Chapter 1 General Background

1.1 Plant genetic resources of Ethiopia

The Ethiopian environment is dominated by heavily dissected and rugged extensive mountains and highlands, which are estimated to cover about 45% of the total area (over one million square kilometers) of the country. The major physiographic features are a massive highland complex of mountains and plateaus divided by the Great Rift Valley and surrounded by lowlands along the periphery. The diversity of the terrain is fundamental to regional variations in climate, natural vegetation, soil composition, and settlement patterns. The presence of wide altitudinal range (120 m below to 4600 m a.s.l.), substantial temperature, edaphic and rainfall differences created a wide range of agroecological conditions that provided sustainable environments for a broad range of life forms. As a result, Ethiopia is considered as one of the richest genetic resource centers in the world. Vavilov (1997) recognized it is one of the eight crop centers of origin (Figure 1.1). For several economically important cereals such as tetraploid wheat (*Triticum* spp.), barley (*Hordeum* spp.) and sorghum (*Sorghum bicolor* (L.) Moench), Ethiopia is considered as center of diversity (Vavilov 1997; Worede 1997) and also a center of origin for crops like anchote (*Coccinia abyssinica* (Lam.) Cogn.), chat (*Catha edulis* (Vahl) Forsk. ex Endl.), coffee (*Coffea arabica* L.), enset (*Ensete ventricosum* (Welw.) Cheesman), gesho (*Rhamnus prinoides* L' Hérit.), gomenzer (*Brassica carinata* A. Braun), noog (*Guizotia abyssinica* (L.f.) Cass.), Oromo potato (*Plectranthus edulis* (Vatke) Agnew) and tef (*Eragrostis tef* (Zucc.) Trotter) (Harlan 1971).

It is speculated that early immigrants of Hamites, some 5,000 years ago, introduced wheat to Ethiopian highlands and emmer wheat (*T. dicocon* Schrank) was the first to arrive (Helbaeck 1959; Feldman 1979). Hanelt (2001) considers the native Ethiopian tetraploid wheat (*T. aethiopicum* Jakubz.) as subspecies of *T. turgidum* and characterizes them as obviously not yet fully understood in relation to *T. durum* Desf. and *T. turgidum* L. The group went through specific evolution in the Ethiopian highlands. In the last decennia, *T. durum* has been introduced especially from the Mediterranean changing gradually the specific characters of local *T. aethiopicum* by intended (breeding) and unintended (natural introgression) action. *T. aethiopicum* Jacubz. a tetraploid wheat, which is morphologically very similar to *T. aestivum,* is 'endemic' to the Ethiopian highlands (Phillips 1995). Makey (1966) and Löve (1982) regarded it as a morphological variant not even meriting the subspecific rank. For primitive wheats, *Triticum dicoccon* Schrank, *Triticum polonicum* L. and *Triticum spelta* L., Ethiopia

is one of the few refuges where they have survived. The number of crops cultivated and their wild relatives in Ethiopia is more than one hundred (Edwards 1991).

Figure 1.1 Crop centers of origin.

1.2 Origin and phylogeny of wheat

Wheat is one of the earliest domesticated crop species. It was domesticated at least as early as 7500 B.C. in the Near East, somewhere in the Fertile Crescent (which comprises the mountain chains flanking the plains of Mesopotamia and Syrian desert including Iran, Jordan, Syria, Turkey, Israel and Palestine) and also in Anatolia and the Balkans. Vavilov (1964) proposed that wheat spread from western Asia, the primary centre of development, to Europe through the Caucasus and the Balkan Mountains and then to other parts of the world. Today, it is the most widespread crop in the world and the staple food of over a third of the world's population.

All cultivated wheats belong to the genus *Triticum*, to the tribe Triticeae in the family Poaceae (Gramineae) and subfamily Pooideae. Chromosome numbers in the genus *Triticum* is a function of the basic number (x = 7) and the ploidy level. Sakamura (1918) discovered the chromosomal basis of the three natural groups or species of wheat (*Triticum* spp.), which is an allopolyploid plant with chromosome numbers 2n=2x=14 (diploid), 2n=4x=28 (tetraploid) and 2n=6x=42 (hexaploid). Beginning in the 1920s, the method of nuclear genome analysis

based on chromosome pairing behavior in interspecific hybrids (Kihara 1919) provided information on genome constitution, phylogeny and the evolution of *Triticum* species. Based on a cytogenetic study, Lilienfield (1951) designated the genome formulae for einkorn (*T. monococcum* L.), emmer (*T. dicoccon* Schrank) and bread wheat (*T. aestivum* L.) as AA, AABB, and AABBDD, respectively.

