.21 | 50.31 | 38.37 |
| Plant height (cm) | 5 | 1530.23 | 307.43 | 119.64 |
| Spike length (cm) | 5 | 11.82 | 0.12 | 1.41 |
| Number of seeds per spike | 5 | 519.60 | 170.60 | 51.74 |
| Biomass yield (tha-1) | 5 | 51.74 | 3.33 | 1.85 |
| Thousand kernel weight (gm) | 5 | 1946.61 | 104.54 | 21.71 |
| Grain yield (tha-1) | 5 | 17.05 | 1.35 | 0.51 |

D.F= degree of freedom, L*G=Location by genotype interaction

4.1.1. Effects of mixtures of Ethiopian tetraploid wheat genotype collections on grain yield in different locations

The analysis of variance showed significant difference among genotypes for grain yield (tha^{-1}) at all locations at $P < 0.001$ except Geregera which was significant at $p < 0.05$.

Maximum grain yields ($2.7, 2.5$ and 2.3 tha^{-1}) were recorded from mixtures of ten improved varieties, Mikuye and Assasa respectively. A minimum of 0.2 tha^{-1} grain yield was recorded from GG2 of Tigray Region.

At Geregera, maximum grain yields were recorded from Assasa (2.6 tha^{-1}), Mangudo (2.4 tha^{-1}), mixtures of Oromia Region (2.4 tha^{-1}), mixtures of top ten Tigray Region (2.4 tha^{-1}), GEBRE of Oromia Region (2.3 tha^{-1}), TIKURE SINDE of Amhara Region (2.3 tha^{-1}), mixtures of parental lines (2.2 tha^{-1}), and mixtures of Tigray Regions (2.2 tha^{-1}). While minimum grain yield was recorded from mixtures of NAM population (1.5 tha^{-1}) and Mikuye (1.8 tha^{-1}).

Significantly maximum values for grain yield ($3.0, 2.9, 2.7$ and 2.6 tha^{-1}) were recorded from mixtures of Oromia Region, mixtures of ten improved varieties, GEBRE of Oromia Region, Mangudo, Mikuye and Assasa respectively at Debre Zeit. While its' minimum (0.6 tha^{-1}) was recorded from GG2 of Tigray Region.

At Chefe Donsa, GEBRE of Oromia Region had mean maximum grain yield (3.4 tha^{-1}) than other genotypes. While mixtures of parental lines (2.4 tha^{-1}), mixtures of top ten Tigray Region (2.4 tha^{-1}), GG2 of Tigray Region (2.4 tha^{-1}), mixtures of Tigray Region (2.5 tha^{-1}).

1), mixtures of ten Amhara Region (2.4 tha^{-1}) and mixtures of NAM population (2.5 tha^{-1}) had minimum grain yield.

Significantly higher grain yield at Ayiba was recorded from GG2 of Tigray Region (2.6 tha^{-1}) and TIKURE SINDE of Amhara Region (2.6 tha^{-1}), mixtures of the country (2.5 tha^{-1}), GEBRE of Oromia Region (2.5 tha^{-1}), Mikuye (2.5 tha^{-1}) and Mangudo (2.4 tha^{-1}). While its' minimum (1.8 tha^{-1}) was recorded from Assasa improved variety.

Higher grain yield was recorded from GEBRE of Oromia Region (3.8 tha^{-1}) Mikuye (3.7 tha^{-1}), Mangudo (3.7 tha^{-1}) as well as mixtures of parental lines (3.6 tha^{-1}). While its' minimum was recorded from mixtures of ten improved varieties (2.8 tha^{-1}), mixtures of Amhara Region (2.8 tha^{-1}), TIKURE SINDE of Amhara Region (3.0 tha^{-1}) and mixtures of top ten Tigray Region (3.1 tha^{-1}).

Table 4. Mean values for grain yield of Ethiopian tetraploid wheat genotypes at six locations

| Genotypes | Locations | | | | | |
|-----------------|--------------------|----------------------|--------------------|--------------------|-------------------|--------------------|
| | Adet | Geregera | DebreZeit | ChefeDonsa | Ayiba | Hagereslam |
| Mix1 | 1.3 ^{def} | 2.1 ^{bcde} | 1.7 ^{def} | 2.7 ^{cde} | 2.5 ^{ab} | 3.4 ^{cde} |
| Mix2 | 0.9 ^f | 2.2 ^{abcde} | 1.8 ^{de} | 2.4 ^f | 1.4 ^e | 3.2 ^{efg} |
| Mix3 | 1.5 ^{cde} | 2.2 ^{abcde} | 1.2 ^f | 2.5 ^{ef} | 2.2 ^c | 3.6 ^{abc} |
| Mix4 | 1.1 ^{ef} | 1.8 ^{ef} | 2.0 ^{cd} | 2.6 ^{def} | 2.2 ^c | 2.8 ^{gh} |
| Mix5 | 1.9 ^{bc} | 2.4 ^{ab} | 3.0 ^a | 2.7 ^{cde} | 2.4 ^b | 3.3 ^{def} |
| Mix6 | 1.3 ^{def} | 2.4 ^{ab} | 1.5 ^{ef} | 2.4 ^f | 2.0 ^c | 3.5 ^{bcd} |
| Mix7 | 1.3 ^{def} | 1.9 ^{cdef} | 2.3 ^{bc} | 2.5 ^{ef} | 2.4 ^b | 3.1 ^{fg} |
| Mix8 | 1.5 ^{cde} | 1.5 ^f | 1.9 ^{cde} | 2.5 ^{ef} | 2.4 ^b | 3.2 ^{def} |
| Mix9 | 2.7 ^a | 2.2 ^{abcd} | 2.9 ^a | 3.1 ^b | 1.6 ^d | 2.8 ^{gh} |
| Sole1 | 1.3 ^{def} | 2.3 ^{abcd} | 1.9 ^{cde} | 2.7 ^{cde} | 2.6 ^a | 3.0 ^{gh} |
| Sole2 | 1.4 ^{def} | 2.3 ^{abc} | 2.9 ^a | 3.4 ^a | 2.5 ^{ab} | 3.8 ^a |
| Sole3 | 0.2 ^g | 1.9 ^{cdef} | 0.6 ^g | 2.4 ^f | 2.6 ^a | 3.5 ^{bcd} |
| Sole4 | 2.3 ^{ab} | 2.6 ^a | 2.6 ^{ab} | 2.8 ^{cd} | 1.8 ^d | 3.4 ^{cde} |
| Sole5 | 2.5 ^a | 1.8 ^{def} | 2.7 ^{ab} | 2.8 ^{cd} | 2.5 ^{ab} | 3.7 ^{ab} |
| Sole6 | 1.6 ^{cd} | 2.4 ^{ab} | 2.9 ^a | 2.9 ^c | 2.4 ^{ab} | 3.7 ^{ab} |
| GM | 1.5 | 2.1 | 2.1 | 2.7 | 2.2 | 3.3 |
| P value | 0.001 | 0.05 | 0.001 | 0.001 | 0.001 | 0.001 |
| S.E. | 0.3 | 0.3 | 0.3 | 0.1 | 0.1 | 0.1 |
| C.V. (%) | 20.2 | 12.5 | 12.9 | 5.1 | 4.6 | 4.4 |

GM =Grand mean, S.E= standard error, C.V=coefficient of variation

4.1.2. Genotype plus genotype by environment interaction (GGE)-biplot analysis

The fitted GGE-biplot model indicated that the first two principal components explained 78.43 % of variation for genotype plus genotype by environment interaction (Figure 7).

The polygon view of the GGE- biplot was drawn by connecting the vertex genotypes with straight lines so that all other genotypes were contained within the polygon and these genotypes were more responsive for environmental change and considered as specifically adapted genotypes.

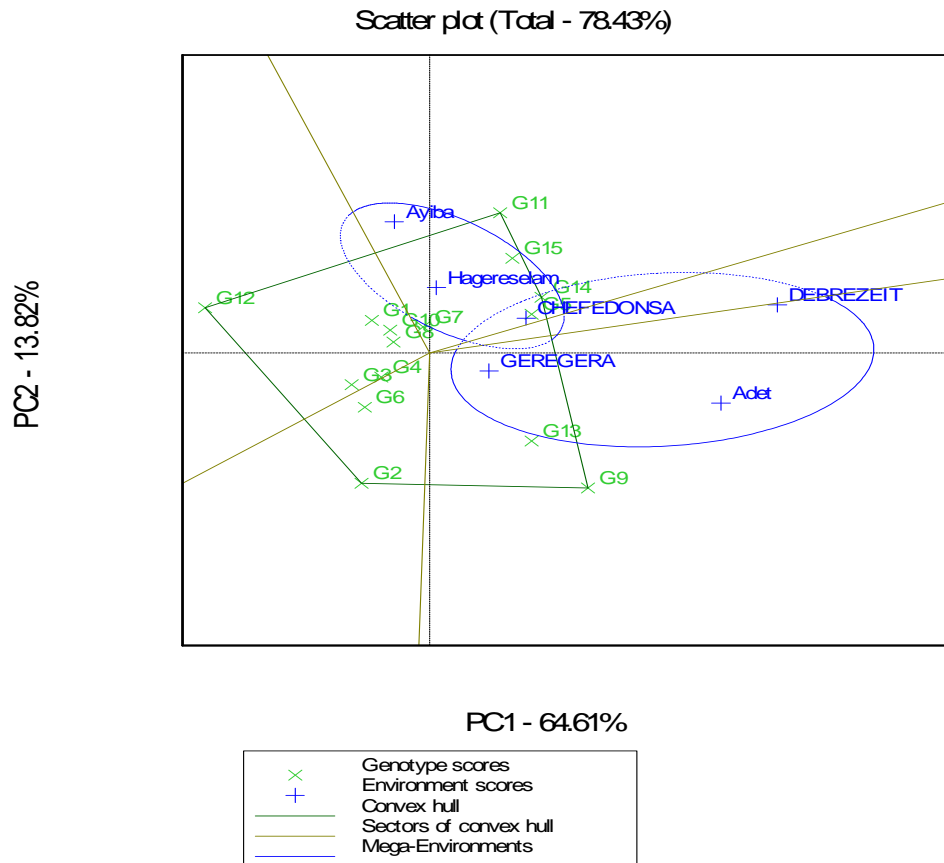
The vertex genotypes were G11 (GEBRE of Oromia Region), G14 (Mikuye), G9 (mixtures of ten improved varieties), G12 (GG2 of Tigray Region) and G2 (mixtures of parental lines) which had the largest distance from the origin (Figure 4).

