Genetic Diversity and Grain Protein Composition of Tetraploid Wheat (*Triticum durum* Desf.) Germplasm from Ethiopia

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Abstract

The extent and patterns of genetic diversity in landraces of tetraploid wheat germplasm collected from major wheat-producing regions of Ethiopia were assessed with the use of agro-morphological, phenol-qualitative traits, grain proteins and molecular markers. The extent of genetic erosion of landraces in tetraploid wheat germplasm from Ethiopia was also evaluated.

Field evaluation of agro-morphological characters and laboratory analysis of grain proteins and molecular markers (ISSR) revealed the presence of broad genetic variation among accessions grown in different regions of Ethiopia. Based on the agro-morphological traits, all accessions included in the study were clustered into 15 clusters, with nine accessions remaining solitary. The first 5, 4 and 3 principal component analyses were involved in explaining most of the variation between region of origin, species and altitudinal classes, respectively. Biomass yield per plot, nature of awn, days to heading, lower glume shoulder width, kernel (seed) colour and stand count at emergence were consistently important in explaining the variation in all accessions studied across region of origin, species and altitude class. Of the phenologic and qualitative traits evaluated in all accessions across the region of origin, the highest Shannon-Weaver diversity index (H′) was mainly due to plant height, a major agronomic character in durum wheat, except for landraces from Arsi and Bale, where it was due to awn colour.

Characterisation of Ethiopian tetraploid wheat germplasm for glutenin sub-unit composition resulted in identification of novel alleles at the *Glu*-1 locus. About 39% of the durum wheat studied contained Glu-A1x sub-units, which are rare in other durum wheats. Although there is monomorphism in a number of accessions for gliadin and glutenin subunits, in this study the B genome was found to be more polymorphic than the A genome.

Analysis of DNA polymorphism with ISSR primers produced 128 polymorphic bands and allowed a separation of 60 accessions of tetraploid wheat genotypes. The Nei genetic distance for all accessions varied from 0.0090 to 0.8574 and that for region of origin from 0.045 to 0.138. Molecular characterisation of four species of *Triticum* using ISSR revealed that these species were clearly separated, with *T. durum* being the most diverse, followed by *T. turgidum*, *T. aethiopicum* and *T. dicoccon* in that order. *Triticum durum* was more closely related to *T. turgidum* than to the other species.

The broad diversity in tetraploid Ethiopian wheat germplasm demonstrated here can be utilised in genetic improvement of the crop through selection and hybridisation. However, this broad genetic diversity is being threatened by genetic erosion. For conservation and practical application reasons, it is necessary to embark on more comprehensive and systematic germplasm collection from all over Ethiopia, with *ex situ* conservation and appropriate *in situ* conservation at the site of origin of landraces. This would also help to prevent genetic erosion arising from landraces being replaced by improved varieties of hexaploid and/or tetraploid wheat.

Keywords: Accessions, Ethiopia, genetic diversity, genetic erosion, germplasm, grain protein, landraces, markers, tetraploid wheat.

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Dedication

This thesis is dedicated to the memory of the late Professor Arnulf Merker, my brother, the late Kassa Hailu and my sister, the late Tsige Hailu.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Hailu, F., Merker, A., Harjit-Singh, Belay, G. & Johansson, E. 2006. Multivariate analysis of diversity of tetraploid wheat germplasm from Ethiopia. Genetic Resources & Crop Evolution 53(6), 1089-1098

II. II. Hailu, F., Johansson, E. & Merker A. 2010. Patterns of phenotypic diversity for phenologic and qualitative traits in Ethiopian tetraploid wheat germplasm. Genetic Resources & Crop Evolution 57(5), 781-790

III. Hailu, F., Johansson, E., Merker, A., Belay, G., Harjit-Singh, & Zeleke, H. 2006. Composition of and variation in high- and low-molecular weight glutenin subunits, and omega gliadins in Ethiopian tetraploid wheat germplasm Plant Genetic Resources 4(2), 134-143

IV. Hailu, F., Merker, A., Belay, G. & Johansson, E. 2005. Molecular diversity and phylogenic relationships of tetraploid wheat species as revealed by inter-simple sequence repeats (ISSR) from Ethiopia. Journal of Genetics & Breeding 59, 329-338

V. Hailu, F., Johansson, E. & Persson Hovmalm H. (manuscript). Assessment of genetic erosion: A case study of tetraploid wheat landraces from Ethiopia.

The contribution of Faris Hailu to the papers included in this thesis was as follows:

I Planned the experiment together with the supervisor, requested germplasm, planted the seed at DebreZeit Agricultural Research Centre, Akaki substation, harvested the plants, collected and analysed the data and wrote the manuscript with input from the co-authors.

II Planned the experiment together with the co-authors, requested germplasm, planted the seed at DebreZeit Agricultural Research Centre, Akaki substation, harvested the plants, collected data both in the laboratory and in the field, analysed the data and wrote the manuscript with input from the co-authors

III Planned the experiment together with the supervisor, milled seed samples into flour at the laboratory, prepared SDS-PAGE and evaluated the storage protein composition of the samples, and wrote the manuscript guided by Eva Johansson.

IV Planted seed samples in the greenhouse, collected young leaf tissues for DNA extraction, carried out all laboratory work and wrote the manuscript with input from the co-authors.

V Planned the experiment together with the main supervisor, set up questionnaires, distributed these to farmers and collected them, carried out on-farm observations, analysed the data and wrote the manuscript guided by Eva Johansson and Helena Persson Hovmalm.