The phylogeny of wheat (*T. aestivum*) is a two step evolutional event (Figure 1.2). *T. urartu,* a diploid species with genome AA crossed with another species with genome BB (Dvorak et al. 1988, 1993). The study of the behavior of chromosomes at meiosis in cells of a plant containing different genomes in hybrids or polyploids is the basis of genome analysis. Chromosomes that have a pairing affinity at meiosis are evolutionary more closely related than those that do not. Molecular evidence is indicating that the B genome of tetraploid wheat was donated by *Ae. speltoides* (Dvorak and Zhang 1990). Following chromosome doubling, the amphiploid (AABB) crossed with *Aegilops tauschii* L., a diploid species with a genome DD and spontaneously doubled to form the hexaploid species *T. aestivum* (AABBDD). McFadden and Sears (1944, 1946) and Kihara (1944) demonstrated that *Ae. tauschii* was the D-genome donor of bread wheat, which arose from a hybridization of a tetraploid wheat and *Ae. tauschii* ssp. *strangulat* (Eig) Tzvel., about 7 000 years ago (see Dvorak et al. 1998 for review).

1.3 A Microsatellite Map of Wheat

Wheat has an extremely large genome of 16×10^9 bp/1C (Bennett and Smith 1976) with more than 80% repetitive DNA. The genomes of all eukaryotes contain a class of sequences, termed microsatellites (Litt and Luty 1989) or simple sequenced repeats (SSRs) (Tautz et al. 1986). Microsatellites with tandem repeats of a basic motif of < 6 bp have emerged as an important source of ubiquitous genetic markers for many eukaryotic genomes (Wang et al. 1994). The analysis of microsatellites is based on the polymerase chain reaction (PCR), which is much easier to perform than RFLP analysis and is highly amenable to automation. In plants, it has been demonstrated that microsatellites are highly informative, locus-specific markers in many species (Condit and Hubbell 1991; Akkaya et al. 1992; Lagercrantz et al. 1993; Bell and Ecker 1994; Rongwen et al. 1995). Microsatellites show a much higher level of polymorphism and informativeness in wheat than any other marker system (Plaschke et al. 1995; Röder et al. 1995; Bryan et al. 1997). Only 30% of all primer pairs developed from microsatellite sequences are functional and suitable for genetic analysis (Röder et al. 1995;