As shown in figure 4, the perpendicular lines drawn in each side of the polygon which divides the biplot into five sectors and the six testing environments into four sectors in which all sectors contained genotypes. Adet and Geregera as a first sector; Chefe Donsa and Debre Zeit as a second sector; Ayiba as a third sector and Hagereselam as fourth sector.

G11 (GEBRE of Oromia region) was the winner for Hagereselam as well as Chefe Donsa and Debre Zeit sectors with G7 (mixtures of top ten Tigray Region), G15 (Mangudo) and G5 (mixtures of Oromia Region) as another options of high yielding for these sectors. G14 (Mikuye) was the winner for Chefe Donsa and Debre Zeit group with G5 (mixtures of Oromia Region) as another option of high yielding genotype.

G9 (mixtures of ten improved varieties) was the winner for Adet and Geregera sector with an option of G13 (Assasa) for high yielding. G12 (GG2 of Tigray Region) was the winner for Ayiba sector with G11 (GEBRE of Oromia Region) as an option for this sector. Whereas G2 (mix cropped parental lines of farmers' varieties) was not the best yielding at all locations.

G8 (mixtures of NAM population) was found near to the origin and had little contribution to significance of treatment by location interaction. This can be taken as genotype with general (wide) adaptation. Whereas G12 and G9, with the greatest distance from the origin of the biplot, had the highest contribution to significant genotype by location interactions.



G1=mixtures of the country, **G2**= mixtures of parental lines, **G3**= mixtures of Tigray Region, **G4**= mixtures of Amhara Region, **G5**=mixtures of Oromia Region, **G6**=mixtures of top ten Amhara Region, **G7**= mixtures of top ten Tigray Region, **G8**=mixtures of NAM population, **G9**=mixtures of top ten improved varieties, **G10**= TKURE SINGE of Amhara Region, **G11**=GEBRE Oromia Region, **G12**=GG2 of Tigray Region, **G13**= Assasa, **G14**= Mikuye and **G15**=Mangudo (NOTE: Genotype codes were changed here for convenience of GGE-biplot analysis)

Figure 7. Polygon view of the GGE-biplot showing the “which-won-where” using standardized data including 15 genotypes

4.1.3. Effects of mixtures of Ethiopian tetraploid wheat genotype collections on yield related traits in different locations

The analysis of variance showed significant difference among genotypes for yield related traits like plant height (cm), spike length (cm), seeds per spike, biomass yield and thousand seed weight (tha^{-1}) in all locations (Appendix 1 and 2). The highest location mean in plant height (97.9 cm) was obtained at Geregera (Figure 8) and at this location, the maximum plant height was recorded from mixtures of ten improved varieties (105.4 cm), mixtures of Tigray Region (104.9 cm) and mixtures of parental lines (103.7 cm). While minimum location mean in plant height was obtained at Debre Zeit and at this location, minimum plant height (73.6 cm) was recorded from Mikuye improved variety (Appendix 1).

The highest location mean in spike length (6.9 cm) was obtained at Debre Zeit (Figure 8) and at this location, significantly maximum spike length was recorded from mixtures of Amhara Region (8.4 cm) and mixtures of the country (8.3 cm). While its' minimum location mean (5.5 cm) was obtained at Chefe Donsa and at this location, minimum spike length was recorded from Mangudo (4.5 cm) and Assasa (4.6 cm) improved varieties (Appendix 1).

In terms of number seeds per spike, maximum location mean (38 seeds) was obtained at Hagereselam (Figure 8) and at this location, significantly higher number of seeds per spike was recorded from mixtures of NAM population (45 seeds), mixtures of parental lines (44 seeds) and mixtures of top ten Tigray Region. While its' minimum location (32 seeds) was recorded at Adet and at this location, minimum number of seeds per spike was recorded from GEBRE of Oromia Region (27 seeds) (Appendix 2).

The highest location mean in biomass yield (9.2 tha^{-1}) was obtained at Hagereslam and significantly higher biomass yield (11.1 tha^{-1}) was recorded from mixtures of the country (Appendix 2). While its' minimum location mean (3.4 tha^{-1}) was obtained at Debre Zeit and significantly minimum biomass yield was recorded from mixtures of ten improved varieties (2.3 tha^{-1}) and Mikuye improved variety (2.3 tah^{-1}) (Appendix 1).

In terms of thousand kernel weight, its' maximum location mean (5.1 gm) was recorded at Ayiba and at this location, significantly higher thousand kernel weight was recorded from Assasa (54.7 gm) and mixtures of parental lines (52.7 gm) (Appendix 2). While its' minimum location mean (31.5 gm) was obtained at Adet and at this location, statistically the least amount of thousand kernel weight was recorded from mixtures of parental lines (25.4 gm) (Appendix 1).

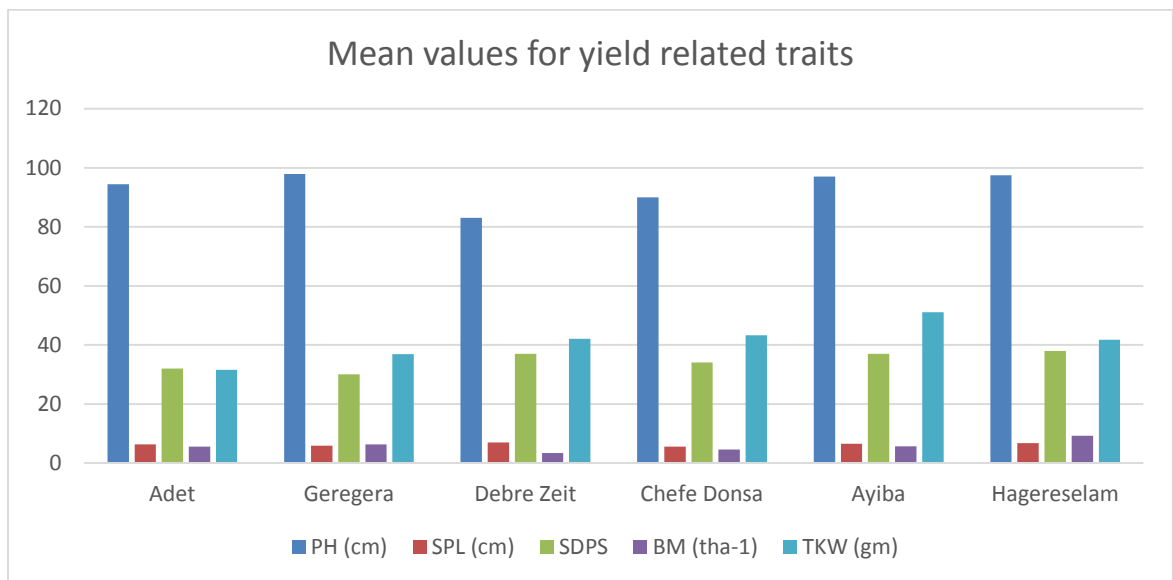


Figure 8. Location mean of yield related traits at six locations

4.1.4. Effects of mixtures of Ethiopian tetraploid wheat genotype collections on morphological traits in different locations

The analysis of variance showed significant difference among genotypes for morphological traits like days to booting days to heading and days to maturity in all locations except day to booting at Ayiba and Hageresalam (Appendix 3 and 4). Minimum location mean in days to booting (36 days) was obtained at Debre Zeit (Figure 9) and at this location, significantly minimum days to booting was recorded from Assasa (29 days), Mikuye (30 days) and Mangudo (30 days) improved varieties (Appendix 3). While maximum location mean in days to booting (71 days) was obtained at Geregera and at this location, significantly highest number of days to booting was recorded from GEBRE of Oromia Region (78 days).

In terms of days to heading, minimum location mean (71 days) was obtained at Adet and at this location, significantly minimum number of days to heading was recorded from Mikuye improved variety (59 days) (Appendix 3). While its' maximum location mean (76 days) was obtained at Geregera and at this location, maximum number of days to heading was recorded from GEBRE of Oromia Region (84 days).

Minimum location mean in days to maturity (87 days) was obtained at Debre Zeit (Figure 9) and at this location, significantly minimum number of days to maturity was recorded from Mikuye (79 days), Assasa (81 days) and Mangudo (81 days) improved varieties. While its' maximum location mean to days to maturity (146 days) was obtained at Geregera and at this location, significantly maximum days to maturity (150 days) was recorded from GEBRE of Oromia Region and mixtures of ten improved varieties.