1. Introduction

1.1 General background

Wheat is one of the most important cereals world-wide and it is grown in many areas (Curtis, 2002; Briggle & Curtis, 1987). During the past four decades, wheat has made a significant contribution to the increase in global food production. This is due to the use of higher-yielding, water and fertiliser responsive, and disease-resistant cultivars, combined with strengthened input delivery systems, tailored management practices and improved marketing (Ortiz *et al*., 2008; Dixon *et al*., 2006). A rapid increase in global wheat production has taken place during the last five decades, mainly due to increased productivity rather than an expansion of the cultivated area, with average global yields having risen from 1 t/ha in the 1950s to about 2.5 t/ha at the turn of the century (Curtis, 2002). According to estimates produced by USDA (2011), GMR (2011) and CSA (2010), wheat is one of the major cereal crops grown world-wide in terms of total production tonnage used for food. Wheat is currently second to rice and ahead of maize as the main human food crop. However, maize is more extensively used in animal feed. In Ethiopia, wheat is the third most grown crop, after teff and sorghum, both in terms of acreage and production volumes (Table 1). In global terms, wheat is the leading source of cereal proteins in human food, having higher protein content than maize or rice. In total, 16% and 26% of total dietary calories in developing and developed countries, respectively, come from wheat (Ortiz *et al*., 2008). Wheat is the most traded food crop internationally and is used as an emergency food in aid for developing countries. Wheat grain is a staple food used to make flour for leavened, flat and steamed breads, biscuits, cookies, breakfast cereal, pasta, noodles, bio-fuel, and for fermentation to make alcoholic beverages such as beer and liquors (Tsegaye & Berg, 2007a). Its importance may vary in different countries, partly as a result of the diversity in different species of wheat. For example, tetraploid wheat is widely grown by farmers on the heavy black clay soils (vertisols) of the Ethiopian Highlands, at altitudes of 1500-3000 metre above sea level (m.a.s.l.) during the main cropping season. Most of the tetraploid wheat under cultivation consists of a mixture of types (Tesemma & Belay, 1991). However, the most suitable range of altitude is 1900-2700 m.a.s.l., where the annual rainfall range is between 600 and 2000 mm (Gebre-Mariam, 1991).

Table 1. Production of wheat world-wide and in Ethiopia during the period 2007-2011 and an estimated value for 2011-2012 (Sources: USDA, 2011; GMR, 2011; CSA, 2010)

Year	World-wide			Ethiopia		
	Area (million hectares)	Yield (metric tons per hectare)	Production (million metric tons)	Area (million hectares)	Yield (metric tons per hectare)	Production (million metric tons)
2011/12*	227.31	3.01	684.40	**	**	**
2010/11	222.48	2.91	648.20	1.61	1.71	2.75
2009/10	222.63	3.05	678.12	1.68	1.83	3.08
2008/09	222.76	3.06	683.41	1.45	1.75	2.54
2007/08	222.55	3.02	607.00	1.43	1.73	2.20

*Predicted production on September 2011; ** Actual value not yet available

Within the total area of wheat under cultivation in Ethiopia, it has been reported that tetraploid and hexaploid species each occupy approximately 50% of the area (Gorfu *et al*., 2001). However, a change in the relative proportions of wheat types grown has been reported more recently, with *e.g*. tetraploid and hexaploid species occupying approximately 30% and 70%, respectively, of the total wheat area under cultivation (CSA, 2010).

Due to the introduction of productive semi-dwarf cultivars, improved varieties, the tetraploid wheat landraces that were widely cultivated in the early 20th century are now increasingly being replaced. This has resulted in the loss of genetically diverse, locally well-adapted but unimproved landraces and in an extinction of on-farm genetic variability (Royo *et al*., 2009). Thus, the level of genetic diversity in tetraploid wheat is being affected by the high selection pressure applied in breeding programmes and the relatively small number of varieties currently in cultivation (Skovmand *et al*., 2005). Pedigree analysis has revealed that the genetic background of the modern pool of elite durum wheat varieties is narrow (Maccaferri *et al*., 2005). A study including 51 cultivars derived from the CIMMYT/ICARDA breeding programme found that the same 15 ancestors were present in the pedigree of at least 80% of the cultivars, with five being present in all of them (Autrique *et al*., 1996). Research shows that narrowing of the gene pool of tetraploid wheat leads to an increased risk of vulnerability to diseases and pests (Frankel *et al*., 1995). It also leads to a decrease in abiotic stress tolerance, particularly to the drought and high temperatures that are typical of many regions growing tetraploid wheat (Mondini *et al*., 2010).

1.2 Origin and distribution of tetraploid wheat

The genetic origin of wheat is of interest, since it is a classic example of how closely related species may be combined in nature into a polyploid series. The species of *Triticum* (*T.*) and their close relatives can be divided into diploid, tetraploid and hexaploid groups, with chromosome numbers of 2n = 14, 28 and 42, respectively, in which the basic chromosome number of wheat is x = 7. The wild species are diploids (2n = 2x = 14), *e.g.* with the genome designation AA (*T. monococcum*), DD (*T. tauschii,* syn. *Aegilops squarossa*), and SS (*T. speltoids*), or tetraploids (2n = 2x = 28), *e.g.* with the genomes AABB (*T. durum* or *T. turigidum*) or AAGG (*T. timopheevii*). *Triticum durum* originated thousands of years ago from a hybridisation between the wild diploid *T. monococcum* L. (A genome donor) and the donor of the B genome which, according to morphological, geographical and cytological evidence, has been recognised as *T. speltoides* (Tausch) Gren. or a closely related species (Colomba & Gregorini, 2011; von Buren, 2001; Friebe & Gill, 1996) (Fig. 1).