Bryan et al. 1997). The majority of such markers is inherited in a codominant manner and, in most cases, they are chromosome-specific. This is a useful feature in a hexaploid genome. The linkage map is shown in Appendix 1. Along the individual linkage groups, the mapped markers were evenly distributed with no significant clustering except in the centromeric regions of some chromosomes. Thus, microsatellites are useful for complete coverage of the wheat genome. Wheat microsatellites are mainly genome-specific and that microsatellite primer sets usually amplify only a single locus from one of the three genomes.
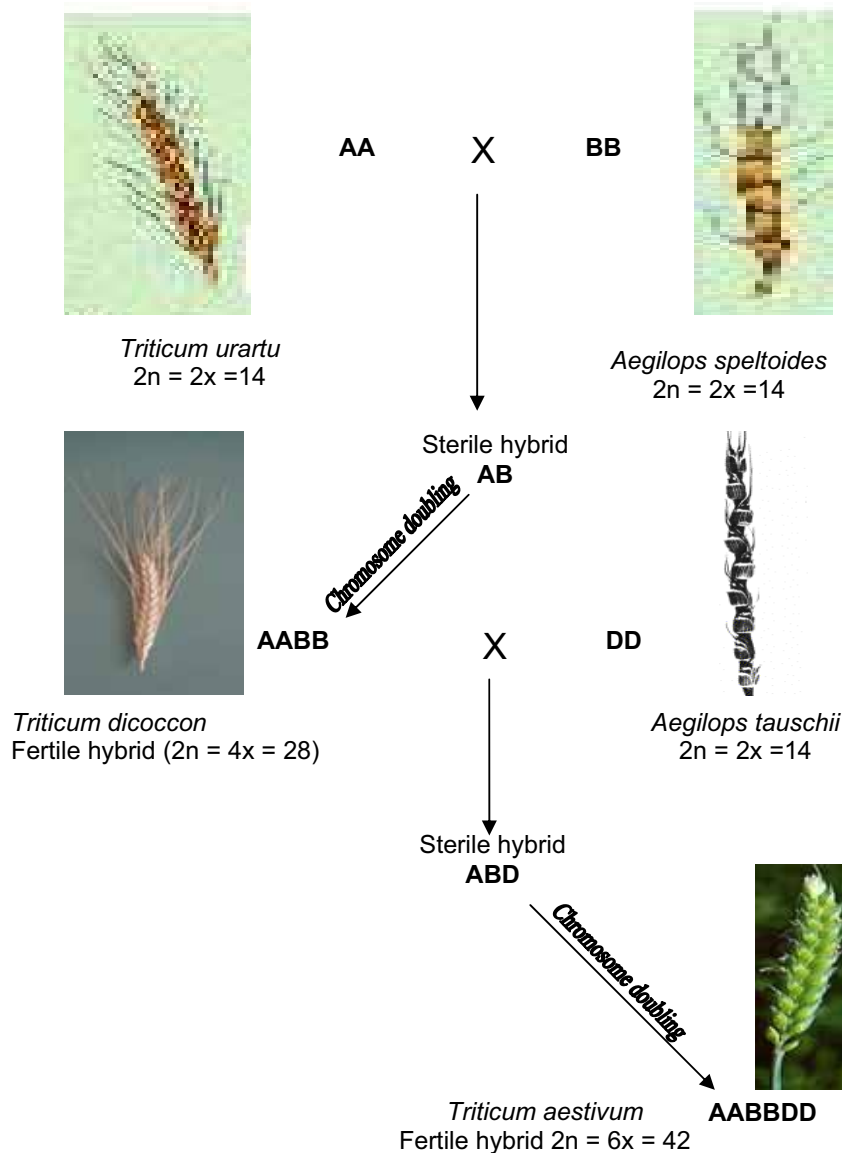

Figure 1.2 Phylogeny of wheat.

Most of the published molecular maps of wheat include only a few mutant loci and agronomically important genes. The main reason for this is that the use of RFLPs and isozyme markers for mapping has been inefficient because of a low level of allelic variation (< 10%) among cultivated varieties (Kam-Morgan et al. 1989). In addition, RFLP assays require large quantities of DNA and are technically demanding and laborious, and the most common detection method uses radioisotopes. A variety of DNA analysis techniques is available for genome analysis in cereals, such as RFLP, RAPD, EST, SCAR and AFLP. However, microsatellites have been reported to be useful to analyze the structure of germplasm collections, because they are co-dominant, abundant, of high reproducibility, highly polymorphic, detect heterogeneity and heterozygotes, evenly distributed over the genome, are PCR based assays and require only small amounts of genomic DNA for analysis (Gupta et al. 1996; Röder et al. 1998). Therefore, they are highly suitable as genetic markers in wheat for mapping agronomically important genes. Furthermore, the analysis of microsatellites can easily be automated and applied to large plant numbers, as has been shown for analysis in the human genome (Mansfield et al. 1994). In wheat, microsatellites have been successfully used in a wide range of applications such as in genotype identification (Hammer et al. 2000), diversity studies (Ben Amer et al. 2001; Fahima et al. 1998; Prasad et al. 2000; Huang et al. 2002), gene and quantitative trait locus analysis (Peng et al. 1999; Huang et al. 2003), and marker-assisted breeding (Huang et al. 2000).