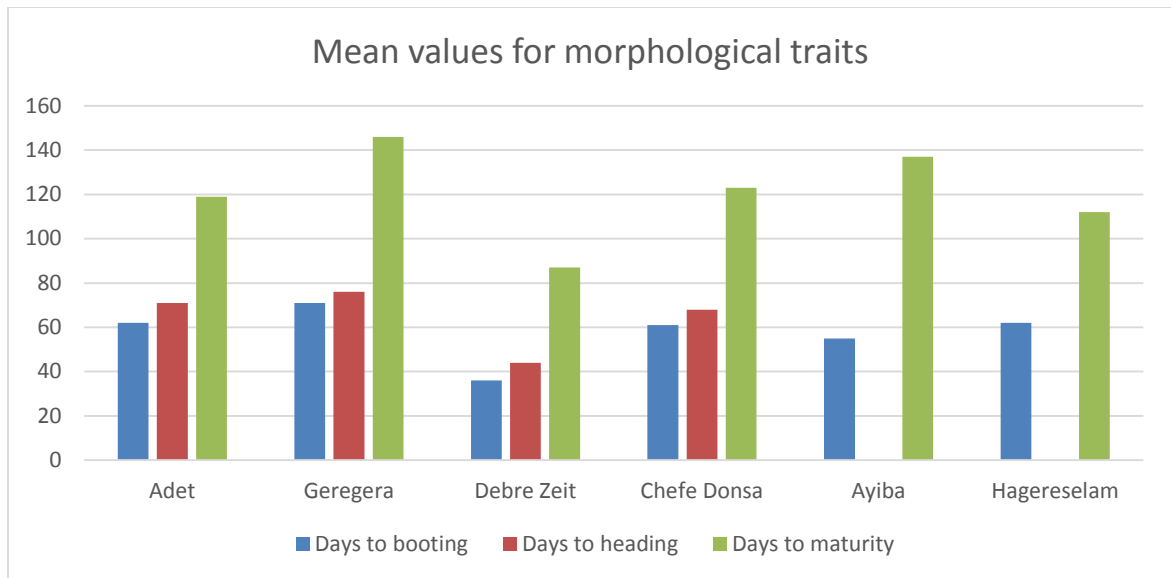


Figure 9. Location mean of morphological traits at six locations

4.2. Effects of mixtures of Ethiopian tetraploid wheat genotype collections on disease traits

Disease recording was done at these three locations (Geregera, Debrezeit and Chefe Donsa) only because there was no disease infestation at Adet, Ayiba and Hagereslam. Only septoria disease was observed and recorded at Geregera and there was significance difference ($p < 0.05$) among genotypes for septoria disease severity (%) (Table 5). Relatively maximum septoria severity were recorded from mixtures of top ten Tigray Region (4.4%), TIKURE SINDE of Amhara Region (4.3%), mixtures of Tigray Region (4.0%) and other six treatments. While minimum values for septoria severity were recorded from GEBRE of Oromia Region (1.3%) and Mikuye (1.5%).

Leaf rust was the only disease observed and recorded at Debrezeit and the analysis of variance showed significance difference ($p < 0.001$) among genotypes. Maximum leaf rust coefficient of infection (55.0) was recorded GG2 of Tigray Region and its' mean minimum coefficient of infections (4.0 and 5.3) were recorded from Assasa and mixtures of top ten

Amhara region respectively (Table 10). At Chefe Donsa, both leaf and yellow rust were recorded. But since the level of disease infestation was very low, there was no significance difference among genotypes for their severity.

Table 5. Mean values for septoria, leaf and yellow rust diseases of Ethiopian tetraploid wheat genotypes

| Genotypes | Geregera | Debrezeit | Chefe Donsa | |
|-----------|----------------------|--------------------|-------------------|-------------------|
| | SPDS | LRCI | LRCI | YRCI |
| Mix1 | 2.7 ^{abcde} | 29.3 ^{bc} | 0.71 ^a | 0.42 ^a |
| Mix2 | 3.6 ^{abcd} | 29.3 ^{bc} | 0.42 ^a | 0.19 ^a |
| Mix3 | 4.0 ^{abc} | 29.3 ^{bc} | 0.55 ^a | 0.63 ^a |
| Mix4 | 2.2 ^{def} | 16.7 ^{bc} | 0.55 ^a | 0.32 ^a |
| Mix5 | 2.9 ^{abcde} | 16.7 ^{bc} | 0.55 ^a | 0.35 ^a |
| Mix6 | 3.2 ^{abcd} | 26.7 ^{bc} | 0.39 ^a | 0.32 ^a |
| Mix7 | 4.4 ^a | 5.3 ^d | 0.55 ^a | 0.32 ^a |
| Mix8 | 2.8 ^{abcde} | 14.0 ^c | 0.42 ^a | 0.32 ^a |
| Mix9 | 2.3 ^{de} | 13.3 ^c | 0.21 ^a | 0.16 ^a |
| Sole1 | 4.3 ^{ab} | 34.7 ^b | 0.60 ^a | 0.63 ^a |
| Sole2 | 1.3 ^f | 14.7 ^{cd} | 0.55 ^a | 0.03 ^a |
| Sole3 | 3.4 ^{abcd} | 55.0 ^a | 0.39 ^a | 0.21 ^a |
| Sole4 | 2.3 ^{de} | 4.0 ^d | 0.55 ^a | 0.32 ^a |
| Sole5 | 1.5 ^{ef} | 12.0 ^c | 0.05 ^a | 0.00 ^a |
| Sole6 | 2.6 ^{cde} | 16.1 ^{bc} | 0.19 ^a | 0.16 ^a |
| GM | 2.9 | 21.1 | 0.4 | 0.3 |
| P value | 0.05 | 0.001 | NS | NS |
| S.E. | 1 | 11.1 | 0.2 | 0.3 |
| C.V. (%) | 34.2 | 52.3 | 53.8 | 88.4 |

GM= Grand mean, **S.E** =Standard error, **C.V.**= coefficient of variation, **SPDS**=Septoria disease severity, **LRCI**=Leaf rust coefficient of infection, **YRCI**=Yellow rust coefficient of infection

4.3. Effects of mixtures of Ethiopian tetraploid wheat genotype collections on nutritional quality as seen at Chefe Donsa

Proximate as well as mineral like Fe and Zn analysis was undertaken for Chefe Donsa grain samples only and the analysis of variance showed significant difference among treatments for crude protein (%), fat (%), crude fiber (%), total carbohydrate (%), Fe (mg per 100gm.) and Zn (mg per 100gm.) at 0.001 probability level and for available carbohydrate (%) at 0.01 probability level. However, the analysis of variance showed no significant difference among treatments for moisture (%) and ash (%) contents (Table 6).

Maximum crude protein contents were recorded from Mangudo (13.0%), mixtures of top ten Amhara Region and TIKURE SINDE of Amhara Region (11.9% respectively). While the mean minimum crude protein (8.1 %) was obtained from GEBRE of Oromia Region.

Significantly higher amounts of fat contents were recorded from TIKURE SINDE of Amhara Region (2.9%), mixtures of top ten Amhara Region (2.6%) and Mangudo (2.5%). While minimum fat contents were recorded from mixtures of parental lines (1.4%), Assasa (1.5%) and mixture of the country (1.7%).

In terms of crude fiber content, maximum crude fiber content were recorded from Assasa (3.4%), GEBRE of Oromia Region (3.3%) and mixtures of top ten Amhara Region (3.3%). While minimum crude fiber content (2.5%) was obtained from mixtures of top ten Tigray Region which was significantly similar with mixtures of ten improved varieties (2.6%) and mixtures of Amhara Region (2.7%).

Significantly higher total and available carbohydrates (78.9 and 75.8 % respectively) were obtained from GEBRE of Oromia Region and their mean minimum (73.0 and 70.0 % respectively) were obtained from Mangudo, TIKUR SINDE of Amhara Region (73.9 and

71.0% respectively) and mixtures of top ten Amhara Region (73.9 and 70.8% for total and available carbohydrates).

In terms of Fe and Zn contents, the highest significant amounts of Fe were obtained from mixtures of parental lines (3.4 gm/100gm), mixtures of top ten Tigray Region (3.4 mg/100gm) and mixtures of ten improved varieties (3.3 mg/100gm). While minimum Fe contents were obtained from Assasa (1.1mg/100gm), GEBRE of Oromia Region (1.1mg/100gm), mixtures of Oromia Region (1.1mg/100gm) and Mangudo (1.3 mg/100gm).

Maximum Zn contents were obtained again from mixtures of parental lines (2.7gm/100gm), mixtures of Tigray Region (2.6gm/100gm), TIKUR SINDE of Amhara Region (2.6gm/100gm), mixtures of Oromia Region (2.5mg/100gm) and GG2 of Tigray Region (2.5gm/100gm). While its' minimum (1.9gm/100gm) was recorded from Assasa.

Table 6. Mean value of nutritional quality parameters of Ethiopian tetraploid wheat genotypes at Chefe Donsa