Wheat may have been the first crop to be domesticated and cultivated by human beings. The process began possibly between 18,000 and 12,000 BC, when wheat was apparently grown in the Middle East and Abyssinian region. The first domesticated wheat recorded is from the Tigris-Euphrates Valley. Human beings have used wheat as food since prehistoric times and some of the evidence indicates that it was first used in a parched form (Reif *et al*., 2005a; Bechere *et al*., 2000; Reitz, 1967). Within tetraploid wheat, cultivated emmer (*T. dicoccum*) was the first to be domesticated. Others such as *T. durum, T. turgidum, T. aethiopicum* and *T. polonicum* might have originated from cultivated emmer through mutation(s) that reduced the toughness of the glumes to attain free-threshing (Morris & Sears, 1967).

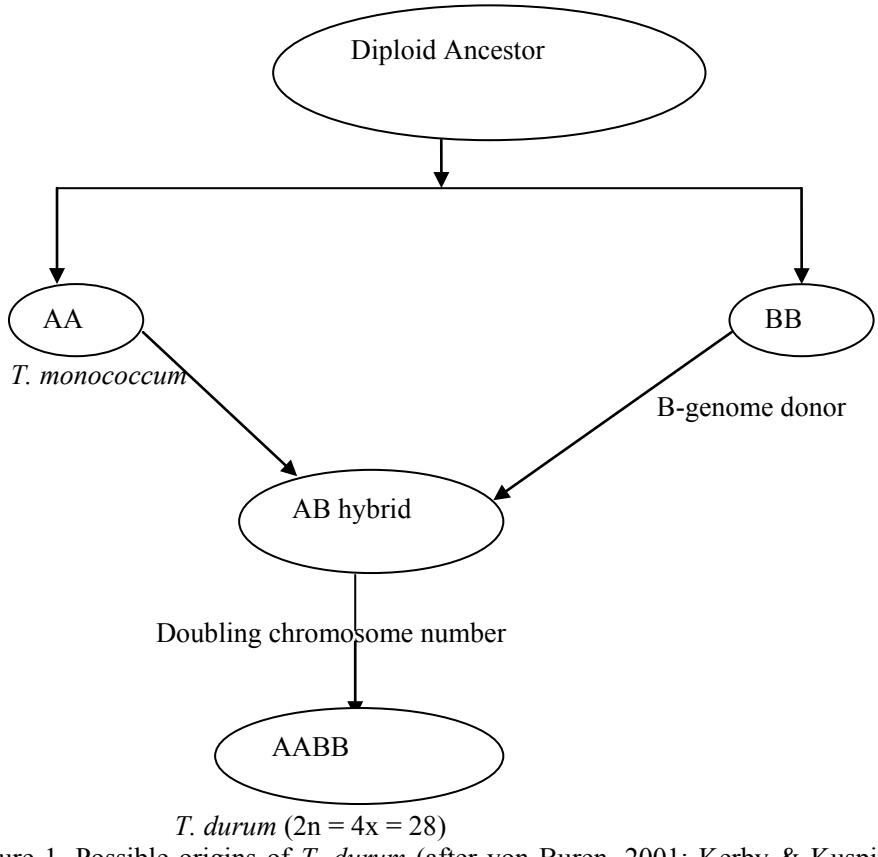

Figure 1. Possible origins of *T. durum* (after von Buren, 2001; Kerby & Kuspira, 1987). Each capital letter represents a genome composed of seven chromosomes.

1.3 Genetic diversity and structure

Knowledge about the distribution and extent of genetic variation within and between populations (accessions) is essential in order to: 1) Estimate any possible loss of genetic diversity; 2) trace the available genetic variability and its potential in breeding programmes; 3) select possible population(s) (accession(s)) for *in situ* and or *ex situ* conservation; and 4) show evidence of the evolutionary forces influencing natural populations (accessions) (Semagn, 2002). Measurements of genetic diversity were initially based on co-ancestry and pedigree records (Kim & Ward, 1997). There are now different means that can be used to assess the genetic diversity. The most widely employed measures of variation within populations are gene diversity or heterozygosity, the number of alleles per locus, the percent of polymorphic loci, allele and genotypic frequencies. For any ploidy level and reproductive system, the Nei (1973) gene diversity index can be used to estimate the diversity that exists in a population.

Based on evaluated sample z marker matrix of traits states that can be used to calculate pair-wise genetic distance matrix, an estimate of genetic proximity involves estimation of distance matrices and multivariate statistics (Harch *et al*., 1997; Brown, 1991; Smith and Smith, 1987). Of the different methods used for calculating the genetic distance between two samples, the Nei genetic distance index (Nei, 1973) is commonly used to analyse genetic diversity for any ploidy level. In order to assess the level of genetic diversity present in germplasm pools and detect genetic relationships among different germplasms and to predict heterosis, a suitable choice of similarity s or dissimilarity coefficient $d = 1 - s$ is important and depends on factors such as: a) the properties of the marker system used, b) the objective/s of the study, c) the operational taxonomic unit (OTU) of the germplasm, d) the genealogy of the germplasm and e) necessary preconditions for subsequent multivariate analyses (Reif *et al*., 2005b). However, the Jacquard and Dice coefficient is commonly used in binary matrix for band presence or absence because it gives more weight to matches than to mismatches and is less biased by the occurrence of a given level of artifactual bands, with a direct biological meaning in estimating the expected proportion of amplified fragment shared by two samples if there is a common ancestor inheritance (Lamboy, 1994; Sneath & Sokal, 1973). A dendrogram/ phenogram linking together clusters of samples that is more genetically similar to each other than to samples in other clusters is produced from the resulting distance matrix, mostly using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) or the Neighbour Joining Method. A process of division, in which all individuals start by being alone in groups of one and then closer groups are merged gradually until finally all individuals form a single group, is used to form clusters.