1.4 Statement of the problem

Agriculture in Ethiopia is predominantly traditional and thus mainly landraces are grown (Tessema and Bechere 1998). This is primarily attributed to problems of adaptability and stability of improved varieties to the adverse farming conditions that prevail on small peasant farms and also due to the unavailability of seeds of improved varieties to farmers in sufficient quantity (Tessema and Bechere 1998). Landraces are the most diverse populations of cultivated plants (Frankel et al. 1995). Harlan's (1975) classic definition of landraces describes them as "balanced populations – variable, in equilibrium with both environment and pathogens and genetically dynamic…the result of millennia of natural and artificial selections." Besides being adapted to their natural and man-made environments, landraces tend to be co-adapted. Genetic variation within a landrace may be considerable (Qualset et al. 1997). Hence, landraces are the genetic bases for further breeding works.

World population is expected to increase by 2.6 billion over the next 45 years, from 6.5 billion today to 9.1 billion in 2050. Ethiopia is one of the nine countries predicted to account for the 2.6 billion increases. There is a pressing need for an astonishing increase in food production to feed this population. Wheat is among the major cereal crops grown in Ethiopia. It grows on an area of about 1.69 million hectares, and ranks third in area and second in total production (FAO 2005). It is an important commodity crop, which could contribute a major part in achieving the country's agricultural objective of food grain self-sufficiency (Teklu 1997). Despite the country having potential environments for wheat culture and being the centre of diversity for tetraploid wheats, the average national yield of wheat is low (1.8 t/ha). The major wheat yield limiting factors in Ethiopia which resulted in such low yield levels, compared to any other part of the world, are diseases, weeds, poor soil fertility, lack of cultivar choice, frost occurrence in the highlands, terminal drought stress and water logging in the intermediate altitudes, and drought stress in the lowlands (Eshetu 2002). Moreover, many of the variability studies (Belay et al. 1992; Belay et al. 1993; Belay et al. 1996; Bechere et al. 1996; Negassa 1986; Tessema et al. 1991; Tessema et al. 1993; Tessema and Bechere 1998) conducted so far are based on morphological traits, which are largely influenced by environmental factors. The few studies performed using microsatellites (Alamerew et al. 2004; Messele 2001), isozymes (Tsegaye et al. 1994; 1996), and glutenine and gliadine storage protein and AFLP (Messele 2001) considered either few accessions or focused mainly in the central highlands of Ethiopia. Thus, it was felt that because wheat landraces have not been adequately evaluated, their genetic resource remains largely unexploited. Therefore, broader characterization of the genetic diversity present in Ethiopian tetraploid wheat landraces is important to maximize the utilization of these materials in breeding for yield and quality traits.

In crop improvement, it is not only working with the existing genetic variation that is essential but also parallel and periodic assessment of the threat of loss of diversity is necessary. Detecting and assessing genetic erosion has been suggested as the first priority in any major effort to arrest loss of genetic diversity. In Ethiopia, even if there are several reports signalling the reduction in the number and area of tetraploid wheat landraces grown (Worede 1983; FAO 1996a; Bechere et al. 2000), the extent to which allelic diversity has been lost hasn't been properly documented. Therefore, this study was conducted with the following objectives. To:

1. investigate the extent and pattern of quantitative traits diversity in Ethiopian tetraploid wheat accessions vis-à-vis characters, regions of origin and altitude,

2. determine the magnitude of genetic variability and relationships between yield and its components and identify major traits attributing to the variation in phenotypic diversity,

3. assess the relationship of Ethiopian tetraploid wheat species using SSR markers,

4. study the regional patterns of microsatellite variation of Ethiopian tetraploid wheat landraces,

5. review genetic diversity studies in Ethiopian tetraploid wheat and explore breeding opportunities and strategies,

6. compare Ethiopian emmer with emmer gene pools from 11 countries in centers of origin and secondary centers of diversity,

7. examine the association between diversity indices based on pheno-morphic and agronomic traits and microsatellites, and

8. to quantify the extent of genetic erosion in tetraploid wheats, investigate the causes of genetic erosion and assess the measures that are being taken to reduce the problem of genetic erosion.