| GPs | MC (%) | Protein (%) | Fat (%) | Ash (%) | Fiber (%) | T.Carb (%) | A.Carb (%) | Fe (mg /100gm) | Zn (mg /100 gm) |
|------------|-------------------|----------------------|-------------------------------|------------------|------------------------------|---------------------|---------------------|-----------------------|------------------------|
| Mix1 | 10.2 ^a | 10.4 ^{cde} | 1.7 ^{d^{ef}} | 1.3 ^a | 3.1 ^b | 76.6 ^{bcd} | 73.5 ^{bc} | 2.1 ^{de} | 2.5 ^{bcd} |
| Mix2 | 10.2 ^a | 10.7 ^{bcd} | 1.4 ^f | 1.4 ^a | 2.8 ^{de} | 76.3 ^{cd} | 73.5 ^{bc} | 3.4 ^a | 2.7 ^a |
| Mix3 | 10.6 ^a | 10.4 ^{cde} | 2.0 ^{cd} | 1.3 ^a | 2.78 ^{ef} | 75.9 ^d | 73.2 ^{bcd} | 1.5 ^f | 2.6 ^{ab} |
| Mix4 | 10.2 ^a | 9.6 ^e | 1.9 ^{d^e} | 1.3 ^a | 2.7 ^{efg} | 77.2 ^{bc} | 74.6 ^{ab} | 3.0 ^b | 2.4 ^{cd} |
| Mix5 | 10.3 ^a | 10.6 ^{cde} | 1.8 ^{d^e} | 1.5 ^a | 2.7 ^{ef} | 76.1 ^d | 73.3 ^{bcd} | 1.1 ^g | 2.5 ^{ab} |
| Mix6 | 10.3 ^a | 10.1 ^{de} | 1.8 ^{d^e} | 1.7 ^a | 2.5 ^g | 76.3 ^{cd} | 73.8 ^{bc} | 3.4 ^a | 2.3 ^d |
| Mix7 | 10.1 ^a | 11.9 ^{ab} | 2.6 ^{ab} | 1.7 ^a | 3.3 ^a | 73.9 ^{ef} | 70.8 ^{ef} | 2.5 ^c | 2.3 ^d |
| Mix8 | 10.1 ^a | 11.05 ^{bcd} | 2.4 ^{bc} | 1.5 ^a | 2.9 ^{cd} | 76.0 ^d | 73.6 ^{bc} | 1.8 ^e | 2.5 ^{bcd} |
| Mix9 | 10.1 ^a | 10.2 ^{de} | 2.05 ^{cd} | 1.4 ^a | 2.6 ^{f^g} | 76.3 ^{cd} | 73.8 ^{bc} | 3.3 ^a | 2.3 ^d |
| Sole1 | 10.2 ^a | 11.9 ^{ab} | 2.9 ^a | 1.3 ^a | 3.1 ^b | 73.9 ^{ef} | 71.0 ^{ef} | 2.7 ^c | 2.6 ^{ab} |
| Sole2 | 10.2 ^a | 8.1 ^f | 1.8 ^{d^e} | 1.1 ^a | 3.4 ^a | 78.9 ^a | 75.8 ^a | 1.1 ^g | 2.4 ^{cd} |
| Sole3 | 10.4 ^a | 11.5 ^{ab} | 2.4 ^{bc} | 1.1 ^a | 3.0 ^{bc} | 74.7 ^e | 71.9 ^{de} | 2.0 ^{de} | 2.5 ^{ab} |
| Sole4 | 10.2 ^a | 9.4 ^e | 1.5 ^{ef} | 1.4 ^a | 3.4 ^a | 77.6 ^b | 74.5 ^{ab} | 1.1 ^g | 1.9 ^e |
| Sole5 | 10.2 ^a | 10.1 ^{de} | 2.4 ^{bc} | 1.5 ^a | 3.1 ^b | 75.9 ^d | 72.9 ^{cd} | 2.2 ^d | 2.3 ^d |
| Sole6 | 10.3 ^a | 13.0 ^a | 2.5 ^{ab} | 1.3 ^a | 3.0 ^{bc} | 73.0 ^f | 70.0 ^f | 1.3 ^{fg} | 2.4 ^{cd} |
| GM | 10.2 | 10.6 | 2.1 | 1.4 | 2.9 | 75.9 | 73.1 | 2.2 | 2.4 |
| P value | NS | 0.001 | 0.001 | NS | 0.001 | 0.001 | 0.01 | 0.001 | 0.001 |
| SE | 0.4 | 0.8 | 0.4 | 0.4 | 0.1 | 0.7 | 0.7 | 0.3 | 0.3 |
| CV (%) | 1 | 5.2 | 9.7 | 12.4 | 2.7 | 0.7 | 0.9 | 5.3 | 3.6 |

GM= grand mean, **S.E**= standard error, **C.V**= coefficient of variation and **Carb**= carbohydrate

4.4. Pearson's Correlation Analysis of yield and nutritional quality as seen at Chef Donsa

Pearson's correlation analysis among grain yield and nutritional quality parameters was done from the grain samples obtained at Chefe Donsa. The correlation analysis showed significant negative associations between grain yield and protein content ($r=-0.27$), grain yield and ash content ($r=-0.35$), grain yield and Fe content ($r=-0.38$) and grain yield and Zn content ($r=-0.36$). Whereas grain yield had no significant correlation with other grain quality parameters like moisture, fat, fiber and carbohydrate (Table 7).

The analysis for correlation also showed significance negative association between protein and total carbohydrate ($r=-0.92$) and between protein and available carbohydrate ($r=-0.62$). But protein showed positive correlations with Fe content ($r=.08$), Zn content ($r=0.37$) and fat ($r=0.52$). Significant negative correlation between total or available carbohydrate and fat ($r=-0.69$ and $r=0.62$ respectively) was also observed in this analysis.

Table 7. Correlations among yield and nutritional quality parameters

| | GY (tha ⁻¹) | Moisture (%) | Protein (%) | Fat (%) | Ash (%) | Fiber (%) | T. Carb (%) | A. Carb (%) | Fe (mg. /100gm) | Zn (mg. /100gm) |
|----------|----------------------------|----------------------|----------------------|----------------------|---------------------|---------------------|---------------------|---------------------|-----------------------|-----------------------|
| GY | 1 | | | | | | | | | |
| Moisture | 0.004 ^{ns} | 1 | | | | | | | | |
| Protein | -0.27 ^{**} | -0.12 ^{ns} | 1 | | | | | | | |
| Fat | 0.009 ^{ns} | -0.07 ^{ns} | 0.52 ^{**} | 1 | | | | | | |
| Ash | -0.35 [*] | -0.45 [*] | 0.08 ^{ns} | -0.03 ^{ns} | 1 | | | | | |
| Fiber | 0.25 ^{ns} | -0.005 ^{ns} | -0.05 ^{ns} | 0.16 ^{ns} | -0.21 ^{ns} | 1 | | | | |
| T. Carb | 0.16 ^{ns} | -0.002 ^{ns} | -0.92 ^{***} | -0.69 ^{***} | -0.11 ^{ns} | 0.05 ^{ns} | 1 | | | |
| A. Carb | 0.09 ^{ns} | -0.07 ^{ns} | -0.87 ^{***} | -0.62 ^{***} | -0.05 ^{ns} | -0.07 ^{ns} | 0.96 ^{***} | 1 | | |
| Fe | -0.38 [*] | -0.16 ^{ns} | 0.08 [*] | 0.03 ^{ns} | 0.28 ^{ns} | -0.45 [*] | -0.11 ^{ns} | -0.03 ^{ns} | 1 | |
| Zn | -0.36 [*] | 0.17 ^{ns} | 0.37 [*] | 0.06 ^{ns} | -0.23 ^{ns} | -0.28 ^{ns} | -0.23 ^{ns} | -0.2 ^{ns} | 0.09 ^{ns} | 1 |

* Significant at 0.05 probability level, ** Significant at 0.01 probability level, *** Significant at 0.001 probability level, ns=non-significant, **GY**=Grain yield, **T. Carb**=Total carbohydrate, **A. Carb**=Available carbohydrate

CHAPTER FIVE

5. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

5.1.1. Effects of mixtures of Ethiopian wheat genotypes collections on agronomic traits

The result of this study showed significant difference among mixed and sole cropped genotypes in terms of phenotypic, yield and yield related traits in all locations. Mixtures of Ethiopian tetraploid wheat collections responded differently for days to booting, heading, flowering and maturity in different locations. This shows the genetic diversity of Ethiopian tetraploid wheat collections which might be useful for selection of parents with diverse genetic background. Similar results on Ethiopian tetraploid wheat showed regional variations in different agro-morphological traits (Faris Hailu, 2010). Differences in phenotypic traits in different regions show the genetic diversity of Ethiopian tetraploid wheat collections (Yifru Teklu and Karl, 2008; Jemanesh Haile et al., 2013)

In terms of plant height and spike length mixtures showed better performances than sole genotypes. This might be because genotypes in the mixture have different behavior in utilizing nutrients and sun light. It shows the opportunities of mixtures as source of parental lines for improvement programs.

From all over performance, sole improved varieties were better in terms of thousand kernel weight which might be because these varieties were selected and released based on their thousand kernel weight and yield performance. However, some mixed genotypes scored higher weight than the locations' mean and even in Ayiba and Hagereselam the highest thousand kernel weight were obtained from mixtures of parental lines and mixtures of the

country respectively. This might show that mixtures of Ethiopian tetraploid wheat collections can be used as crop improvement program for high yielding varieties.

Mixtures of Ethiopian tetraploid wheat collections gave better or comparative biomass and grain yield in all locations. In all locations, the maximum values of biomass (6.6, 4.3, 7.2, 5.3, 7.1 and 11.1 tha^{-1}) were obtained from mixtures of farmers' varieties like mixtures of top ten Amhara Region, mixtures of Tigray Region, mixtures of the country, mixtures of Oromia Region and other mixed genotypes like mixtures of top ten Tigray Region. While its' minimum or lower values were recorded from sole cropped improved varieties and it shows the performance of mixtures with regards to biomass yield which might be very important for farmers' preference to animal feed, straw. This result is in agreement with Sarandon & Sarandon (1995) who found a total aerial biomass production which is eight percent higher than the most productive cultivars with higher ears per meter square and harvest index from their mixed treatments.

The overall performances of mixtures as well as pure stands of Ethiopian tetraploid wheat collections showed better and comparative yield with regards to improved varieties. This performance of mixtures might come from their different mechanism like complementarity which occurs when genotypes are grown together in a diverse population which have different profiles of resource use, compensation which occurs when the yield of one component increases while the other decreases without affecting the overall yield of the mixtures and facilitation which happens when a component cultivar may benefit another component directly by improving microclimate (Khalifa and Qualset, 1974; Willey, 1979).

The result of this study is in agreement with Finckh et al. (2000) and Kiær et al. (2009) who reported that increasing the number of components in mixtures of winter barley and wheat resulted in significantly increased yield benefits above the mean of the monoculture components.

5.1.2. Which won where pattern

The two mega environments that resulted from clustering of the six environments by the GGE-biplot analysis gave an idea of the relatedness of the environments by predicting their yield based on their existing yield data. Thus, Ayiba and Hagerselam were clustered as one mega environment and Debre Zeit, Chefe Donsa, Geregera and Adet as another one mega environment. G 12 (GG2 of T igray Region), G 11 (GEBRE of O romia R egion), G 15 (Mangudo) are best fit for mega environment one (Ayiba and H agereselam) with G 5 (mixtures of Oromia Region), G14 (Mikuye) and G7 (mixtures of top ten Tigray Region) as an options. Whereas for mega environment two, G 14, G 5, G 13 (Assasa) and G 9 (mixtures of ten improved varieties) are best fit. The GGE-biplot diagram is a data visualization tool, which graphically displays genotype by environment interaction in a two-way table (Yan et al., 2000).

