Genetic Erosion and Morphological and Molecular Markers Diversity in Ethiopian Tetraploid Wheat Landraces: Implications for Conservation and Utilization

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Dedication

This dissertation is dedicated to my beloved mother Mrs. Atnaf Bogale who gave me everything she has to raise, educate and let me succeed in life.

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Abbreviations, Acronyms, and signs used

AFLP	Amplified Fragment Length Polymorphisms
CA	Cluster Analyses
CIMMYT	International Maize and Wheat Improvement Center
DFA	Discriminant Function Analysis
DST	between-accessions diversity
EARO	Ethiopian Agricultural Research Organization
EST	Expressed Sequence Tags
FAO	Food and Agricultural Organization
GA	Expected Genetic advance
GCV	Genotypic Coefficients of Variation
GST	Nei's coefficient of genetic differentiation
G×E	Genotype by Environment Interaction
h^2B	Heritability in broad sense
HS	Within-Accessions Diversity
HT	Total Genetic Variability
IBC	Institute of Biodiversity Conservation
IPK	Institute of Plant Genetics and Crop Plant Research
MoA	Ministry of Agriculture
MSE	Mean Squares of Experimental Error
MSG	Mean Squares of Genotypes
MSGY	Mean Squares of Genotypes x Year Interaction
NGO	Non Governmental Organizations
NTSYS-pc	Numerical and Taxonomy and Multivariate Analysis system
PCA	Principal Components Analysis
PCs	Principal Components
PCR	Polymerase Chain Reaction
PCV	Phenotypic coefficients of variation
PIC	Polymorphic Information Content
QTLs	Quantitative Traits Loci
RAPD	Random-Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphisms

SAHN	Sequential, Agglomerative, Hierarchical, and Nested
SCAR s	Sequence Characterized Amplified Regions
SG-2000	Sasakawa Global 2000
SIMQUAL	Similarity for Qualitative Data
SPSS	Statistical Package for the Social Sciences
SSR	Simple Sequence Repeats
STS	Single Nucleotide Polymorphism
UPGMA	Un-weighted Pair Group Method Analysis
Vg, σ_g^2	Genotypic Variances
Vph, σ_p^2	Phenotypic Variance
Xgwm	Gatersleben Wheat Microsatellite

Publications

This dissertation is based on the following article based chapters that are cited in the text as follows.

1. Teklu Y. and Hammer K. 2006a. Multivariate analysis of quantitative trait variation in tetraploid wheat landraces. Euphytica (under review).
2. Teklu Y. and Hammer K. 2006b. Genetic variation and association of metric traits in Ethiopian tetraploid wheat germplasm. Journal of Agricultural Sciences (Cambridge) (under review)
3. Teklu Y. and Hammer K. 2006c. Diversity of Ethiopian tetraploid wheat germplasm: Breeding opportunities for improving grain yield potentials and quality traits. Plant Genetic Resources (Cambridge) (under review).
4. Teklu Y. and Hammer K. 2006d. Farmers Perception and genetic erosion of Ethiopian tetraploid wheat landraces. Genetic Resources and Crop Evolution (in press).
5. Teklu Y., Hammer K., Huang X.Q. and Röder M.S. 2006a. Analysis of microsatellite diversity in Ethiopian tetraploid wheats. Genetic Resources and Crop Evolution (in press).
6. Teklu Y., Hammer K., Huang X.Q. and Röder M.S. 2006b. Regional patterns of microsatellite diversity in Ethiopian tetraploid wheat landraces (in press).
7. Teklu Y., Hammer K. and Röder M.S. 2006c. Simple sequence repeats marker polymorphism in emmer wheat (*Triticum dicoccon* Schrank): Analysis of genetic diversity and differentiation. Genetic Resources and Crop Evolution (in press).
8. Teklu Y., Hammer K. and Röder M.S. 2006d. Comparative analysis of diversity indices based on morphological and microsatellite data in tetraploid wheats. Journal of Genetics and Breeding (in press).

Summary

A better characterization and understanding of genetic diversity and its distribution is essential not only to efficiently exploit the available genetic resources in breeding programs but also to design collecting trips and conservation projects. To this end, the genetic diversity present in Ethiopian tetraploid wheat landraces was assessed using morphological and molecular markers. In morphological analysis, 271 accessions were evaluated for 13 pheno-morphic and agronomic traits using several statistical procedures such as Shannon Weaver diversity index and multivariate techniques of clustering, ordination and discriminant function analysis. Results of principal components analysis indicated that the first five principal components with eigenvalues > 1 were able to explain 75.42% of the total variation in the entire accessions. Both cluster analysis and scatter plot 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 function analysis has succeeded in differentiating accessions, with a medium correct classification rate of 60.9 %. Major variables integrating in the DFA were days to heading and spike length. The overall Shannon Weaver diversity index for all traits was 0.74. The partitioning of the total phenotypic diversity into within- and among-regions diversity indicated that the within region diversity was 0.71 and the between diversity was 0.29. In general, phenotypic diversity showed considerable differences for each trait in different geographical regions and altitudinal classes.

Knowledge of the magnitude of genetic variability and relationships between yield and its components would facilitate crop improvement. As a result, phenotypic and genotypic coefficients of variation, heritability, genetic advance, correlation and path coefficient analysis were computed. Phenotypic coefficients of variation were larger than genotypic coefficients of variation for all the traits. The highest phenotypic and genotypic coefficients of variations were recorded for kernels per spike and kernels per plant, respectively. Broad sense heritability among the quantitative traits studied ranged from 18% (spikelets per spike) to 97.59 % (days to maturity). Correlation analysis showed that most genotypic correlations among the thirteen metric traits were higher than the phenotypic correlations. High correlation values, which are positive and highly significant ($P \leq 0.01$) both at genotypic and phenotypic levels, were obtained between

grain yield per plant and biomass yield per plant and also harvest index. Path analysis was computed on six selected traits. The highest direct effect was exhibited by harvest index followed by days to maturity, TKW and kernels per plant. The study indicated that Ethiopian tetraploid wheat could be improved better by selecting genotypes for high harvest index, TKW and kernels per plant than by using grain yield *per se* as a selection marker.

The extent and patterns of microsatellite diversity of 133 Ethiopian tetraploid wheat landraces and 8 introduced cultivars were analyzed using 29 SSR markers. A total of 383 alleles was detected with an average value of 13.14 alleles per locus. Relatively higher number of alleles was observed on B genome than on A genome. A high level of polymorphism and a large number of alleles unique for each species were detected. Accessions collected from the same region were pooled together and number of alleles and gene diversity were calculated over the 29 SSRs for each region to determine regional patterns of diversity in the Ethiopian materials. The highest average gene diversity value was found in Shewa (0.65), followed by Gondar (0.64). No significant correlation was observed between geographic distance and genetic distance. Out of the total 383 different alleles detected, 93 (24.4%) region specific alleles were observed. Ethiopian emmer wheat accessions were compared with 73 emmer accessions collected from 11 countries with a set of 29 simple-sequence repeat markers. High value of mean number of alleles per locus was found in Ethiopian materials (6.95) followed by Iran (4.86), Morocco (4.10) and Armenia (4.03). Ethiopian accessions were clustered in the same group with Yemen and Spain gene pools, indicating its close relationships with these genepools.

Euclidean and Nei genetic distance estimates were calculated from binary phenotypic and molecular data matrices, respectively and the relationships between the two distances were assessed using simple linear correlation, mantel test, cluster analysis and principal component analysis. Results from the Mantel test and simple linear correlation analysis proved that the genetic variability based on microsatellites is not significantly correlated with the variability based on morphological characters. When the two distances were plotted against each other, the resulting scatter plot depicted the presence of a triangular shaped relationship between them. Both cluster diagram and bi-plot of the first two axis

of PCA showed the presence of different patterns of geographical variation between microsatellites and phenotypic evaluations. The lack of concordance between molecular and phenotypic measures of genetic variation suggests molecular measures of genetic diversity cannot exactly predict or substitute phenotypic genetic variability.

Assessing the threat of genetic erosion is very important in order to save the landraces, which are row materials for breeding and are irreplaceable if lost. Nevertheless, even if there are several reports signaling the reduction in the number and area of tetraploid wheat landraces grown in Ethiopia, the extent to which allelic diversity has been lost have not been properly documented. Therefore, genetic erosion was assessed in three districts in eastern Ethiopia. Using the calculation scheme: gene erosion = 100%-gene integrity, i.e., the still extant landraces, a genetic erosion up to 100% was calculated in accessions belonging to the relationships of *T. durum*, *T. turgidum and T. dicoccon*. Number of farmers growing landraces of tetraploid wheats drastically decreased in all surveyed areas in the past decades. Displacement of landraces by other crops was the prominent factor for ending landrace cultivation. Farmers' preference to yield potential and cash crops subsequently reduced the chance of maintaining landraces. The problem of loss of genetic variation through inappropriate maintenance of ex situ collections was also recognized.

Generally, the present works showed the presence of ample genetic variation both at phenotypic and DNA levels. Hence, it is useful to devise appropriate breeding strategies to effectively utilize the readily utilizable genetic polymorphism existing in Ethiopian tetraploid wheats. In addition, it is important to give a due emphasis for collection and conservation of the endangered landraces for future breeding works.

Zusammenfassung

Ein besseres Verständnis der genetischen Diversität und ihrer Verteilung ist nicht nur für eine effektive Nutzung der genetischen Ressourcen in Züchtungsprogrammen notwendig, sondern auch für die Gestaltung von Sammelreisen und Erhaltungsprojekten. Dazu wurde die Diversität bei äthiopischen tetraploiden Weizenlandsorten mittels morphologischer und molekularer Marker untersucht. Für die morphologische Analyse wurden 271 Akzessionen auf 13 morphologische und agronomische Merkmale untersucht. Zur Anwendung kamen statistische Verfahren wie der Shannon Weaver Diversitäts-Index und multivariate Techniken (Clustering, Ordination, Diskriminanz-Analyse). Die Ergebnisse der Hauptkomponenten – Analyse ergaben, dass die ersten fünf Hauptkomponenten mit Eigenvalues >175, 42% der Gesamtvariation aller Akzessionen bestimmen. Sowohl die Cluster-Analyse als auch Scatter-Plot von PC1 gegenüber PC2 zeigten das Fehlen eines klaren Musters einer regionalen Gruppenierung, da die Akzessionen verschiedener Gebiete über viele Cluster verteilt waren. Mit der Diskriminanz-Analyse konnten die Akzessionen mit einer mittelren korrekten Klassifikationsrate von 60,9% differenziert werden. Wichtige Variablen der Diskriminanz-Analyse waren Tage bis zum Ährenschieben und Ährenlänge. Der Shannon-Weaver Diversitäts-Index für alle Merkmale betrug 0,74. Die Aufteilung der phänotypischen Gesamt-Diversität in eine Diversität zwischen den und in eine solche innerhalb der Regionen ergab Werte von 0,71 bzw. 0,29. Im allgemeinen zeigte die phänotypsche Diversität beträchtliche Unterschiede für jedes Merkmal in verschiedenen geographischen Regionen und Höhenklassen.

Die Kenntnis des Ausmaßes an genetischer Variabilität und den Beziehungen zwischen dem Ertrag und seinen Komponenten würde die Pflanzenzüchtung erleichtern. Folglich wurden phänotypische und genotypische Variation, Heritabilität, genetischer Fortschritt, Korrelationen und Pfadkoeffizienten errechnet. Die phänotypischen Variationskoeffizienten waren für alle Merkmale größer als die genotypischen. Die höchsten Variationskoeffizienten, sowohl phänotypisch als auch genotypisch, wurden für Körner je Ähre und Körner je Pflanze gefunden. Heritabilität im weiteren Sinne reichte für die untersuchten quantitativen Merkmale von 18% (Ährchen je Ähre) bis 97,59% (Tage bis zur Reife). Die Korrelationsanalyse zeigte, dass die meisten der genotypischen Korrelationen bei den 13 metrischen Merkmalen höher waren als bei den phänotypischen

Korrelationen. Hohe Korrelationswerte, positiv und hochsignifikant ($P \leq 0,01$), sowohl im genotypischen als auch im phänotypischen Bereich, wurden zwischen Kornertrag je Pflanze und Biomasse-Ertrag je Pflanze sowie auch dem Harvest-Index erhalten. Die Pfadanalyse wurde für sechs ausgewählte Merkmale durchgeführt. Die höchsten direkten Effekte zeigte der Harvest-Index, gefolgt von Tagen bis zur Reife, TKM und Körnern je Pflanze. Die Ergebnisse zeigen, dass äthiopischer tetraploider Weizen besser durch die Selektion von Genotypen auf hohen Harvest-Index, TKM und Körner je Pflanzen verbessert werden kann als durch die Selektion auf den Ertrag direkt.

Das Ausmaß und die Verteilung von Diversität bei den 133 äthiopischen tetraploiden Weizen-Landsorten und 8 eingeführten Sorten wurde mit Hilfe von 29 Mikrosatelliten-Markern untersucht. Insgesamt wurden 383 Allele gefunden mit einem Mittelwert von 13,14 Allelen je Locus. Die relative Anzahl der Allele beim B Genom war höher als die beim A Genom. Es wurde ein hoher Grad von Polymorphismus und eine große Anzahl von Allelen spezifisch für jede Art gefunden. Um die regionalen Muster der Diversität in dem äthiopischen Material zu bestimmen, wurden Akzessionen der gleichen Regionen zusammengefasst und die Anzahl der Allele sowie die genetische Diversität über die 29 SSR Marker für jede Region berechnet. Der höchste Wert für genetische Diversität wurde in Shewa gefunden (0.65), gefolgt von Gondar (0.64). Keine signifikante Korrelation wurde zwischen geographischer Entfernung und genetischer Distanz gefunden. Unter den insgesamt 383 verschiedenen Allelen waren 93 (24.4%) regionsspezifisch. Äthiopischer Emmerweizen wurde mit 73 Emmer-Proben aus 11 Ländern verglichen. Dazu wurden 29 SSR Marker herangezogen. Hohe Werte für die Anzahl von Allelen je Locus wurden im äthiopischen Material gefunden, gefolgt von Iran (4.86), Marokko (4.10) und Armenien (4.03). Die äthiopischen Proben clusterten in der selben Gruppe mit jemenitischem und spanischem Material. Das ist ein Hinweis auf die nahe Verwandtschaft dieser Genpools.

Maße der euklidischen und der genetischen Distanz nach Nei wurden aus binären phänotypischen und molekularen Daten-Matrizen kalkuliert und die Beziehungen zwischen diesen zwei Distanzmaßen wurden untersucht durch einfache lineare Korrelation, Mantel-Test, Cluster-Analyse und Hauptkomponentenanalyse. Die Ergebnisse aus dem Mantel-Test und der einfachen linearen Korrelationsanalyse zeigten, dass die genetische Variabilität auf der Basis von Mikrosatelliten nicht signifikant

korreliert ist mit derjenigen auf der Basis von morphologischen Merkmalen. Die zusammengefasste graphische Darstellung beider Distanzmaße zeigt die Form eines Dreiecks. Sowohl das Cluster-Diagramm als auch der Di-Plot der ersten zwei Achsen der PCA zeigten unterschiedliche Muster der geographischen Variation zwischen Mikrosatelliten und phänotypischen Evaluierungen. Das Fehlen von Konkordanz zwischen molekularen und phänotypischen Maßen der genetischen Variation lässt vermuten, dass molekulare Maße der genetischen Diversität die phänotypische Variabilität nicht genau vorhersagen oder ersetzen können.

Die Erfassung der Generosion ist sehr wichtig für die Rettung der Landsorten, die Rohmaterial für die Züchtung darstellen und deren Verlust nicht ersetzt werden kann. Trotzdem es viele Berichte gibt, die eine Reduzierung in Anzahl und Fläche der äthiopischen tetraploiden Weizenlandsorten signalisieren, ist der Verlust an allelischer Diversität wenig dokumentiert. Deshalb wurde die Generosion in drei Distrikten Ost-Äthiopiens untersucht. Nach dem Schema Generosion = 100% - genetische Integrität, d.h. der noch vorhandenen Landsorten, wurde eine Generosion bis zu 100% für die tetraploiden Weizen berechnet. Die Anzahl der Bauern, die noch Landsorten der tetraploiden Weizen anbauen, hat in den letzten Jahrzehnten in allen Untersuchungsgebieten drastisch abgenommen.

Die Verdrängung der Landsorten durch andere Fruchtarten war dafür der ausschlaggebende Faktor. Die Vorliebe der Bauern für hohes Ertragspotential und Cash-Crops verringerte folglich die Möglichkeit zur Erhaltung von Landsorten. Das Problem des Verlusts an genetischer Variation durch unzureichende Erhaltungsprogramme in den Ex-situ Sammlungen wurde auch erwähnt.

Insgesamt zeigten die Untersuchungen umfangreiche genetische Variationen sowohl auf dem phänotypischen als auch auf dem DNA Niveau. Deshalb ist es nützlich, angemessene Züchtungsstrategien für die effektive Nutzung der genetischen Diversität der äthiopischen tetraploiden Weizen zu entwickeln. Zusätzlich ist es wichtig, den Wert der Sammlung und Erhaltung der Landsorten für zukünftige Züchtungsarbeiten zu betonen.

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 x 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.

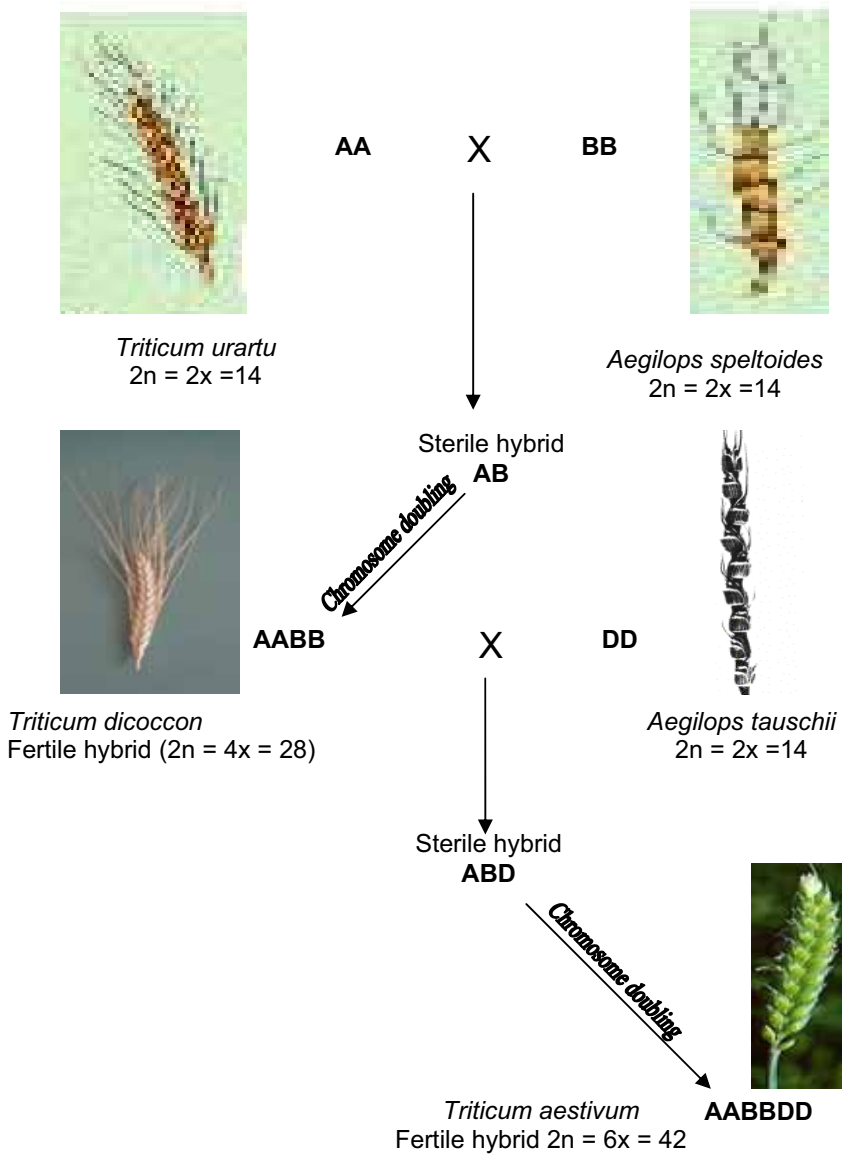


Figure 1.2 Phylogeny of wheat.

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

Table 2.1. Collecting region, number, and accession number of Ethiopian tetraploid wheat landraces used in the study.

Regions of Collections	N	Accession Number
Arsi	24	7021, 7045, 7072, 8298, 216078, 219072, 219076, 222371, 222392, 222393, 222410, 222417, 222427, 222435, 222555, 226865, 226935, 226972, 231231, 231233, 232230, 236974, 236979, 236980
Bale	24	5008, 5229, 204351, 214351, 222299, 222319, 222322, 222328, 226165, 226814, 230675, 230690, 231238, 231467, 231470, 238865, 238885, 238893, 239695, 239698, 239700, 239708, 239712, 239713
Gamugofa	7	7222, 7224, 8284, 8299, 8302, 8310, 222743
Gojam	19	5390, 5487, 5546, 6915, 6916, 6923, 6924, 6928, 6932, 6947, 6953, 6954, 6977, 6980, 7692, 203922, 219510, 226836, 238102
Gondar	27	6843, 6846, 6856, 6917, 7267, 7270, 7274, 7301,7395, 7415, 7935, 8292, 206662, 206672, 214263, 216457, 216491, 216514, 216556, 216557, 216563, 216587, 216610, 222202, 222702, 226244, 226958
Hararghe	29	5101, 5317, 5386, 7329, 7333, 7337, 7345, 7347, 7458, 7460, 7615, 7892, 7893, 7898, 7899, 7901, 7902, 7904, 7911, 7912, 7913, 7920, 7922, 226188, 216831, 219253, 219265, 219268, 231602
Illubabor	1	8482
Kefa	6	8483, 240507, 240508, 240509, 240510, 240511
Shewa	75	5250, 5252, 5314, 5550, 5585, 5605, 5898, 5971, 6137, 6138, 6222, 7113, 7114, 7135, 7156, 7158, 7162, 7213, 7215, 7216, 7220, 7884, 7930, 7931, 7958, 7977, 8013, 8057, 203766, 203964, 208255, 208302, 208782, 209063, 214305, 216070, 216666, 216692, 222195, 222197, 222460, 222497, 226201, 226225, 226238, 226241, 226242, 226272, 226286, 226288, 226293, 226301, 226313, 226326, 226332, 226347, 226378, 226381, 226383, 226391, 226396, 226962, 226968, 229266, 231474, 231511, 231516, 231556, 237867, 238105 2002/Pn 43#165 2002/Pn 55#235 2002/Pn 61#157, Pn 106#104, Pn 61#150
Sidamo	6	8169, 227029, 227030, 227033, 227034, 227037
Tigray	26	5632, 7947, 7948, 7956, 7957, 203931, 204703, 207849, 213307, 216063, 216451, 216452, 216455, 216623, 216629, 216633, 221741, 222608, 238114, 238117, 238118, 238126, 238129, 238130, 238135, 238468
Welega	10	7749, 8480, 8481, 204939, 214528, 214586, 214587, 222474, 222477, 222478
Welo	17	7365, 7370, 7372, 7469, 7508, 7512, 7559, 7563, 7951, 7952, 8245, 8474, 8475, 216619, 222818, 223262, 226942

presented in Figure 2.1. The accessions have also been classified based on the four-altitudinal classes: I (≤ 2000 meters above sea level (masl)), II (2001 –2500 masl), III (2501-3000 masl), and IV (>3000 masl). The number of accessions belonging to these four altitudinal classes is

47, 123, 89, and 12 accessions, respectively. In Ethiopia, wheat is mainly grown under rainfed condition at altitude ranging from 1800-2800 masl. In some parts of the country, it is known to grow at above 3000masl. Consequently, more number of accessions were sampled from altitude class two and three in this study. The experiment was conducted during 2002/2003 and 2003/2004 main cropping seasons at Alemaya University research site (Rare), which is located at 1980masl. A randomized complete block design with two replications was used. Each plot consisted of two rows each 1 m long and 20 cm apart. The distance between blocks and the spacing between plots were 1.5 m and 0.5m, respectively. Fertilization of experimental plots and all other cultural management were done following the recommended cultural practices.

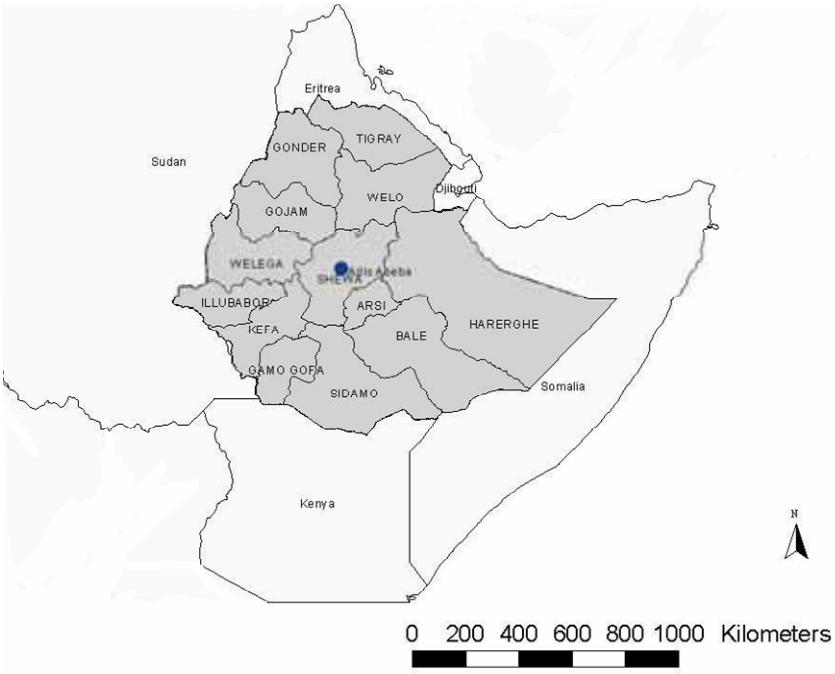


Figure 2.1. Map showing the different regions of Ethiopia and its neighbors.

Data were recorded for a set of 13 quantitative traits. Data for days from emergence to anthesis (DTH) and from emergence to maturity (DTM) were determined on plot basis and grain filling period (GFP) was determined as days between these two phenological traits. Plant height (PH) (cm) and spike length (SL) in cm and spikes per plant (SP), spikelets per spike (SS), kernels per spike (KS), and kernels per plant (KP) in numbers were determined based on five randomly selected plants per plot. Grain yield per plant (GY) and biomass yield per plant (BY), both in grams, and harvest index were assessed on ten randomly selected

plants per plot and 1000-kernel weight (TKW) in gram was determined from dried samples of 1000 grains.

Statistical analysis

Data were analyzed by numerical taxonomic techniques using the procedure of PCA, CA and DFA (Sneath and Sokal 1973) using means of the two years. PCA and CA were performed using values that were standardized to mean zero and a variance of unity to avoid differences in the scale of measurements of the different traits. After data were standardized, the average Euclidean distance was calculated for each accession pair. The resulting distance matrix was subjected to UPGMA to generate a dendogram using SAHN module. Both numerical taxonomic analyses were performed using the computer program NTSYS-pc (Numerical and Taxonomy and Multivariate Analysis system) version 2.0 (Rohlf 1998). PCA was computed using the MINITAB statistical computer package (MINITAB 2000) to provide variable independence and balanced weighting of traits, which leads to an effective contribution of different characters on the basis of respective variation. Only those principal components whose eigenvalues are greater than 1 were chosen. Components with an eigenvalue of less than 1 account for less variance than did the original variable (which had a variance of 1), and so are of little use. Clear guidelines do not exist to determine the significance or importance of a trait coefficient, i.e., eigenvector (Ayana and Bekele 1999). However, Johnson and Wichern (1988) suggested that coefficient or eigenvector greater than half divided by the standard deviation of the eigenvalue of the respective PC is important. This rule was used for weighing the relative significance of different traits constituting the PCs. Discriminant function analysis was conducted using Minitab to verify the validity of classification of accessions based on regions of origin and altitude.

2.3 Results

Principal component analysis

The eigenvectors, eigenvalues and percent of total variance of the five principal components, which is computed using the 271 accessions represented by rows and the values of the 13 traits arranged in the columns as variables, was reported in Table 2.2. The first five components with eigenvalues greater than 1 were able to explain 75.42% of the total variation. In particular, the first component, which explained 25.29% of the total variation, was associated mainly with thousand kernel weight, grain yield, biomass yield, number of spikes per plant, and kernels per plant. The second component, which explained 18.42 % of

the total variation, was attributed mainly due to variations in days to heading, plant height, and days to maturity. The third PC extracted about 14% of the total variation. The main plant traits that accounted for this variation was days to maturity. The fourth and the fifth principal components accounted for 9.4 and 8.3 % of the total variation, respectively. Number of spikelets per spike and harvest index were the most important traits in the fourth and fifth principal components, respectively (Table 2.2).