The proportion of genetic variation distributed into different hierarchies can be quantified using the Nei (1973) parameter Gst, which is equivalent to Wright's Fst (Hamrick & Godt, 1989). These statistical parameters are based on allele frequencies for co-dominant molecular markers. However for dominant markers, the AMOVA principle is used to extract variance components and analogs of F-statistics from a matrix of squared Euclidean distances (Semagn, 2002). The distribution of variation among and within a population or genetic structure is estimated based on total genetic diversity (Ht), genetic diversity within population (Hs), and genetic diversity between populations (Dst). In a species, the pattern of variation can be measured in terms of levels of diversity in different populations and genetic distances between different populations.

Vavilov (1951, 1929) and Zohary (1970) reported the presence of high genetic diversity in cultivated tetraploid wheat and considered Ethiopia to be a centre of diversity and the site of origin of tetraploid wheat. Most of the studies to date on Ethiopian wheat landraces have been based on the diversity of agro-morphological characters, which are highly heritable (Eticha *et al*., 2005; Bechere *et al*., 1996;

Pecetti & Damania, 1996; Abebe & Giorgis, 1991; Tesemma *et al*., 1991; Tesemma & Belay, 1991; Perrino & Porceddu, 1991; Spagnoletti-Zeuli & Qualset, 1987; Negassa, 1986; Jain *et al*., 1975; Porceddu *et al*., 1973), on isozymes (Tsegaye *et al*., 1994) and on cytological markers (Belay & Merker, 1999). There are specific endemic characters for some of the tetraploid wheat species in Ethiopia, such as violet grain and beardless or half-bearded hard durum wheat, which shows great variation from place to place (Teklu & Hammer, 2006; Belay *et al*., 1993; Tesemma *et al*., 1991; Jain *et al*., 1975). The variation in the Ethiopian landraces includes some significant values such as resistance to diseases, pests and various stress conditions. For wheat landraces collected from the central highlands of Ethiopia, Tesemma *et al*. (1991) have found regional diversity, whereas Bechere *et al*. (1996) have reported the absence of such differences between seeds collected from the north central highlands. Messele (2001) has also shown the presence of high diversity across regions and altitudes using morphological, chromosomal and molecular markers. However, in most of these previous studies samples from all regions were not included, since some studies examined samples from the central highlands, whereas others studied samples from the north central highlands. Furthermore, the research was conducted outside the natural habitat of the landraces and qualitative and quantitative traits and different wheat types were lumped together. The studies mentioned above have contributed to the identification of useful materials for plant breeding purposes and to a better understanding of the crop in designing appropriate collection and conservation strategies. However, despite these studies, knowledge of the variation in Ethiopian wheat landraces for biochemical and molecular markers remains limited, which indicates there is still a need for more information on population structure and on potential inputs for breeding.

Harlan (1975) indicated that genetic variation can be attributed to human activities, environmental factors and the dynamic process of hybridisation, segregation and selection. Hence, the variation may be the result of the interaction between abiotic and biotic factors and population characteristics. Physical and biotic environmental factors exert selection pressure on variants, leading to the establishment of specific types, which indicates that the genetic variation is different from place to place depending on differences in the environment. For instance, Porceddu *et al.* (1988) observed a significant differentiation in agro-morphological traits among Ethiopian tetraploid wheat landraces collected from the same regions, but from different places which varied in their edaphic and climatic conditions.

As regards the biological characteristics of a species, population structure and mating system determine the level and pattern of genetic variation. In general, outbreeding increases gene flow, resulting in higher polymorphism and genetic diversity, while selfing restricts gene flow (Hamrick & Godt, 1989). According to Clegg (1980), plant population heterotic selection maintains outcrossing. As hybridisation results in introgression, it is an important factor in the evolution of tetraploid wheat in Ethiopia (Asins & Carbonell, 1989).

1.4 Assessing biodiversity and/or quality

The diversity of *Triticum* species can be determined in different ways, such as the use of agro-morphological markers, molecular markers and chromosomal morphology. The quality of wheat can be regarded as the ability to produce a product suitable for the end-user and the quality is dependent on genetic and environmental factors. There are several variables determining wheat quality, such as grain size, starch content and structure, seed storage protein percentage and composition.