The polygon view of the GGE-biplot indicates the best genotype(s) in each environment and group of environments based on the principle “which-won-where” pattern of the multi environment trial data (Yan & Hunt, 2001). The polygon is formed by connecting the markers or vertexes of the genotypes that are furthest away from the biplot origin such that all other genotypes are contained in the polygon. These genotypes were the best or poorest

in some or all of the environments because they were farthest from the origin of biplot (Yan and Kang, 2002).

Genotypes located near the biplot origin are less responsive to the change of environments in contrast to far located ones (Yan and Kang, 2002). Therefore, G8 (mixtures of NAM population) was found near to the origin and had little contribution to significance of treatment by location interaction. This can be taken as genotype with general (wide) adaptation.

5.1.3. Effects of mixtures of Ethiopian wheat genotypes collections on disease traits

In this study, the disease pressure in all locations other than Debre Zeit was very low to get a significance difference among genotypes and even to record the infestation of rust diseases.

In Debrezeit, where there was high infestation of leaf rust, mixtures of Ethiopian tetraploid wheat collections reduced coefficient of infection of the disease similar to resistant improved varieties. While the highest infestation was observed on sole cropped Ethiopian tetraploid wheat collections. Mundt et al. (1994) and Mundt et al. (1995) also reported similar result to this study in which yield was increased by 7% in the presence of disease and mixtures reduced the severity of yellow rust relative to their component pure stands by 53%.

With regarding to septoria which was recorded in Geregera, some mixtures like mixtures of Amhara Region reduced the disease severity. While some mixtures like mixtures of top

ten Tigray Region and mixtures of Tigray Region were highly infested by the disease. This shows again the comparative performance of mixtures with resistance improved varieties.

However, for both rust and septoria diseases, there was no that much significant difference recorded among mix cropped and sole cropped farmers' varieties when compared to resistant improved varieties. This might be because susceptible and resistance farmers' varieties were not differentiated and mixed as well. This result is supported by Garrett and Mundt (1999) who suggested to use a simple mixture of susceptible and very resistance genotypes in deployment of disease resistance for a particular host to pathogen interactions.

Yellow and leaf rust can cause up to 60% yield loss and stem rust can cause 100 percent yield loss (Park et al., 2007). While severe epidemics of septoria tritici blotch can reduce wheat yields by 35 to 50% (Ponomarenko et al., 2011). Thus, it is very important to mix genotypes which have resistance response to diseases with susceptible but high yielding farmers' varieties to reduce disease infestation at farmers' field.

5.1.4. Effects of mixtures of Ethiopian wheat genotypes collections on proximate and selected minerals as seen at Chefe Donsa

In this study, even though there was no significance difference among genotypes, the moistures contents were in the range of 10.1 to 10.6% which is ideal for storage. It is reported that wheat or flour with high moisture content (greater than 14.5 percent) attracts mold, bacteria and insects, all of which cause deterioration during storage (Hoseney, 1994). The flour moisture content ranged from 10.1 to 10.6% in this study and in agreement with Nasir et al. (2003) who report that having 9% moisture content followed by flour having 10% moisture content were suitable for extended shelf life.

With regards to protein content, results from this study showed the range of protein from 8.1 to 13.0% with location mean of 10.6%. This is in agreement with the finding of Kumar et al. (2011) who reported the range of protein from 8 to 15% and with the finding of Blanco et al. (2006) who reported that under normal cropping conditions, wheat genotypes show protein content which is between 10 and 14%.

Mixtures of Ethiopian tetraploid wheat collections showed the highest protein content and as a result, the protein content of mixtures of top ten Tigray Region (11.9%) is in agreement with an average mixed wheat whole grain protein content (11.3%) reported by EHNRI (1997) and an average of wheat grain (11.3%) which is not mixed (Koehler and Wieser, 2013). This indicates that mixtures can give comparative protein content with sole cropped high yielding varieties. This result is in agreement with Sarandon & Sarandon (1995) who also found in their research that mixtures' grain protein percentage was similar to that of the pure stand cultivar with better grain quality.

In terms of fat and ash, an average of 2.10% was reported by Kumar et al. (2011). In this study, the range of fat content was from 1.4 to 2.9%. Mixtures of genotypes showed fat contents which are below this average except mixtures of top ten Tigray Region (2.6%). But they are above the average of Ethiopian wheat whole grain mixtures (1.8%) which is reported by EHNRI (1997). Other mix cropped genotypes showed relatively low amount of fat content. Mixtures were also not significantly different from improved varieties in terms of ash content.

In this study, the range for fiber content was from 2.5 to 3.4% and mixtures as well as sole cropped Ethiopian tetraploid wheat collections showed better performance in providing the

nutrient. For instance, GEBRE of Oromia Region and mixtures of top ten Tigray Region gave the highest amounts (3.4 and 3.3% respectively). These values were above the average fiber content of Ethiopian wheat whole grain mixtures (3.0%) reported by EHNRI (1997). Rave et al. (2007) demonstrated the beneficial effects of fiber consumption in protection against heart disease and cancer, normalization of blood lipids, regulation of glucose absorption and insulin secretion and prevention of constipation and diverticular disease. Thus, the result of this study showed that mixtures as well as pure stands of Ethiopian tetraploid wheat collections can be used as a good source of fiber,

Total and available carbohydrates ranged from 73.0 to 78.9% and 70.0 to 75.8% respectively and their maximums were recorded from the same genotype GEBRE of Oromia Region and this is higher than the average carbohydrate of mixed wheat (75.3%) which is reported by EHNRI (1997). While their minimums were recorded from the same genotype Mangudo improved variety and this shows sole cropped farmers' varieties are superior in their carbohydrate contents than improved varieties.

From the result of this study, higher amount of Fe and Zn content were obtained from mixtures of Ethiopian tetraploid wheat collections. An amount of 3.4 and 3.3 mg/100gm Fe contents were found from mixtures of parental lines, mixtures of top ten Amhara Region and mixtures of ten improved varieties respectively. This is comparable with the average of Fe content of mixed wheat grain (3.7%) which is reported by EHNRI (1997). Meanwhile Zn content which was the highest from all genotypes was obtained from mixtures of parental line. While their minimums were obtained from Assasa improved variety. This result revealed that mixtures can be used as diversified diet to reduce micronutrient deficiency and as source of Fe and Zn contents for improving high yielding improved

varieties. This was in agreement with Cakmak et al. (2004) who indicated very promising gene sources of tetraploid wheat genotypes as for improving Fe and Zn contents of modern varieties' seeds.

Deficiencies of Zn and Fe cause various serious health complications such as stunting, increased susceptibility to infectious diseases, impaired brain function and mental development, poor birth outcomes and anemia (Kennedy et al., 2003; Hotz and Brown, 2004). Low dietary intake of Zn and Fe has been discussed as a major reason for the high prevalence of micronutrient deficiencies in human populations (Bouis, 2003; White and Broadley, 2009). The result of this study strengthen the idea of Welch (2002), Welch and Graham (2002) and Cakmak et al. (2004) who reported that the large increment in the percentage of people suffering from micronutrient malnutrition over the last four decades was associated with the global expansion of high yielding and input responsive cereal cultivars and high consumption of diets with little diversity consisting one or two staple foods.

5.1.5. Correlation among yield and grain quality traits at seen at Chefe Donsa

Correlation analysis provides a method to measure the strength of a linear relationship between two numeric variables. The result from the correlation analysis among grain yield and grain quality traits in this study revealed both negative and positive associations. Grain yield was negatively correlated Fe ($P < 0.05$) and Zn ($P < 0.05$). For instance, mixtures of parental lines had the highest (3.4 mg/100gm) Fe content and 2.7mg/100gm Zn content. However the genotype had the lowest yield (2.4 t ha^{-1}) at the location (Chefe Donsa). While the high yielding GEBRE of Oromia Region (2.4 t ha^{-1}) had minimum (1.1 and 2.4

gm/100gm) Fe and Zn contents. This might be because of the dilution effect of the starch on minerals or this is an effect of concentration dilution by the dry matter accumulated in grain (Peterson et al., 1983). This result is in agreement with other studies (Graham et al., 1999; Murphy et al., 2008) who reported the negative association between grain yield and minerals. In addition, Ficco et al. (2009) and Zhao et al. (2009) reported negative correlation between Zn and grain yield but non correlation between Fe and grain yield.

In this study, protein was positively correlated with Fe ($P < 0.05$) and Zn ($P < 0.05$) contents and negatively with grain yield ($P < 0.01$). Similar results were reported by Oury et al. (2006) and Blanco et al. (2012). This association might show again the dilution effect by dry matter accumulation on both protein and mineral concentrations as they are controlled by the same genes and it might show protein as a sink for Fe and Zn (Peterson et al., 1986).

5.2. Conclusion

The analysis in each location shows mixtures as well as pure stands of Ethiopian tetraploid wheat genotypes performed well and showed comparative and even better grain yield and grain quality traits. GGE-biplot analysis was done to support the result obtained from each locations' yield analysis and it followed 'which won where' pattern to select the best performing genotypes in each location.

Based on the yield results of these analyses, mixtures of top ten Amhara Region, mixtures of ten improved varieties, Assasa and Mikuye are best genotypes for Adet and Geregera of Amhara Region. Mixtures of Oromia Region, GEBRE of Oromia Region, Assasa and Mikuye are best genotypes for Debre Zeit and Chefe Donsa of Oromia Region. Whereas

mixtures of top ten Tigray Region, GG2 of Tigray Region, GEBRE of Oromia Region and Mangudo are best genotypes for Ayiba and Hagereselam of Tigray Region.

Since GGE-biplot analysis showed genotypes which fit in more than one environment, this came true for GEBRE Oromia Farmers' variety which proved to be the best for Ayiba, Hagereselam, Debrezeit and Chefe Donsa locations. From this analysis it can be indicated that mixtures are not that much responsible for genotypes by location interaction as all the vertexes which are responsible for significant genotype by location interaction were sole cropped genotypes. This shows that mixtures can be used anywhere regardless of location specificity and farmers' can exchange seeds for diversity maintenance of the crop.