Table 2.2. Eigenvalues, percent of total variance explained, cumulative variance and eigenvectors of the first five principal components of 13 metric traits.

	Eigenvectors				
Trait	PC1	PC2	PC3	PC4	PC5
Grain Yield	0.759	0.106	0.179	-0.125	0.333
Biomass Yield	0.714	0.427	0.035	-0.204	-0.238
Harvest Index	-0.250	-0.536	0.089	0.139	0.723
Days to Maturity	-0.237	0.573	0.586	0.420	-0.002
Days to Heading	-0.343	0.788	0.276	0.405	0.078
Grain Filling Period	0.521	0.005	0.454	-0.201	-0.277
Plant height	0.277	-0.575	0.447	-0.088	-0.152
Spiklets per spike	-0.320	0.470	-0.016	-0.608	0.223
Spiklet length	0.305	0.087	0.390	0.069	0.154
Kernels per spike	0.484	-0.485	0.238	0.436	-0.098
Spikes per plant	0.692	0.377	-0.433	0.076	0.258
Kernels per plant	0.838	0.166	-0.307	0.229	0.216
Thousand kernel weight	0.078	0.028	0.680	-0.363	0.298
Eigenvalue	3.287	2.395	1.821	1.22	1.081
Percent of total variance explained	25.285	18.422	14.009	9.387	8.312
Cumulative percent of total variance	25.285	43.707	57.716	67.104	75.416

Principal component analysis was also computed using the means of regions of origin and means of altitudinal class for the 13 quantitative characters in order to examine the regional and altitudinal pattern of variation. On regional bases, the first three principal components with eigenvalues greater than 1 explained 77.8% of the total variation (Table 2.3). The first and second principal components collectively explained 66.5 % of the multivariate variation among the regions. The first principal component accounted for 40.84% of this variation,

indicating its greater importance in differentiating the regions of origin of the accessions. The percentage of variation explained by the first principal component and the vector loadings for each agronomic character for the eleven regions of origin and the four altitudinal classes are given in Table 1.4. Considering only those PCs with eigenvalues more than 1, five PCs accounted for about 85, 87, and 91% of total variation in Arsi, Gojam, IKS and Shewa regions, respectively. Except Gondar, which had 6 PCs with eigenvalues greater than 1, the total variation of all the remaining regions were explained by four PCs each.

Table 2.3. Eigenvalues, percent of total variance explained, cumulative variance and eigenvectors of the first three principal components computed based on the 11 regions of origin.

Regions	PC1	PC2	PC3
Arsi	0.26	0.27	0.31
Bale	0.20	0.94	-0.64
Gamo Gofa	0.62	0.07	-0.18
Gojam	-0.14	-0.53	-0.64
Gondar	-0.02	-0.09	0.36
Hararghe	-0.25	0.06	-0.11
IKS	0.92	-0.88	0.02
Shewa	0.72	0.20	0.27
Tigray	-1.00	-0.50	0.21
Welega	-0.98	0.05	-0.13
Welo	-0.34	0.42	0.34
Eigenvalue	4.05	2.51	1.15
Percent of total variance explained	40.84	25.30	11.64
Cumulative percent of total variance	40.84	66.15	77.78

The first PC accounted for about 34.91, 33.75, 39.42, 35.61, 24.56, 27.91, 35.79, 26.32, 36.28, 41.32 and 31.54 % of the variance of Ethiopian tetraploid wheat accessions from Arsi, Bale, Gamugofa, Gojam, Gondar, Hararghe, IKS, Shewa, Tigray, Welega, and Welo, respectively (Table 2.4). The traits that have accounted for the variations in this PC are different from region to region, implying the importance of different traits in determining the variation of Ethiopian tetraploid wheat accessions in the different regions. For example, number of kernels per spike and plant height are among the most important traits governing

the variation in Shewa, where as both traits haven't attributed to the variation of the first PC in Bale. Likewise, number of spiklets per spike is the most important character separating characters on PC1 in Welo but it has a non significant contribution in all the remaining regions excluding Gamugofa, Tigray and Welega. Generally, however, traits like grain yield, biomass yield, number of kernels per spike, number of spikes per plant and number of kernels per plant have high loadings with significant contribution to the variation of accessions along the first principal axis in most regions. Differentiation in these traits was therefore a primary source of overall variation in regional groupings.

Cluster Analysis

Cluster analysis was also performed to examine the pattern of genetic diversity in the 271 Ethiopian tetraploid wheat accessions. CA grouped the 271 accessions into ten main clusters (Table 2.5). Cluster VI and V contained the least (17) and the highest (35) number of accessions, respectively. Mahalanobis distances (Mahalanobis 1936), which is the measure of the extent of genetic diversity between the cluster, was computed. Since Mahalanobis distances is measured in terms of standard deviations from the centroid, therefore a case which is more than 1.96 Mahalanobis distances units from the centroid has less than 0.05 chance of belonging to the group represented by the centroid. Using this statistics, it was found that all the clusters were different from each other. Minimum inter-cluster distance between II and IV (2.16) indicated close relationship among genotypes falling in these clusters. Maximum inter-cluster distance was found between cluster III and VI (6.01) and followed by clusters III and VII (5.74), VII and IX (5.69) and VI and VII (5.18) suggesting wide diversity between these clusters (Appendix 2). Thus, hybridization among accessions drawn from these widely divergent clusters would likely to produce heterotic combinations with high yield potential and improved quality traits. On the whole, there was no clear pattern of regional grouping as accessions of the different regions were distributed patchily over many clusters. For example, accessions from Harerge and Shewa were grouped in all the ten clusters. Accessions from Arsi, Bale, and Gonder were distributed over 9 clusters, while accessions from Gojam, IKS, and Welo were grouped in eight clusters. Even in regions like Welega and Gamugofa where few accessions were represented compared to other regions, accessions were distributed over many clusters indicating the presence of high variation among accessions within a particular region. The overlapping of the clustering patterns of accessions of many of the regions was an indication of lack of strong regional differentiation, which could be partly ascribed to gene flow (Ayana and Bekele 1999).

Table 2.4. Eigenvectors and eigenvalues of the first principal component of the 271 Ethiopian tetraploid wheat accessions based on regions and altitude of collection sites.

Trait	Eigenvectors														
	Regions											Altitudinal Class			
	Arsi	Bale	Gamougofa	Gojam	Gondar	Hararghe	IKS	Shewa	Tigray	Welega	Welo	I	II	III	IV
GY	0.78	0.81	0.86	0.78	0.85	0.75	0.75	0.79	0.80	0.93	0.21	0.74	0.77	0.79	0.50
BY	0.59	0.92	0.94	0.71	0.55	0.88	0.92	0.90	0.78	0.53	-0.13	0.88	0.61	0.81	0.75
HI	0.18	-0.54	-0.18	-0.10	0.15	-0.61	-0.88	-0.38	-0.19	0.14	0.39	-0.52	-0.08	-0.23	-0.60
DTM	-0.41	0.38	0.05	-0.49	-0.28	-0.22	0.16	0.18	-0.09	-0.05	-0.53	-0.21	-0.35	0.08	-0.51
DTH	-0.73	0.40	0.07	-0.46	-0.11	-0.08	0.34	0.21	-0.36	-0.59	-0.85	-0.19	-0.49	-0.04	-0.52
GFP	0.29	0.47	0.56	0.79	0.61	0.60	0.82	0.54	0.68	0.75	0.67	0.62	0.59	0.43	-0.20
PH	0.82	-0.24	-0.06	0.10	-0.16	-0.25	-0.41	-0.09	0.31	0.87	0.57	-0.02	0.38	0.22	-0.19
SS	-0.55	0.11	-0.42	-0.02	0.07	-0.06	0.23	0.06	-0.52	-0.63	-0.85	-0.57	-0.20	-0.21	-0.19
SL	0.39	0.44	0.64	0.33	0.27	0.41	0.31	0.22	0.63	0.40	0.74	0.52	0.31	0.30	-0.03
KS	0.82	0.18	0.70	0.63	0.66	0.32	-0.61	0.15	0.57	0.72	0.81	0.52	0.44	0.43	0.09
SP	0.41	0.94	0.88	0.82	0.85	0.73	0.76	0.77	0.83	0.56	-0.05	0.81	0.65	0.68	0.91
KP	0.78	0.94	0.89	0.87	0.91	0.80	0.63	0.81	0.90	0.91	0.29	0.88	0.82	0.80	0.95
TKW	0.46	0.08	0.72	0.69	0.72	-0.13	0.08	0.37	-0.49	0.58	0.33	0.01	0.01	0.35	-0.40
Eigenvalue	4.54	4.39	5.13	4.63	3.19	3.63	4.65	3.42	4.72	5.37	4.10	4.34	3.24	3.09	3.73
% of variance explained	34.91	33.75	39.42	35.61	24.56	27.91	35.79	26.32	36.28	41.32	31.54	33.41	24.93	23.80	28.67
Cumulative % of total variance	84.86	80.32	94.74	86.77	85.13	73.32	90.82	75.82	74.70	90.55	82.54	84.46	76.26	76.12	90.71
No. of PCs with eigenvalues > 1	5	4	4	5	6	4	5	5	4	4	4	4	5	5	5

Table 2.5. Regional distributions of the 271 tetraploid wheat landraces over ten clusters

Regions	Clusters										Total
	I	II	III	IV	V	VI	VII	VIII	IX	X	
Arsi	1	3	1	3	6	2	2	2	4	1	25
Bale	8		2	1	2	4	1	1	4		23
Gamugofa	1		1	2	1	1	1				7
Gojam	2		2	1	7		3	1	1	2	19
Gondar		3	1	2	5	2	1	7	5	1	27
Hararghe	9	2	4	1	4	1	1	2	3	2	29
IKS	2	1	3	4	1			1	1		13
Shewa	4	21	8	17	4	5	4	3	8	1	75
Tigray	2	1	3		2		6	6		6	26
Welega	2				1		1	2	2	2	10
Welo		1	1		2	2	2	1	4	4	17
Total	31	32	26	31	35	17	22	26	32	19	271

The clustering pattern on the 271 accessions grouped according to the four altitudinal class of collection also showed a scattered distribution over the ten clusters (Appendix 3). Accessions belonging to the altitudinal class I, II and III have been found in all ten clusters indicating the presence of high diversity within each altitudinal classes. Thus, the study does not suggest any distinct pattern of clinal grouping in Ethiopian tetraploid wheat germplasm.

Accessions belonging to the same regions were pooled to obtain means of each character for the eleven regions of origin and compute cluster analysis. The UPGMA dendogram, which was constructed using Euclidean distance, differentiated all regions of collection (Figure 2.2). It revealed the presence of close relationship between Arsi and Gondar, Harerge and Welo, and Tigray and Welega. IKS was distantly clustered from the other regions indicating the distinctiveness of accessions from this region which might be

attributed to the fact that it contains accessions collected from wider range of geographical areas, i.e., from Illubabor, Keffa and Sidamo.

Table 2.6. Summary of discriminant analysis of the 271 Ethiopian tetraploid wheat accessions based on region of origin

Put into region			True region										
		N	1	2	3	4	5	6	7	8	9	10	11
1	Arsi	24	7	2	0	0	0	1	0	11	3	0	0
2	Bale	24	4	15	0	0	0	1	0	4	0	0	0
3	Gamougofa	7	0	1	4	0	0	0	0	1	1	0	0
4	Gojam	19	1	1	0	10	3	1	0	1	2	0	0
5	Gondar	27	0	3	0	0	15	2	0	1	6	0	0
6	Hararghe	29	2	1	0	0	3	19	0	2	2	0	0
7	IKS	13	0	1	0	0	0	2	5	4	1	0	0
8	Shewa	75	0	1	0	0	0	5	0	64	5	0	0
9	Tigray	26	0	0	0	0	0	6	0	6	14	0	0
10	Welega	10	0	2	2	0	0	2	0	0	1	3	0
11	Welo	17	0	2	1	0	0	1	0	1	3	0	9
% of correct regional classification			29.2	62.5	57.1	52.6	55.6	65.5	38.5	85.3	53.8	30.0	52.9

From the PCA results based on means of region for the 13 metric characters, a two dimensional canonical plot of PC1 versus PC2 was constructed (Figure 2.3). All regions were spatially separated in the two-dimensional scatter plot. Particularly, IKS, Tigray and Welega were ordinated far from others showing their distinctness form the other regions. In general, the PCA result confirmed those obtained by cluster analysis. As with the dendogram, the PCA plot placed accessions from IKS, Tigray and Welega distantly from the others in the loading plots.

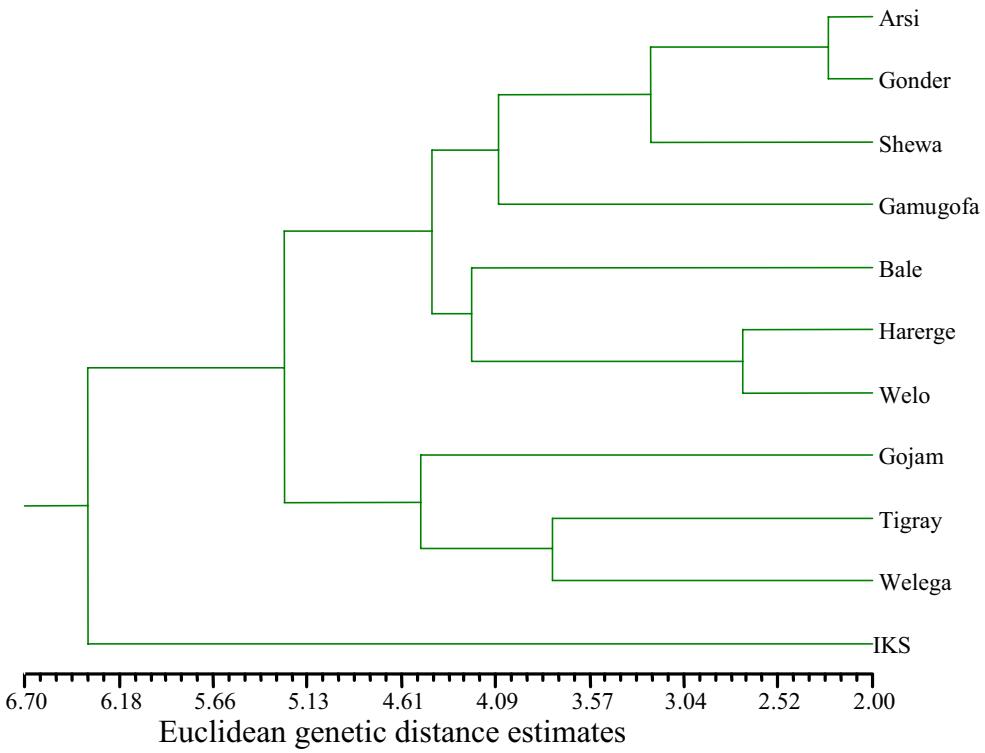


Figure 2.2. Phenogram showing the clustering patterns of the eleven regions of origin of Ethiopian tetraploid landraces.

Discriminant analysis

Discriminant analysis, also known as supervised classification (Ripley 1996), was used to assess the degree of separation of the accessions by multivariate measurements and to examine the impact of individual variables on the discrimination. The mathematical objective of discriminant analysis is to weigh and linearly combine the discriminating variables in some fashion so that the groups are forced to be as statistically distinct as possible (McLachlan 1992). In the analysis, variables measured on individuals whose grouping is known are combined to construct a new variable that can be used to classify accessions. The number of accessions from a given region that are placed in the correct respective region, where the original collection was made, by discriminant analysis is termed the rate of correct classification. In this study, the analysis was applied to the means of 271 accession and 13 measured phenotypic traits (predictors) and generated a

medium discriminatory function, allowing the correct classification of 60.9%. The groups that displayed the highest values of correct classifications were the accessions that were collected from Shewa and Harerghe (85.3 and 65.5%, respectively). The lowest value was displayed by the group of accessions from Arsi and IKS (29.2 and 38.5%, respectively) (Table 2.6). In all the remaining regions, the percent of correct classification greater than 50% was obtained. Although there is no an established standard of accuracy that has been defined for effective predictive value of discriminant analysis in differentiating accessions in genetic diversity studies, a discriminant function which ensures a prediction probability of more than 50% is acceptable. Therefore, the percent of correct classification obtained in this study is sufficient enough in differentiating accessions.

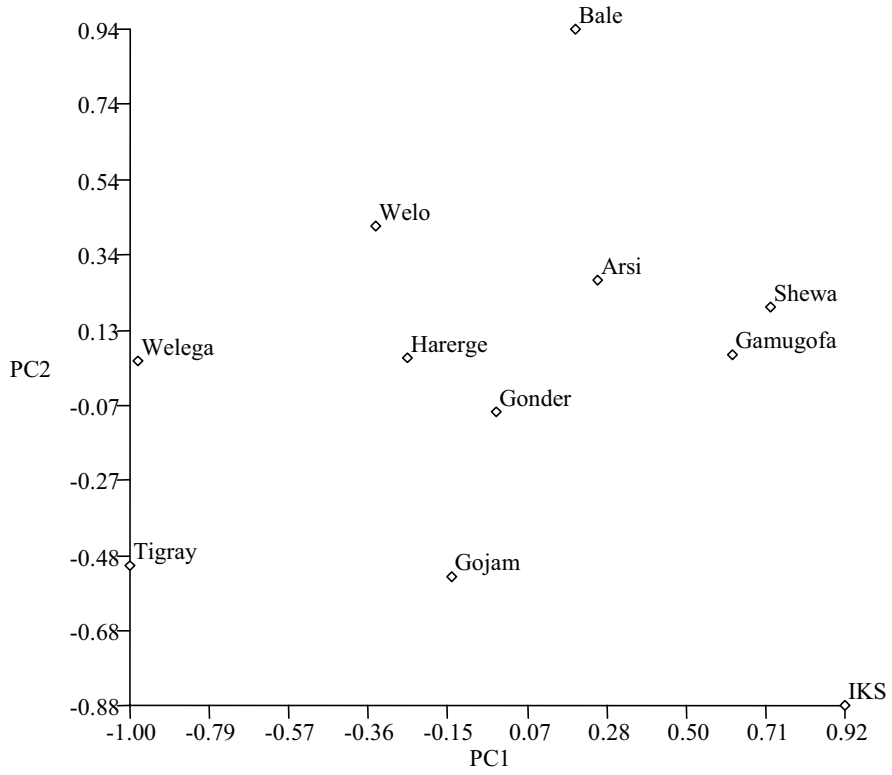


Figure 2.3. Two-dimensional representation of grouping patterns among the 11 regions of origin derived from principal component analysis of the 13 quantitative traits.

Discriminant analysis, using the altitudinal classes as a classifying variable, revealed that 78.6 % of the 271 accessions were correctly placed in their respective altitudinal classes (Table 2.7). A correct classification rate of 83.0, 75.6, 80.9, and 75.0% was detected for altitudinal class I, II III and IV, respectively.

Table 2.7. Summary of discriminant analysis of 271 Ethiopian tetraploid wheat accessions by altitude.

			True altitudinal class classification			
Put into altitudinal class		N	I	II	III	IV
1	I	47	39	5	2	1
2	II	123	13	93	17	0
3	III	89	2	9	72	6
4	IV	12			3	9
% of correct altitudinal class classification			83.0	75.6	80.9	75.0

2.5 Discussion

Characterization of accessions for important traits will facilitate efficient synthesis of breeding populations that are designed to accomplish specific objectives. Knowledge of the extent of variability for plant traits and association of specific traits with geographic origin will help to define needs and locations for future collection of wheat germplasm. Multivariate techniques of clustering, ordination, and discriminant function analysis were used to investigate the diversity present among the 271 Ethiopian tetraploid wheat accessions. The first two components of the 271 accessions have explained 43.71% of the total variation. Traits like thousand kernel weight, grain yield, biomass yield, number of spikes per plant, and grain filling period in the first principal component and days to heading, plant height, and days to maturity in the second component have high loadings their importance for wheat as descriptors. The traits attributed for the variation of the first principal component in the different regions are different. The heterogeneous distribution of diversity over regions and the uniqueness of certain geographical regions suggest that collection of germplasm should include as much area as possible rather than

concentrating on limited sites (Engels 1994). Similar findings were reported in tef by Kebebew et al. (2003) and mentioned the importance of taking such fact in future germplasm collection and conservation programs.

Both cluster analysis and the two-dimensional scatter plot of PC1 versus PC2 revealed the absence of clear pattern of regional grouping as accessions of the different regions were distributed patchily over many clusters. According to Bekele (1984), this phenomenon was attributed to presence of high level of migration (gene flow) among regions where wheat grows as a polymorphic population, a factor increasing hybridization rate, resulting in population genetic interconnections, and thereby building up a wealth of genetic variation with similarities and dissimilarities depending on regional activities of evolution. Geographical separation of populations, a parameter usually considered important when collecting germplasm, also did not predict genetic differences very well in the study of Fahima et al. (1999) and del Rio et al. (2001). The absence of remarkable differences in diversity among the different provinces is in agreement with the findings of several morphological diversity studies in Ethiopian wheats (Bekele 1984; Negassa 1986, Bechere et al. 1996; Bechere et al. 1996; Pecetti and Damania 1996). It also concurs with the observations in Ethiopian sorghum (Teshome et al. 1997; Ayana and Bekele 1999).

In Ethiopia, tetraploid wheats are an indigenous crop that has been under cultivation since ancient times. The wide diversity for phenotypic traits in different regions may also be attributed to the long histories of its cultivation under diverse agro-climatic condition that have resulted in accumulation of changes as a result of sexual recombination, migration and perhaps mutation, with subsequent selection by farmers in geographical isolation for adaptability under various agro-ecological regimes and cropping systems. Major variables integrating in the discriminant function analysis, which yielded 60.9% of correct classification, were days to heading and spike length. Characters such as days to 50% flowering and maturity and plant height are also more important in determining sorghum adaptation to a particular ecological zone (Ayana and Bekele 1999). These authors explained the greater importance of such adaptive characters like plant height and

days to 50% flowering in differentiating accessions of different regions in principal component analysis and the grouping together of accessions belonging to regions having similar agro-climatic conditions in cluster analysis as the indication of the relevance of environmental factors in governing the structure of morphological variation.

As with the present study, Pecetti and Damania (1996) found a higher percentage of correct classification in wheat from Shewa. In sorghum, Ayana and Bekele (1999) observed a greater percentage of correct classification in Shewa. Shewa is a region in Ethiopia where tetraploid wheats are widely cultivated. It has favorable climatic conditions for wheat cultivations. However, Jaradat (1991) found that the correct classification in a discriminant analysis of durum wheat landrace genotypes from Jordan was highest in the driest districts of collection. Contrary to the present study, classification based on altitude resulted in less percentage of correct classification than regional classification in tef germplasm populations (Kebebew et al. 2003). The higher proportion of correct regional than clinal classification can presumably be attributed to the association of greater misclassification with an increase in the diversity of the group (Pecetti and Damania 1996). Nevertheless, Ayana and Bekele (1998) found that the discrimination of sorghum accessions was more pronounced when discriminant analysis was based on adaptation zones rather than regions of origin.

Overall, the discriminant analysis result of the present study is medium. An increase in number of predictor markers could result in a decrease in misclassification. Hence, future analysis should use more characters (predictors) to increase the percentage of correct predictions. As the number of phenotypic traits increases in a comparison of breeding pools, the number of genes involved in the control of phenotypic traits would increase accordingly and, thereby, improve the utility of phenotypic diversity in predicting genotypic diversity.

The genetic diversity available worldwide should be exploited by seeking sources of desirable attributes for use as donors in breeding programmes. Their use for crop improvement would justify the high investments in time, effort and finance for the

collection and preservation of landraces and old cultivars (Damania et al. 1997). Knowledge of adaptive traits linked to certain ecological conditions helps in choosing sites for *in situ* conservation that need to be integrated with *ex situ* conservation (Ayana and Bekele 1998; Hammer 2004). Thus, it is important to examine the association of useful phenological, agronomic and morpho-physiological traits with eco-geographical variables in Ethiopian tetraploid wheats. Such information could be helpful to effectively utilize the ample wheat genetic diversity in the country.

Chapter 3 Genetic variation and association of metric traits in Ethiopian tetraploid wheat germplasm

3.1 Abstract

Knowledge of the magnitude of genetic variability and relationships between yield and its components would facilitate crop improvement. As a result, phenotypic and genotypic coefficients of variation, heritability, genetic advance, correlation and path coefficient analysis were computed for thirteen quantitative traits in 271 Ethiopian tetraploid wheat landraces. The experiment was conducted for two main cropping seasons. Combined analysis of variance over years revealed the presence of significant variations for all characters. Phenotypic coefficients of variation were larger than genotypic coefficients of variation for all the traits. The highest phenotypic variations were recorded for kernels per spike followed by thousand kernel weight (TKW), kernels per plant, and harvest index. Genotypic coefficient of variation was higher in kernels per plant followed by TKW, days to maturity and kernels per spike. Only TKW, kernels per plant, kernels per spike and the three phonological traits possessed more than 10% variation both phenotypic and genotypic levels. Broad sense heritability among the quantitative traits studied ranged from 18% (spikelets per spike) to 97.59 % (days to maturity). To predict the selection effects precisely, heritability accompanied with genetic advance (GA) is more useful than heritability alone. Yield components such as kernels per spike, spike length, and TKW have possessed moderately high heritability combined with high genetic advance. Correlation analysis showed that most genotypic correlations among the thirteen metric traits were higher than the phenotypic correlations. High correlation values between grain yield per plant with biomass yield per plant and harvest index that were positive and highly significant ($P \leq 0.01$) were obtained both at genotypic and phenotypic levels. Path analysis was computed on six selected traits. The highest direct effect was exhibited by harvest index followed by days to maturity, TKW and kernels per plant. Plant height showed negative direct effect on yield, which could be ascribed due to the negative indirect effect through spikes per plant and days to maturity. The present study indicate that Ethiopian tetraploid wheat could be improved better by selecting

genotypes for high harvest index, TKW and kernels per plant than using grain yield *per se* as a selection marker.

3.2 Introduction

Tetraploid wheat is an important commodity crop in Ethiopia, which could contribute a major part in achieving the country's agricultural objective of securing food grain self-sufficiency (Teklu 1997). The crop is wholly utilized. The grain is used for the manufacture of flour for different purposes. Bread, biscuits and pasta products such as macaroni and spaghetti are some of the industrial products. Wheat grain is known to be a major source of energy and protein in the country for subsistence farmers. Traditionally, it is used for making “dabo”, “dabokolo”, “ganfo”, “kinche” and other types of food (Bechere et al. 2000). The straw is a good source for animal feed and it is also used for thatching roofs (Teklu 1997). The potential of wheat will be that it will enter export market if production is expanded and productivity is increased.

In spite of its important economic values, there are many reports indicating the decline of population size and area of tetraploid wheats (Hailu 1991; FAO 1996a, Teklu and Hammer, 2006d). Displacement of landraces by improved varieties of bread wheat was the prominent factor for ending landrace cultivation (Teklu and Hammer 2006d). Heisey and Brennan (1991) reported that most of the studies have come to a conclusion that yield (or yield potential) is the most important criterion for the choice of a variety by a farmer. Grain yield is a complex character and is the multiplicative end product of many yield components as it is polygenically controlled. Therefore, for efficient genetic improvement of cultivars through selection, information on nature and magnitude of variation in the breeding materials, association of characters with yield and among themselves and the extent of environmental influence on the expression of these characters are necessary. Since economically important plant characters are largely, if not entirely, quantitatively inherited, they can be evaluated by using biometrical techniques (Araujo and Coulman 2004). Biometrical evaluation permits the estimation of population genetic parameters such as means, genetic and environmental variances, heritability, genetic and phenotypic correlation coefficients, and expected genetic advances from

selection (Araujo and Coulman 2004). Heritability is an approximate measure of the expression of a character. The genotypic and phenotypic correlation coefficients are measures of the degree of closeness of the linear relationship between pairs of variables that provide information about yield components and their genetic association with one another. Although correlation coefficient is very important to determine traits that directly affect grain yield, they are insufficient to determine indirect effects of these traits on grain yield (Bhatt 1973). Therefore, it is essential to determine the effects of yield components on grain yield using path coefficient analysis. Path analysis enables breeders to rank the genetic attributes according to their contribution (Dewey and Lu 1959). To increase yield, study of direct and indirect effects of yield components provides the basis for its successful breeding programme and hence the problem of yield increase can be more effectively tackled on the basis of performance of yield components and selection for closely related characters (Choudhry et al. 1986).