Chapter 2 Multivariate analysis of quantitative traits variation in Ethiopian tetraploid wheats landraces

2.1 Abstract

For effective utilization of genetic diversity it is important to know its nature and structure of genetic variation. Multivariate techniques of clustering, ordination and discriminant function analysis were used to investigate the pattern of diversity present in 271 Ethiopian tetraploid wheat accessions vis-à-vis regions of origin and altitude. Major traits attributing to the variation in phenotypic diversity of tetraploid wheats have also been determined. The accessions were evaluated for 13 pheno-morphic and agronomic traits at Alemaya University research site (Rare) during two main cropping seasons. Results of PCA indicated that the first five (PCs) with eigenvalues greater than 1 were able to explain 75.42% of the total variation in the entire accessions. The first PCA axis, which explained 25.29% of the total variation, was closely related to variations in thousand kernel weight, grain yield, biomass yield, and number of spikes per plant. PCA was also computed using the means of regions of origin for the 13 quantitative characters in order to study the regional pattern of variation. The first three principal components with eigenvalues greater than 1 have explained 77.8% of the total variation. In all regions, the total variance explained in the PCA is greater for PC1 than PC2. Cluster analysis allocated entire accessions to ten clusters. The clusters did not include all the accessions from the same or nearby sites in the same group. Mahalanobis distance was used as a measure of the extent of genetic diversity between the clusters. As with the cluster analysis, Plots of PC1 versus PC2 showed the absence of clear pattern of regional grouping as accessions of the different regions were distributed patchily over many clusters. Discriminant analysis has succeeded in differentiating accessions, with a medium correct classification rate of 60.9 %. Major variables integrating in the discriminant function were days to heading and spike length. The implications of the above findings to wheat improvement, collection and conservation activities have been discussed.

2.2 Introduction

Plant genetic resources constitute the foundation upon which agriculture and world food securities are based and the genetic diversity in the germplasm collections is critical to the world's fight against hunger. They are the raw material for breeding new plant varieties and are a reservoir of genetic diversity. The future food supply of all societies depends on the exploitation of genetic recombination and allelic diversity for crop improvement, and many of

the world's farmers depend directly on the harvests of the genetic diversity they sow for food and fodder as well as the next seasons seed (Smale et al. 2004).

Although Ethiopia is centre of diversity for tetraploid wheat, the average national yield of wheat is low, 1.8 t/ha, (FAO 2005). A better characterization and understanding of genetic diversity and its distribution is essential not only to efficiently exploit the available genetics resources in breeding programs but also to design collecting trips and conservation projects. Morphological characterization, which estimate diversity and evaluate germplasm phenotypically, is the first step in the description and classification of germplasm (Smith and Smith 1989). Various multivariate analysis techniques have been successfully used to classify and measure the pattern of phenotypic distribution in relation to collection regions and adaptation zones on germplasm accessions from *ex situ* conservation of cultivated crops and on traits that are direct targets of human selection (Pecetti et al. 1992). Multivariate methods are useful for characterization, evaluation and classification of plant genetic resources when a large number of accessions are to be assessed for several characters of agronomic and physiological importance (Peeters and Martinelli 1989). In Ethiopia, genetic diversity of wheat (*Triticum* spp.) has been assessed using multivariate techniques (Bekele 1984; Damania et al. 1996; Elings 1991; Pecetti et al. 1992). However, most of these studies haven't represented accessions collected from the whole regions of the country. It is useful to estimate genetic diversity based on collections from a large range of geographical areas to get more reliable information. Broader characterization of the genetic diversity present in Ethiopian tetraploid wheat landraces is important to maximize the utilization of these materials in breeding for yield and quality traits. As a result, 271 tetraploid wheat accessions collected from all over the country were used to study phenotypic diversity using multivariate techniques in this study. The objectives were (i) to determine the extent and regional patterns of diversity of tetraploid wheat accessions, (ii) to identify the traits accounting for the gross phenotypic diversity of wheat germplasm and for regional differentiation, and (iii) to examine the validity of the classification of accessions based on regions of collections. Results are also discussed in relation to wheat breeding, collection and conservation programs.

2.3 Materials and Methods

Plant materials and data collection

A total of 271 tetraploid wheat landraces collected from all geographical regions of Ethiopia were used (Table 2.1). A map showing the different regions of Ethiopia and its neighbors is