With regarding to nutritional qualities, low yielding genotypes showed high nutritional contents than high yielding ones. For instance, highest amount of Fe and Zn were recorded from mixtures of parental lines which was not performing well in its' grain yield. In contrast, the minimum amount of protein was recorded from the high yielding GEBRE of Oromia Region. In spite of this negative correlation among protein, micro nutrients and grain yield, mix as well as sole cropped farmers' varieties showed better grain quality than sole cropped improved varieties. This result indicates that supplementing the diversity diets through mixtures is very vital to reduce micronutrient deficiency in areas where monotype staple crops dominating the diet. However, GEBRE of Oromia Region can be a good source of carbohydrate and fiber contents as it was high yielding and has no significant correlation between its' high grain yield and these nutrients.

From this study, it can be concluded that farmers' can maintain the diversity of Ethiopian tetraploid wheat while they are benefiting from its' high yielding and high nutritive value

through mixing different genotypes which have different merits. The same approach can be adopted by researchers to enhance long standing farmers' practices and will create a platform for farmer research networks.

5.3. Recommendations

Based on the result of this study, the following points are forwarded as recommendations:

- As both quality and grain yield benefits can be obtained from mixtures, farmers can maintain Ethiopian tetraploid wheat by growing different mixtures and the research system can design encourage plans for farmers
- For disease and other pests reduction, susceptible and resistance genotypes should be mixed based on the desire of the growers rather than mixing genotypes with the same behavior
- Since grain yield and most grain quality parameters have negative association, genotypes mixtures should be considered a better solution to attain both high yield and grain quality
- Formal or informal seed exchange among farmers in different local areas should be encouraged as genotypes which are not best fit in one area could be best for others and this can help to reduce genetic erosion of the crop
- Researchers should be encouraged to use genetic materials from mixtures of Ethiopian tetraploid wheat genotypes, which are a good source of grain quality as well as yield and yield related traits
- Ethiopian tetraploid wheat genotype mixtures could be evaluated across locations in dry land agro ecological zones for possible isolation of climate smart mixtures to cope with shifts in climate patterns

- Farmers should get the chance to compare and options for selecting mixtures brought from different areas so that they can select spikes they like to maintain the diversity and get benefit out of it
- Mixtures from Oromia, Tigray and Amhara regions, which have better yield and nutritional quality performances in this experiment should be given to farmers

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Appendices

Appendix 1. Mean values for yield related traits of Ethiopian tetraploid wheat genotype collections ($P < 0.001$) at Adet, Geregera and Debre Zeit

| Genotypes | Adet | | | | | Geregera | | | | | Debre Zeit | | | | |
|-----------------|---------|----------|------|-------------------------|-----------|----------|----------|----------|-------------------------|-----------|------------|----------|-------|-------------------------|-----------|
| | PH (cm) | SPL (cm) | SDPS | BM (tha ⁻¹) | TKW (gm.) | PH (cm) | SPL (cm) | SDPS | BM (tha ⁻¹) | TKW (gm.) | PH (cm) | SPL (cm) | SDPS | BM (tha ⁻¹) | TKW (gm.) |
| Mix1 | 100.5ab | 7.2abc | 29cd | 5.5bc | 28.0de | 101.8bc | 6.3bc | 28fg | 7.1a | 36.3cdef | 85.2bcd | 8.3a | 36cd | 3.6bcd | 42.6d |
| Mix2 | 104.3a | 6.7d | 29cd | 5.6bc | 25.4e | 103.7ab | 6.5b | 29efg | 6.5ab | 37.5bcde | 79.4efg | 7.6bc | 35de | 3.6bcd | 40.2fgh |
| Mix3 | 103.2ab | 7.3ab | 28cd | 6.0ab | 30.8cd | 104.9a | 7.3a | 31cde | 7.0ab | 37.3bcdef | 82.8cde | 6.9d | 30gh | 4.0ab | 38.4h |
| Mix4 | 89.7e | 6.9cd | 28cd | 5.5bc | 27.7de | 101.1c | 5.9cd | 24i | 6.1abc | 35.4ef | 80.3def | 8.4a | 33def | 3.9abc | 38.5gh |
| Mix5 | 102.8ab | 7.0bcd | 31c | 6.4ab | 26.0de | 97.9de | 6.2bc | 24i | 6.7ab | 32.8g | 91.0a | 7.6bc | 34de | 3.1de | 39.2fgh |
| Mix6 | 99.3abc | 7.0bcd | 29cd | 6.6ab | 29.1de | 100.8c | 6.1bc | 29efg | 6.8ab | 36.1cdef | 84.1cde | 7.6bc | 32efg | 4.3a | 38.7gh |
| Mix7 | 99.1abc | 7.4a | 29cd | 5.6abc | 28.2de | 97.3e | 6.3bc | 30def | 5.3cde | 36.8bcdef | 86.3abc | 7.9ab | 32efg | 3.4bcde | 43.2d |
| Mix8 | 97.0bcd | 5.2f | 31bc | 4.9cd | 35.3bc | 102.2bc | 5.4de | 31cde | 4.9de | 38.3bc | 86.8abc | 6.0ef | 39c | 3.4bcde | 43.9cd |
| Mix9 | 99.9ab | 5.8e | 43a | 6.8a | 36.9b | 105.4a | 5.9cd | 30.8cdef | 5.9bcd | 39.0b | 90.2ab | 5.9ef | 46b | 2.3f | 45.3c |
| Sole1 | 98.1abc | 7.0bc | 30cd | 4.9cd | 27.7de | 100.0cd | 6.2bc | 27gh | 7.2a | 35.6def | 85.6bc | 7.6bc | 34def | 3.1de | 40.3efg |
| Sole2 | 91.3de | 5.7e | 27d | 6.4ab | 27.3de | 88.2f | 5.6d | 24hi | 6.9ab | 35.2f | 74.4gh | 6.3e | 31fgh | 3.4bcde | 40.7ef |
| Sole3 | 6.2e | 6.9cd | 20e | 2.9e | 27.4de | 100.6c | 6.3bc | 32bcd | 6.7ab | 37.8bcd | 86.4abc | 7.3cd | 29h | 3.3cde | 40.7ef |
| Sole4 | 92.7cde | 5.1f | 41a | 5.8abc | 39.0b | 96.1e | 5.0e | 33bc | 6.3abc | 36.9bcdef | 83.5cde | 5.3g | 46b | 3.8abcd | 47.4b |
| Sole5 | 77.9f | 5.1f | 42a | 5.9abc | 38.9b | 85.8f | 4.9e | 34b | 4.7e | 36.3cdef | 73.6h | 5.6fg | 50a | 2.3f | 42.2de |
| Sole6 | 73.6f | 4.5g | 35b | 3.9de | 44.3a | 82.1g | 4.2f | 37a | 6.5ab | 41.9a | 75.3fgh | 5.3g | 44b | 2.8ef | 49.7a |
| GM | 94.4 | 6.3 | 32 | 5.5 | 31.5 | 97.9 | 5.9 | 30 | 6.3 | 36.9 | 83 | 6.9 | 37 | 3.4 | 42.1 |
| S.E. | 4 | 0.2 | 2.2 | 0.7 | 3.1 | 1.4 | 0.3 | 1.8 | 0.7 | 1.4 | 3 | 0.3 | 1.8 | 0.4 | 1.1 |
| C.V. (%) | 4.2 | 2.7 | 7 | 12.4 | 9.9 | 1.4 | 5.1 | 5.9 | 10.9 | 3.7 | 3.6 | 4.1 | 5 | 10.5 | 2.6 |

PH =Plant height, **SPL**=spike length, **SDPS**= Seeds per spike, **BM**= Biomass yield, **TKW**=thousand kernel weight, **GM** =Grand mean, **S.E**= standard error, **C.V**=coefficient of variation

Appendix 2. Mean values for yield related traits of Ethiopian tetraploid wheat genotype collections at Chefe Donsa, Ayiba and Hageresalam