There is renewed interest in wheat landraces and primitive cultivars as important sources of genetic variation (Brush 1995) mainly because of the trend toward greater uniformity that has narrowed the genetic base of modern wheat cultivars, thus increasing their vulnerability to biotic and abiotic stresses (Moghaddam et al. 1997). Ethiopian tetraploid wheats are characterized by low but stable yield (Tessema 1991). Lakew et al. (1997) confirmed the presence of individual genotypes within landraces which have a yield potential comparable with the best breeding lines and sources of disease resistance justifying the need for developing a routine methodology to use the large collection of landraces available in breeding programs. Because landrace cultivation is still predominant in the country (Bechere et al. 1996; Tessema et al. 1993), the first step in breeding should be to maximize the utilization of these indigenous materials (Tessema et al. 1993). To alleviate the problem of displacement of landraces by improved varieties of bread wheat, tef and other crops, development of high yielding varieties of tetraploid wheat is essential to compete economically with improved varieties of other crops and thereby boost the production trend of this crop (Bechere et al. 2000; Teklu and Hammer, 2005d). According to Tarekegn (1994), cultivar development has contributed a lot in improving yield potential of wheat in Ethiopia. He also found the absence of a yield

plateau and indicated the possibly of further gains in grain yield potential. The key to such genetic improvement of grain yield potential, however, is effective utilization of genetic variability that requires clear understanding of the magnitude of variances in the breeding material. The present study was, therefore, undertaken using 271 Ethiopian tetraploid wheat accessions that represent the entire geographical regions of the country with the following objectives: (i) to estimate variability, heritability and genetic advance for yield and yield contributing traits, ii) to determine the association between yield and yield components, and iii) to quantify the direct and indirect effects of selected yield components on grain yield. Results have been discussed in relation to crop improvement.

3.3 Materials and methods

Plant materials and data collection

Plant materials used, data collection and other experimental procedures are as described in Teklu and Hammer (2005a).

Data Analysis

All measured variables were subjected to analysis of variance procedures to assess differences among varieties. Analysis of variance was carried out following the standard procedure given by Gomez and Gomez (1984). The homogeneity of error mean squares of separate analysis of variance for year was performed using Hartleys test (Rangaswamy 1995). Mean value over the two years for each character was used to determine descriptive statistics like the range, arithmetic means and standard errors of means for each of the variables. Heritability in broad sense (h^2B) was estimated as the ratio of the genotypic variance (V*g*) to the phenotypic variance (V*ph*) on accession mean basis following the procedure given by Allard (1999) and Fehr (1987). Mean squares from the combined analyses of variance over the two years were used to estimate phenotypic variance (σ_p^2) and genotypic variances (σ_g^2) as follows.

$\sigma_g^2 = \left[\text{MSG} - \left(\text{MSGY} - \text{MSE}\right)/\text{r} - \text{MSE}\right]/\text{ry}$, and

$\sigma_p^2 = \sigma_\text{g}^2 + \sigma_\text{gy}^2/\text{r} + \sigma_\text{e}^2/\text{ry}$

Where: MSG, MSGY and MSE are the mean squares of genotypes, genotype x year interaction, and experimental error; r and y are the numbers of replications and years; and σ^2_{gy} and σ^2_e are genotype x year interaction and error variances, respectively.

Procedures given by Johanson et al. (1955) was followed to calculate phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) as:

$$PCV = (\sigma_p / \bar{x}) \times 100$$

$$GCV = (\sigma_g / \bar{x}) \times 100$$

where σ_p, σ_g and $\bar{x}$ are the phenotypic, genotypic standard deviations, and grand mean of character from the combined analysis of variance

c) Expected Genetic advance (GA) and GA (as per cent of the mean) assuming selection of the superior 5% of the genotypes were estimated as per Fehr (1987) as:

GA = K (σ_p) (h^2B), and

GA (as% of the mean) = (GA/$\bar{x}$) × 100

where K is a constant (with 5% selection intensity it is equalt to 2.06), σ_p is the phenotypic standard deviation, h^2B is the broad sense heritability value, and $\bar{x}$ refers to the grand mean of the character. Correlation coefficient were determined as described by Singh and Chaudhry (1979) whereas path coefficient analysis was made according to Dewey and Lu (1959).

3.4 Results

Descriptive statistics and analysis of variance

Descriptive statistics such as the minimum, maximum, mean and standard error of a mean and mean squares from the combined analysis of variance for the 13 quantitative traits measured were presented in Table 3.1. The combined analysis of variance results revealed that the genotypic differences among genotypes were highly significant ($P \leq 0.05$) for all the characters. Also, the range of variation, which is the difference between the maximum and minimum values of each character, with regards to 13 quantitative traits indicated the presence of wide variation for all quantitative variables. Among traits,

grain yield per plant ranged from 3.7 to 9.2 gm/plant with a mean value of 8.3 gm. Days to maturity, days to heading, and grain filling period have shown range values of 30, 31, and 22 days, respectively. Similarly, the differences between the maximum and minimum mean values in biomass yield per plant, harvest index, plant height, spikelets per spike, spike length, kernels per spike, spikes per plant, kernels per plant, and TKW were 18.5 gm, 32.45, 29.7 cm, 11.4, 5.2, 26.8, 5.9, 168.7, and 28.8gm.

Table 3.1. Summary of descriptive statistics and mean squares of characters from combined analysis of variance.

Trait	Descriptive statistics				Mean squares		
	Minimum	Maximum	Mean	S.E. of a mean	Genotype (270)§	Genotype x Year (270)	Error (540)
GY	3.70	9.20	7.93	0.05	8.15**	4.06	2.11
BY	11.50	30.00	24.35	0.10	17.44**	9.15	5.90
HI	15.86	48.31	36.78	0.20	47.01**	24.25	6.50
DTM	127.0	157.00	119.17	0.16	107.62**	2.59	2.40
DTH	86.00	117.00	98.16	0.18	46.54**	4.61	2.70
GFP	32.00	54.00	41.04	0.11	28.59**	9.32	4.16
PH	70.20	99.90	91.08	0.20	55.66**	20.50*	10.35
SS	16.00	27.40	20.26	0.08	7.50**	4.92**	1.95
SL	9.10	14.30	8.89	0.04	4.36**	1.50**	0.48
KS	25.00	51.80	10.53	0.18	10.09**	4.28**	2.70
SP	3.00	8.90	5.99	0.05	4.86**	3.49	2.99
KP	75.00	243.70	133.50	2.11	142.00**	29.00	9.70
TKW	23.10	51.90	35.11	0.14	43.98**	13.62	6.56

§ Numbers in parenthesis represent degrees of freedom

*,** Mean squares of characters were significant at the probability level of 0.05 and 0.01, respectively.

Genotypic and phenotypic variability

Phenotypic coefficients of variation were larger than genotypic coefficients of variation for all the traits indicating the influence of environmental effect (Table 3.2). A narrow difference between these two variances was recorded for days to maturity (0.47), days to

heading (1.17), spike length (4.22), kernels per plant (5.43) and plant height (5.62) indicating less environmental influence on the phenotypic expression of these characters. On the other hand, a wide difference between PCV and GCV was observed for spikes per plant (14.56), Harvest Index (16.48), and kernels per spike (24.41) indicating higher influence of environment on these characters and thus, selection on the phenotypic basis would not be effective for the genetic improvement of such traits. Traits having low values for the difference between phenotypic and genotypic coefficients of variation suggest that they are primarily due to genetic effects (Ahmed et al. 2003). The highest phenotypic variations were recorded for kernels per spike followed by TKW, kernels per plant, and harvest index. Genotypic coefficient of variation was higher in kernels per plant followed by TKW, days to maturity and kernels per spike. Only TKW, kernels per plant, kernels per spike and the three phonological traits possessed more than 10% variation both at phenotypic and genotypic levels. In agreement with the present results, Ehdaie and Waines (1989) reported high genotypic and phenotypic coefficients of variation for number of spikes per plant, number of grains/spike, TKW, harvest index and grain yield/plant, respectively.

Heritability and genetic advance

A character can be improved only if it is highly heritable. Hence, heritability estimates (broad sense) is used to determine the heritable proportion of the total genetic variation. Broad sense heritability estimates ranged from 18 (spikelets per spike) to 97.59 % (days to maturity). Of the remaining traits, heritability estimate greater than 50% was detected in kernels per plant (78.09 %), TKW (66.33%), grain filling period (64.17 %), spike length (61.05%), plant height (59.47%) and kernels per spike (53.95%). To predict the selection effects precisely, heritability accompanied with genetic advance (GA) is more useful than heritability alone (Johnson et al. 1955). Therefore, genetic advance was also computed as percentage of mean. The results indicated that maximum genetic advance was found in kernels per spike (16.09%), followed by spike length (13.88%), TKW (12.38%) and spikes per plant (9%) (Table 3.2). The GA values suggest that population means for most of the characters evaluated may be improved substantially by selecting the superior 5% of the materials considered. Interestingly, grain yield components such as

kernels per spike, spike length, and TKW have possessed moderately high heritability combined with high genetic advance. On the other hand, days to heading and maturity, which have high heritability values, have also moderately high genetic advance suggesting the possibility of further gains through selection. This concurs with the findings of Belay et al. (1993) who reported intermediate to high order estimate combinations of GCV, heritability and GA (as % of the mean) for grain yield per plant, number of kernels per spike, harvest index and TKW. In this study, traits like spikelets per spike exhibited low values of both heritability and genetic advance. Low genetic advance as percentage of mean indicated little scope for further improvement through individual plant selection.

Table 3.2. PCV and genetic GCV, components of variance, h^2B, GA and GA as percent of the mean of characters.

			Estimates of components of variance			h^2B		GA
Trait	PCV	GCV	vp	Vg	Vgl	(%)	GA	(% of mean)
GY	20.10	8.73	1.79	0.78	0.97	43.46	1.20	13.43
BY	16.24	6.85	3.95	1.67	1.63	42.15	1.73	7.09
HI	25.92	9.44	9.53	3.47	8.88	36.41	2.32	6.30
DTM	19.32	18.85	26.88	26.23	0.10	97.59	10.42	7.49
DTH	11.61	10.44	11.40	10.24	0.96	89.89	6.25	6.37
GFP	15.84	10.17	6.50	4.17	2.58	64.17	3.37	8.21
PH	13.88	8.26	12.65	7.52	5.08	59.47	4.36	4.78
SS	6.19	0.12	1.25	0.02	1.48	18.21	0.46	2.27
SL	10.83	6.61	0.96	0.59	0.51	61.05	1.23	13.88
KS	41.42	17.01	4.36	1.79	2.04	53.95	1.69	16.09
SP	19.25	4.69	1.15	0.28	0.25	24.37	0.54	9.00
KP	24.78	19.35	33.09	25.84	9.65	78.09	9.25	6.93
TKW	28.80	19.10	10.11	6.71	3.53	66.33	4.35	12.38

Correlation between traits

In crop improvement, it is useful to know the genetic interrelationship of various traits to identify a trait with higher heritability that can be used as selection criteria for higher yield. Consequently, the association between yield and yield components were determined both at genotypic and phenotypic levels (Table 3.3). High correlation values between grain yield per plant with biomass yield per plant and harvest index that were positive and highly significant ($P \leq 0.01$) were obtained both at genotypic and phenotypic levels indicating strong inherent relationships of grain yield with these traits. In the same way, high values of positive and significant genotypic and phenotypic correlation values were obtained between biomass yield and grain filling period, biomass yield and spike length, harvest index and kernels per spike, and between days to heading and spikes per plant were obtained. As with the present findings, Belay *et al*. (1993) found a strong positive association between days to heading and days to maturity. Among spike characteristics, high genotypic and phenotypic correlation values were obtained for spikelets per spike and kernels per spike, and kernels per spike and kernels per plant. In most cases, the magnitudes of the genetic and phenotypic correlations were nearly equal, implying that the influence of the environment on these correlations was minimal (Falconer 1989). Grain yield per plant was negatively and significantly associated with days to heading, and grain filling period. A similar association was observed between biomass yield per plant and days to maturity. Likewise, harvest index was negatively and significantly associated with days to heading and days to maturity. A relatively high and significant genotypic but low phenotypic correlation was found between grain yield per plant and spikelets per spike indicating high association of additive genes controlling the pair of characters. The low phenotypic association may be ascribed to environmental effects.

Path coefficient analysis

Path coefficient analysis permits a critical examination of components that influence a given correlation and can be helpful in formulating an efficient selection strategy

Table 3.3. Phenotypic (P) and genetic (G) correlation coefficients among all possible pairs of the 13 characters.

Traits	Coefficient type	BY	HI	DTM	DTH	GFP	PH	SS	SL	KS	SP	KP	TKW
GY	P	0.72**	0.61**	0.40**	-0.62**	-0.42**	0.13	0.23*	0.60**	0.33**	0.27*	0.44**	0.31**
	G	0.75**	0.67**	0.43**	-0.41**	-0.47**	0.18	0.56**	0.58**	0.39**	0.32**	0.45**	0.41**
BY	P		0.39**	-0.49**	0.51**	0.61**	0.16	0.21	0.62**	0.43**	0.31**	0.21	0.33**
	G		0.41**	-0.34**	0.57**	0.69**	0.19	0.27*	0.77**	0.45**	0.37**	0.27*	0.34**
HI	P			-0.23*	-0.49**	-0.10	-0.09	0.27*	-0.16	0.66**	0.08	0.42**	0.13
	G			-0.33**	-0.52**	-0.17	-0.10	0.29*	-0.17	0.74**	-0.11	0.54**	0.14
DTM	P				0.35**	-0.14	0.19	-0.55**	0.45**	0.12	-0.15	-0.26*	-0.29*
	G				0.37**	-0.25*	0.30**	-0.57**	0.43**	-0.21	-0.16	-0.31**	-0.23*
DTH	P					-0.18	0.38**	0.65**	0.49**	0.44**	0.71**	0.39**	0.09
	G					-0.23*	0.41**	0.70**	0.52**	0.48**	0.89**	0.51**	0.13
GFP	P						0.37**	0.47**	0.67**	-0.06	0.40**	-0.29*	0.22
	G						0.39**	0.52**	0.63**	-0.13	-0.50**	-0.41**	0.24*
PH	P							-0.16	0.60**	-0.22	-0.10	0.49**	0.27*
	G							-0.19	0.71**	-0.36	-0.25	0.51**	0.34**
SS	P								0.49**	0.73**	0.33**	0.52**	0.04
	G								0.69**	0.82**	0.45**	0.65**	0.11
SL	P									0.39**	0.26*	0.10	0.23*
	G									0.28**	0.24*	0.14	0.18
KS	P										0.31**	0.64**	-0.16
	G										0.55**	0.68**	-0.24*
SP	P											-0.15	-0.11
	G											-0.08	-0.19
KP	P												0.16
	G												0.23*

(Scheiner et al. 2000). This approach is based on *a priori* assumptions, which traits are to be included in the analysis (Baye and Becker 2005). Hence, traits like days to maturity, spikes per plant, kernels per spike, TKW, plant height, and harvest index which have

been reported as important components of yield (Akio et al. 2004; Sidwell et al. 1976; Ahmed et al. 2003) have been selected to compute path analysis. Results of analysis of the direct and indirect effects of these traits on grain yield are presented in Table 3.4. The highest direct effect was exhibited by harvest index followed by days to maturity, TKW and kernels per plant. Plant height showed negative direct effect on yield, which could be ascribed due to the negative indirect effect through spikes per plant and days to maturity. Likewise, Ahmed et al. (2003) found a negative direct effect of plant height on grain yield unlike Ashraf et al. (2002) who reported a positive direct effect for plant height on yield. Among the traits Ahmed et al. (2003) considered, the highest direct effect was produced by harvest index.

Table 3.4. Analysis of direct and indirect effects of eight characters on the grain yield in Ethiopian tetraploid wheat accessions.

Traits	Direct effect	Indirect effect through						Genotypic correlation with grain yield per plant (gm)
		HI	DTM	SL	PH	KP	TKW	
HI	0.556	-	-0.004	-0.03	-0.006	-0.019	0.143	0.41**
DTM	0.487	-0.004	-	0.069	0.042	-0.047	-0.129	0.67**
SL	-0.166	0.101	-0.203	-	-0.054	0.079	0.027	0.58**
PH	-0.15	0.024	-0.138	-0.06	-	0.162	0.011	0.32**
KP	0.166	-0.065	-0.139	-0.079	0.146	-	0.07	0.45**
TKW	0.253	0.315	-0.247	-0.018	-0.068	0.045	-	0.41**

3.5 Discussion

Greater knowledge of the magnitude of genetic variability for yield and yield components in Ethiopian tetraploid wheats and relationships among the grain yield component traits will facilitate the breeding improvement of the species. As a result, a study was conducted to assess genetic variation, heritability and genetic advance for yield and its attributes and quantify relationships among those traits. Results of analysis of variance indicated that the genotypes differed significantly for all the traits revealing the presence of a considerable diversity, which could be utilized in developing high yielding cultivars

through selection breeding, in Ethiopian tetraploid wheats for all plant characters studied. The characters having high genotypic coefficient of variation indicate high potential for effective selection (Burton and De Vane 1953). In the present study, the genotypic coefficient of variation was higher in kernels per plant followed by TKW, days to maturity and kernels per spike. Results of this study concur with the findings of Moghaddam et al. (1997) who reported that the PCV was generally higher than the GCV for most of the characters, but in many cases, the two values differed only slightly. Moreover they found the lowest values for the developmental characters and plant height, and the highest values were shown by number of tillers and spikes per plant, and straw biomass, followed by number of grains per spike, TKW, and grain yield. Selection efficiency, however, is related to magnitude of heritability and genetic advance (Johnson et al. 1955). Knowledge of heritability is fundamental as it influences the effectiveness of selection. The highest heritability estimates for days to maturity (0.97) per plant indicate that a large proportion of the total variance was attributable to the genotypic variance. Such characters, which have heritability of one or close to one in quantitative genetic terms, are the types often preferred as attributes for characterization of germplasm collections. Heritability was low particularly in spikelets per spike and spikes per plant, which could be attributed to the large phenotypic variances indicating large environmental influence. Across the 13 measured quantitative traits, the heritability estimate differed considerably. This is expected because estimates of heritability may differ widely in the same crop and same trait (Hill et al. 1998; Rasmuson 2002), because heritability always refers to a defined population and specific experimental setup (Holland et al. 2002; Nyquist 1991).

High heritability does not necessarily mean high genetic gain (Baye and Becker 2005). Johnson et al. (1955) and Panse (1957) suggested that the estimates of heritability and expected genetic advance, a parameter that indicates the degree of gain in a character obtained under a particular selection pressure, should always be considered jointly. In the present study, grain yield per plant has moderate values of both heritability and genetic advance. Similarly, Belay et al. (1993) reported moderate heritability estimate coupled with moderate genetic advance (as % of the mean) for grain yield per plant. On the

contrary, Moghaddam et al. (1997) found lowest heritability estimate with a relatively intermediate value for expected genetic advance was reported for grain yield. Sidwell et al. (1976) and Ehdaie and Waines (1989) also reported moderate to low values of heritability and expected genetic advance for grain yield in wheat. Ahmed et al. (2003) found high heritability coupled with high genetic advance for plant height, biological yield, spike length, harvest index and grain yield. Likewise, Khan (1990) reported high heritability along with high genetic advance for yield and its components. High heritability coupled with high genetic advance (GA) for kernels per spike, spike length, and TKW indicate the predominance of additive gene effects on such traits. Higher genetic advance associated with high heritability value indicated additive gene effect in controlling the characters and had considerable value to the breeder for plant selection (Panse 1957). Those traits with high heritability coupled with high genetic advance can be utilized through mass selection even if they are under polygenic control (Chauhan and Nanda 1983).

Grain yield is a complex character and is the multiplicative end product of different yield components as it is polygenically controlled. Hence, information on the association between grain yield and its components is important for breeding. In this study, grain yield was positively and significantly correlated both at genotypic and phenotypic levels with all traits except the three phenological traits and plant height. As with the present finding, the genetic correlation between grain yield with the developmental characters was reported by Belay et al. (1993). A non-significant association between grain yield and plant height was also reported by Ahmed et al. (2003) and Moghaddam *et al*. (1997). According to Ehdaie and Waines (1989), grain yield and the developmental characters were not correlated in wheat landraces collected from southwestern Iran. However, they reported a negative correlation between grain yield and plant height. In the studies conducted by Sinha and Sharma (1979) and Belay et al. (1993), grain yield was positively correlated with each of the three primary grain yield components, with either positive or negative correlation between grain yield and plant height. Khan et al. (1999) and Chowdhry et al. (2000) also found a significant and positive association between grain yield and tillers per plant, grains per spike and TKW.

Since correlation coefficients doesn't give clear information about the interrelationship between yield and its components, the genetic correlation coefficient estimates were partitioned into direct and indirect effects using path analysis to establish the intensity of effects of yield components on yield. The highest direct effect was exhibited by harvest index followed by days to maturity, TKW and kernels per plant. The positive direct effect of harvest index on yield per plant obtained in this study supports the statement of Wallace et al. (1993) that breeding for increased harvest index remains the most effective method of breeding for high yield. Among the traits, which have exerted higher direct effects, only days to maturity was negatively correlated with all of the remaining traits. To improve the yield components with negative association, suitable recombination which can be obtained through biparental mating, mutation breeding or diallel selective mating by breaking undesirable linkages could be applied (Ahmed et al. 2003).

Heritability estimates for grain yield are usually lower than some of its components suggesting that environmental effects constitute a major portion of the total phenotypic variation for this character. Given that, selection of superior genotypes on the basis of yield *per se* would not be as effective as selection for its primary components (Moghaddam et al. 1997). Hence, it is useful to consider harvest index, TKW and kernels per plant as selection markers in selection breeding of Ethiopian tetraploid wheat landraces. According to the results of yield potential comparisons of improved varieties of durum wheat over years of release, yield potential of wheat has significantly increased and this was mainly attributed to an increase in harvest index (Tarekegn 1994). His results demonstrate that increasing the harvest index of wheat would be a more efficient way to improve grain yield potential of wheat. The present findings partly agree with result of Moghaddam et al. (1997) who reported that landraces could be improved by intercrossing the promising genotypes identified in this study, with simultaneous selection for earliness, fewer number of spikes per plant, greater number of grains per spike and heavier grains. In a similar study in Ethiopian tetraploid wheats, TKW were positively correlated with grain yield per plant and had intermediate direct effects (Belay et al. 1993). They suggested that improvement of the Ethiopian wheat landraces may be

possible through indirect selection for TKW and tiller number or direct selection for grain yield *per se*.

Chapter 4 Analysis of microsatellite diversity in Ethiopian tetraploid wheat landraces

4.1 Abstract

The extent and patterns of microsatellite diversity in 141 Ethiopian tetraploid wheat landraces were analyzed using 29 microsatellite markers. A high level of polymorphism and a large number of alleles unique for each species were detected. Compared to emmer (*T. dicoccon*) and accessions belonging to the relationships of poulard (*T. turgidum*) wheats, a higher genetic diversity was observed in accessions that belong to the relationship of *T. durum*. The A-genome was more polymorphic than the B-genome in all the three species. Microsatellites with $(GA)_n$-repeats had a higher number of alleles than $(GT)_n$-repeats. A species pairwise comparison was made to determine the percentage of shared alleles and a large number of common alleles among species were observed. Average gene diversity, across the 29 microsatellite loci, was 0.684 for *T. durum*, 0.616 for *T. dicoccon* and 0.688 for *T. turgidum*. Genetic distances were lower between *T. durum* and *T. turgidum* (0.26) than *between T. durum* and *T. dicoccon* (0.34) or between *T. turgidum* and *T. dicoccon* (0.38). A significant correlation ($P < 0.01$) was found between the number of alleles per locus and the gene diversity in all the three species. Allelic frequency variation was highest between *T. turgidum* and *T. dicoccon* (10.62%) and lowest between *T. durum* and *T. turgidum* (4.86%). A genetic similarity coefficient of 0.34, 0.46 and 0.37 was found in *T. durum*, *T. dicoccon*, *and T. turgidum*, respectively. The dendogram, which was constructed on the basis of a similarity matrix using the UPGMA algorithm, distinguished all accessions represented in the study.

4.2 Introduction

Assessing the overall patterns of genetic diversity and the distribution of genetic variability in a crop species is useful in conservation and also facilitates the selection of parents with diverse genetic background and thereby make crop improvement more efficient. Several 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) were conducted for morpho-physiological traits in Ethiopian

tetraploid wheat landraces and it was found that they posses many useful traits. For example, they have valuable traits such as early ripening, short culm, long coleoptiles, low tillering (Porceddu et al. 1973); resistance to powdery mildew and glume blotch (Negassa 1986), Hessian fly (Amri et al. 1990), stripe rust (Belay et al. 1992), and moderate resistance to pH and drought (Porceddu et al. 1973). Vavilov (1951) found Ethiopian tetraploid wheat that had 20% protein.

The genetic variability in Ethiopian tetraploid wheat has also been assessed using microsatellites (Alamerew et al. 2004; Messele 2001), isozymes (Tsegaye et al. 1994; 1996), glutenine and gliadine storage protein and AFLP (Messele 2001). The few studies performed using molecular markers considered only few accessions representing mainly the central highlands of Ethiopia. Compared to morphological evaluation and characterization of accessions that are largely influenced by environmental factors (Perera et al. 2003; Prasad et al. 2000), molecular markers can reveal differences among accessions at the DNA level and thus provide a more direct, reliable, and efficient tool for germplasm conservation and management. In Ethiopia, because wheat landraces cultivation is predominant (Bechere et al. 1996; Belay et al. 1993; Tessema et al. 1993; Tessema and Bechere 1998), it is very important to maximize the utilization of these materials in breeding. Therefore, a reliable characterization and the accurate estimation of the genetic diversity present in Ethiopian tetraploid wheat landraces are necessary for sustainable future wheat breeding and genetic resources conservation programs. Thus, this study was conducted to determine the extent of genetic variation and assess the relationship of Ethiopian tetraploid wheat species using SSR markers.

4.3 Materials and Methods

Plant material

141 tetraploid wheat accessions consisting of 65 landraces belonging to the relationships of *Triticum durum* Desf. and 5 exotic *T. durum* cultivars, 35 landraces that belong to the relationships of *T. turgidum* L. and 2 exotic *T. turgidum* cultivars, and 33 *T. dicoccon* Schrank landraces and one *T. dicoccon* cultivar from Yemen were used in the study. In addition, the hexaploid *T. aestivum* varieties ‘Chinese Spring’ (CS) and ‘Aztec’ were

included as standards. The nomenclature of species was according to Szabó and Hammer (1996). Accessions were obtained from DZARC, IBC, Ethiopia, and IPK, Gatersleben, Germany. Lists of the accession and their country of origin are presented in Table 4.1.

DNA extraction and PCR amplification

For each accession, total genomic DNA was extracted from pooled leaves of six plants of three weeks old seedlings. The extraction was performed according to Fulton et al. (1995) with extraction buffer described in Plaschke et al. (1995). PCR amplifications were performed as described by Röder et al. (1998). PCR reaction contained 50-100 ng template DNA, 250 nM cy5-labelled forward primer, 250 nM unlabelled reverse primer, 0.2 mM dNTPs, 2.5µl PCR buffer (10 x), 1.5 mM $MgCl_2$, 1 U *Taq* DNA polymerase in a total volume of 25 µl. Fragment detection was performed as described by Röder et al. (1998). Fragments were detected by an Automated Laser Fluorescence (ALF express) sequencer (Amersham Biosciences) and fragment sizes were calculated using the computer program Fragment Analyzer 1.02 (Amersham Biosciences) by comparison with internal size standards. In the case of weak or no fragment products, PCR amplifications were repeated to exclude failed PCR reaction as the cause of the null allele.

Microsatellite loci

Microsatellites that are highly polymorphic and gave strong amplification bands in wheat in earlier studies (Fahima et al. 1998; Huang et al. 2002; Plaschke et al. 1995; Röder et al 2002) were used to analyze the pattern of genetic diversity in 141 tetraploid wheat accessions and 2 hexaploid standards. Twenty-eight Gatersleben Wheat Microsatellites (GWM) and one microsatellite from a pseudogliadine gene, Taglgap, representing two markers for each chromosomes of the A and B genomes were used in the study. The microsatellite primers used were described by Röder et al. (1998) and for the primer Taglgap by Devos et al. (1995). Microsatellite loci *Xgwm157-2D* and *Xgwm161-3D* that represent the D genome were used to check for the presence of mixtures of hexaploid accessions in the Ethiopian tetraploid wheat landraces. These two primers failed to amplify fragments from all lines except for the hexaploid standards.

Table 4.1. List of accession numbers and their origin.