1.4.1 Wheat grain proteins

Previous studies have attempted to determine the content, composition and structure of the grain proteins in wheat (Özbek *et al*., 2011; Bradová & Štočková, 2010; Shewry *et al*., 2002; Belton *et al*., 1998; Johansson, 1996; Johansson *et al*., 1993; Shewry & Tatham, 1990; Uhlen 1990; Shewry *et al*., 1986; Osborne, 1907). Mature wheat grains contain 8-20% protein. Albumins (extractable in water), globulins (extractable in saline water), gliadins (extractable in aqueous alcohol) and glutenins (extractable in dilute acid or alkaline) are components of seed storage proteins in wheat (Bonfil *et al*., 1997; Wall, 1979). The major storage proteins are gliadins and glutenins, which interact in the presence of water to form gluten, the protein complex responsible for the visco-elastic properties that make durum wheat superior for pasta making (Wieser, 2000; Peña *et al*., 1994). The gluten proteins, the gliadins and glutenins, constitute up to 80-85% of total flour protein (Shewry *et al*., 1995). Differences in wheat properties are mainly due to variations in structure, amount and proportion of the different gluten proteins (Moragues *et al*., 2006; Shewry *et al*., 1986). The gliadins have been proposed to be responsible for gluten viscosity. Gliadins are mostly monomeric proteins, and can be divided into α, β, γ and ω on the basis of their relative mobility in polyacrylamide gel electrophoresis (PAGE) (Porceddu *et al*., 1998). Gliadins are encoded by the complex loci *Gli*-1 (γ *and* ω) and *Gli*-2 (α and β), located on the short arm of the homoeologous chromosomes 1 and 6, respectively. Glutenins are aggregated proteins linked by intermolecular disulphide bonds and non-covalent forces. They have high molecular weights of up to several million and determine gluten strength and elasticity. Glutenins consist of high molecular weight glutenin subunits (HMW-GS), *i.e*. A subunits, and low molecular weight glutenin subunits (LMW-GS), which can be further divided into B, C and D subunits (Wieser, 2000; Shewry *et al*., 1986). High molecular weight glutenin subunits are encoded by the complex loci *Glu*-1, located on the long arm of chromosome 1, whereas most of the LMW-GS are encoded by the complex loci *Glu*-3 that is found very closely linked to G*li*-1. Separate loci coding for some intermediate and fast moving B subunits are present on chromosome 1B (Liu & Rathjen, 1996; Clarke *et al*., 1993).

As strong gluten durum semolina is required for production of pasta (spaghetti, macaroni and noodles), the estimation of gluten strength is usually an important selection criterion in a breeding programme. Several methods can be used in order

to determine gluten strength in wheat; one of the cheapest and easiest is the sodium dodecyl sulphate (SDS) sedimentation test (Clarke *et al*., 1993). This test is simple to perform but highly correlated with other tests for gluten strength, can be performed in an early filial generation and requires as little as 1 g of ground grain (Dick & Quick, 1983). Research reports indicate that gliadin band 45 (determined by SDS-PAGE) is associated with high gluten strength, while gliadin band 42 tends to be associated with weaker gluten and poor visco-elastic properties in durum wheat (Kosmolak *et al*., 1980).

Flour containing a large amount of gluten is generally of better quality than flour with low gluten content and is thus suitable for the preparation of pasta and bread (Ruiz & Carrillo, 1995; Clarke *et al*., 1993; Payne & Lawrence, 1983). The disappearance of one band or the presence of a new and the observation of a noticeable change in band intensity help to define qualitative and quantitative effects, respectively (Payne *et al*., 1984).

1.4.2 Agronomic and morphological criteria as markers

Agro-morphological criteria such as the colour and structure of seeds, glume nature, spike density, days to maturity and heading, plant height, thousand kernel weight, *etc*. can be used to study the variation among the tetraploid wheat varieties. For successful conservation and utilisation, knowledge on the nature and extent of the available variation is important. Different studies have dealt with the variability in the Ethiopian wheat landraces for agronomic and morphological characters (Messele, 2001; Belay *et al*., 1993; Pecetti *et al*., 1992; Jain *et al*., 1975). In most cases the studies showed the presence of variation within and between populations and geographical regions.

1.4.3 Molecular markers

During the past few years, detailed genetic and physical chromosome maps have been produced for a variety of organisms. This has led to improvements in the efficiency of conventional plant breeding through the use of indirect selection by molecular markers, linked to the traits of interest. Traditional morphological and agronomic traits are gradually being replaced or complemented by molecular markers. The reason for this change is that molecular markers are virtually unlimited, cover all the genome, are not influenced by the environment and are less time-consuming for the characterisation of new hybrids. In plant systems the DNA markers can be used for germplasm characterisation, genetic diagnostics, characterisation of transformants, study of genome organisation and phylogenetic analyses (Rafalski *et al*., 1996). Extensive genetic maps using molecular markers were initially prepared for tomato and maize, but have also been developed for a variety of other plant systems, including several cereal crops such as rice, barley and wheat (Garcia *et al*., 2004). Since chromosome manipulation is easy in wheat, the crop can be used as a model system to study polyploidy cytogenetics (Gupta *et al*., 1999). Several molecular marker studies on wheat have been published using techniques such as RFLP (Marino *et al*., 1996; Devos & Gale, 1993; Xie *et al*.,

1993; Chao *et al*., 1989), microsatellites (Alamerew *et al*., 2004; Messele, 2001; Röder *et al*., 1998), and AFLP (Messele, 2001; Khan *et al*., 2000; Bohn *et al*., 1999).

Inter Simple Sequence Repeats (ISSR)

Inter Simple Sequence Repeats (ISSR) are semi-arbitrary markers amplified by polymerase chain reaction (PCR) with a microsatellite primer. According to Spooner *et al*. (2005), with the help of ISSR about 10-60 fragments from multiple loci are generated simultaneously, separated by gel electrophoresis, and scored as the presence or absence of fragments of a particular size. ISSR is a simple and quick method that combines most of the advantages of microsatellites and amplified fragment length polymorphism with the universality of random amplified polymorphic DNA (Gupta *et al.,* 1994; Wu *et al*., 1994). As it is a molecular marker that can be used to screen a large portion of the genome without prior sequence knowledge of DNA, ISSR can be used to study genetic diversity and phylogenetic relationships in crop plants, clone and strain identification, as well as in gene mapping (Godwin *et al*., 1997; Zietkiewicz *et al*., 1994).