| Genotypes | Chefe Donsa | | | | | Ayiba | | | | | Hageresalam | | | | |
|-----------------|-------------|----------|--------|-------------------------|----------|----------|----------|---------|-------------------------|----------|-------------|----------|---------|-------------------------|-----------|
| | PH (cm) | SPL (cm) | SDPS | BM (tha ⁻¹) | TKW (gm) | PH (cm) | SPL (cm) | SDPS | BM (tha ⁻¹) | TKW (gm) | PH (cm) | SPL (cm) | SDPS | BM (tha ⁻¹) | TKW (gm.) |
| Mix1 | 92.7bcd | 6.0bc | 29g | 4.9ab | 41.3hi | 106.1ab | 7.7ab | 35.4de | 6.8ab | 49.1cde | 102abc | 7.0bcd | 37cde | 9.4bcd | 47.2a |
| Mix2 | 88.2f | 5.4de | 32def | 4.3bcd | 41.1i | 112.1a | 5.8efg | 36.1cde | 6.0abcd | 52.7ab | 98bc | 6.7bcde | 44ab | 7.9de | 36.4f |
| Mix3 | 99.5a | 6.4a | 32de | 5.3a | 42.6gh | 94.5e | 5.3fg | 44.1a | 5.1def | 51.9abcd | 85de | 6.8bcde | 30f | 11.1a | 43.2bc |
| Mix4 | 88.9ef | 5.7cd | 31efg | 4.8ab | 40.3ij | 97.7cde | 7.8a | 33.8de | 6.7abc | 50.5bcde | 99bc | 6.1efg | 41abcd | 7.1e | 37.2def |
| Mix5 | 93.9bc | 5.9bc | 32de | 5.0ab | 40.5ij | 105.8ab | 7.6ab | 36.1cde | 7.1a | 50.0bcde | 107a | 6.5cdef | 38cde | 9.3bcd | 43.6abc |
| Mix6 | 89.4ef | 6.1bc | 32de | 4.7ab | 40.3ij | 86.0fg | 6.1def | 37.7bcd | 4.6ef | 48.6e | 100bc | 7.4abc | 34ef | 10.6ab | 42.8bc |
| Mix7 | 89.7def | 5.9bc | 31defg | 4.7ab | 43.3fg | 83.3g | 6.8bcd | 35.5de | 6.0abcd | 51.2bcde | 82e | 5.3g | 43abc | 8.5cd | 37.1ef |
| Mix8 | 94.8b | 5.2ef | 36c | 4.4bc | 44.8de | 92.2ef | 6.4de | 31.8e | 5.5cde | 52.1ab | 104ab | 5.7fg | 45a | 9.8abc | 42.4c |
| Mix9 | 94.5b | 5.2ef | 41b | 4.4bc | 47.4b | 86.2fg | 6.2def | 41.0abc | 4.0f | 52.0abc | 103abc | 7.4ab | 38bcde | 9.3bcd | 46.4ab |
| Sole1 | 89.3ef | 6.0bc | 30fg | 4.4bc | 39.5j | 96.2de | 5.7efg | 41.2ab | 4.6ef | 50.5bcde | 97c | 6.4def | 42abcd | 8.5cd | 40.4cde |
| Sole2 | 84.2g | 5.0fg | 29g | 5.2a | 40.3ij | 104.9abc | 7.4abc | 33.8de | 6.7abc | 48.9de | 98bc | 7.9a | 33ef | 10.5ab | 43.4bc |
| Sole3 | 90.8cdef | 6.1ab | 33d | 4.7ab | 44.0ef | 92.2ef | 6.5cde | 43.1a | 5.8bcde | 51.7abcd | 97c | 6.4def | 38cde | 8.9bcd | 43.4bc |
| Sole4 | 91.5cde | 4.6h | 41b | 4.8ab | 46.6bc | 93.4ef | 4.9g | 34.4de | 4.6ef | 54.7a | 90d | 6.3def | 36def | 8.0cde | 40.9cd |
| Sole5 | 80.8h | 4.9gh | 44a | 3.9cd | 45.9cd | 102.5bcd | 7.4abc | 35.9de | 5.2def | 50.3bcde | 99bc | 7.1abcd | 37cde | 10.1abc | 40.0cdef |
| Sole6 | 81.4gh | 4.5h | 40b | 3.7d | 51.2a | 102.3bcd | 6.5cde | 33.8de | 5.5bcde | 52.2ab | 101bc | 7.1abcd | 39abcde | 8.4cde | 40.5cde |
| GM | 90 | 5.5 | 34 | 4.6 | 43.3 | 97 | 6.5 | 37 | 5.6 | 51.1 | | 6.7 | 38 | 9.2 | 41.7 |
| P value | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.05 | 0.05 | 0.01 | 0.001 | 0.05 | 0.001 | 0.001 | 0.01 | 0.05 | 0.001 |
| S.E. | 1.9 | 0.2 | 1 | 0.3 | 0.8 | 2.8 | 4.4 | 2.9 | 0.7 | 1.8 | 2.7 | 0.5 | 3.7 | 1 | 2.2 |
| C.V. (%) | 2.1 | 3.6 | 3 | 7.4 | 1.9 | 2 | 4.6 | 8 | 13 | 3.5 | 2.4 | 7.7 | 9.7 | 11.1 | 5.3 |

Appendix 3. Mean values for morphological traits of Ethiopian tetraploid wheat genotype collections ($P < 0.001$) at Adet, Geregera and Debrezeit

| Genotypes | Adet | | | Geregera | | | Debre Zeit | | |
|-----------|------|------|---------|----------|--------|--------|------------|-----|--------|
| | DB | DH | DM | DB | DH | DM | DB | DH | DM |
| Mix1 | 62e | 74cd | 120bcde | 72bc | 78bc | 145def | 39bcd | 47b | 89bcde |
| Mix2 | 61e | 73cd | 117efg | 74b | 79b | 144efg | 37d | 49b | 90bcd |
| Mix3 | 65d | 74c | 123ab | 73b | 78bc | 145def | 39bcd | 47b | 92bc |
| Mix4 | 58f | 74c | 122abc | 73b | 78bc | 143efg | 42ab | 42c | 88cde |
| Mix5 | 61e | 71de | 118cdef | 69cde | 76bcde | 148abc | 37d | 44c | 87de |
| Mix6 | 68c | 75c | 120bcde | 71bcd | 77bcd | 144efg | 41bc | 47b | 93b |
| Mix7 | 61e | 75c | 116fg | 72bc | 77bcd | 146cde | 38d | 47b | 88de |
| Mix8 | 56fg | 66f | 123ab | 69cde | 74de | 145def | 28e | 38d | 83fg |
| Mix9 | 54gh | 61gh | 120bcd | 68de | 73ef | 150a | 28e | 38d | 82fg |
| Sole1 | 61e | 68ef | 115g | 71bc | 77bcd | 143fg | 38cd | 42c | 81g |
| Sole2 | 73b | 80b | 119cdef | 78a | 84a | 150a | 39bcd | 47b | 86ef |
| Sole3 | 82a | 87a | 124a | 73b | 78bc | 142g | 44a | 55a | 106a |
| Sole4 | 55gh | 61gh | 118defg | 69cde | 75cde | 147bcd | 29e | 37d | 81g |
| Sole5 | 53h | 59h | 117defg | 67ef | 73ef | 145def | 30e | 38d | 79g |
| Sole6 | 55gh | 63g | 120bcd | 65f | 70.0f | 149ab | 30e | 39d | 81g |
| GM | 62 | 71 | 119 | 71 | 76 | 146 | 36 | 44 | 87 |
| S.E. | 1.5 | 1.7 | 1.8 | 0.3 | 2.2 | 1.6 | 1.8 | 1.3 | 2.3 |
| C.V. (%) | 2.5 | 2.4 | 1.5 | 2.6 | 2.9 | 1.1 | 5.1 | 3.1 | 2.7 |

DB =Days to booting, **DH**=Days to heading, **DM** =Days to maturity, **GM** =Grand mean, **S.E**= standard error, **C.V**=coefficient of variation

Appendix 4. Mean values for morphological traits of Ethiopian tetraploid wheat genotype collections at Chefe Donsa, Ayiba and Hageresalam

| Genotypes | Chefe Donsa | | | Ayiba | | | Hageresalam | | |
|-----------|-------------|-------|--------|-------|-------|--------|-------------|--------|---------|
| | DB | DH | DM | DB | DF | DM | DB | DF | DM |
| Mix1 | 64bc | 68cd | 125abc | 56bcd | 81ab | 139ab | 62a | 67def | 107f |
| Mix2 | 64b | 70b | 124bc | 53bcd | 75bc | 136abc | 66a | 68bcd | 117a |
| Mix3 | 64bc | 69bcd | 126a | 52bcd | 72cd | 134c | 65a | 70abc | 113abcd |
| Mix4 | 64bc | 68cd | 121d | 51bcd | 75bc | 140a | 62a | 67def | 116ab |
| Mix5 | 63c | 67d | 124bc | 59bc | 75bc | 139abc | 63a | 70ab | 108ef |
| Mix6 | 63bc | 69bc | 123bc | 71a | 79abc | 139abc | 62a | 6bcde | 112bcde |
| Mix7 | 63bc | 67d | 121d | 49cd | 72cd | 137abc | 62a | 67def | 110def |
| Mix8 | 53e | 66e | 124bc | 58bcd | 78abc | 136abc | 59a | 64g | 111cdef |
| Mix9 | 53e | 65e | 123cd | 47d | 68d | 137abc | 61a | 65efg | 114abc |
| Sole1 | 63c | 67d | 112e | 61ab | 80ab | 135bc | 62a | 68bcde | 110cdef |
| Sole2 | 67a | 73a | 125ab | 52bcd | 83a | 140a | 61a | 65fg | 114abc |
| Sole3 | 68a | 74a | 125ab | 56bcd | 72cd | 135bc | 63a | 72a | 113abcd |
| Sole4 | 55d | 67d | 125abc | 53bcd | 80ab | 136abc | 61a | 66defg | 117a |
| Sole5 | 55d | 68cd | 124bc | 57bcd | 83a | 136abc | 61a | 66defg | 114abcd |
| Sole6 | 55d | 67d | 125ab | 55bcd | 78abc | 136abc | 62a | 67cdef | 108ef |
| GM | 61 | 68 | 123 | 55 | 77 | 77 | 62 | 67 | 67 |
| p value | 0.001 | 0.001 | 0.001 | NS | 0.01 | 0.05 | NS | 0.01 | 0.01 |
| S.E. | 0.8 | 0.9 | 1.3 | 6.5 | 4.2 | 4.2 | 2.7 | 1.8 | 1.8 |
| C.V. (%) | 1.3 | 1.3 | 1 | 12 | 5 | 5 | 4.3 | 2.6 | 2.6 |

Appendix 5. Level of significance of variance for nutritional quality traits

| Location | Variance Source | Traits with mean squares and level of significance | | | | | | | | |
|-------------|-----------------|--|---------------------|---------------------|---------------------|---------------------|---------------------|--------------------|----------------------|----------------------|
| | | Moisture (%) | Protein (%) | Fat (%) | Fiber (%) | Ash (%) | Total Carb (%) | Available Carb (%) | Fe (mg/100gm) | Zn (mg/100gm) |
| Chefe Donsa | Treatment | 0.021 ^{ns} | 1.48 ^{**} | 0.35 ^{***} | 0.13 ^{***} | 0.043 ^{ns} | 2.83 ^{***} | 2.8 ^{**} | 1.22 ^{***} | 0.068 ^{***} |
| | Replication | 0.46 ^{***} | 0.056 ^{ns} | 0.033 ^{ns} | 0.048 [*] | 0.065 ^{ns} | 0.065 ^{ns} | 1.37 ^{ns} | 0.003 ^{ns} | 0.012 ^{ns} |
| | Block | 0.007 ^{ns} | 1.038 [*] | 0.034 ^{ns} | 0.01 ^{ns} | 0.012 ^{ns} | 0.43 ^{ns} | 0.31 ^{ns} | 0.0034 ^{ns} | 0.0034 ^{ns} |
| | Error | 0.0099 | 0.3 | 0.04 | 0.01 | 0.029 ^{ns} | 0.26 | 0.43 | 0.013 | 0.0074 |