Accession Number	Country of origin	Accession Number	Country of origin	Accession Number	Country of origin
Triticum dicoccon Schrank		6856	Ethiopia*	223262	Ethiopia*
TRI 18073	Yemen+	6916	Ethiopia*	226242	Ethiopia*
5008	Ethiopia**	6917	Ethiopia*	226396	Ethiopia*
5386	Ethiopia*	6928	Ethiopia*	227112	Mexico
6138	Ethiopia*	6932	Ethiopia*	231516	Ethiopia*
6222	Ethiopia*	6953	Ethiopia*	238118	Ethiopia*
6843	Ethiopia*	7021	Ethiopia*	238130	Ethiopia*
7329	Ethiopia*	7045	Ethiopia*	238135	Ethiopia*
7337	Ethiopia*	7072	Ethiopia*	TRI 5851	Iran+
7347	Ethiopia*	7156	Ethiopia*	TRI 2192	Turkey+
7458	Ethiopia*	7158	Ethiopia*	*Triticum turgidum L.*	
7559	Ethiopia*	7222	Ethiopia*	5101	Ethiopia*
7615	Ethiopia*	7345	Ethiopia*	5252	Ethiopia*
7692	Ethiopia*	7370	Ethiopia*	5605	Ethiopia*
7913	Ethiopia*	7372	Ethiopia*	7114	Ethiopia*
7920	Ethiopia*	7415	Ethiopia*	7162	Ethiopia*
8284	Ethiopia*	7460	Ethiopia*	7213	Ethiopia*
8302	Ethiopia*	7512	Ethiopia*	7893	Ethiopia*
203766	Ethiopia*	7892	Ethiopia*	7899	Ethiopia*
206672	Ethiopia*	7901	Ethiopia*	7931	Ethiopia*
214263	Ethiopia*	7902	Ethiopia*	7957	Ethiopia*
219253	Ethiopia*	7935	Ethiopia*	7958	Ethiopia*
219510	Ethiopia*	7947	Ethiopia*	8013	Ethiopia*
222478	Ethiopia*	7951	Ethiopia*	8057	Ethiopia*
222555	Ethiopia*	7956	Ethiopia*	216063	Ethiopia*
226347	Ethiopia*	8292	Ethiopia*	216587	Ethiopia*
226865	Ethiopia*	8310	Ethiopia*	216633	Ethiopia*
226935	Ethiopia*	8481	Ethiopia*	216666	Ethiopia*
226942	Ethiopia*	8482	Ethiopia*	216692	Ethiopia*
232230	Ethiopia*	8483	Ethiopia*	222202	Ethiopia*
237867	Ethiopia*	200872	Mexico *	222460	Ethiopia*
238893	Ethiopia*	203922	Ethiopia*	222818	Ethiopia*
239698	Ethiopia*	203931	Ethiopia*	226201	Ethiopia*
239708	Ethiopia*	204703	Ethiopia*	226241	Ethiopia*
239712	Ethiopia*	207849	Ethiopia*	226272	Ethiopia*
Triticum durum Desf.		208255	Ethiopia*	226326	Ethiopia*
2002/P^{n} 43#165	Ethiopia**	210793	Mexico *	226332	Ethiopia*
2002/P^{n} 55#235	Ethiopia**	213307	Ethiopia*	226378	Ethiopia*
2002/P^{n} 61#157	Ethiopia**	214528	Ethiopia*	226391	Ethiopia*
P^{n} 106#104	Ethiopia**	216070	Ethiopia*	226968	Ethiopia*
P^{n} 61#150	Ethiopia**	216078	Ethiopia*	226972	Ethiopia*
5314	Ethiopia*	216452	Ethiopia*	227037	Ethiopia*
5317	Ethiopia*	216455	Ethiopia*	231470	Ethiopia*
5390	Ethiopia*	216623	Ethiopia*	231511	Ethiopia*
5487	Ethiopia*	216629	Ethiopia*	238102	Ethiopia*
5550	Ethiopia*	222197	Ethiopia*	238105	Ethiopia*
5632	Ethiopia*	222322	Ethiopia*	TRI 5948	Iran+
6846	Ethiopia*	222497	Ethiopia*	TRI 1623	Greece+

*, Accessions were obtained from the Institute of Biodiversity Conservation and Research, Ethiopia.
**, Accessions were obtained from the Debre Zeit Agricultural Research Center, Ethiopia.
+, Accessions were obtained from the Institute of Plant Genetics and Crop Plant Research, Germany.

Statistical analysis

Evaluation of the number of alleles per locus was based on the tetraploid wheat accessions. The fragment sizes in 'CS' and 'Aztec' were taken as controls to check the uniformity in the different runs. Gels were scored as binary data matrix. The presence (1) and absence (0) of alleles for each microsatellites marker were recorded for each accessions. The binary data were used to compute a pairwise similarity coefficients using the DICE similarity index (Dice 1945). The similarity matrix was subjected to cluster analysis using UPGMA algorithm on NTSYS, version 2.0 (Rohlf 1998). Gene diversity was calculated according to the formula of Nei (1973). Genetic distance, which is the measure of qualitative changes or presence/absence of alleles among pair of groups, was computed as described by Nei and Li (1979). The degree of allelic frequency variation (Axy), which is indicator of quantitative changes in allelic frequencies of different loci, was determined according to the formula of Khlestkina et al. (2004). Both genetic distances and allelic frequency variations were calculated separately for the 29 microsatellites loci, over the different genomes, homoeologous chromosome groups and species.

4.4 Results

SSR polymorphism

The 29 SSR markers revealed altogether 320, 202, and 271 alleles in *T. durum*, *T. dicoccon* and *T. turgidum* landraces, respectively. The average number of alleles per locus was 11.03 in *T. durum*, 6.97 in *T. dicoccon* and 9.345 *in T. turgidum*. The number of alleles varied widely with microsatellite loci in all three species. In *T. durum* the number of alleles ranged from 1 (*Xgwm415*) to 31 (*Xgwm312*), while it ranged from 1 (*Xgwm415*, *Xgwm619* and *Xgwm631*) to 17 (*Xgwm312* and *Xgwm268*) *in T. dicoccon*. In *T. turgidum*, the number of alleles ranged from 2 (*Xgwm619*) to 25 (*Xgwm312*) (Table 4.2).

A relatively higher number of alleles was found in *T. durum* than in *T. turgidum* and *T. dicoccon* (Table 4.3). In *T. durum* the average number of alleles per genome A and B is

11.36 and 10.27, respectively, whereas, it is 7 for the A-genome and 6.99 for the B-genome in *T. dicoccon*. The number of alleles recorded in *T. turgidum* for genomes A and B was 9.36 and 9.33, respectively. Comparison of number of alleles across the seven homoeologous groups of chromosomes in the different species indicated that the highest number of alleles (14 alleles) was found on homologous group 2 in *T. durum*, on homologous group 6 (8.5 alleles) in *T. dicoccon* and on homologous group 4 (12.75 alleles) in *T. turgidum* (Appendix 4).

The number of alleles was also compared across motifs of the microsatellites. A higher number of alleles was observed in *T. durum* for both $(GT)_n$ and $(GA)_n$ motifs than *in T. dicoccon* and *T. turgidum*. The $(GA)_n$ motif produced an average number of 11.27, 7.27 and 10.00 alleles per locus, respectively, in *T. durum*, *T. dicoccon* and *T. turgidum*. For the $(GT)_n$ motif, an average number of 9.71, 5.14, and 7.00 alleles in *T. durum*, *T. dicoccon and T. turgidum* were observed. Comparing microsatellite markers with the different repeat motifs, those with $(GA)_n$-repeats had more alleles than $(GT)_n$-repeats. Except in *T. durum,* the average number of compound microsatellites alleles over genome was higher than the average number of simple microsatellites alleles (Table 4.3).

In some accessions, no amplification was detected for some primers. Such alleles, where locus specific primers give no PCR products are referred to null alleles. Even though the number of these null alleles differed, their occurrence is fairly dependent on the microsatellites. In all three species, null alleles for locus *Xgwm459* were observed. Except this locus, all other primers amplified in all accessions of *T. dicoccon*. In *T. durum* and *T. turgidum* the loci *Xgwm312* and *Xgwm601* gave null alleles and *Xgwm698* was not amplified in two accessions in *T. turgidum* (Table 4.2).

Gene diversity

Gene diversity was used for the evaluation of genetic diversity in the three Ethiopian tetraploid wheat species. Average gene diversity, across the 29 microsatellite loci, was

Table 4.2. Chromosomal location, size range of alleles, number of alleles and genetic diversity of the three species.

Microsatellite	Chromosomal location	*T. durum*			*T. dicoccon*			*T. turgidum*		
		Size Range of alleles (bp)	Number of alleles	Gene diversity	Size Range of alleles (bp)	Number of alleles	Gene diversity	Size Range of alleles (bp)	Number of alleles	Gene diversity
Xgwm752	1AS	116-160	11	0.76	118-136	5	0.53	118-138	6	0.7
Xgwm357	1A(C)	118-146	9	0.79	102-126	5	0.60	118-132	7	0.7
Xgwm95	2AS	108-130	8	0.77	108-126	4	0.63	116-130	8	0.73
Xgwm312	2AL	null, 185-297	31	0.91	183-275	17	0.90	null,185-303	25	0.94
Xgwm720	3AS	126-166	17	0.88	126-162	10	0.84	124-164	14	0.88
Xgwm155	3AL	127-147	11	0.73	129-143	6	0.35	125-145	9	0.72
Xgwm601	4AS	null, 143-169	12	0.87	151-163	7	0.79	null, 149-165	8	0.8
Xgwm160	4AL	179-191	5	0.53	179-185	4	0.47	179-185	4	0.43
Xgwm415	5AS	133	1	0	133	1	0	131-135	3	0.2
Xgwm186	5AL	98-138	8	0.75	98-148	8	0.75	100-138	10	0.74
Xgwm459	6AS	null, 134-180	15	0.9	null, 132-158	11	0.86	null, 128-162	14	0.84
Xgwm1089	6AL	116-168	14	0.65	120-160	9	0.84	120-150	8	0.67
Xgwm631	7AS	189-199	4	0.18	191	1	0	189-199	4	0.28
Xgwm698	7AL	154-216	13	0.73	160-218	10	0.85	null, 152-208	11	0.74
Xgwm18	1BS	176-190	7	0.68	180-190	6	0.63	180-190	6	0.65
Xtaglgap	1BS	212-290	17	0.85	233-282	7	0.51	212-284	13	0.83
Xgwm268	1BL	180-272	16	0.82	182-312	17	0.88	182-274	21	0.91
Xgwm148	2BS	142-174	10	0.76	138-148	5	0.67	142-164	7	0.73
Xgwm619	2BL	129-155	7	0.17	135	1	0	135-143	2	0.1
Xgwm389	3BS	116-142	11	0.88	116-132	8	0.84	118-142	10	0.84
Xgwm655	3BL	159-197	13	0.79	169-189	6	0.61	155-197	10	0.69
Xgwm898	4BS	105-121	8	0.3	103-123	6	0.60	105-119	6	0.61
Xgwm513	4BL	135-147	6	0.6	139-145	4	0.69	133-147	7	0.66
Xgwm540	5BS	114-140	9	0.75	124-132	5	0.49	114-142	7	0.66
Xgwm408	5BL	136-200	12	0.79	148-202	10	0.78	146-202	11	0.77
Xgwm680	6BS	123-149	8	0.68	123-155	11	0.74	123-145	8	0.69
Xgwm219	6BL	155-179	9	0.63	155-175	3	0.51	153-179	8	0.77
Xgwm46	7B(C)	147-175	13	0.86	157-171	7	0.71	157-183	12	0.81
Xgwm577	7BL	122-162	15	0.83	122-154	8	0.79	null, 128-162	12	0.87

0.684 *for T. durum*, 0.616 for *T. dicoccon* and 0.688 for *T. turgidum*. *Xgwm312* was the most polymorphic locus. The gene diversity for this primer was 0.91, 0.90 and 0.94 in *T. durum, T. turgidum* and *T. dicoccon*, respectively. Significant correlation coefficients of 0.662 ($P < 0.001$), 0.782 ($P < 0.001$) and 0.756 ($P < 0.001$) were found between gene diversity and the number of alleles per locus in *T. durum*, *T. dicoccon and T. turgidum,* respectively (Figure 4.1).

Table 4.3. Number of alleles and gene diversity in different genomes, and motifs across 29 loci.

	T. durum		*T. dicoccon*		*T. turgidum*	
	Number of alleles	Gene diversity	Number of alleles	Gene diversity	Number of alleles	Gene diversity
Genome						
A	11.36	0.68	7	0.6	9.36	0.67
B	10.27	0.65	6.9	0.63	9.33	0.71
Mean	10.82	0.67	6.95	0.62	9.35	0.69
Motif						
(GT)n	9.71	0.6	5.14	0.554	7	0.63
(GA)n	11.27	0.67	7.27	0.6	10	0.67
Mean	10.49	0.635	6.205	0.577	8.5	0.65
(CA)j(TA)k	11.75	0.79	7.75	0.73	10.25	0.78
(CT)j(GT)k	11	0.88	8	0.84	10	0.84
(GT)j(GA)k	8	0.68	11	0.74	8	0.69
Mean	10.25	0.783	8.92	0.77	9.42	0.77

Distribution of alleles across the three species

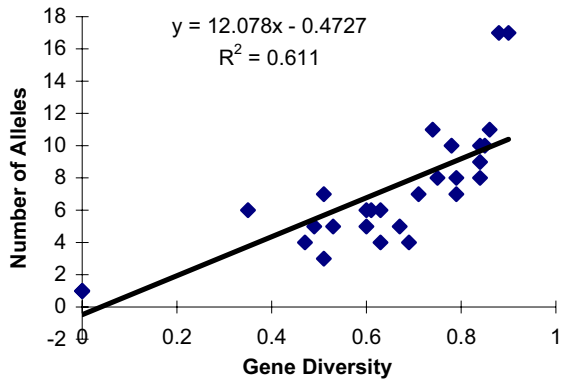
A large number of species specific alleles was detected. Of the total number of 320 alleles detected over the 29 loci in *T. durum*, 73 alleles were found to be specific to *T. durum*. Over the 29 microsatellites loci, a total of 46 and 24 alleles that are specific to *T. turgidum* and *T. dicoccon*, respectively, were observed. With a pairwise comparison

between species the percentage of shared alleles among species was determined. In *T. durum* and *T. turgidum* 213 (36 %) common alleles were observed out of the 591 alleles found in the two species, and 170 (33%) common alleles were detected in both *T. durum* and *T. dicoccon* out of the 522 total alleles. A comparison of *T. dicoccon* and *T. turgidum* showed that 142 (30%) of alleles are common to both species out of a total of 473 (Appendix 5).

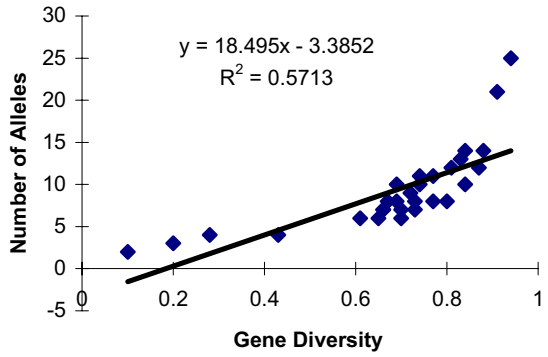
In addition, qualitative and quantitative differences of shared alleles were compared over genomes and species. Genetic distances were lowest between *T. durum* and *T. turgidum* (0.26) and highest between *T. turgidum* and *T. dicoccon* (0.38). The genetic distance between *T. durum* and *T. dicoccon* was 0.34. The allelic frequency variation, which is the measure of the difference in changes of frequency of shared alleles, is higher between *T. turgidum* and *T. dicoccon* (10.62%) than between *T. durum* and *T. dicoccon* (8.44%) or between *T. durum* and *T. turgidum* (5.41%) (Table 4.4). Comparison of allelic frequency variation between *T. durum* and *T. turgidum* over genomes indicated the presence of a higher allelic frequency variation in the B-genome than in the A-genome, implying that shared alleles between the two species appeared with closer frequencies on the A-genome than the B-genome. On contrary the allelic frequency variation between *T. durum* and *T. turgidum* or between *T. turgidum* and *T. dicoccon* was higher on the A-genome.

Analysis of relationship

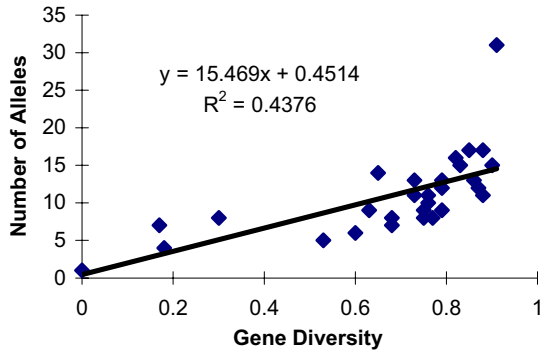
The genetic similarity (GS) coefficients for all possible 2415, 561, and 666 pairs *of T. durum*, *T. dicoccon* and *T. turgidum* accessions, respectively, were calculated. It ranged from 0.06 to 0.73 with an average of 0.34 in *T. durum*. In *T. dicoccon*, it ranged from 0.13 to 0.79 with an average of 0.46, while it ranged from 0.06 to 0.90 with an average value of 0.37 in *T. turgidum*. The cluster diagram delineated all accession used in the study into two major clusters (Figure 4.2). Cluster one contained only two accessions: Chinese Spring and A-5948 an Iranian *T. turgidum* accession. All the remaining 141 accessions were grouped in the second cluster, which is further partitioned into six sub clusters. In cluster IIa eight accessions were included of which three are Ethiopian *T. durum* landraces (Accn. No. 216666, 226972 and 227037) and the remaining are the



(a)



(b)



(c)

Figure 4.1. Correlation between gene diversity and the number of alleles, over 29 microsatellite loci, in a) *T. durum* b) in *T. dicoccon* and c) in *T. turgidum*.

hexaploid wheat (Aztec) and four of the eight introduced tetraploid wheat cultivars. Thirty-five accessions were grouped in sub cluster IIb that consists of 29 Ethiopian *T.*

dicoccon accessions, 5 *T. durum* and one *T. turgidum* accessions. The fact that 85% of *T. dicoccon* accessions represented in the study are grouped in the same cluster (IIb) indicated the distinctiveness of this group from *T. turgidum* and *durum* landraces. Similarly, in cluster IIc 36% of the *T. durum* accessions used in this study were included besides one Ethiopian *T. dicoccon* and three *T. turgidum* landraces. The remaining *T. durum* landraces are scattered over the different groups. Sub cluster IID contained a large number of accessions of *T. turgidum* and of *T. durum*. It is further differentiated into different groups. Only three Ethiopian *T. durum* landraces were included in sub cluster IIe and two *T. turgidum* landraces were grouped in sub cluster 11f. Cluster IIf is also connected with the other clusters at the lowest similarity level indicating its distinctness from other clusters.

Table 4.4. Genetic distance (GD_{I-II}) and Allelic Frequency Variation (A_{I-II}) between different species of Ethiopian tetraploid wheats across genomes.

Genome	$GD_{T.\ durum\text{-}T.\ turgidum}$	$GD_{T.\ durum\text{-}T.\ dicoccon}$	$GD_{T.\ turgidum\text{-}T.\ dicoccon}$	$A_{T.\ durum\text{-}T.\ turgidum}$ (%)	$A_{T.\ durum\text{-}T.\ dicoccon}$ (%)	$A_{T.\ turgidum\text{-}T.\ dicoccon}$ (%)
A	0.26	0.29	0.41	5.05	9.3	11.19
B	0.26	0.38	0.35	5.77	7.58	10.05
Mean	0.26	0.34	0.38	5.41	8.44	10.62

4.5 Discussion

Knowledge about germplasm diversity and genetic relationships among breeding materials is an invaluable aid in crop improvement strategies. Molecular markers can reveal differences among accessions at the DNA level and thus provide a more direct, reliable, and efficient tool for germplasm conservation and management. Twenty-nine SSR markers were used to determine the extent and patterns of microsatellite diversity in Ethiopian tetraploid wheat accessions representing three species. The 29 microsatellites displayed a high level of polymorphism in all the three species. Comparison of the results

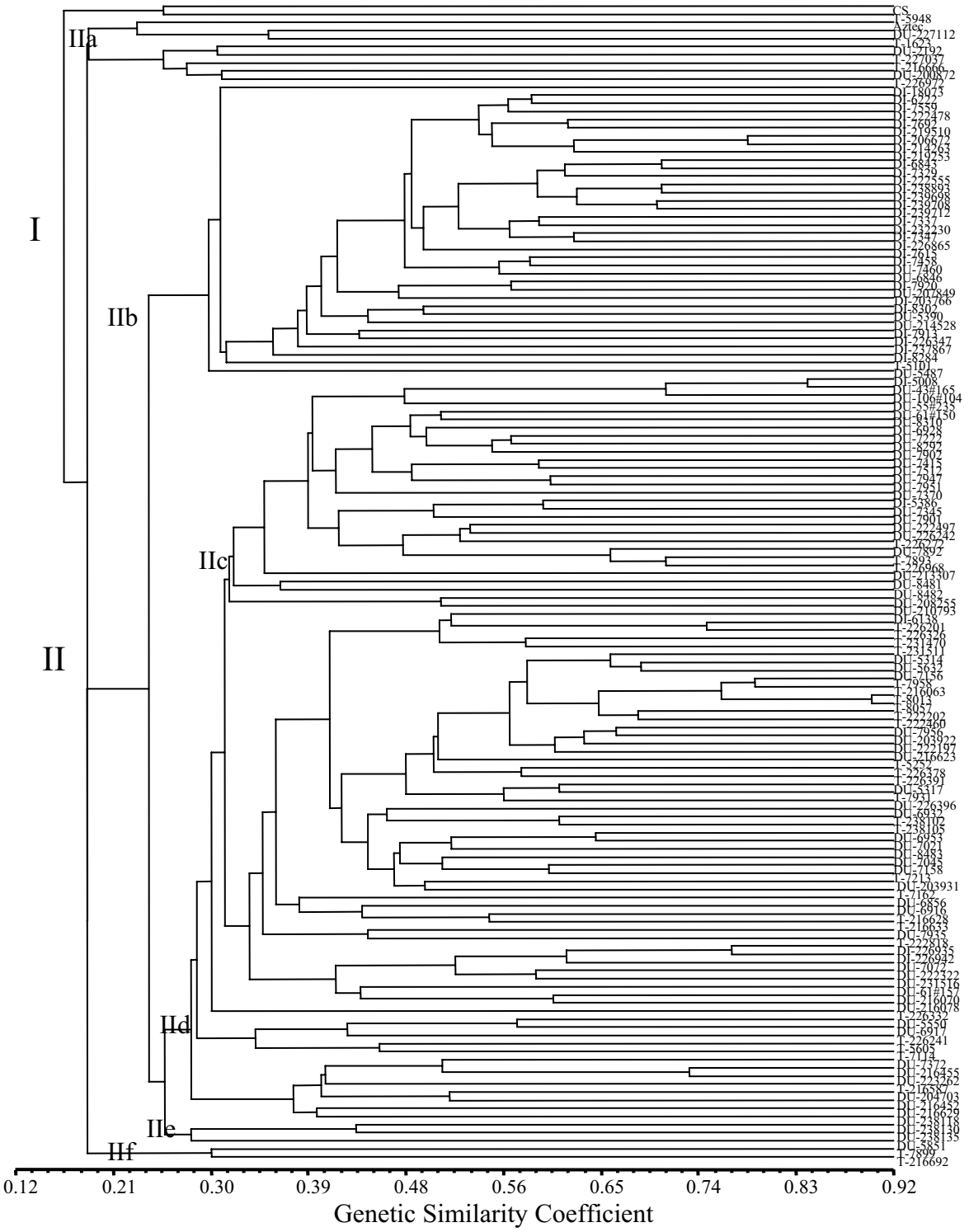


Figure 4.2. Dendogram resulting from cluster analysis of the Dice genetic similarity matrix among 141 Ethiopian tetraploid wheat landraces and 2 standard accessions. Prefixes T-, DU- and DI are added to accessions numbers to indicate that the accession belongs to *T. turgidum, T. durum and T. dicoccon* species group, respectively.

obtained in the present study with those published earlier for Ethiopian tetraploid wheats (Alamerew et al. 2004; Messele 2001; Tsegaye et al. 1996) indicates that the average number of alleles per locus found in the present study was relatively higher. Alamerew et al. (2004) analyzed the genetic diversity of 135 wheat accessions of Ethiopian gene bank collection maintained at IPK using 22 wheat microsatellites and detected 286 alleles, ranging from 4 to 26 per loci. For the three species *T. aestivum*, *T. aethiopicum* and *T. durum* on average 9.9, 7.9 and 7.9 alleles per locus, respectively, were observed. The extent of polymorphism in 26 Ethiopian tetraploid wheat accessions and four cultivars was assessed using 12 SSR markers and only 96 polymorphic bands with number of alleles per locus ranging from 3 to 17 was detected by Messele (2001). The same accessions were characterized using AFLP and a total of 84 polymorphic bands were scored, with an average of 9.3 polymorphic bands per primer combination, ranging from 2 to 19. Diversity and relationships among ten tetraploid wheat landrace populations collected from different localities in the central highlands of Ethiopia were studied using isozyme marker and a total of 18 alleles were detected at nine polymorphic loci (Tsegaye et al. 1996). Genetic diversity of 26 Ethiopian tetraploid wheat accessions and 6 cultivars were also studied using glutenine and gliadine storage protein and a total of 49 polymorphic bands were identified (Messele 2001). The high gene diversity observed in this experiment may be attributed to a higher sample size, which contains accessions collected from diverse geographical areas, used and also to the more informativeness of SSR markers compared to AFLP, isozyme or glutenine and gliadine storage protein markers. Huang et al. (2002) evaluated the genetic diversity of 998 wheat accessions using 24 microsatellite loci and detected a total of 470 alleles with an average number of 18.1 alleles per locus. Ben Amer et al. (2001) used twenty-four wheat microsatellites to estimate the extent of genetic diversity among 15 Libyan wheat genotypes and detected 116 alleles with an average of 4.5 alleles per locus. Similarly, Prasad et al. (2000) characterized 55 genotypes using 21 loci and detected a total of 155 alleles.

In the present study, *Xgwm312* is the most polymorphic locus. However, Messele (2001) observed the highest number (17) of alleles on WMS577 and the lowest (3) for WMS160. Likewise, Ben Amer et al. (2001) reported that the most polymorphic markers were

WMS619 and Taglgap with 11 alleles each. *Xgwm459* was the most polymorphic locus in the study of Huang et al. (2002).

Genome A is more polymorphic than genome B in all the three species. In contrary to this, the largest number of alleles per locus occurred on the B-genome in the study by Alamerew et al. (2004), Ben Amer et al. (2001), Huang et al. (2002) and Khlestkina et al. (2004). Fahima et al. (1998) reported an average of 10.9 and 9 alleles per locus for A and B-genomes, respectively. Similar to the present findings, Huang et al. (2002) reported that the $(GA)_n$ microsatellites produced greater number of alleles per locus than microsatellites with the $(GT)_n$. In addition, Struss and Plieske (1998) reported the occurrence of more alleles for $(GA)_n$-repeats than for $(GT)_n$-repeats in barley. On the contrary, Prasad et al. (2000) found $(GT)_n$-repeats to be more polymorphic than other simple repeats such as $(GA)_n$ in wheat.

Gene diversity values obtained in the present investigation are comparable with the results of other studies (Huang et al. 2002, Plaschke et al. 1995; Prasad et al. 2000) on genetic diversity of wheat using microsatellites. The polymorphic information content (PIC), which Anderson et al. (1993) described as gene diversity ranged from 0.21 to 0.90 with an average of 0.71 (Prasad et al. 2000) for *T. aestivum* accessions. Plaschke (1995) recorded gene diversity values ranging from 0.29 to 0.79 while a PIC mean value of 0.36 was reported by Bohn et al. (1999). Huang et al. (2002) found an average gene diversity of 0.77. Gene diversity per locus showed a linear correlation with the number of alleles in all the three species. This result is in agreement with the findings of Huang et al. (2002) but not with Prasad et al. (2000) who reported that the PIC value was not correlated with the number of alleles.

Few primer sets failed to give good amplification indicating the presence of null-alleles in some accessions. Such alleles were also found in Ethiopian *T. durum* and *T. turgidum* landraces in an experiment conducted by Messele (2001). Tsegaye et al. (1996) reported the occurrences of null alleles at two loci in Ethiopian *T. durum* landraces. Similarly, null alleles were detected by Prasad et al. (2000) for primers WMC216 and WMC267, in 7

and 14 genotypes, respectively. Manifesto et al. (2001) reported the presence of null alleles at loci *Xpsp3033*, *Xpsp3034*, *Xpsp3050*, and *Xpsp3080* respectively, in 1, 8, 1 and 4 varieties. Null alleles of SSRs are relatively common in wheat (Devos et al. 1995; Plaschke et al. 1995). Null allele is generally attributed to mutation, deletion, insertions or inversions within the binding site for a DNA primer and results due to a primer site too close to the microsatellite. This may either inhibit primer binding, giving a faint band or may completely prevent this binding, leading to the loss of PCR product (Gupta and Varshney 2000; Liu et al 2001). Donini et al. (1998) stated the causes of null alleles as although a null allele can arise from point mutation(s) in one or both of the primer sites, the difference in frequency of nulls between wheat, a polyploid species, and the diploids barley and human suggests that a likelier origin of many of the wheat nulls lies in the occurrence of deletions, which include the chromosomal segment harboring the microsatellite sequences.