2. Objectives of the study

The objectives of this thesis work were to:

1. Make a preliminary evaluation of Ethiopian tetraploid wheat germplasm for morphological and agronomic traits.
2. Assess and examine the extent and pattern of diversity of Ethiopian tetraploid wheat germplasm with respect to origin of regions, species and altitudinal classes.
3. Classify the different sets of tetraploid wheat germplasm based on their similarity by means of hierarchical cluster analysis.
4. Identify the traits that account for a large part of the overall diversity in the tetraploid wheat germplasm studied.
5. Characterise Ethiopian tetraploid wheat germplasm for composition of high molecular weight (HMW) ─ glutenin subunits, low molecular weight (LMW) ─ glutenin subunits and gliadins.
6. Characterise Ethiopian tetraploid wheat germplasm using molecular markers.
7. Evaluate the extent and magnitude of genetic erosion of tetraploid wheat in Ethiopia.

3. Materials and methods

3.1. Plant material and experiments

A total of 121 tetraploid wheat accessions, representing 111 landraces and 10 released varieties were used (Papers I and II). The landraces were obtained from seeds collected by the Ethiopian Institute of Biodiversity Conservation (IBC), while the released varieties were obtained from Debre Zeit Agricultural Research Centre (DZARC), Ethiopia. The landraces are collections from different major wheat-producing regions in Ethiopia (Arsi, Bale, Gojam, Gonder, Shewa, Tigray and Wello), which occur at different altitudes. The tetraploid wheat accessions were sown during the 2003 main season at Debre Zeit Agricultural Research Centre, Akaki sub-station (8º52'N; 38º47'E, approx. 2130 m.a.s.l.), in an 11x11 simple lattice design with plot size of 2 rows of 2 m length, 20 cm intra row spacing and 30 cm inter row spacing. Seed rate and fertiliser dose were as recommended by DZARC.

In Paper III, 120 accessions from Ethiopia were analysed for both glutenins and gliadins. A Swedish hexaploid wheat (cv. Sport) was used as reference for the electrophoretic analyses. The tetraploid wheat cultivars Claro De Balazote, Langdon and Mexicali, obtained from Dr Nieto-Taladriz (Spain), were used as a standard for low molecular weight glutenin subunits.

Sixty of the 120 accessions were chosen randomly within each region of origin and used for DNA polymorphism analysis using ISSR primers (Paper IV). In addition, on-farm observations and comparisons of the number of accessions/landraces collected from the field in 2002 and 2010 were used to study the magnitude of genetic erosion (Paper V).

3.2. Data collection and analysis

Data were collected on both qualitative and quantitative phenotypic characters from the field experiment, based on the descriptive list produced by the International Union for the Protection of New Varieties of Plants (Anonymous, 1994). For analysis of ISSR and storage protein composition, polymorphic bands among the accessions were scored. The procedures used are described in detail in the Papers I-V. In general, all data collected were subjected to the following statistical analyses:

- Multivariate analysis involving clustering was carried out to determine the pattern of genetic similarity of the tetraploid wheat accessions used in the study and principal component analysis to identify the traits accounting for much of the general variation.

- Analysis of variance and variance components was carried out to study the diversity and to partition the overall diversity into components.
- Frequencies of qualitative traits and molecular data were used in the form of descriptive statistics to estimate accession/population parameters.
- Relative Shannon-Weaver diversity and Nei's gene diversity index were computed to measure genetic diversity both within and among accessions/populations.
- Interviews and questionnaire surveys were conducted with farmers and on-farm observations were made to study the magnitude of genetic erosion in tetraploid wheat.

4. Summary of results and discussion

4.1. Phenotypic diversity

The landraces were found to have a high amount of genetic diversity, which is useful for conservation and also to facilitate the selection of parents with diverse genetic backgrounds, thereby making crop improvement more efficient (Papers I-IV). Different levels of diversity were observed in the different accessions/populations (Paper I). On the basis of agro-morphological traits, the tetraploid wheats were clustered into 15 groups, consisting of 2 up to 37 accessions. However, nine accessions from Bale, Gojam, Gonder, Tigray and Wello remained solitary (Paper I), which shows that tetraploid wheat is a very diverse crop species in Ethiopia.

The variation in the landraces mainly occurred among the accessions (populations), within and between regions (Papers I-IV). Previous studies on Ethiopian tetraploid wheat also showed the presence of regional variation in different traits (Yifru *et al*., 2006; Pecetti & Damania, 1996; Negassa, 1986). This finding is in agreement with results by Hamrick & Godt (1989) indicating that self-pollinating species maintain high genetic diversity at their polymorphic loci and that most of the variation is found among accessions. Teklu & Hammer (2008) also indicated the presence of a high genetic variation among accessions of Ethiopian tetraploid wheat.

The first 5, 4 and 3 principal components explained a large proportion of the variation among region of origin, species and altitude classes, respectively. Most of the characteristics relevant for the agro-morphological performance of cultivars are complex quantitative traits regulated by several genes. Al-Hakimi *et al.* (1997) developed genotypes with an extreme expression for the number of grains per spike, grains per spikelet and spike length from a cross between *T. durum* and *T. polonicum* and other alien donors. Although the different combinations of agro-morphological traits were found to be important in explaining much of the variation among the accessions according to region of origin, species and altitudinal classes, biomass yield per plot, nature of awn, days to heading, lower glume shoulder width, kernel (seed) colour and stand count at emergence were consistently important in explaining the variation in all the accessions examined here.