Ns=not significant* Significant at 0.05 probability level, *** Significant at 0.001 probability level, *Carb*=Carbohydrate

Appendix 6. Accession and SNP code for mixtures of top ten Tigray, Amhara, Tigray Regions and ten improved varieties

| Top 10 Tigray mixtures | | Top 10 amhara mixtures | | Tigray mixtures | | Ten improved varieties mixtures | |
|------------------------|----------------|------------------------|----------------|-----------------|----------------|---------------------------------|----------------|
| SNP Code | Accession name | SNP Code | Accession name | SNP Code | Accession name | SNP Code | Accession name |
| 121ET | 222541 | 133ET | 222786 | 175ET | 236300 | 343ET | Quamy |
| 090ET | 214874 | 143ET | 226094 | 286ET | 236297 | 482ET | Selam |
| 365ET | 208316 | 085ET | 214357 | 408ET | 238133 | 484ET | Metaya |
| 450ET | 238120 | 076ET | 210796 | 185ET | 238125 | 344ET | Ude |
| 305ET | 238137A | 453ET | 208252 | 172ET | 236291 | 483ET | Megenagna |
| 328ET | 238579 | 422ET | 208294 | 024ET | 206556 | 390ET | Hitosa |
| 423ET | 231572 | 140ET | 222859 | 141ET | 223257 | 464ET | GINCHI |
| 036ET | 208192 | 438ET | 214881 | 183ET | 238119 | 463ET | Mangudo |
| 191ET | 238522 | 372ET | 208264 | 186ET | 238126 | 341ET | Assassa |
| 219ET | 208215A | 424ET | 208239 | 305ET | 238137A | 472ET | Mukiye |

Note: Mixtures of top ten in Tigray are not collection only from Tigray Region, but also from Amhara and Oromia Regions (Accession 222541, 238579, 208192 and 208215A are from Amhara, 208316 from Oromia, 238137A from Tigray region)

Appendix 7. Accession number and SNP code for mixtures parental lines

| SNP Code | Accession name | SNP Code | Accession name | SNP Code | Accession name |
|----------|----------------|----------|----------------|----------|----------------|
| 277ET | 228763A | 079ET | 210825 | 370ET | 222856 |
| 023ET | 206554 | 022ET | 206551 | 015ET | 8210A |
| 455ET | 208780 | 240ET | 210806A | 475ET | GG2 |
| 158ET | 228768 | 335ET | 6856A | 173ET | 236295 |
| 354ET | 236271 | 101ET | 222298 | 362ET | 234498 |
| 051ET | 208279 | 266ET | 226834B | 416ET | 208312 |
| 392ET | 210795 | 165ET | 231580 | 032ET | 208167 |
| 319ET | 238552A | 071ET | 208365 | 093ET | 215276 |
| 149ET | 226840 | 013ET | 8205 | 083ET | 213310 |
| 322ET | 238555A | 170ET | 236282 | | |

Appendix 8. Accession number and SNP code for mixtures of Amhara Region

| SNP Code | Accession name | SNP Code | Accession name | SNP Code | Accession name | SNP Code | Accession name | SNP Code | Accession name | SNP Code | Accession name |
|----------|----------------|----------|----------------|----------|----------------|----------|----------------|----------|----------------|----------|----------------|
| 265ET | 226834A | 139ET | 222855 | 138ET | 222841 | 163ET | 231565 | 038ET | 208196 | 449ET | 208162 |
| 121ET | 222541 | 196ET | 238561 | 299ET | 236987 | 087ET | 214571 | 176ET | 236301 | | 208168 |
| 122ET | 222542 | 206ET | 206518A | 027ET | 206700 | 025ET | 206567 | 259ET | 222537A | 380ET | 222500 |
| 119ET | 222499A | 126ET | 222641 | 328ET | 238579 | 161ET | 229251 | 030ET | 208155 | 431ET | 231597 |
| 081ET | 212561 | 078ET | 210821 | 129ET | 222707A | 042ET | 208229 | 210ET | 208131A | 385ET | 222858 |
| 008ET | 7960 | 292ET | 236312A | 242ET | 210812A | 339ET | 8333A | 028ET | 208136 | 386ET | 222682 |
| E509ET | 238567 | 148ET | 226839 | 264ET | 222816B | 131ET | 222730 | 425ET | 208173 | 445ET | 206666 |
| 177ET | 236313 | 105ET | 222346 | 276ET | 228593B | 037ET | 208194 | 437ET | 208184 | 441ET | 228600 |
| 036ET | 208192 | 091ET | 214877 | 045ET | 208242 | 033ET | 208175 | 440ET | 234514 | 357ET | 208200 |
| 136ET | 222811 | 329ET | 239081A | 132ET | 222752 | 035ET | 208190 | 388ET | 222655 | 360ET | 222366 |
| 287ET | 236303A | 272ET | 227056B | 324ET | 238564A | 247ET | 210818 | 411ET | 234513 | 427ET | 238570 |
| 254ET | 215268BI | 197ET | 238576 | 164ET | 231573 | 180ET | 236986 | 415ET | 222854 | 326ET | 238573 |
| 375ET | 208243 | 433ET | 210896 | 447ET | 214366 | 219ET | 208215A | | | | |

Appendix 9. Accession number and SNP code for mixtures of Oromia Region

| SNP Code | Accession name | SNP Code | Accession name | SNP Code | Accession name | SNP Code | Accession name | SNP Code | Accession name |
|----------|----------------|----------|----------------|----------|----------------|----------|----------------|----------|----------------|
| 007ET | 5679 | 257ET | 222441A | 189ET | 238492B | 070ET | 208334 | 369ET | 226887 |
| 160ET | 228862 | 118ET | 222464 | 117ET | 222457 | 295ET | 236979A | 421ET | 226928 |
| | 208327 | 355ET | 238507 | 110ET | 222413 | 018ET | 204353 | 452ET | 238498 |
| 159ET | 228771 | E506ET | 226894 | 308ET | 238504C | 020ET | 204530 | | 208931 |
| 114ET | 222434 | 108ET | 222387 | 311ET | 238531 | 202ET | 204493A | 409ET | 214303 |
| 235ET | 208746A | 201ET | 204470B | 088ET | 214585 | 239ET | 210805A | 399ET | 208321 |
| 055ET | 208286 | 307ET | 238504B | 112ET | 222426 | 066ET | 208328 | 365ET | 208316 |
| 192ET | 238528 | 361ET | 222432 | 204ET | 204562A | 281ET | 231547A | 391ET | 215411 |
| 009ET | 8019 | 059ET | 208293 | 306ET | 238504A | 011ET | 8034B | 414ET | 228753 |
| 144ET | 226191 | 310ET | 238525B | 063ET | 208315 | 262ET | 222787B | 432ET | 208262 |
| 074ET | 208486 | 179ET | 236982 | 057ET | 208290 | 444ET | 208336 | 367ET | 208280 |
| 115ET | 222435 | 102ET | 222326 | 001ET | 5300 | 435ET | 238516 | 378ET | 208320 |
| 116ET | 222440 | 188ET | 238492A | 238ET | 210798B | 417ET | 226371 | 407ET | 222462 |
| 134ET | 222805 | 123ET | 222554 | 190ET | 238519 | E513ET | 222327A | 420ET | 202458 |
| 124ET | 222578 | 056ET | 208288 | 053ET | 208283 | 398ET | 208277 | 050ET | 208273 |
| 145ET | 226363 | 490NET | 203889 | 135ET | 222806 | 382ET | 231524 | 333ET | 5578A |
| 097ET | 222197A | 096ET | 222190 | 003ET | 5588 | 454ET | 208281 | 494NET | 214502 |
| 109ET | 222408 | 111ET | 222419 | 065ET | 208322 | 418ET | 208258 | | 236988 |
| 152ET | 227029 | 496NET | 208278 | | | | | | |

Appendix 10. Accession number and SNP code for mixtures from other regions

| SNP Code | Accession name | SNP Code | Accession name |
|----------|----------------|----------|----------------|
| 092ET | 215111 | 017ET | 8436 |
| 402ET | 210801 | 231ET | 208373A |
| 244ET | 210816B | 258ET | 222499B |
| E511ET | 210816A | E501ET | 207058 |
| 012ET | 8151 | 004ET | 5611 |
| 229ET | 208276A | 255ET | 216628A |
| 241ET | 210806B | 317ET | 238540D |
| 297ET | 236985A | 499NET | 202012 |
| 280ET | 229251B | 154ET | 227058 |
| | 212415 | 458ET | 208151 |
| 429ET | 222780 | 031ET | 208161 |
| | 206548bl | E508ET | 238540 |
| 208ET | 206551A | 436ET | 238537 |
| 234ET | 208383B | | |

Appendix 11. Some pictures taken in the field



Pictures at grain maturity stage



Pictures taken during field visit by Professor Zemedu Asfaw

Appendix 12. Some pictures taken in the laboratory



a. Fiber determinator



b. Fat extractor



c. Furnace



d. Atomic Absorption spectrophotometer



e. Kjeldahl protein digester



f. Kjeldahl protein analyzer

Declaration

I, the undersigned declare that this Thesis is my original work and it has not been presented in other universities, colleges or institutes for a degree or other purpose. All sources of the materials used have been duly acknowledged.

Name: _____ Signature: _____ Date: _____

This work has been done under my supervision.

Name: _____ Signature: _____ Date: _____

_____ Signature: _____ Date: _____