As revealed by number of alleles and gene diversity values, *T. durum* is more diverse than *T. turgidum*. This is in agreement with the findings of Messele. (2001) who reported that variation is higher in *T. durum* than in *T. turgidum*. Moreover, Asins and Carbonell (1989) have noted high isozyme variation in the *T. durum* collections of an Ethiopian origin. The high gene diversity in *T. durum* in the present study might be attributed to the higher sample size of *T. durum* represented in the experiment.

The presence of large number of species-specific alleles is the reflection of the existence of divergent population structure and rich genetic diversity within each species that would be useful for breeding. Alamerew et al. (2004) also detected species-specific alleles with numbers of 124 (without the D genome loci 75), 17 and 24 for *T. aestivum*, *T. aethiopicum* and *T. durum*, respectively.

Low average genetic similarity coefficients calculated for all the three species also revealed the presence of high genetic diversity in the Ethiopian tetraploid wheat accessions. These results are comparable with genetic similarity coefficients reported by various authors such as 0.52 by Huang et al. (2002), 0.31 (Plaschke et al. 1995), 0.57 (Bohn et al. 1999) and 0.23 by Prasad et al. (2000) for hexaploid *T. aestivum* accessions.

In general, this study demonstrated the presence of a high amount of genetic diversity in Ethiopian tetraploid wheat landraces. Because landraces prevail in farmers' fields, collection and conservation strategies in Ethiopia should focus on these resources in order to save them from the threat of genetic erosion they faced as a result of mainly displacement by improved bread wheat varieties and other crops. Our study also showed that microsatellites can be used for landrace identification and reliably estimating diversity and relationships among accessions and so to improve the efficiency of germplasm collection and properly plan the *in situ* and *ex situ* conservation programs. Many SSR loci might be significantly linked to agronomically important traits. Hence, Ethiopian tetraploid wheats deserve broader characterization at molecular levels, including mapping of important traits that can be used in future crop improvement programs.

Chapter 5 Regional patterns of microsatellite diversity in Ethiopian tetraploid wheat accessions

5.1 Abstract

This study was conducted to assess regional patterns of diversity of Ethiopian tetraploid wheat accessions and to identify areas of diversity that can be used as source of new germplasm for developing high yielding and stable varieties. A set of 133 Ethiopian tetraploid wheat accessions and 8 introduced cultivars was analyzed using 29 wheat microsatellite markers. A total of 383 alleles were detected with an average value of 13.14 alleles per locus. Relatively more number of alleles was observed on B genome than on A genome. Gene diversity indices ranged from 0.08 to 0.95 with a mean value of 0.72. Accessions collected from the same region were pooled together and number of alleles and gene diversity were calculated over the 29 SSRs for each region. Higher numbers of alleles were detected in Shewa (8.72) followed by Tigray (5.86) and Hararghe (5.76). The highest average gene diversity value was found in Shewa (0.65), followed by Gondar (0.64). No significant correlation was observed between geographic distance and genetic distance. Out of the total 383 different alleles detected, 93 (24.4 %) region specific alleles were observed. Region specific alleles were found across all chromosomes except for *Xgwm752*, *Xgwm155* and *Xgwm148*. Genetic similarities coefficients were estimated for all possible 55 pairs of regional comparisons. It ranged from 0.16 to 0.52, with a mean value of 0.50. All provinces were differentiated in the UPGMA cluster diagram.

5.2 Introduction

Genetic diversity varies geographically across a species range. It is, therefore, of prime importance for theory of conservation and application to compare and contrast populations across the entire range of geographical distribution of a species (Nevo 1998). Information on the extent and patterns of distribution of genetic variation of a crop species is essential for effective utilization of germplasm in plant breeding programmes (Hayward and Breese 1993). It is also useful to elucidate the taxonomy, evolution and origin of the crop (Diederichsen and Hammer 2003). Plant genetic resource conservation

strategies, supported by an understanding of the geographical distribution of genetic variation, are likely to result in a wider representation of conserved diversity in the *ex situ* gene banks and *in situ* genetic reserves. Sampling is more efficient if collecting trips have clearly defined target areas and habitats (Ferguson et al. 1998). The development of high yielding and stable varieties requires a continuous supply of new germplasm as a source of desirable genes and/or gene complexes (Ayana and Bekele 1998). The primary sources of such genes are landraces, introductions, weedy, and wild relatives of crop plants (Harlan 1992). The availability of such germplasm requires the identification of areas of diversity of various characters of agronomic importance, especially within the centers of diversity (Bekele 1984).

In Ethiopia, phenotypic diversity studies in relation to geographical regions on wheat (Bechere et al. 1996, Bekele 1984, Negassa 1986) demonstrated the wide diversity that prevails in the country. Unlike evaluation and characterization of accessions based on morphological and agronomic data, molecular markers provide the best estimate of genetic diversity being independent of confounding effects of environmental factors. It is useful to estimate genetic diversity based on collections from a large range of geographical areas and with as many markers as possible to get precise information. So far, no detailed study has been performed in Ethiopia to determine the regional patterns of diversity of Ethiopian tetraploid wheats using molecular markers. Therefore, the objective of the present study is to investigate the regional patterns of microsatellites diversity of Ethiopian tetraploid wheat landraces.

5.3 Materials and Methods

Plant material

Lists of the accession used and their region of collection/country of origin are presented in Table 5.1. Sources of accessions are presented in Teklu et al. (2006a).

Experimental procedures and data analysis

DNA extraction and PCR amplification procedures, microsatellite loci used and fragment scoring methods are as mentioned in Teklu et al. (2006a). The data were analyzed using

the SIMQUAL (Similarity for Qualitative Data) routine to generate Dice similarity coefficients (Dice 1945). These similarity coefficients were used to construct dendrograms using the UPGMA employing the SAHN from the NTSYS-pc, version 2.0 (Rohlf 1998). Gene diversity was calculated according to formula of Nei (1973): $Gene\,\text{diversity} = 1 - \sum \text{Pij}^2$ where Pij is the frequency of the jth allele for ith locus summed across all alleles for the locus. Genetic distances among pair of groups were computed as $G\text{Dxy} = 1 - \left({}^{2\text{Nxy}}\!/_{\text{Nx} + \text{Ny}} \right)$ where Nx and Ny are the number of alleles in groups X and Y, respectively, and Nxy is the number of alleles shared between the two groups (Nei and Li 1979).

5.4 Results

SSR Polymorphism and Genetic diversity

Genetic diversity of 133 Ethiopian tetraploid wheat accessions and 8 introduced cultivars were assessed using 29 highly polymorphic SSR markers (Plaschke et al. 1995, Röder et al. 1998). A total of 383 alleles were detected in the 133 Ethiopian tetraploid wheat accessions with an average value of 13.14 alleles per locus. The number of alleles observed ranged from 2 to 42 (Table 5.2). Comparison of number of alleles across genomes indicated that slightly more number of alleles was observed on B (13.27) genome than on A genome (13.00). Gene diversity indices ranged from 0.08 to 0.95 with a mean value of 0.72 (Table 5.2).

Regional patterns of SSR polymorphism and genetic diversity

Accessions collected from the same region were pooled together and number of alleles and gene diversity were calculated over the 29 SSRs for each region (Table 5.3). Because only one accession represented each region of Ilubabur, Kefa and Sidamo, respectively, accessions from these three regions were pooled together and the three provinces were designated as IKS. A high number of alleles was recorded in accessions collected from Shewa (8.72) followed by accessions from Tigray (5.86), Harerghe (5.76), and Gondar (5.03). The lowest number of alleles was recorded in Welega (Table 5.3). Of the different provinces, the highest average gene diversity value over the 29 SRRs is found in Shewa

Table 5.1. Collecting region, number, accession number (Acc. no) and altitude in meters of the collecting sites for Ethiopian tetraploid wheat landraces.

Region	Number	Acc. no	Altitude	Region	Number	Acc. no	Altitude	Region	Number	Acc. no	Altitude
		7021	2435			5101	2200			6138	2270
		7045	2620			5317	1980			6222	2700
		7072	2495			5386	2210			7114	2700
		216078	2990			7329	1990			7156	2735
Arsi	9	222555	2465			7337	2110			7158	2235
		226865	2590			7345	2310			7162	2210
		226935	2815			7347	2350			7213	2470
		226972	2440			7458	1800			7931	2600
		232230	1690	Harerghe	18	7460	1800			7958	2600
		5008	1669			7615	2500			8013	2800
		222322	2080			7892	2240			8057	2260
		231470	2450			7893	2240			203766	3100
Bale	7	238893	2400			7899	1660			208255	2515
		239698	2100			7901	1660			216070	2370
		239708	2440			7902	2320			216666	2190
		239712	2670			7913	2220			216692	2880
		7222	2340			7920	2400			222197	2275
Gamugofa	4	8284	1820			219253	2100			222460	2600
		8302	2780			5632	3067	Shewa	43	222497	2570
		8310	2760			7947	1960			226201	2300
		5390	2767			7956	2150			226241	2900
		5487	2050			7957	2600			226242	2900
		6916	2110			203931	2367			226272	3020
		6928	2660			204703	2310			226326	2590
		6932	1500			207849	2150			226332	2835
Gojam	10	6953	2500			213307	2340			226347	2190
		7692	2540	Tigray	18	216063	2487			226378	2860
		203922	2145			216452	2400			226391	2100
		219510	2400			216455	2340			226396	2510
		238102	2300			216623	2367			226968	2780
		6843	2580			216629	2600			231511	2400
		6846	2580			216633	2150			231516	2350
		6856	1760			223262	1945			237867	2110
		6917	2640			238118	1980			238105	2550
		7415	2720			238130	2280			2002/P^n 43#165	2720
Gonder	11	7935	2000			238135	2470			2002/P^n 55#235	2700
		8292	3120			7370	2500			2002/P^n 61#157	2600
		206672	2550			7372	1790			P^n 106#104	2630
		214263	2720			7512	2815			P^n 61#150	2600
		216587	2660	Welo	7	7559	2610			200872	
		222202	2100			7951	2440			210793	
Illubabor		8482	1820			222818	3250			227112	
Keffa	3	8483	2140			226942	1960	Exotic	8	5851	
Sidamo		227037	2240			5252	2510	cultivars		1623	
		8481	2030	Shewa		5314	1771			5948	
Welega	3	214528	2350			5550	3300			2192	
		222478	2090			5605	2600			18073	

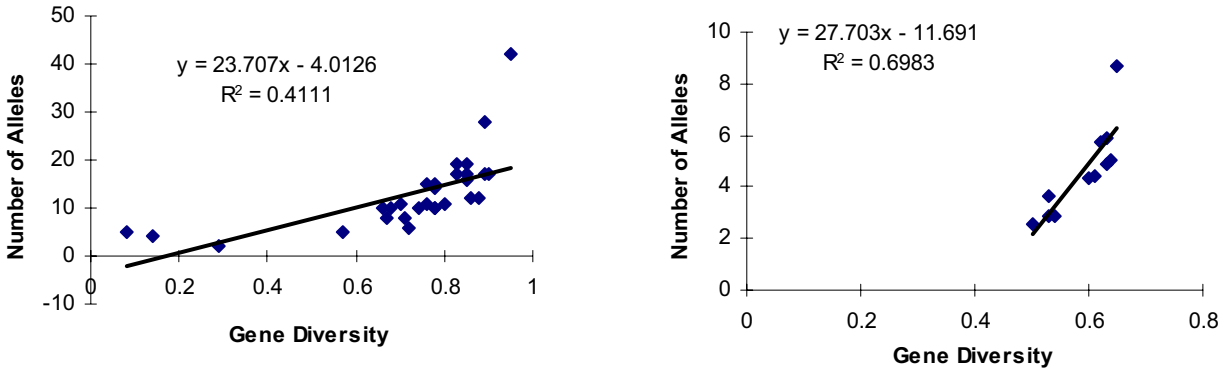
(0.65), followed by Gondar (0.64), Tigray (0.63), Gojam (0.63), Harerghe (0.62), Arsi 0.61) and Welo (0.60) and the lowest in Welega (0.50) (Table 5.3). Significant correlation coefficient of 0.836 ($P < 0.001$) was found between gene diversity and the number of alleles per locus calculated based on the different Ethiopian provinces (Figure 5.1b).

Table 5.2. Chromosomal location, size range, number of alleles and genetic diversity determined based on 133 Ethiopian tetraploid wheat landraces.

Microsatellite	Chromosomal location	Number of alleles	Gene diversity
*Xgwm*752	1AS	8	0.71
Xgwm357	1A(C)	10	0.78
*Xgwm*95	2AS	10	0.78
*Xgwm*312	2AL	42	0.95
*Xgwm*720	3AS	17	0.89
*Xgwm*155	3AL	11	0.80
*Xgwm*601	4AS	12	0.86
*Xgwm*160	4AL	5	0.57
*Xgwm*415	5AS	2	0.29
*Xgwm*186	5AL	10	0.74
*Xgwm*459	6AS	17	0.90
*Xgwm*1089	6AL	15	0.76
*Xgwm*631	7AS	4	0.14
*Xgwm*698	7AL	19	0.83
*Xgwm*18	1BS	6	0.72
Xtaglgap	1BS	19	0.85
*Xgwm*268	1BL	28	0.89
*Xgwm*148	2BS	11	0.76
*Xgwm*619	2BL	5	0.08
*Xgwm*389	3BS	12	0.88
*Xgwm*655	3BL	15	0.78
*Xgwm*898	4BS	10	0.66
*Xgwm*513	4BL	8	0.67
*Xgwm*540	5BS	10	0.68
*Xgwm*408	5BL	16	0.85
*Xgwm*680	6BS	14	0.78
*Xgwm*219	6BL	11	0.70
*Xgwm*46	7B(C)	17	0.83
*Xgwm*577	7BL	17	0.85

Altitudinal classes' diversity

All accessions occurring in an altitude class were also pooled together and their genetic diversity was determined. High average number of alleles per loci (10.52) and average gene diversity (0.72) was observed in altitude class II (2000 – 2500) followed by altitudinal class III (2501 to 3000) and altitude class I (< 2000). The lowest average number of alleles and gene diversity was observed in altitude class VI (>3000), which is attributed to its small sample size (Table 5.4).



(a) (b)

Figure 5.1. Correlation between gene diversity and the number of alleles, over 29 microsatellite loci, in (a) the total Ethiopian wheat landraces and (b) in different accession groups constructed based on regions of collections.

Distribution of region-specific alleles

Region-specific alleles were examined across all microsatellite loci for each region. Out of the total 383 different alleles detected in the Ethiopian tetraploid wheat accessions, 93 (24.4 %) region-specific alleles were observed. Region-specific alleles were found across all chromosomes except for *Xgwm* 752, 155 and 148. Shewa, Harerghe, Tigray, Gojam and Gondar are rich in region specific alleles (29, 11, 11, 10, and 10 respectively) (Table 5.3).

Table 5.3. Number of alleles, gene diversity and number of region-specific alleles calculated for the 29 microsatellite loci for the different regions.

Region	Number of alleles	Gene diversity	Number of region-specific alleles
Arsi	4.41	0.61	4
Bale	3.62	0.53	3
Gamugofa	2.86	0.54	5
Gojam	4.86	0.63	10
Gondar	5.03	0.64	10
Harerghe	5.76	0.62	11
IKS	2.86	0.53	5
Shewa	8.72	0.65	29
Tigray	5.86	0.63	11
Welega	2.59	0.50	1
Welo	4.34	0.60	4
Exotic	5.76	0.73	-

Genetic relationship

Assessment of genetic similarities coefficients estimates of all possible 8778 pairs of 133 Ethiopian tetraploid wheat accessions ranged from 0.06 to 0.90, with a mean value of 0.30. These results concur with genetic similarity coefficients reported by Plaschke et al. (1995) and Prasad et al. (2000). Genetic similarities coefficients were also estimated for all possible 55 pairs of regional comparisons. It ranged from 0.16 (between Arsi and Shewa) to 0.52 (between Arsi and Gojam), with a mean value of 0.50 (Appendix 6). All provinces were differentiated in the UPGMA cluster diagram, which had two major clusters (Figure 5.2). Cluster I contained only Gondar, implying that it is the most distinct group. All the remaining provinces including the exotic group were clustered in Cluster II. The relationship between Welega or IKS with others should be viewed with caution as a low number of accessions is represented from these two regions. The UPGMA analysis revealed that the genetic similarity among accessions collected in different geographic

regions, as in the case of Gojam and Arsi, Shewa and Tigray or Hararghe and Welega, was higher than that among neighboring regions. Differentiation among regions was weak.

Table 5.4. Number of alleles and gene diversity of Ethiopian tetraploid wheat landraces computed based on altitude classes.

Class	Altitude (m)	Number	No of alleles	Gene diversity
I	<2000	18	6.62	0.67
II	2001-2005	63	10.52	0.72
II	2501-3000	45	9.9	0.70
VI	>3000	6	3.97	0.62

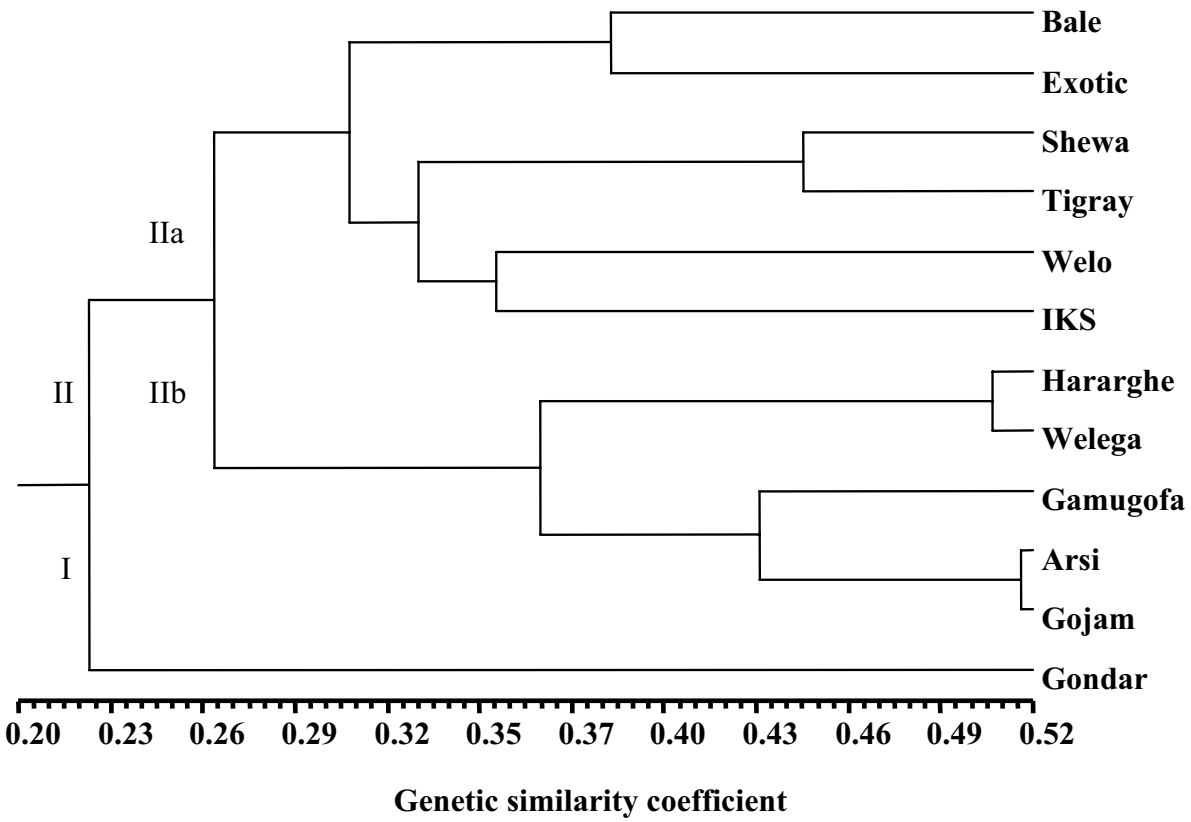


Figure 5.2. UPGMA dendogram generated based on mean Dice similarity coefficients among the accession groups of the 11 provinces and introduced cultivars.

5.5 Discussion

The regional and local genetic and ecological patterns provide the best guidelines for regional and local sampling strategies and *in situ* and *ex situ* conservation of genetic resources for use in breeding (Nevo 1998). In this study, regional patterns of genetic diversity of 133 Ethiopian tetraploid wheat accessions were assayed using 29 microsatellite markers. The number of alleles scored is comparable with those reported in similar studies in wheat. Fahima et al. (2002) found a mean of 18 allelic variants per locus in wild emmer wheat populations profiled with 20 *Xgwm* loci. Stachel et al. (2002) and Röder et al. (2002) detected a total of 202 and 199 alleles, respectively. Autrique et al. (1996) analyzed a collection of 113 durum wheat accessions using RFLPs and scored an average of 4.3 variants per probe. The extent of polymorphism in 26 Ethiopian tetraploid wheat accessions and four cultivars was assessed using 12 SSR markers and 96 polymorphic bands with number of alleles per locus ranging from 3 to 17 was detected (Messele 2001). Diversity and relationships among ten tetraploid wheat landrace populations collected from different localities in the central highlands of Ethiopia were studied using isozyme marker and a total of 18 alleles were detected at nine polymorphic loci (Tsegaye et al. 1996). Genetic diversity of 26 Ethiopian tetraploid wheat accessions and 6 cultivars were also studied using glutenine and gliadine storage protein and a total of 49 polymorphic bands were identified (Messele 2001). The high gene diversity observed in our experiment may be attributed to the use of large number of landraces collected from all over the country.

In this study, the B genome was more polymorphic than the A genome. The B genome chromosomes appear to be relatively richer than the A genome ones, in various classes of repetitive DNA, particularly microsatellites (Cuadrado and Schwarzacher 1998). According to the findings of Huang et al. (2002) and Zhang et al. (2002), microsatellite loci of the B genome are more variable than those of the A genome. On the contrary, the D genome presents a greater number of alleles than the B genome, while the greatest number was found in the A genome (Roussel et al. 2004). Stachel et al. (2000) observed the highest number of alleles on the A genome (5.2), closely followed by the B genome (5.1).

Gene diversity indices for the 29 microsatellite loci ranged from 0.08 to 0.95 with a mean value of 0.72. This value is analogous with the mean diversity values of 0.662, 0.71 and 0.77 which were reported by Roussel et al. (2004), Prasad et al. (2000) and Huang et al. (2002), respectively. A significant correlation coefficient of 0.641 ($P < 0.001$) was found between gene diversity and the number of alleles per locus for the 133 Ethiopian tetraploid wheat landraces (Figure 5.1a). This result is in agreement with the findings of Huang et al. (2002) and Roussel et al. (2004). On the contrary, Prasad et al. (2000) reported that the PIC value was not correlated with the number of alleles.

Regional comparison of SSR polymorphism showed that a high number of alleles was recorded in accessions from Shewa. Messele (2001) analyzed 26 Ethiopian tetraploid wheats that were collected from Shewa province using 12 *Xgwm* loci and revealed 7.9 alleles per locus. The higher number of alleles in Shewa in this study may be attributed to a higher number of accessions that covered most districts of the region. Evaluations of the association between genetic and geographic distances allow one to evaluate the *relative* influences of gene flow and drift on genetic diversity within and between regions. The correlation between genetic distance and geographic distance was low and non significant ($r = -0.15$, $P = 0.740$), indicating the presence of high level of migration (gene flow) among regions. Similar finding were reported in evaluations based on pheno-morphic and agronomic traits (Teklu et al. 2006a). Geographical separation of populations, a parameter usually considered important when collecting germplasm, also didnot predict genetic differences very well in the study of del Rio et al. (2001), del Rio and Bamberg (2002) and Fahima et al. (1999). The absence of remarkable differences in gene diversity among the different provinces is in agreement with the findings of several morphological diversity studies in Ethiopian wheats. Negassa (1986) characterized 293 Ethiopian wheats (*Triticum spp*.) (both tetraploid and hexaploid) that were collected from eleven provinces and reported a non significant Shannon weaver diversity index for differences among provinces but significant differences within provinces. Similarly, Bechere et al. (1996) examined the patterns of eleven phenotypic characters in 27 tetraploid wheat landraces population consisting of 2876 entries collected from the north and north central regions of

Ethiopia. They found significant differences, for the overall characters, only among populations within regions and altitude groups, but not among regions or altitude groups. Likewise, Bekele (1984) and Pecetti and Damania (1996) reported that the magnitude of the contribution of different components of hierarchy to the total quantitative phenotypic diversity is high at the lowest level (within localities, being followed by the differences among the population in a region and among regions). Microclimatic conditions indeed generated both protein (Nevo et al. 1996) and DNA (Ouwor et al. 1997) patterns of polymorphism that paralleled macro scale environmental conditions. Compared to Ethiopian provinces, gene diversity of the introduced cultivars group was high. This might be due to the fact that these accessions were from different countries covering a wide range of edaphic and climatic condition attributing to high genetic diversity.

Similar to our findings, high frequencies of rare alleles have also been reported in *Triticum urartu* (Moghaddam et al. 2000), in *Ae. tauschi* (Dudnikov 1998), in European wheat varieties (Röder et al. 2000) and in Gatersleben wheat collections (Huang et al. 2002). Similarly, Roussel et al. (2004) reported on the average, about 72% of the total number of alleles were observed with a frequency of less than 5% and were considered to be rare alleles. The relative abundance of rare alleles means that they make a greater contribution to the overall genetic diversity of the collection (del Rio et al. 2001, Roussel et al. 2004). Hence, it is important to include region-specific or rare alleles for maximizing the genetic variations in the gene bank collections and to utilize them in breeding. Region-specific alleles were found in all provinces implying that the different provinces require special attention in order to capture the whole range of region-specific alleles.

Genetic erosion is threatening the genetic base of tetraploid wheat landraces (FA0 1996; Worede 1983; IBCR 2002; Teklu and Hammer 2006d). Conservation and utilization programs should maximize sampling strategies by following the ecological-genetic factors, allozyme and DNA markers as effectively predictive guidelines (Nevo 1998). The presence of ample genetic diversity within each region indicated the importance of each region for future germplasm collection. Even in regions where fewer accessions

were represented and thereby lower numbers of alleles were recorded, some region specific alleles that might be linked to useful trait were found. Hence, it is important to consider these regions as well in future germplasm collection mission in order to save the irreplaceable landraces, if lost. Comparison of accessions based on altitude demonstrates that areas in altitudinal classes II and III are more suitable for future *in situ* conservation and germplasm collection.

Zhang et al. (2002) assayed 43 Chinese wheat varieties with 90 polymorphic SSR to determine the minimum number of SSR alleles required to detect genetic relationships in their accessions. They suggested that 350 to 400 alleles were needed to detect genetic relationships among common wheat varieties. Likewise, Roussel et al. (2004) reported that the 609 alleles which were detected from 559 French bread wheat accessions (landraces and registered varieties) using a set of 41 wheat microsatellite markers were large enough to indicate the robustness of their results. Therefore, the 383 alleles recorded in this study are sufficient enough to indicate the effectiveness of microsatellites in differentiating the Ethiopian tetraploid wheat landraces.

Chapter 6 Simple sequence repeats marker polymorphism in emmer wheat (*Triticum dicoccon* Schrank): Analysis of genetic diversity and differentiation

6.1 Abstract

Genetic diversity was investigated in 73 accessions of emmer wheat (*Triticum dicoccon* Schrank) from 11 geographical regions using a set of 29 simple-sequence repeat (SSR or microsatellite) markers, representing at least two markers for each chromosome. The SSR primers amplified a total of 357 different alleles with an average of 12.31 alleles per locus. The number of fragments detected by each primer ranged between 6 (*Xgwm1066*) and 21 (*Xgwm268*). Null alleles were detected in nine of the 29 primers used. A high level of gene diversity index was observed. Across the 29 primers, gene diversity ranged from 0.60 (*Xgwm46*) to 0.94 (*Xgwm655*), with a mean of 0.82. There was a highly significant correlation ($r = 0.882$; $P < 0.01$) between gene diversity index and the number of loci, showing the number of loci *per se* is a strong indicator of diversity. Analysis of genetic diversity within and among eleven geographical regions revealed most of the genetic diversity of the total sample resided within regions. The coefficient of gene differentiation (Gst = 0.27) showed that the genetic variation within and among the 11 geographical regions was 73 and 27%, respectively. High value of mean number of alleles per locus was found in Iran (4.86) followed by Morocco (4.10) and Armenia (4.03). On the contrary, lower mean number of alleles per locus was detected in Yemen (2.83). The average gene diversity index across regions ranged from 0.52 (Slovakia) to 0.67 (Morocco) with an average of 0.60. Multivariate techniques of principal component analysis and clustering were employed to examine genetic relationship among the 73 emmer wheat accessions vis-à-vis geographical regions of collections. The genetic distance coefficients for all possible 55 pairs of regional comparisons ranged from 0.63 (between Iran and Armenia, Georgia and Azerbaijan, Georgia and Slovakia) to 0.97 (between Morocco and Yemen, Spain and Georgia, and Turkey and Iran) with a mean of 0.82. From the PCA results, a two dimensional plot of PC1 versus PC2 was constructed. The scatter plot of the first two principal components which explained altogether 27 % of the total variation depicted the presence of a clear pattern of geographical differentiation except in few cases like accessions from Caucasian region. Similar pattern of genetic

relationships among accessions was observed in cluster analysis. The study provided genetic information of emmer wheat in relation to geographical regions of origin. The information could be utilized in crop improvement, germplasm conservation programs, and in further investigation.