The use of phenologic and qualitative traits resulted in the highest Shannon-Weaver diversity index (H′) for awn colour in the Arsi and Bale regions of origin, whereas for the remaining regions of origin the highest H′ was mainly due to plant height (Paper II). Plant height, though it is affected by changes in the environment and interaction of genes with the environment, is a major agronomic character in durum

wheat. The high plant height which is associated with lodging is not considered as a problem in Ethiopia, as the straw can be used for animal feed. The analysis of variance of H′ values showed a significant variation among regions in days to maturity, lower glume shoulder shape, glume hairiness, spike length, kernel length, beak shape and seed texture, whereas significant differences among altitude classes were found for days to heading and glume colour. Ethiopian farmers believe that broad genetic variability is valuable to buffer against adverse environmental conditions in a locality. Thus, by combining two or more superior selected plants of wheat, a cultivar mixture with better yield stability and disease resistance in the locality can be obtained. For the intended use, similar seed colour, glume colour, height and maturity are important. Farmers tend to prefer such a mixture, especially if the grain is white, because it obtains a higher price on the market than mixed-colour seed (personal observation). Analysis of H′ variance among species also showed significant difference in lower glume shoulder shape, awn length and seed texture. This is in agreement with Belay *et al*. (1997), who observed H′ spanning the range from completely monomorphic to highly polymorphic at the population level, but ranging from intermediate to high at the regional level.

4.2. Storage protein composition

Characterisation of Ethiopian tetraploid wheat germplasm for glutenin subunit composition resulted in the identification of novel alleles at the *Glu*-1 locus (Paper III). About 39% of the Ethiopian durum wheat accessions studied contained the Glu-A1x subunit, which is reported to be rare in other durum wheat. Although monomorphism was observed in a number of accessions for gliadin and glutenin subunits in this study, the B genome was found to be more polymorphic than the A genome. This finding is in agreement with results obtained by Alamerew *et al*. (2004) using microsatellites on Ethiopian tetraploid and hexaploid wheat. Furthermore, Ben Amer *et al*. (2001) reported that the microsatellite loci of the B genome are more variable than those of the A genome. Previous researchers have also reported novel alleles of the *Glu*-1 and *Glu*-3 loci (Payne *et al*., 1984, 1980; Randhawa *et al*., 1997, 1995). These novel alleles may be associated with high gluten strength, as gluten content and strength determine the quality of durum wheat. As indicated by Rajaram & van Ginkel (2001), novel alleles observed in germplasm collections can be introduced into cultivated wheat via marker-assisted intergeneric hybridisation followed by introgression or by genetic transformation.

In Ethiopia, at present about 13 500 accessions of wheat are maintained at the Institute of Biodiversity Conservation (IBC). These are mainly landraces, locally adapted genotypes that have evolved because of natural and artificial selection forces over the millennia, which are one of the invaluable heritages that farmers have preserved for many generations and are still using. Although these landraces make up the majority of wheat production in Ethiopia, knowledge about their industrial quality potential is lacking. As Ethiopia is a centre of diversity and origin for tetraploid wheat, useful novel variability for various traits may exist in the

germplasm. Novel alleles encoding gliadins and glutenins may be present and when transferred to hexaploid wheat these might be helpful in improving the bread-making quality of wheat. In addition, there may be variability for other quality traits, such as carotenoid pigments, in the tetraploid wheat germplasm (Hailu & Merker, 2008). Until recently, wheat-breeding efforts in Ethiopia were mainly directed towards improvement and stabilisation of production and productivity. With changing consumer demands, breeding for quality is now gaining importance. One approach for improving the quality of wheat cultivars is by utilising genes from the wild relatives, landraces and primitive forms that can provide valuable genes for disease resistance, high protein content, tillering, drought tolerance and other economically desirable attributes. Thus, progenitors of cultivated species or landraces can be an important source of variability for broadening the genetic base of cultivated crops.

The relationship between storage protein composition and end-use quality of wheat has been well established. Because of its kernel size, hardiness and golden amber colour, durum wheat is well known to produce superior pasta and macaroni products. An essential element of pasta cooking quality is the ability of protein components to interact during pasta processing, resulting in insoluble aggregates and a visco-elastic complex that entraps starch granules, limiting surface disintegration of pasta during cooking. Studies have also indicated that the visco-elasticity of cooked pasta and macaroni is correlated with protein content and type (Royo *et al*., 2009; Damidaux *et al*., 1980; Galterio *et al*., 1993; Kosmolak *et al*., 1980). Pasta produced from high grain protein content (with at least a minimum semolina protein concentration required) and strong gluten durum semolina has superior end-use quality with better nutritive value. Due to their high cooking firmness and increased tolerance to overcooking, durum cultivars with high protein concentration are demanded by modern industries that produce pasta (Liu & Rathjen, 1996).

4.3. Molecular diversity of tetraploid wheat

Determination of Nei (1973) gene diversity index indicated that most of the variation in the Ethiopian tetraploid wheat studied here is among accessions/populations, which is typical of self-pollinating populations. The relatively high value of the total gene diversity (H_T) and the gene diversity among accessions/populations (D_{ST}), combined with the low value of gene diversity within accessions (H_S), indicate that the genetic diversity in self-pollinating species is maintained among accessions (Table 2). Our results show that the use of a qualitative trait as a marker system provides better results than those obtained using other markers. Previous studies also reported that quality traits can be used to study the diversity in tetraploid wheat (Teklu & Hammer, 2008; Belay *et al*., 1997). However, most of the qualitative traits are affected by variation in the environment.