6.2 Introduction

Emmer wheat (*Triticum dicoccon* Schrank) is an allopolyploid species (2n = 4x = 28) with the genome formula AABB. It is one of the first cereals ever domesticated and belongs to the oldest crops of the world (Damania et al. 1992; Nebsitt and Samuel 1995; Hammer et al. 2004). It is originated in the mountains of the Fertile Crescent: in Iran, Turkey, Iraq, Jordan, Syria, Israel and Palestine, where its wild progenitor (*T. dicoccoides* Koern. ex Asch. et Graebn.) Schweinf. still thrives (Harlan and Zohary 1966; Perrino et al. 1996). According to Szabo´ and Hammer (1996) and Filatenko et al. (2001), domesticated emmer was widely distributed from Northern Africa through most parts of Europe and the Mediterranean area to Central Asia. Emmer was the only or by far the principal wheat until the emergence of the tetraploid naked species in the 1st millennium BC and in most of Europe competition from other wheat species was significant up to about the birth of Christ (Helbaek 1959). With the development of agriculture, however, the cultivation of emmer wheat was replaced by durum wheat and soft wheat and became increasingly limited. At present emmer wheat is under cultivation in marginal farming areas of Oman, Slovakia, Yemen, Italy, the Balkan Peninsula, Turkey, Ethiopian highlands, Morocco, India, and in some parts of Spain (Hammer et al. 2004; Hammer and Perrino 1984; D'Antuono 1989; Peña-Chocarro 1995).

Emmer wheat is grown for human consumption and cattle feed. It is one of the high protein containing cultivated wheat species. Its protein levels as threshed range from 5%-35% higher than oats or barley, while the protein of the grain kernels range from 18.5%-21.5% (Stallknecht et al. 1997). With emmer's high protein content and smooth, easily digested starch, the gruel is especially favored by nursing mothers. One of the most valuable characters of emmer is the occurrence of disease resistance forms. It is non susceptible to rust and powdery mildew (Vavilov 1931). A high degree of allelic

variation was evident for the seed storage proteins (glutenins and gliadins) (Pflüger et al. 2001; Piergiovanni and Blanco 1999). This wide polymorphism can be used to transfer new quality genes to bread and durum wheats, and to widen its genetic basis. Emmer wheat is thought to be the base population from which the founder genotypes of durum wheat populations were derived, and thus it represents a genetic resource for durum wheat cultivars, providing good genes for resistance to biotic and abiotic stress (Corazza et al. 1986; Damania et al. 1992).

A renewed interest has occurred during the last decade toward local varieties of emmer wheat (Barcaccia et al. 2002). The revival of traditional food has increased the interest in hulled wheats over the last few years. This is due to the low-input techniques used for their management (D'Antuono 1989), the increasing demand for unconventional foods, and the therapeutic properties attributed to their derivatives (Auricchio et al. 1982). The potential of emmer wheat resides mainly in its genetic potential to obtain new products with high digestibility and non-toxicity for coeliac disease (Pflüger et al. 2001). In addition, the variability of emmer wheat could be a useful gene reservoir for the breeding programs of durum and bread wheats (Sharma et al. 1981; Srivastava and Damania 1989). Currently, emmer is a candidate crop for sustainable agriculture in Italy (Figliuolo and Perrino 2004). Pre-breeding and breeding work aimed at improving yield stability and quality traits has been started (Laghetti et al. 1999), and recently two new cultivars have been selected and registered. To effectively utilize the crop, Perrino et al. (1996) suggested the importance of carrying out a study on hulled wheat germplasm regarding phenotypic and genotypic variability and diversification in the center of cultivation of emmer wheat.

The estimation of genetic diversity at the DNA level improves the identification and characterization of primary and secondary centers of diversity (Chowdhury and Slinkard 2000; Serret et al. 1997). Knowledge of diversity patterns will also allow breeders to better understand the evolutionary relationships among accessions, to sample germplasm in a more systematic fashion, and to develop strategies to incorporate useful diversity in their breeding programs and bring optimal improvement of the quality and productivity

of crops (Bretting and Widrlechner 1995). Despite this, the genetic characterization at the DNA level which is a key tool for modern exploitation of local varieties of neglected crops such as emmer wheat are lacking (Barcaccia et al. 2002). Therefore, SSR primers were used in this study to investigate the genetic diversity of emmer wheat collected from eleven different geographical regions. In order to study the relationship of the eleven gene pools with Abyssinian emmer wheat accessions, 26 SSR primers that are common in this experiment and in the study by Teklu et al. (2006c) were considered.

6.3 Materials and Methods

Plant materials

A total of 73 accessions of *Triticum dicoccon* Schrank germplasm held at IPK, Gatersleben, Germany were used. The accessions were collected from 11 different countries of Near East, Middle East, Central Asia, Europe and North Africa. Besides, the hexaploid *T. aestivum* varieties 'Chinese Spring' (CS) and 'Aztec' were included as standards. Lists of the accession and country of origin are presented in Table 6.1.

DNA extraction and PCR amplification

For each accession, nine seeds were pooled and DNA was isolated following the procedure in Fulton et al. (1995) with extraction buffer described in Plaschke et al. (1995). PCR amplifications were performed as described by Röder et al. (1998; unpublished). Fragments were detected following the method described in Teklu et al. (2006a; 2006b).

Microsatellite loci

The microsatellites used for analysis are listed in Table 6.2. Twenty-eight Gatersleben Wheat Microsatellites and one microsatellite from a pseudogliadine gene, Taglgap, representing two markers for each chromosomes of the A and B genomes were used. The microsatellite primers used were described by Röder et al. (1998) and for the primer Taglgap by Devos et al. (1995).

Table 6.1. Country of origin, sample size (N), and accessions number of plant materials used in the study.

Country	N	Accessions Number
Armenia	9	Tri 9542, Tri 9543, Tri 11167, Tri 16207, Tri 17698, Tri 18039, Tri 18041, Tri 18042, and Tri 18043
Azerbaijan	7	Tri 18092, Tri 18093, Tri 18094, Tri 18095, Tri 18097, Tri 18098, and Tri 18099
Slovakia	9	Tri 9867, Tri 9868, Tri 10063, Tri 10064, Tri 10066Tri 10069, Tri 10070, Tri 10071, and Tri 10317
Georgia	6	Tri 13158, Tri 16608, Tri 16609, Tri 18193, Tri 18197, and Tri 18206
Iran	11	Tri 6141, Tri 6158, Tri 7305, Tri 18080, Tri 18081, Tri 18082, Tri 5861, Tri 18084, Tri 18085, Tri 18087, and Tri 18089
Israel	4	Tri 3424, Tri 16877, Tri 16879, and Tri 16880
Italy	6	Tri 16723, Tri 16726, Tri 16798, Tri 16803, Tri 16807, and Tri 18243
Yemen	4	Tri 28004, Tri 28027, Tri 28072, and Tri 18073
Morocco	6	Tri 2884, Tri 4313, Tri 17429, Tri 18167, Tri 18168, and Tri 18169
Spain	6	Tri 16885, Tri 18114, Tri 18115, Tri 18156, Tri 18161, and Tri 18164
Turkey	5	Tri 584, Tri 17023, Tri 17038, Tri 17040, and Tri 17058
Total	73	

Statistical analysis

SSR data scoring, estimation of gene diversity and genetic distances among pair of groups, were conducted following the procedures applied in Teklu et al. (2006a; 2006b; 2005c). For a single locus, gene diversity ranges from 0 (monomorphic) to 1 (very higly discriminative with many alleles in equal frequencies). In measuring the Nei's coefficient of genetic differentiation (GST) (Nei 1973; Nei and Chesser 1983), the total genetic variability (HT) was first subdivided into within-accessions (HS) and between-accessions (DST) components, following postulations by Nei (1973). *HS*, the within-group diversity, was calculated as the mean of the diversity values obtained for the separate regions. GST, which ranges from 0 (all genetic variation maintained within regions) to 1 (all genetic

variation among regions) was calculated as (HT- HS)/HT. This coefficient indicates how large a proportion of the overall variation is the result of differences within and between geographical regions of origin. The Pearson's correlation coefficient between the gene diversity and number of loci has been computed. Genetic relationships between germplasm pools were examined following the multicariate procedures used in Teklu and Hammer (2005a).

6.4 Results

Genetic diversity

On the whole, the 29 microsatellites produced 357 alleles in the 73 emmer wheat accessions, with an average of 12.31 alleles per locus. The number of fragments detected by each primer ranged between 6 (*Xgwm1066*) and 21 (*Xgwm268*). Null alleles were detected in ten of the 29 primers used. A high level of gene diversity index was observed. Across the 29 primers, gene diversity ranged from 0.60 (*Xgwm46*) to 0.94 (*Xgwm655*), with a mean of 0.82. Number of alleles and gene diversity values calculated for the Ethiopian emmer are 6.95 and 0.62%, respectively (Table 6.2).

Examination of genetic diversity present within and among the eleven geographical regions revealed Nei's coefficient of genetic differentiation value of 0.27 (Table 6.2). This indicated that 27% of the total genetic diversity existed among regions, while 73% existed within regions, which suggests that the genetic diversity of emmer wheat detected in the present study is mainly lying within regions. The distribution of variability between- and within-regions, however, varies between primers. For example, *Xgwm160, Xgwm631* and *Xgwm680* detected most variability between regions (41, 41 and 55%, respectively), whereas primers *Xgwm95, Xgwm1066* and *Xgwm1089* detected most variation within populations, in which each detected 82% (Table 6.2).

Genetic diversity and geographical differentiation

The 73 accessions were grouped into 11 groups according to their country of origin and analysis of genetic diversity was performed (Table 6.3). High value of mean number of alleles per locus was found in Iran (4.86) followed by Morocco (4.10) and Armenia

(4.03). On the contrary, low mean number of alleles per locus was detected in Yemen (2.83). Heterozygosity, defined as the identification of more than one allele for a given marker in a single accession (Röder et al. 2002), was also employed for evaluating

Table 6.2. Microsatellites, chromosomal location, product size range, number of alleles and genetic diversity and gene differentiation values determined based on 73 emmer wheat accessions.

Microsatellite	Chromosomal location	Product size range (bp)	Number of alleles	Ht*	Hs	DST	Gst
*Xgwm*752	1AS	110-148	8	0.73	0.58	0.15	0.21
Xgwm357	1A(C)	120-134	7	0.76	0.51	0.25	0.33
*Xgwm*95	2AS	116-134	8	0.73	0.59	0.14	0.19
*Xgwm*312	2AL	null, 201-251	20	0.92	0.67	0.25	0.27
*Xgwm*720	3AS	null, 120-174	20	0.93	0.70	0.23	0.25
*Xgwm*155	3AL	113-159	15	0.91	0.70	0.21	0.23
*Xgwm*601	4AS	121-169	18	0.90	0.71	0.19	0.21
*Xgwm*160	4AL	179-191	7	0.74	0.44	0.30	0.41
*Xgwm*415	5AS	null, 125-135	7	0.78	0.51	0.27	0.35
*Xgwm*186	5AL	null, 120-148	15	0.90	0.64	0.26	0.29
*Xgwm*334	6AS	null, 107-135	14	0.89	0.70	0.19	0.21
*Xgwm*1089	6AL	124-174	10	0.80	0.66	0.14	0.18
*Xgwm*631	7AS	185-205	10	0.78	0.46	0.32	0.41
*Xgwm*1066	7AL	120-140	6	0.60	0.49	0.11	0.18
*Xgwm*18	1BS	180-212	14	0.87	0.67	0.20	0.23
Xtaglgap	1BS	null, 215-260	13	0.80	0.58	0.22	0.28
*Xgwm*268	1BL	null, 180-242	21	0.94	0.73	0.21	0.22
*Xgwm*148	2BS	140-170	14	0.89	0.65	0.24	0.27
*Xgwm*619	2BL	null, 135-173	14	0.86	0.69	0.17	0.20
*Xgwm*389	3BS	116-148	13	0.83	0.59	0.24	0.29
*Xgwm*655	3BL	155-177	10	0.80	0.63	0.17	0.21
*Xgwm*898	4BS	103-117	8	0.79	0.64	0.15	0.19
*Xgwm*513	4BL	137-153	9	0.76	0.48	0.28	0.37
*Xgwm*213	5BS	150-180	12	0.75	0.54	0.21	0.28
*Xgwm*408	5BL	null,148-190	11	0.79	0.54	0.25	0.32
*Xgwm*680	6BS	123-151	8	0.71	0.32	0.39	0.55
*Xgwm*219	6BL	155-195	15	0.89	0.66	0.23	0.26
*Xgwm*46	7B(C)	145-175	12	0.73	0.57	0.16	0.22
*Xgwm*577	7BL	null, 128-166	18	0.91	0.62	0.29	0.32
Mean			12.31	0.82	0.60	0.22	0.27

*, Ht refers to the entire diversity; HS, the within-group diversity; DST, the between group diversity, and Gst to the Nei's coefficient of genetic differentiation.

geographical patterns of genetic diversity. The value of mean heterozygosity expressed as percent for the eleven regions was 19.96%. The minimum heterozygosity value of 12.5% was observed in the accessions from Spain, and the maximum value was detected in

accessions from Azerbaijan and Slovakia which are 28% and 33%, respectively (Table 6.3).

Table 6.3. Regions of origin, number of alleles per locus, gene diversity, heterozygosity and number of region-specific alleles calculated for the 29 microsatellite loci for the 11 geographical regions.

Region	Number of alleles per locus	Gene diversity	Heterozygosity (%)	No of region-specific alleles
Armenia	4.03	0.55	17.1	4
Azerbaijan	3.45	0.52	28.0	6
Slovakia	3.34	0.52	33.0	6
Ethiopia*	6.95	0.62	10.5	-
Georgia	3.90	0.63	15.0	5
Iran	4.86	0.66	16.3	9
Israel	3.03	0.56	14.8	9
Italy	3.62	0.64	23.8	11
Yemen	2.83	0.58	15.9	13
Morocco	4.10	0.67	26.9	13
Spain	3.59	0.61	12.5	5
Turkey	3.38	0.60	16.3	7

*, From Teklu et al. (2006a).

Region specific alleles (present in one region but absent in the other) was observed in all regions (Table 6.3). A total of 88 region specific alleles were found which comprises about 26.7% of the total number of alleles detected in the entire data set. Accessions from Armenia have the lowest number (4) whereas accessions from Morocco and Yemen have the highest (13) number of regions specific alleles. The average diversity index across regions ranged from 0.52 (Slovakia) to 0.67 (Morocco) with an average of 0.60. Of the remaining regions, higher diversity was recorded in accessions from Iran and Italy with

gene diversity values of 0.64 and 0.66 respectively. In general, in all regions diversity index values of greater than 0.5 were detected.

Table 6.4. Genetic distance estimates among 11 geographical regions.

	Geographical Regions*	1	2	3	4	5	6	7	8	9	10	11
1	Armenia	-										
2	Azerbaijan	0.78	-									
3	Slovakia	0.72	0.73	-								
4	Georgia	0.72	0.63	0.63	-							
5	Iran	0.63	0.73	0.80	0.63	-						
6	Israel	0.81	0.87	0.87	0.87	0.90	-					
7	Italy	0.83	0.88	0.82	0.82	0.75	0.75	-				
8	Yemen	0.88	0.94	0.94	0.87	0.87	0.90	0.79	-			
9	Morocco	0.94	0.84	0.75	0.81	0.94	0.78	0.88	0.97	-		
10	Spain	0.88	0.87	0.94	0.97	0.77	0.81	0.85	0.88	0.94	-	
11	Turkey	0.87	0.83	0.86	0.86	0.97	0.46	0.78	0.93	0.71	0.80	-
	Mean distance to other regions	0.81	0.81	0.80	0.78	0.80	0.80	0.81	0.90	0.86	0.87	0.81

Genetic distance

The genetic distance coefficients for all possible 55 pairs of regional comparison ranged from 0.63 (between Iran and Armenia, Georgia and Azerbaijan, Georgia and Slovakia) to 0.97 (between Morocco and Yemen, Spain and Georgia, and Turkey and Iran) with a mean of 0.82. Although Yemen, Spain and Morocco have the greatest mean distance to the other regions (0.90, 0.87 and 0.86 respectively), mean genetic distance is nearly equal and high in all the remaining regions – always greater than 0.78 (Table 6.4). This also demonstrates that the within region variations have a more pronounced contribution to the overall diversity of emmer wheat accessions observed in the present study. The presence of high between region genetic distance also indicates the existence of wide differentiation among geographical regions.

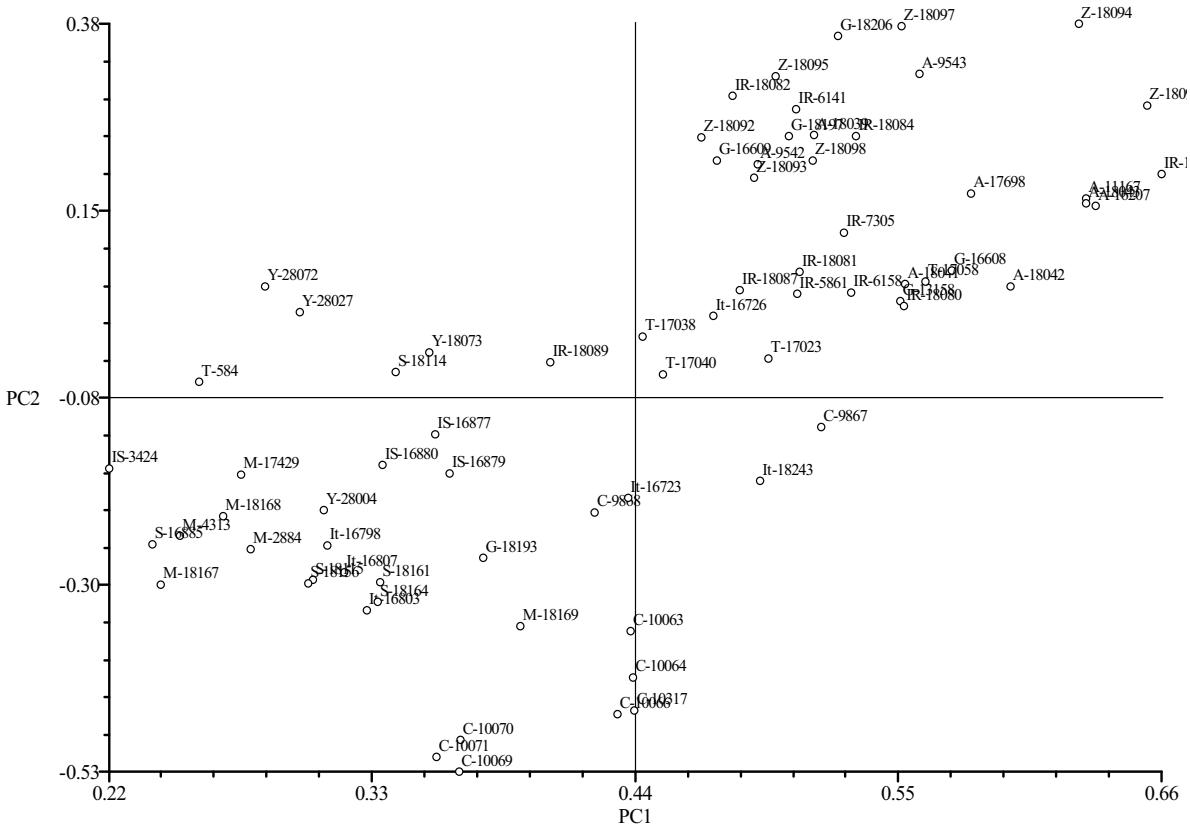


Figure 6.1. Two-dimensional principal co-ordinate analysis based Dice similarity coefficient for 73 emmer wheat accessions. PC1 and PC2 are the first and second principal co-ordinate.

Genetic relationship

Multivariate techniques of principal component analysis and clustering were employed to examine genetic relationship among the 73 emmer wheat accessions vis-à-vis geographical regions of collections. The first and the second principal components accounted for 20.96% and 6.04% of the total variation, respectively. From the PCA results, a two dimensional plot of PC1 versus PC2 was constructed (Figure 6.1). In general a clear pattern of geographical differentiation was observed. With few exceptions, accessions were separated based on regions of origin. For example, the accessions from Morocco were plotted separately from others. Likewise, accessions from

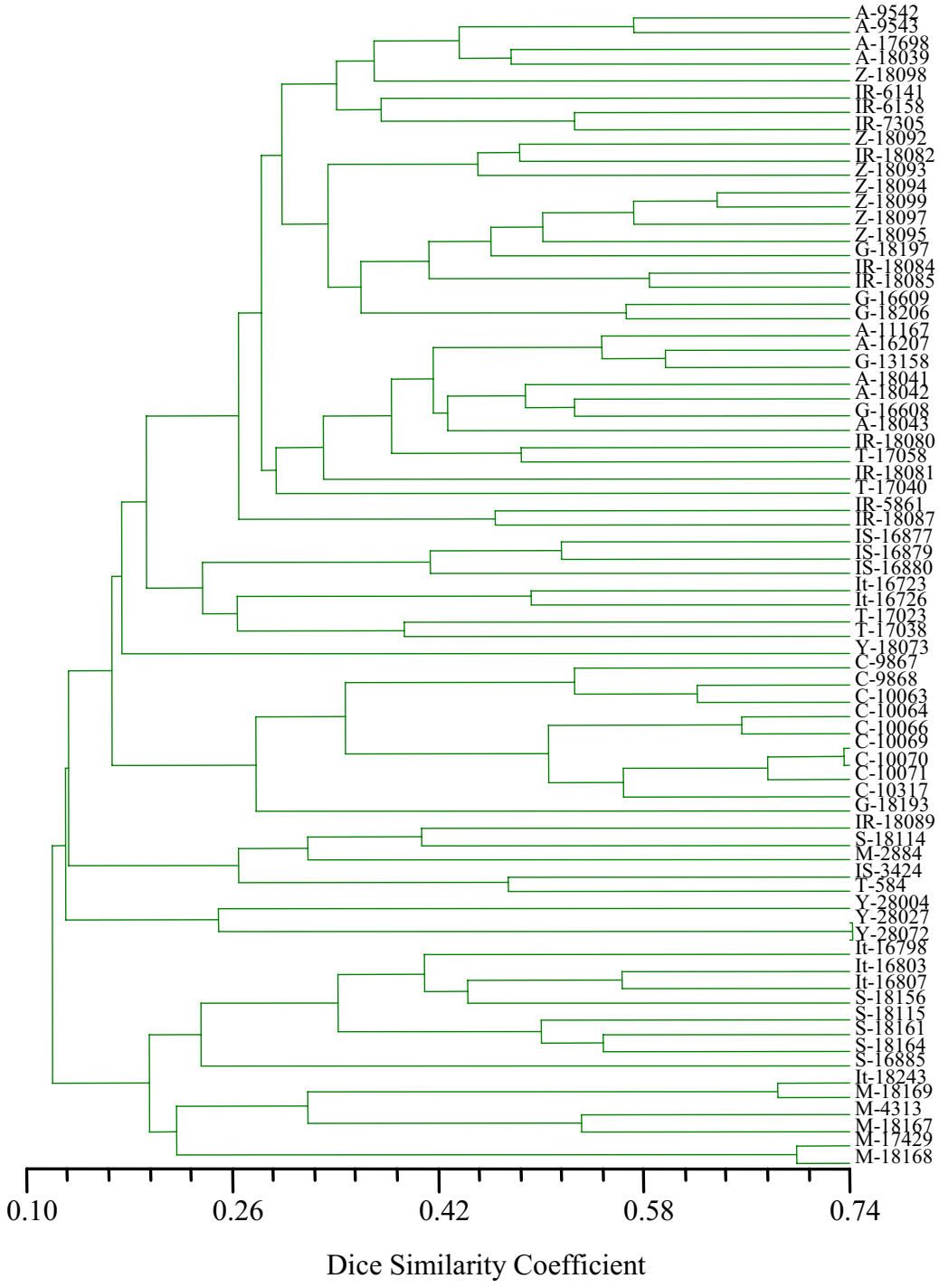


Figure 6.2. UPGMA phenogram based on Dice similarity coefficients of the 73 emmer accessions. Prefixes are added to accessions numbers with the following codes: A- = Armenia; C- = Slovakia; G- = Georgia; Is- = Israel; Ir- = Iran; It- = Italy; M- Morocco; S- = Spain; T- = Turkey; Y- = Yemen; and Z- = Azerbaijan.

Slovakia, Italy, Spain and Yemen were grouped separately. However, accessions from Caucasian region (Azerbaijan, Armenia, and Georgia) are grouped together in the second quadrant. They are mixed with some accessions from Iran and Slovakia.

Cluster analysis was also computed to analyze genetic relationship among the 73 emmer accessions. The resulting dendogram classified all 73 accessions into two major clusters in which the first cluster contains only materials from Spain, Morocco and Italy (Figure 6.2). Close examination of this cluster indicates the clear pattern of geographical differentiation. In cluster 2, relationships between groups based on cluster analysis and geographical origin was observed. For instance, the different accessions from Slovakia are clustered together. A similar pattern was observed in accessions from Morocco, Spain, Italy and Yemen. As with the scatter plot of the principal component analysis, overlapping in grouping was observed in accessions from Caucasian region.

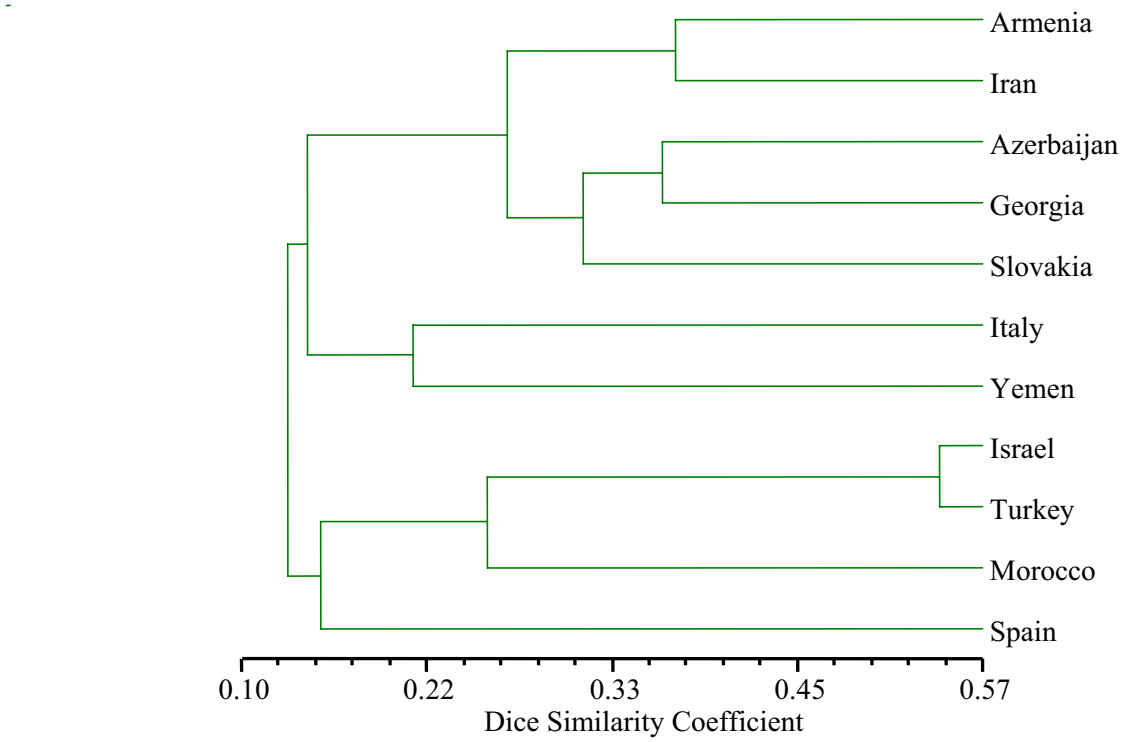


Figure 6.3. A dendogram generated based on Dice similarity coefficients among the 11 geographical regions of origin.

To further elucidate the genetic relationship, a dendogram was constructed based on the eleven accession groups. All regions were differentiated in the cluster diagram, which had two major clusters (Figure 5.3). Spain, which was clustered in cluster one along with Morocco, Israel and Turkey, was the most distinct group. In cluster two, Armenia, Iran, Azerbaijan, and Georgia are clustered in the same sub-cluster while Italy and Yemen were put in the other sub cluster. Except Israel and Turkey, which have been connected by branches whose vertex has a coefficient of 0.54, all regions were connected at the low level of coefficient, implying the presence of high regional variation.

6.5 Discussion

Examining genetic diversity within as well as genetic relatedness among populations from different geographic areas is expected to have a significant impact on the conservation and utilization programs of emmer germplasm. To this end, a diversity study was conducted on 73 emmer wheat accessions collected from 11 geographical regions using 29 SSR markers. High degree of polymorphism was detected as revealed by the high number of alleles per locus (12.31) and gene diversity (0.82) values. In similar studies, Figliuolo and Perrino (2004) analyzed a collection of 194 emmer accessions with 15 SSRs loci and detected an average 7.7 alleles/locus. Barcaccia et al. (2002) studied eleven local varieties of emmer from central and southern Italy using 17 RAPD marker loci and reported an average of 4.25 alleles per primer. In durum wheat, Eujayl et al. (2001) detected within a sample of 64 genotypes an average of 5.5 alleles per locus. Fahima et al. (2002) found a mean of 18 allelic variants per locus in wild emmer wheat populations profiled with 20 SSR loci. Bertini et al. (2001) found an average of 5.2 alleles / locus from 30 genotypes of spelt wheat analyzed using 17 microsatellite loci. Röder et al. (2002) characterized 502 European wheat varieties using 19 microsatellites markers and found an average alleles per locus and PIC of 10.5 and 0.674, respectively. There was a highly significant correlation ($r = 0.882$; $P < 0.01$) between gene diversity index and the number of loci, showing the number of loci *per se* is a strong indicator of diversity. It is interesting to find that genome B was more polymorphic than genome A as this genome determines the resistance of emmer wheat to powdery mildew (Krivchenko et al. 1979). The high gene diversity index and the average number of alleles per SSR

marker reported herein indicate the presence of potentially utilizable polymorphism for breeding.

Ten primers failed to give good amplification indicating the presence of null-alleles. This is in agreement with the findings of Röder et al. (2002) who reported the occurrence of null alleles in 11 out of 19 markers. Across geographical regions, a non significant correlation ($r = 0.581$; $P < 0.05$) between gene diversity and the number of alleles, indicating the importance of both variables for explaining diversity. For example, high diversity is obtained from accessions from Yemen although low number of alleles per locus was scored. Despite the large difference in number of alleles between Georgian and Italian gene pools, their gene diversity values are nearly equal. Close observation of the alleles amplified for many of the accessions revealed the presence of heterozygosity or heterogeneity in which a single SSR marker locus amplified two or more alleles within a single bulk template of an accession. Evidence of internal heterogeneity in wheat, which is a self pollinating plant, was also reported by Röder et al. (2002). They found the presence of internal heterogeneity in some of the 502 European wheat varieties characterized using 19 microsatellites markers. The presence of heterozygosity might also indicate plants comprising the template bulk contained mixtures for the particular SSR marker locus.

A relatively high level of gene diversity was found in Morocco suggesting that this pool was the most diverse among the 11 germplasm pools in our study. The presence of a relatively high level of diversity in Morocco, and countries like Spain, Georgia and Italy indicated the importance of secondary centers of diversity. Harlan (1955) studied the distribution of variability in crop plants and concluded that there exist several centers of diversity in different crops which could not be regarded as centers of origin. The centers of diversity are not confined exclusively to centers of origin. Due to differences in ecological conditions and human activities in establishing new ties for introgressive hybridization, the centers of variability may differ considerably in frequency of different genes in different geographical regions. The potential breeding value of the secondary centers of variability is no less important than that of the primary centers (Harlan 1971).

Dvorak et al. (1998) suggested that the regions around the Caspian sea, such as Iran and Armenia, were the geographical place of origin of *T. aestivum* and thus large genetic variation should exist in the origin sites. However, comparative analysis of microsatellite diversity of 998 bread wheat germplasm among the regions of Africa, America, the Near East, the Middle East, North Europe, Southeast Europe and Southwest Europe indicated that the greatest genetic diversity was found in the Near East (Huang et al. 2002).

The total genetic diversity observed in the present study was explained by high variation within geographical regions than among regional differentiation. Nevertheless, it should be noted that there exists wide differentiation among the different regions as revealed by mean genetic distance estimates, cluster and principal component analysis. High within population genetic diversity (≥ 93%) was also found in barley (Dai and Zhang 1989). Besides, most of the total genetic diversity of grass pea occurred within the various geographic regions in the study by Chowdhury and Slinkard (2000). On the contrary, Koenig and Gepts (1989) reported low intrapopulation genetic diversity (Hs = 0.006) in common bean. Environmental heterogeneity among the different countries could have affected natural selection forces and thereby caused significant genetic differentiation with in each region. Variation in anthropogenic factors (human selection forces) might have also caused a significant regional differentiation. The high within region variation coupled with the occurrence of region specific alleles, which might be linked to useful traits, in the different region, may implicate that each region may contain distinct alleles and thus it is important to give a due attention for breeding and germplasm collection for each region. Local varieties have the highest genetic variation and adaptation to the natural and anthropological environment in which they originated. They represent an irreplaceable bank of highly co-adapted genotypes (Barcaccia et al. 2002).

Genetic relationships between accessions were assessed using principal coordinate analysis. The scatter plot of the first two principal components which explained altogether 27% of the total variation depicted the presence of clear pattern of geographical differentiation except in Caucasian gene pools. The absence of clear pattern of geographical variation in the Caucasian region could be explained by the possible

existence of germplasm exchange among the neighboring regions. Similarities in climate and culture could also play important role. Above all, in the Caucasian region the race of emmer that was mainly grown belongs to subspecies *asiaticum*. Based on geographical distribution and morphological and ecological characteristics, four subspecies were recognized in *T. dicoccon*: 1) subsp. *maroccanum* Flaskb. (Moroccan emmer); 2) subsp. *abyssinicum* Vav. (Ethiopian emmer); 3) subsp. *dicoccon* (subsp. *europaeum* Vav.) (European emmer), and 4) subsp. *asiaticum* Vav. (Eastern emmer) (Gökgöl 1955; Dorofeev et al. 1979; Szabo´ and Hammer 1996.). As in PCA, similar pattern of genetic relationships among accessions was observed in cluster analysis. In order to examine the relationship of the eleven gene pools considered in the present study with Abyssinian emmer wheat accessions, a dendogram was constructed based on 26 SSR primers that are common in the present experiment and the study by Teklu et al. (2006a). The general relationship between the different gene pools is similar to that described in Figure 6.3. Ethiopian accessions were clustered in the same group with Yemen and Spain gene pools, although they are connected at the lowest genetic similarity coefficient level indicating the presence of wide genetic differences among them. The grouping of Yemen and Ethiopian emmer accessions in the same cluster is expected since in both countries Ethiopian emmer (subsp. *abyssinicum*) was widely distributed (Hammer et al. 2004). It is known that East Africa in the narrow sense was affected by Semitic civilizations and trades through the Arabian trade and supply routes since Sumerian time (3000 B.C.) (Cavalli-Sforza et al. 1994). It is speculated that early immigrants of Hamites, some 5,000 years ago introduced emmer wheat (*T. dicoccon*) to Ethiopia (Helbaeck 1959; Feldman 1979). According to Figliuolo and Perrino (2004), germplasm from Italy and Ethiopia appears to belong to a more primitive gene pool. In the present study, however, germplasm from Italy were intermediate between other gene pools, whereas accessions from Spain are the most differentiated ones.

On the whole, the result showed the utility of microsatellites for germplasm characterization. Because diversity is a raw material for developing new varieties, more characterization using morphological and molecular markers is needed for better understanding of the nature and pattern of genetic diversity of emmer wheat, a crop

which has a renewed interest and endowed with rich genetic diversity and potential to be utilized for the development of more products and also to be used in improving disease resistance, agronomic and quality traits of bread and durum wheat varieties.

Chapter 7 Comparative analysis of diversity indices and genetic relationships based on agronomic traits and microsatellites in Ethiopian tetraploid wheats

7.1 Abstract

Comparative analysis of genetic polymorphism and relationship in 133 Ethiopian tetraploid wheat accessions was conducted using morphological descriptors and molecular technique. Molecular variation was estimated using 29 polymorphic microsatellite loci, and quantitative variation was measured using 13 metric traits. Phenotypic and microsatellites diversity were estimated using the Shannon Weaver and Nei gene diversity indices, respectively. Diversity index estimated based on the entire quantitative and microsatellites data is 0.79 and 0.71, respectively. The Nei's coefficient of gene differentiation was equal to 0.15 for both phenotypic and microsatellites data implying that the genetic variation within and among the 11 geographical regions was 85 and 15%, respectively. Euclidean and Nei genetic distance estimates were calculated from binary phenotypic and molecular data matrices, respectively. The relationships between the two distances were assessed using simple linear correlation, mantel test, cluster analysis and principal component analysis. Results from the Mantel test and simple linear correlation analysis proved that the genetic variability based on microsatellites is not significantly correlated with the variability based on morphological characters. When the two distances were plotted against each other, the resulting scatter plot depicted the presence of a triangular shaped relationship between them. Both cluster diagram and bi-plot of the first two axis of PCA showed the presence of different patterns of geographical variation between microsatellites and phenotypic evaluations. The lack of concordance between molecular and quantitative measures of genetic variation suggests that molecular measures of genetic diversity cannot exactly predict or substitute quantitative genetic variability.

7.2 Introduction

The genetic diversity among and within landraces makes them a valuable resource as potential donors of genes for the development and maintenance of modern crop varieties, and for direct use by farmers (Solcri and Smith 1995). The study of phenotypic and

genetic diversity is important for making decisions related to the selection of sites and populations for *in situ* conservation, for evaluating pre-breeding and breeding germplasm, and for determining the uniqueness and distinctness of the phenotypic and genetic constitution of genotypes with the purpose of protecting the breeder's intellectual property rights (Franco et al. 2001). The genetic variability in Ethiopian tetraploid wheat has also been assessed using various methods. Several studies reported the presence of large morphological variation in Ethiopian tetraploid wheat (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; Vavilov 1951). Different molecular markers such as microsatellites (Alamerew et al. 2004; Messele 2001; Teklu et al. 2006a and b), isozymes (Tsegaye et al. 1994; 1996), glutenine and gliadine storage protein and AFLP (Messele 2001) have also confirmed the presence of appreciable genetic polymorphism in these materials.

Information about the relationship between molecular and phenotypic distances has several practical applications (Burstin and Charcosset 1997). It is fundamental for designing optimal germplasm collection, management practices and for developing an index for parental selection (Tsegaye et al. 1996). From a genetic resource conservation point of view, such knowledge may be useful to know whether or not two individuals or populations that are phenotypically similar display gene combinations. Such information is also interesting for the protection of owners right since marker analysis allows the identification of lines that are likely to share similar alleles at the QTLs among a set of lines with similar phenotypes (Burstin and Charcosset 1997). This can be also used for genetic resources conservation (Burstin and Charcosset 1997). As a result, numerous studies have been conducted to investigate the relationship between diversity at marker loci and morphological differentiation (Ayele et al. 1999; Autrique et al. 1999; Bar-Hen et al. 1995; Burstin and Charcosset 1997; Crouch et al. 2000; Hundera 2004; Knapp and Rice 1998; Lefebvre et al. 2001; Moser and Lee 1994; Ntundu et al. 2004; Ramakrishnan et al. 2004; Schut et al. 1997; Senior et al. 1998; Vanhala et al. 2004).

In Ethiopia, although several independent variability studies using both molecular and morphological techniques have been conducted, only Tsegaye et al. (1996) have studied the relationships among Ethiopian tetraploid wheat landrace populations using isozyme markers and agronomic traits. Hence, there is lack of information about the association between morphological and molecular markers characterization in Ethiopian tetraploid wheats. This study was, therefore, conducted to examine the relationship between patterns of genetic variation based on morphological characterization and microsatellites data.

7.3 Materials and Methods

Morphological experiment

Plant materials and data collection

In order to conduct the comparison between morphological and molecular analysis of genetic diversity with the same set of accessions, means of 133 accessions (Table 5.1) that were also analyzed using microsatellites were selected from the combined analysis of variance of data from field experiment conducted with a total of 271 tetraploid wheat landraces at Alemaya University research site (Rare) during 2002/2003 and 2003/2004 main cropping seasons using randomized complete block design with two replications were used. Experimental procedures including field layout and data collection are described in Teklu and Hammer (2005a).

Molecular Evaluation

Plant materials, DNA extraction and PCR amplification

For this comparison molecular data from Teklu et al. (2006a; b) were used. Hence, plant materials, DNA extraction, PCR amplification, fragment detection, the microsatellites used for analysis and their description are given in Teklu (2005a; 2005b).

Statistical analysis

The Shannon and Weaver (1949) Diversity Index (H'_c) was used as a measure of phenotypic diversity for each trait after their transformation into classes. It was estimated using $H'_C = -\sum_{i=1}^{n} p_i \log_e p_i$ where for a given character *C*, *n* is the number of phenotypic classes and *p* is the proportion of observation in the i^{th} class. The index was estimated for each character over all accessions and for each character within a region. Due to its additive property (Kent and Coker 1992), Shannon-Weaver Diversity Indices obtained for each character were pooled for each region over the respective number of accessions. To avoid the effect of the different numbers of phenotypic classes while comparing indices obtained for the different characters, a standardized index, SDIc, was calculated as $\text{SDIc} = H'_C / \log e^n$. For molecular data, evaluation of the number of alleles per locus, calculation of gene diversity, Nei's coefficient of genetic differentiation and partitioning of gene diversity into componenets was done as described in Teklu et al. (2006a; 2006b; 2006c).

Phenotypic data was standardized to have mean zero and a standard deviation of 1and phenotypic distances were calculated as Euclidean distances. Genetic distances were calculated from the SSR data following the standard procedure given by Nei and Li (1979). The correspondence between the two distance matrices was assessed using simple linear correlation, Mantel test, cluster analysis, and principal component analysis. Simple Linear Correlation was computed using SPSS 12 (SPSS 2003). Mantel correlation static (Mantel 1967) was computed using XLSTAT (WIN) PRO 7.5. In cluster analysis, SSR data were analyzed using the SIMQUAL routine to generate matrix of genetic similarity based on the Nei index (Nei and Li 1979). Euclidean distance matrix of was computed from phenotypic data. These matrices were used to construct dendograms using the UPGMA algorithm. The TREE procedure was used to visualize the tree diagrams (dendograms) (Page 1996). PCA was computed using NTSYS-pc statistical software. Scatter plots were constructed from the PCoAs results by plotting the scores on the first two principal coordinates.

7.4 Results

Phenotypic diversity estimates

Phenotypic diversity was estimated using the Shannon Weaver Diversity Index. The over all diversity index estimated based on the entire data set is 0.79 (Table 7.1). Among regions, the range of mean index of phenotypic diversity varied from 0.41 for accessions from Wellega and IKS to 0.87 for accessions from Shewa and Gonder (Table 7.3). Partitioning the total genetic diversity into its components and estimating genetic differentiation helps to determine the contribution of the different levels of variability to the total diversity available in a given crop or in a given area. This information could be used in germplasm collection and *in situ* conservation. In the present study, the Nei's genetic differentiation value was 0.15 implying that 85% and 15% of the total variation was attributed to the within and between regions variation, respectively (Table 7.1).

Table 7.1. Partitioning of the phenotypic diversity within and between geographical regions of collections.

Characters	Ht*	Hs	DST	Gst
Grain Yield	0.69	0.54	0.15	0.22
Biomass Yield	0.77	0.69	0.08	0.11
Harvest Index	0.78	0.71	0.07	0.09
Days to Maturity	0.81	0.78	0.03	0.04
Days to Heading	0.84	0.83	0.01	0.02
Grain Filling Period	0.80	0.74	0.06	0.08
Plant height	0.84	0.82	0.02	0.02
Spiklets/spike	0.90	0.85	0.05	0.06
Spike length	0.77	0.72	0.05	0.06
Kernels/spike	0.72	0.67	0.05	0.07
Spikes/plant	0.80	0.66	0.14	0.18
kernels/plant	0.75	0.68	0.07	0.10
TKW	0.79	0.11	0.68	0.86
Mean	0.79	0.68	0.11	0.15

*, Ht refers to diversity based on the entire data , Hs and Ds, the proportion of diversity within regions and between regions, respectively. Gst refers to Nei's genetic differentiation.

SSR genetic diversity

Twenty nine informative SSR markers generated a total of 385 fragments across all accessions (Table 7.2). The number of amplified fragments per locus varied from 3

(*Xgwm415*) to 42 (*Xgwm312*) with an average of 13.28 fragments per loci. Primers such as *Xgwm631*, *Xgwm160*, and *Xgwm619* detected few numbers of alleles which are 3, 5, and 5 respectively. The most polymorphic primers included *Xgwm698* (19), *Xtaglgap* (19), *Xgwm898* (19) and *Xgwm268* (28). Null alleles were detected in five of the 29 primers used. The gene diversity ranged from 0.06 (*Xgwm415*) to 0.95 (*Xgwm312*), with a mean of 0.72. A high level of genetic diversity obtained in the present study could be due to the representation of accessions covering large geographical and climatic conditions. It might also be the result of polymorphic microsatellites used. The choices of highly polymorphic markers contribute to the enhancement of PIC values (Struss and Plieske 1998). As with phenotypic diversity, the Nei's coefficient of genetic differentiation value (0.15) indicated that total genetic diversity was mainly explained by the within regions diversity which accounted for 85% of the existing variation (Table 7.2). The high phenotypic diversity value compared to genetic diversity might be attributed the high power of a statistical test to detect differences between quantitative characters than for polymorphic neutral or near-neutral genes (Lewontin 1984).

Comparison of distance matrices

Euclidean and Nei and Li (1979) genetic distance indices for each of the 55 pairs of regional comparisons were calculated from binary phenotypic and molecular data matrices, respectively (Table 7.3). Phenotypic (Euclidean) distances between all pairs of the regions varied between 0.15 (between Hararghe and Shewa) to 1.32 (between Tigray and Welo), with average of 0.49; whereas, Nei and Li (1979) genetic distance estimates ranged from 0.51 (between Gamugofa and IKS) to 0.95 (between Gojam and Gonder), with a mean value of 0.74 (Table 7.3). Based on phenotypic diversity indices, mean distances estimate of regions relative to others is greatest for Tigray (0.84). However, it is Gojam (0.82) which had the highest SSR mean genetic distance and thus quite different from the remaining regions.

Table 7.2. Microsatellites, chromosomal location, product size range, number of alleles, genetic diversity and gene differentiation values determined based on 133 Ethiopian tetraploid wheat accessions.

Microsatellites	Chromosomal location	Product size range (bp)	Number of alleles	Ht	Hs	DST	Gst
Xgwm752	1AS	116-138	8	0.714	0.629	0.085	0.119
Xgwm357	1A(C)	102-146	10	0.775	0.648	0.127	0.164
Xgwm95	2AS	108-130	10	0.776	0.665	0.111	0.143
Xgwm312	2AL	124-166	42	0.945	0.817	0.128	0.135
Xgwm720	3AS	null, 183-303	17	0.893	0.739	0.155	0.173
Xgwm155	3AL	127-145	10	0.792	0.677	0.115	0.145
Xgwm601	4AS	null, 143-169	12	0.859	0.735	0.123	0.144
Xgwm160	4AL	179-191	5	0.575	0.471	0.103	0.18
Xgwm415	5AS	131-135	3	0.059	0.036	0.023	0.393
Xgwm186	5AL	98-138	10	0.74	0.633	0.107	0.144
Xgwm459	6AS	null, 128-164	17	0.904	0.791	0.113	0.124
Xgwm1089	6AL	116-168	15	0.764	0.686	0.077	0.101
Xgwm631	7AS	189-199	4	0.143	0.104	0.039	0.271
Xgwm698	7AL	null, 152-218	19	0.829	0.669	0.16	0.193
Xgwm18	1BS	176-190	7	0.729	0.602	0.127	0.174
Xtaglgap	1BS	212-190	19	0.852	0.744	0.108	0.127
Xgwm268	1BL	180-290	28	0.893	0.742	0.151	0.169
Xgwm148	2BS	138-174	11	0.759	0.707	0.052	0.069
Xgwm619	2BL	129-155	5	0.084	0.083	0.001	0.014
Xgwm389	3BS	116-142	12	0.879	0.768	0.111	0.127
Xgwm655	3BL	159-197	13	0.785	0.701	0.084	0.107
Xgwm898	4BS	103-123	19	0.658	0.56	0.098	0.149
Xgwm513	4BL	133-147	7	0.686	0.599	0.087	0.127
Xgwm540	5BS	114-142	10	0.681	0.556	0.125	0.184
Xgwm408	5BL	136-202	16	0.847	0.759	0.087	0.103
Xgwm680	6BS	123-155	12	0.748	0.654	0.094	0.126
Xgwm219	6BL	153-179	10	0.704	0.618	0.086	0.123
Xgwm46	7B(C)	143-183	17	0.826	0.687	0.139	0.168
Xgwm577	7BL	null, 122-162	17	0.847	0.745	0.103	0.121
Mean			13.28	0.715	0.615	0.101	0.149

To examine the relationships between distances computed from quantitative traits and distances computed with molecular markers, the phenotypic distance matrix was compared to a molecular genetic distance matrix using simple linear (Pearson) correlation. Very low and non significant (r = 0.002, P = 0.989) correlation coefficient value was obtained implying that the relationship between the two distance measures is weak and is not linear. To further clarify the relationship of the two distances, matrices of genetic distances values generated from SSR and morphological data were compared using the Mantel test. A non significant and low mantel correlation value was obtained (r

Table 7.3. Euclidean distance (upper value) and Nei genetic distance (lower value) among the 11 accessions groups based on regions of collection.

Regions	Arsi	Bale	Gamugofa	Gojam	Gonder	Harerge	IKS	Shewa	Tigray	Welega	Welo
Arsi	-	0.2	0.33	0.34	0.56	0.34	0.2	0.31	0.96	0.48	0.42
Bale	0.75	-	0.19	0.19	0.55	0.32	0.29	0.33	0.97	0.56	0.37
Gamugofa	0.63	0.59	-	0.29	0.7	0.48	0.37	0.47	1.11	0.73	0.26
Gojam	0.89	0.88	0.88	-	0.46	0.24	0.34	0.29	0.87	0.56	0.52
Gonder	0.69	0.66	0.66	0.95	-	0.25	0.5	0.28	0.42	0.28	0.91
Harerge	0.74	0.7	0.7	0.86	0.85	-	0.33	0.15	0.66	0.33	0.68
IKS	0.74	0.64	0.51	0.94	0.76	0.66	-	0.22	0.88	0.48	0.5
Shewa	0.61	0.7	0.67	0.88	0.73	0.68	0.68	-	0.68	0.33	0.65
Tigray	0.52	0.87	0.73	0.94	0.7	0.81	0.84	0.68	-	0.55	1.32
Welega	0.74	0.65	0.68	0.86	0.71	0.76	0.67	0.72	0.78	-	0.88
Welo	0.71	0.67	0.64	0.89	0.85	0.75	0.72	0.71	0.71	0.67	-

= 0.013, P = 0.335) confirming that the association between the two distance measures is a non linear. Both statistics showed the lack of correspondence between the two distances. To identify the type of relationships the two distances were plotted against each other. As it is seen from Figure 7.1, there exists a triangularly shaped relationship between the two distances implying that genotypically similar accessions were also

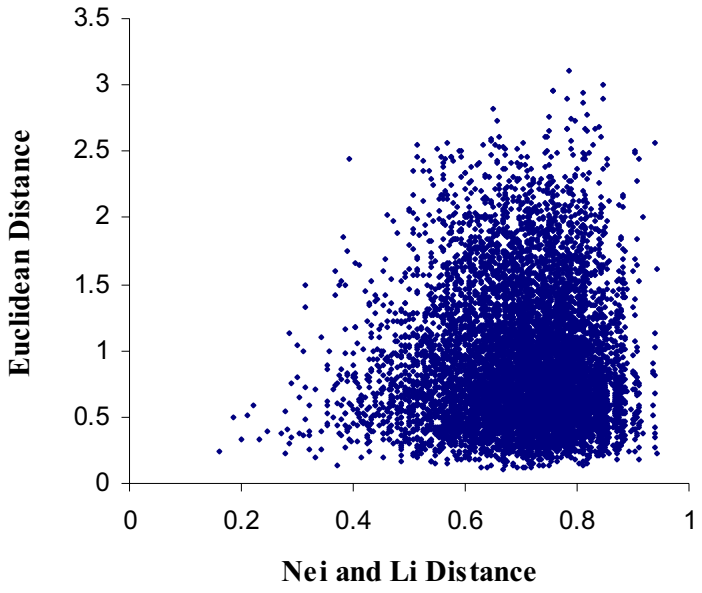


Figure 7.1. Relationship between phenotypic and molecular genetic distances.

phenotypically similar whereas genotypically distant accessions were phenotypically either similar or distant.

Cluster analysis

Clustering analysis was carried out to examine the patterns of variation of morphological and molecular distances. The resulting dendograms (Figure 7.2 a and b) showed different patterns of variation, although in both diagrams the whole regions are grouped into two major clusters. In the dendogram that was constructed based on phenotypic data (Figure 7. 2a), accessions from Tigray are grouped in cluster 1 suggesting that this region was the most distinct region. However, in cluster diagram based on SSR marker (Figure 7.2b), accessions from Gojam were grouped separately in cluster 1 and are the most distinct ones. Different patterns of variations among the remaining regions, which were grouped in cluster two of both diagrams, were observed.

Principal component analysis

Principal component analysis was also employed to further clarify the relationships between phenotypic and molecular distance measures. For phenotypic data, the first and the second principal components accounted for 93.20% and 3.35% of the total variation, respectively. The two dimensional plot based on these two axes (Figure 7.3a), showed a clear pattern of geographical differentiation, although there is overlapping among some regions. For instance, overlapping was observed between Gojam and IKS, and Hararghe and Shewa revealing the existence of close similarity among accessions from these pairs. Tigray was distantly placed from other regions indicating its distinctiveness as revealed by UPGMA diagram. From the PCA results based on molecular data, only PC1 and PC2 had eigenvalues greater than 1 and explained 33.93 and 10.21% of the total variations, respectively. The two dimensional plot of PC1 versus PC2 (Figure 7.3b) based on molecular data also differentiated all accessions with accessions from Bale and IKS being overlapped and Gojam placed distantly from the remaining regions. Generally, differences in the patterns of geographic distribution of genetic diversity between phenotypic and molecular marker data among regions were observed.