Table 2. Nei gene diversity index of tetraploid wheat germplasm with different marker systems. G_{ST} = Coefficient of gene differentiation, H_T = total gene diversity, H_S = gene diversity within accessions/populations, and D_{ST} = gene diversity among accessions/populations

Marker	G_{ST}	H_T	H_S	D_{ST}
ISSR	0.750	0.401	0.100	0.301
Storage protein	0.810	0.189	0.035	0.154
Qualitative traits	0.805	0.572	0.112	0.460

The analysis of DNA polymorphism with ISSR primers produced 128 polymorphic bands and allowed the separation of the 60 accessions of the tetraploid wheat genotypes (Paper IV). The Nei genetic distance varied from 0.0090 to 0.8574 for all accessions and from 0.045 to 0.138 for region of origin. The pattern of UPGMA clustering using the standard genetic distance for all accessions resulted in the formation of eight major clusters consisting of 2 to 35 accessions, and the formation of four clusters based on region of origin. The clustering pattern for region of origin indicated proximity-based trends, implying a more pronounced germplasm exchange among neighbouring regions than among those further apart. Molecular characterisation of four species of *Triticum* using ISSR revealed that the species were clearly separated. The diversity within species was such that *T. durum* was the most diverse, followed by *T. turgidum*, *T. aethiopicum* and *T. dicoccon* in that order. Moreover, *T. durum* was more closely related to *T. turgidum* than to the other species (Paper IV).

Analysis of wheat landraces conducted at the molecular level revealed high levels of genetic diversity and major genetic differences between landraces and improved materials. This demonstrates that the selective pressure from evolution versus modern plant breeding has formed two independent gene pools. Evaluation of these gene pools allows the discovery of additional genetic variability (William *et al.*, 2008; Hao *et al.*, 2006; Zhang *et al.*, 2006; Reif *et al.*, 2005).

4.4. Genetic erosion

On-farm observations, interviews and questionnaires distributed to the farmers were used to determine the magnitude of genetic erosion in Ethiopian tetraploid wheat (Paper V). Using the calculation scheme, genetic erosion = 100% ─ genetic integrity, *i.e.* the still extant landraces, a genetic erosion up to 100% was detected in *T. dicoccon* in the Gojam and Gonder provinces. Similarly, 100% genetic erosion of *T. polonicum* was observed in Tigray and Gojam. In terms of the overall genetic erosion in the country, 32.0%, 35.3%, 55.9%, 84.4% and 84.0% erosion was found for *T. durum*, *T. turgidum*, *T. aethiopicum*, *T. polonicum* and *T. dicoccon*, respectively. The most important factors for loss of landraces were reduction in land size(cultivated area), displacement by released/modern varieties of hexaploid wheat

and teff, reduced benefit from the landraces, and displacement by other crops and chat. Previous studies (Tsegaye & Berg, 2007b; Teklu & Hammer, 2006; FAO, 1996) also reported the problem of genetic erosion in Ethiopian tetraploid wheats. The observed great diversity in tetraploid wheat in Ethiopia faces a major threat from the pronounced genetic erosion.

5. Conclusions and recommendations

Due to its wide agronomic adaptability, ease of grain storage and the wide range of diverse food products that can be made from its flour, wheat is an important source of calories across the world. A rapid increase in global wheat production has taken place during the past five decades, mainly due to increased productivity rather than an expansion of the cultivated area. However, the assessment of genetic diversity among cultivars is indispensable for plant breeding purposes, since it provides an evaluation of the variation available in different germplasm collections.

The results presented in this thesis indicate the presence of a high genetic diversity among the 121 accessions investigated. A number of these accessions possess gluten subunits, which are positively associated with quality of wheat, and this information can thereby be used for the development of wheat cultivars suitable for pasta-making. In a relatively high proportion of the durum wheat accessions evaluated, Glu-A1x encoded proteins were expressed, which until now have only been reported in a small proportion of other durum wheat collections. If the rate of advance in yields of wheat is to be maintained or even increased, our understanding and ability to manipulate the underlying genetic control of complex characters such as yield, end-use quality and abiotic stresses must be improved. This can only be achieved when there is high genetic diversity in the crop. The predicted occurrence of climate change will affect survival of a trait if that trait cannot adapt to the changing climate, which can occur due to narrowing genetic diversity. However, if there is a high genetic diversity the species will likely to cope with environmental change. Thus, maintaining genetic diversity is key for the survival of species, as well as for identifying novel alleles involved in gluten property determination in the future.

The fact that the high genetic diversity of tetraploid wheat in Ethiopia is being threatened by genetic erosion indicates that priority should be given to the collection and conservation of landraces. This plant material is irreplaceable if lost. The best method of conservation is the use of a complementary approach comprising different *ex situ* and *in situ* conservation techniques. Apart from conservation, creation of sustainable agricultural systems that actively use as much biodiversity as possible should remain the major goal. Crop diversity is one of the most fundamental important resources for human life on earth like the air we breathe and the water we drink. Therefore, broadening the genetic base of crops and increasing the range of genetic diversity available to farmers is crucial for the future survival of mankind.

Based on the results in this thesis, the following recommendations can be made:

1. New alleles involved in gluten properties are continually being found in landraces, creating new opportunities and the need for additional analyses of accessions on a large scale.
2. The correlation between the composition of gluten subunit patterns observed and pasta quality remain to be determined through quality evaluation of pasta produced from the different materials studied.
3. As Ethiopia is a centre of diversity for tetraploid wheat, there is great scope for finding useful novel variability for various traits in the germplasm. Consequently, there is a need to evaluate/characterise the accessions in order to help improve their use in the processing industry in Ethiopia, as the country is currently importing durum wheat to fulfil its demands.
4. The best method of conservation is the use of complementary *ex situ* and *in situ* techniques, accompanied by sustainable agricultural systems. Systems that actively use as much biodiversity as possible should be the major target of the government.

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