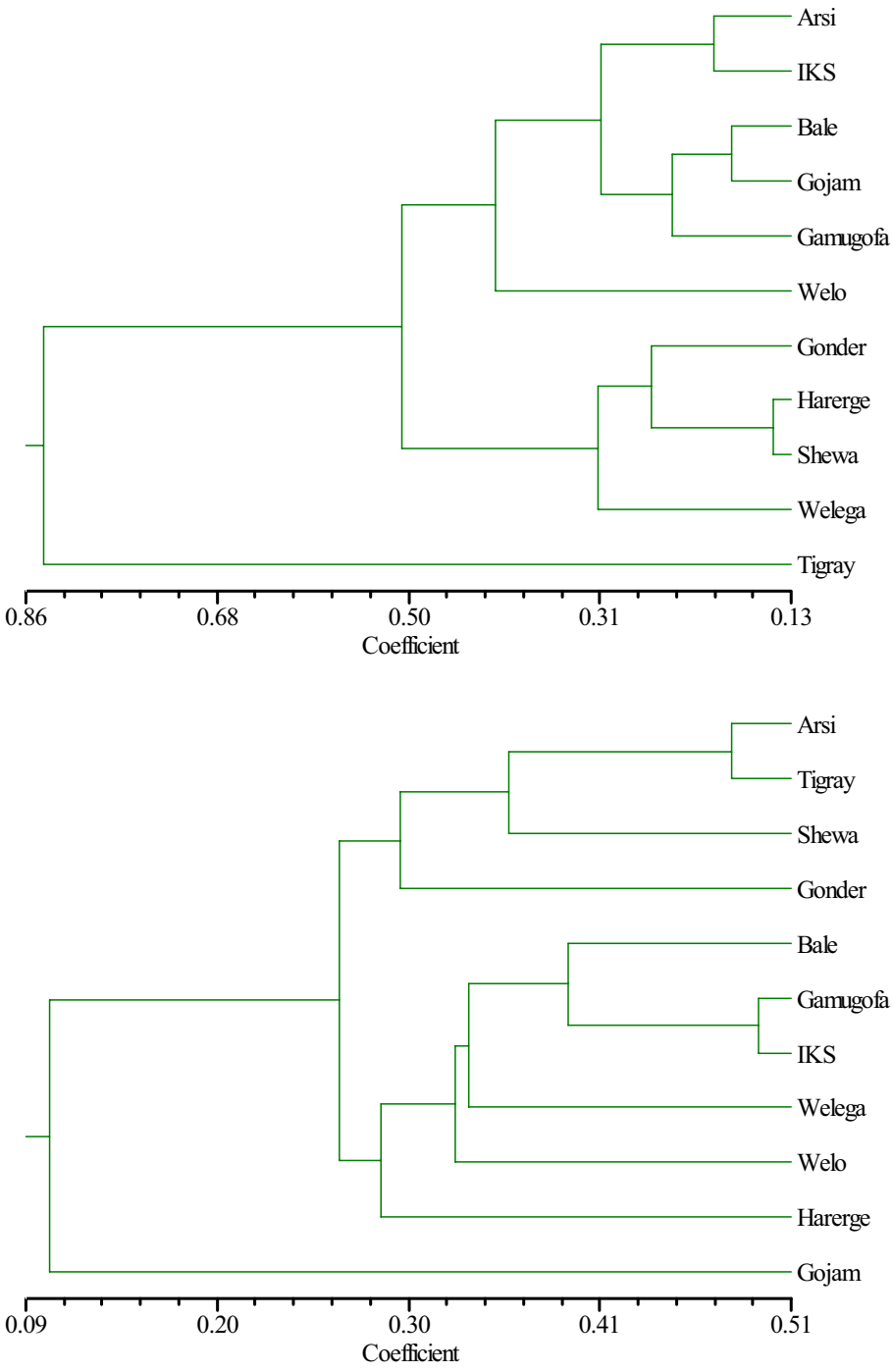


Figure 7.2. Phenogram constructed based on the 11 regions of collections of 133 Ethiopian tetraploid accessions using UPGMA algorithm for (a) phenotypic and b) SSR genetic distance values.

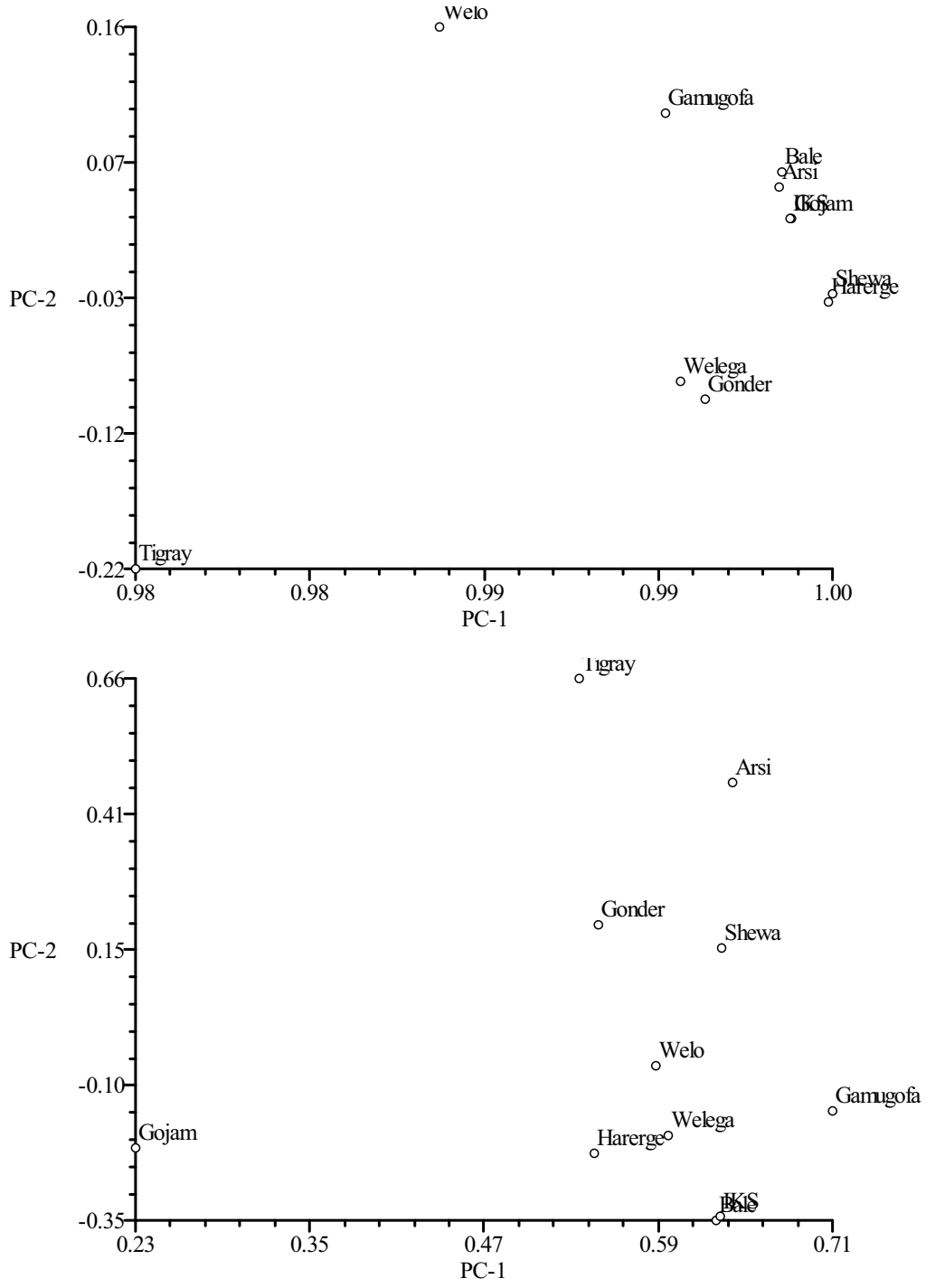


Figure 7.3. Bi-plot of the first two principal components of principal component analysis of a) morphological data b) molecular data.

7.5 Discussion

The relationship between morphological and SSR distances were compared using Pearson correlations, mantel Z statistic, cluster analysis and PCAs. Results from the Mantel test and simple linear correlation analysis proved that the genetic variability based on SSR is not significantly correlated with the variability based on morphological characters. Contrary to this, significant associations between molecular and phenotypic distances were reported in related studies (Hundera 2004; Knapp and Rice 1998; Lefebvre et al. 2001; Ramakrishnan et al. 2004). A linear relationship is expected between mean heterozygosity and the variance for a polygenic trait, provided that all gene action is additive (Falconer 1989). In general, however, a non-significant correlation between phenotypic distances and molecular genetic distances in plants seems to be a widespread phenomenon (Bar-Hen et al. 1995; Roldan-Ruiz et al. 2001). In agreement to the present finding, most studies on the correlation between molecular marker and phenotypic distance measures have revealed weak or no association (Ayele et al. 1999; Autrique et al. 1999; Bar-Hen et al. 1995; Burstin and Charcosset 1997; Crouch et al. 2000; Moser and Lee 1994; Ntundu et al. 2004; Schut et al. 1997; Senior et al. 1998; Vanhala et al. 2004). The lack of congruence between phenotypic distances and molecular distances might be attributed to large number of accessions used for the analysis. According to Engelborghs et al. (1999), good correlations can be obtained between molecular marker diversity and morphotype group when a small number of genotypes are screened. The fact that phenotypic datasets are usually limited in number of traits measured and these traits are more or less directly influenced by the environment, whereas molecular markers are potentially numerous and are not influenced by genotype x environment interaction (Ayele et al. 1999; Reed and Frankham 2001; Singh and Ramanujam 1981; Ghaderi et al. 1984) could also attribute for the poor relationship. In other studies, this lack of relationship has been interpreted as resulting from irrelevant choices of phenotypic traits into account for calculation of the quantitative distance (Partap et al. 1980). The lack of concordance between different distance measures should not be regarded as indicating a weakness or limitation of these systems (Roldan-Ruiz et al. 2001). This result suggests that the molecular marker

distance doesn't exactly represent of the phenotypic genetic distances. However, molecular markers allow to distinguish phenotypically similar cultivars.

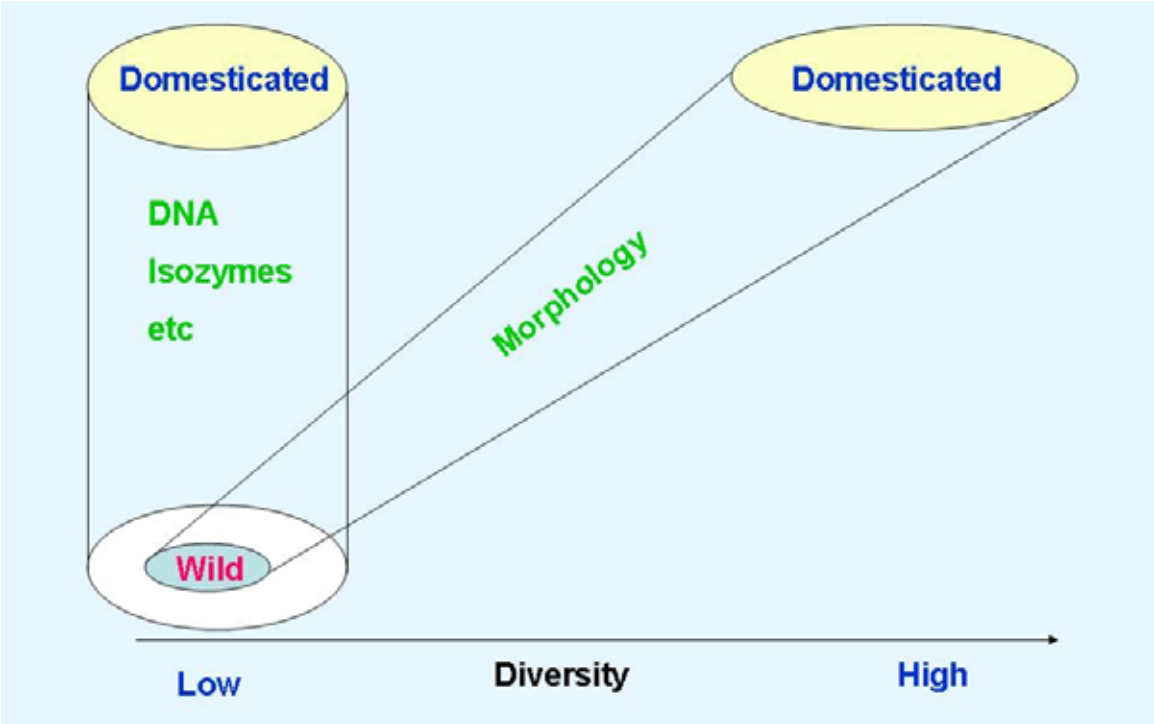


Figure 7.4 Diagram showing that in morphology a domesticated species is different from its wild ancestor and displays much greater diversity, whereas in isozymes, DNA or other molecular markers the diversity remains almost the same.

Source: Lester and Daunay (2002).

The triangular relationship between molecular and phenotypic distances observed in this study is in agreement with the findings of several studies (Dillmann et al. 1997; Lefebvre et al. 2001; Vanhala et al. 2004). Both theoretical and experimental investigations by Burstin and Charcosset (1997) resulted in a triangular relationship between marker distances and phenotypic distances. The triangular shape implies that low molecular marker distances are associated with low phenotypic distances, whereas high molecular marker distances correspond to either low or high phenotypic distances. The triangular shape of the relationship between the marker and phenotypic distances can be explained

by the linkage disequilibrium between the two distances and polygenic inheritance of metric traits as a given quantitative value can be obtained with different gene combinations (Burstin and Charcosset 1997).

While molecular and quantitative trait variation may be theoretically correlated, empirical studies using both approaches frequently reveal discordant patterns. In this study, both UPGMA diagram of cluster analysis and scatter gram of principal component analysis showed the presence of different patterns of geographical variation between SSR and phenotypic methods. Likewise, cluster analyses of the isozyme and agronomic data produced different patterns and groupings in Ethiopian tetraploid wheat (Tsegaye et al. 1996). The authors ascribed this lack of agreement due to the different forces of evolution acting on isozyme markers and agronomic traits since agronomic traits, are the prime target of artificial selection. Roldan-Ruiz et al. (2001) also observed only little agreement on variety relationships between the morphology and the molecular methods, i.e., both the absolute distances and the rankings appeared to be quite different between the three measurement systems (AFLP, STS and morphology). However, the morphological and molecular data led to similar representations of the cultivar relationships in the study by Rotondi et al. (2003). Likewise, similar grouping of varieties was observed in morphological distances and genetic similarity based on SSR markers in Tunisian winter barley (Hamza et al. 2004). In congruence with the present result, the geographical distribution of genetic diversity measured by SSR markers was not completely accordant with that obtained by phenotypic traits in the studies by Gomez et al. (2004) and Yu et al. (2004). Tang and Knapp (2003) also observed modest differences in molecular genetic diversity in domesticated sunflowers despite substantial phenotypic diversity. The contrast between the supposedly selectively neutral or near-neutral nature of microsatellites (Li et al. 2000) and the adaptive value of phenotypic traits (Hill et al. 1998) might in part explain this apparent discrepancy (Gomez et al. 2004). That is, phenotypic traits are subject to both natural and artificial selection since environmental conditions and farmers' selection criteria lead to divergence between landraces. Lester and Duanay (2002) examined the diversity of African vegetable *Solanum* species, and how they have evolved from their wild ancestors and concluded that domestication

process has not only produced cultigens that are very different in morphology from their wild ancestors, but also that these cultigens display a vast range of diversity within themselves. On the other hand, diversity studies based on molecular markers such as isozymes and DNA have revealed that the diversity within the cultigens has been found to be little or no greater than that of the wild ancestor. This situation, which is shown in Figure 7.4, is also exemplified by tomato (*Lycopersicon esculentum*), pepper (*Capsicum annuum*), and maize (*Zea mays*) (Doebley et al. 1987, Lester 1989, Lefebvre et al. 2001). This seems to be a general rule for all domesticated species of plants except perhaps those derived from interspecific hybridization (Lester 1989; Lester and Duanay 2002). This paradox, of the vast and deviant morphological diversity of cultigens compared to their wild ancestors on the one hand, and the minimal increase in diversity of molecular markers such as DNA and isozymes on the other, demands an explanation.

Lefebvre et al. (2001) employed the ratio between the mean sampling standard and mean of the respective genetic distance determining the precision of different genetic distances measures. Following this procedure, lower ratio was obtained for SSR marker (0.139) than phenotypic markers (0.529) implying that estimation of the genetic distance is more precise in SSR marker than phenotypic markers. It should be noted, however, that the distance measures are different for two markers. Ramakrishnan et al. (2004) described phenotypic data as less precise data. Crouch et al. (2000) also reported that classification systems using phenotypic indices based on agronomic characters may not provide accurate taxonomic differentiation. DNA markers have the advantage of being independent of environmental effects and providing direct information on the genome of each individual. Nevertheless, Lefebvre et al. (2001) reported that estimation of the genetic distance is more precise using phenotypes than using molecular markers.

Nei's coefficient of genetic differentiation value of 0.15 was obtained in both phenotypic and genetic diversity implying 85 % of genetic diversity was explained by the within regions diversity. The partitioning of the total genetic variation between landraces and agro-ecological zones by molecular markers indicated that a considerable part of the variation was attributable to the lowest level variation, i.e., differences among landraces

within agro-ecological zones in common bean (Gomez et al. 2004). Tsegaye et al. (1996) also reported that much of the isozyme diversity (85%) of Ethiopian tetraploid wheats, was attributable to the within-population level. Nonetheless, Vanhala et al. (2004) detected different sources of variation between molecular and morphological markers, i.e., genetic variation were larger between populations than within them, whereas for phenotypic measurements variation was larger within populations than between them. High within population genetic diversity (≥ 93%) was also found in barley (Dai and Zhang 1989).

The lack of concordance between molecular and quantitative measures of genetic variation suggests that molecular measures of genetic diversity cannot exactly predict or substitute quantitative genetic variability. In this study, the fact that genetic diversity may be present despite the absence of morphological variation as implied by the triangular relationship between the two diversity measures imply that the measures complement each other. Therefore, the combination of molecular and morphological analyses of genetic diversity is important to unveil pattern of genetic variation and genetic relationship in Ethiopian tetraploid wheats more precisely.

Chapter 8 Diversity of Ethiopian tetraploid wheat germplasm: Breeding opportunities for improving grain yield potentials and quality traits

8.1 Abstract

In this chapter, Shannon Weaver diversity indexes were employed to examine the phenotypic diversity in 271 Ethiopian tetraploid wheat accessions in relation to characters, regions of origin and altitude. Moreover, review of genetic diversity studies in Ethiopian tetraploid wheat was made to explore breeding opportunities. The diversity index varied widely across regions. It varied from 0.40 for accessions from Gamugofa to 0.68 for accessions from Hararghe. Among the four-altitudinal classes, the highest (0.72) and the lowest (0.61) mean diversity indices were noted in altitude class II and IV, respectively. The Diversity Index (H′) showed that most traits are polymorphic. The overall diversity index for all traits was 0.74. The Shannon-Weaver diversity index varied widely across regions, but was relatively even for most traits considered. The partitioning of the total phenotypic diversity into within- and among-regions diversity indicated that the within region diversity was 0.71 and the between diversity was 0.29. Altitudinal wise, 95% of the total variation was attributed to the within region diversity. Principal component analysis was computed on the diversity index of regions of origin and altitudinal class for the 13 quantitative to examine the regional and altitudinal pattern of variation. On regional bases, the first four axes, whose eigenvalues are greater than one, explained about 82 % of the observed phenotypic diversity in the 271 tetraploid wheat accessions. The first and second axis accounted for about 29.8 % and 20.5 of the total variation, respectively. On altitudinal bases, however, only the first two principal components, which produced eigenvalues greater than 1, explained 89.7% of the total variation. In general, phenotypic diversity showed considerable differences for each trait in different geographical regions and altitudinal classes that could be utilized in wheat improvement programs. Breeding opportunities and strategies are suggested.

8.2 Introduction

In its national research strategic plan, the EARO has considered wheat as the number one priority crop among cereals (EARO 2000). The demand for wheat is continuously

increasing. The estimated 2.2 million metric tons national wheat grain requirement is 50% greater than the total production and, therefore, Ethiopia is a net importer of wheat (Eshetu 2002). It means, for Ethiopia to become self-sufficient in wheat grain, the total production has to nearly double. A bilateral strategy, i.e. vertical increase in wheat productivity through development of high yielding varieties and horizontal increase in area under wheat crops through expanding wheat cultivation to new areas, was suggested to meet this demand (EARO 2000). Although Ethiopia has potential environments for expanding wheat culture, meeting expected demands by continued expansion of agricultural production into marginal areas might be difficult as the economic costs of increasing yields by intensification of agronomic infrastructure are high (Skovmand et al. 2001). Therefore, the country has to work more towards increasing productivity and total production thereof. By utilizing the genetic diversity, it is possible to improve productivity of existing wheat cultivars and meet the challenge of feeding the increasing number of population. Modern agricultural development, in which plant breeding plays a major role, has achieved remarkable success in increasing food production to meet the demand of a growing population (Evans 1998). Even in the age of genomics, genetic diversity remains the cornerstone of crop improvement (Sneller et al. 2005). 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 fights against hunger. They are the raw material for breeding new plant varieties and are a reservoir of genetic diversity. Future gains in yield potential will almost certainly require exploitation of the largely untapped sources of genetic diversity housed in collections of wheat landraces and wild relatives (Skovmand et al. 2001).

In Ethiopia, there are large amounts of wheat germplasm (about 12, 000 accessions) that have been collected and maintained mainly in the institute of biodiversity conservation. Landraces constitute the lion's share of these collections. Landraces, which are locally adapted genotypes that have evolved because of natural and artificial selection forces over the millennia, are one of the invaluable heritages that traditional farmers have given us (Myer 1994). Landraces may be used as starting populations for cultivar development (Lakew et al. 1997) or as sources for the introgression of genes and quantitative trait loci

conferring resistance to biotic (Huang et al. 1997) and abiotic stresses (Forster et al. 2000). Despite these valuable features, the use of landraces has been discouraged in many developing countries on the basis that they have low yield potential. However, Lakew et al. (1997) confirmed the presence of individual genotypes within landraces which have a yield potential comparable with the best breeding lines and sources of disease resistance justifying the need for developing a routine methodology to use the large collection of landraces available in breeding programs. Tessema (1991) reported the presence of ample diversity in Ethiopian tetraploid wheat landraces. Various authors (Vavilov 1951; Porceddu et al. 1973; Amri et al. 1990; Belay et al. 1992; Kubo et al. 2004) also confirmed the uniqueness of the Ethiopian tetraploid wheat germplasm for different useful traits. However, it is felt that these collections have not been fully utilized in the breeding programs. Efficient utilization of the genetic potential held in the germplasm collections requires a better knowledge of the collected material including morphological and phenological characterization and evaluation for useful agronomic traits (Zaharieva et al. 2003). Assessment of genetic diversity gives a breeder an opportunity to take up breeding programme for specific agro-ecological conditions. Although several authors (Jain et al. 1975; Bekele 1984; Negassa 1986; Bechere et al. 1996; Pecetti and Damania 1996; Eticha et al. 2005) have conducted different variability studies in Ethiopian tetraploid wheats, there is still a need for more information concerning the diversity present in Ethiopian tetraploid wheat germplasm and its structure, and about their potential interest for breeding. For instance, there is lack of information on the extent and pattern of phenotypic diversity in relation to all geographical regions of the country. Moreover, most of the above studies (Bekele 1984; Negassa 1986; Eticha et al. 2005) are based on qualitative traits accessions collected from limited geographical areas. Hence, the phenotypic diversity present in the 271 Ethiopian tetraploid wheat landraces collected from all over the country has been examined in the present study. Moreover, the breeding opportunities prevailing in Ethiopian tetraploid wheat have been summarized from past findings and strategies to enhance yield and quality traits were suggested.

8.3 Materials and Methods

Plant materials and data collection

Plant materials used, data collection and all other experimental procedures are as described in Teklu et al. (2006a).

Statistical analysis

The Shannon and Weaver (1949) Diversity Index (H'_c) was used as a measure of phenotypic diversity for each trait after transformation into classes. The index was estimated for each character over all accessions and for each character within a region and altitudinal class. Due to its additive property (Kent and Coker 1992), Shannon-Weaver Diversity Indices obtained for each character were pooled for each region and altitudinal class over the respective number of accessions. To avoid the effect of the different numbers of phenotypic classes while comparing indices obtained for the different characters, a standardized index SDIc was used. The diversity index computed based on whole data set (H_t) was partitioned into within region or altitude and between regions of origin and between altitudinal classes following the procedure of Paul et al. (1997). The within region ($H_{r)}$ and the within altitude class (H_a) diversity indices refers to the average diversity index of each character estimated based on regions of origin and altitudinal classes, respectively. The between regions (Dst_r) and between altitudinal classes (Dst_a) diversity index were computed as H_t-H_r and H_t-H_a, respectively. (Dst_r)/H_t and (Dst_a)/H_t are the coefficient of gene differentiation based on regions and altitudinal classes, respectively. Principal component analysis on the diversity index was conducted using the computer program NTSYS-pc version 2.0 (Rohlf 1998).

8.4 Results

Estimates of diversity

Diversity was estimated using the Shannon Weaver Diversity Index (Table 8.1). The over all diversity index estimated based on the entire data set is 0.72 (Table 8.2). Among regions, the range of mean index of diversity varied from 0.68 for accessions from Hararghe to 0.40 for accessions from Gamugofa. The diversity indices differed among regions for specific characters. For instance, traits such as spikelets/spike, spikelet length,

seeds/spike, spikes/plant, and kernels/plant showed high polymorphism in Arsi, Hararghe and Shewa. The highest diversity index (0.75) for plant height was recorded in collections from Arsi. Hararghe and Shewa also exhibited high polymorphism for biomass yield and harvest index (Table 8.1).

Among the three altitudinal classes, the highest (0.72) and the lowest (0.61) mean diversity indices were noted in altitude class II and IV, respectively. The diversity indices differed among regions for specific characters. In altitudinal class I, all characters except grain yield and biomass showed diversity index of more than 0.70. In altitude class II and III most of the traits except grain yield and spikelets per spike in the first and grain yield

Table 8.1. Estimates of the Shannon Weaver diversity index (H′) for the thirteen metric characters across geographical regions and altitudinal classes.

Geographical Regions	GY	BY	HI	DTM	DTH	GFP	PH	SS	SL	KS	SP	KP	TKW	Mean
Arsi	0.47	0.62	0.68	0.51	0.64	0.73	0.75	0.74	0.73	0.71	0.75	0.69	0.75	0.67
Bale	0.60	0.64	0.68	0.67	0.64	0.67	0.60	0.50	0.71	0.19	0.49	0.45	0.41	0.56
Gamugofa	0.23	0.40	0.59	0.37	0.73	0.4	0.37	0.37	0.12	0.47	0.26	0.47	0.37	0.40
Gojam	0.53	0.66	0.65	0.62	0.52	0.53	0.62	0.71	0.73	0.6	0.69	0.64	0.71	0.63
Gondar	0.45	0.60	0.73	0.54	0.62	0.75	0.54	0.63	0.74	0.73	0.73	0.65	0.73	0.65
Hararghe	0.50	0.70	0.70	0.69	0.49	0.63	0.69	0.75	0.73	0.73	0.7	0.75	0.72	0.68
Shewa	0.57	0.71	0.72	0.45	0.48	0.72	0.45	0.73	0.74	0.75	0.74	0.71	0.74	0.65
IKS	0.34	0.52	0.61	0.65	0.57	0.73	0.65	0.71	0.71	0.62	0.51	0.46	0.71	0.60
Tigray	0.53	0.66	0.69	0.38	0.47	0.67	0.38	0.28	0.70	0.58	0.69	0.58	0.70	0.56
Welega	0.52	0.66	0.47	0.61	0.61	0.69	0.61	0.74	0.74	0.67	0.61	0.61	0.74	0.64
Welo	0.42	0.64	0.63	0.63	0.66	0.63	0.49	0.74	0.56	0.74	0.7	0.7	0.49	0.62
Altitudinal Classes (m)														
I	0.56	0.31	0.70	0.74	0.74	0.73	0.75	0.74	0.72	0.74	0.74	0.73	0.74	0.69
II	0.6	0.74	0.73	0.73	0.74	0.75	0.68	0.74	0.75	0.75	0.73	0.72	0.75	0.72
III	0.57	0.71	0.72	0.65	0.70	0.72	0.73	0.75	0.74	0.74	0.74	0.71	0.75	0.71
IV	0.37	0.59	0.53	0.73	0.62	0.73	0.54	0.73	0.37	0.64	0.73	0.57	0.73	0.61

and days to maturity in the later displayed a diversity index value of more than 0.70. In altitude class IV, however, only five traits have exhibited a diversity index of greater than 0.70 (Table 8.1), indicating the low polymorphism for traits in this altitudinal class than others.

Table 8.2. Partitioning of the phenotypic diversity within and between geographical regions of collections and altitudinal classes.

Characters	Geographical Regions of Origin				Altitudinal classes		
	H_t	H_r	Dst_r	Gst_r	H_a	Dst_a	Gst_a
Grain Yield	0.59	0.40	0.19	0.33	0.53	0.06	0.12
Biomass Yield	0.61	0.52	0.09	0.14	0.59	0.02	0.03
Harvest Index	0.74	0.55	0.19	0.26	0.67	0.07	0.10
Days to Maturity	0.73	0.47	0.26	0.36	0.71	0.02	0.03
Days to Heading	0.75	0.49	0.26	0.34	0.70	0.05	0.07
Grain Filling Period	0.74	0.55	0.19	0.26	0.73	0.01	0.01
Plant height	0.73	0.47	0.26	0.35	0.68	0.05	0.07
Spiklets per spike	0.75	0.53	0.22	0.29	0.74	0.01	0.01
Spiklet length	0.74	0.55	0.19	0.25	0.65	0.09	0.13
Kernels per spike	0.75	0.52	0.23	0.30	0.72	0.03	0.04
Spikes per plant	0.75	0.53	0.22	0.29	0.74	0.01	0.02
Kernels per plant	0.73	0.52	0.21	0.30	0.68	0.05	0.07
Thousand kernel weight	0.75	0.54	0.21	0.27	0.74	0.01	0.01
Mean	0.72	0.51	0.21	0.29	0.68	0.04	0.05

Partitioning of phenotypic diversity

Subdividing the variation into its components may assist in genetic resources conservation and utilization, by determining the relative contribution of the different levels of variability to the total diversity available in any one area. This would enable of *in situ* gene conservation, or use of appropriate gene pools in crop improvement for specific plant attributes (Bekele 1984). To determine the significance of the different

regional components, the total variation was partitioned into within- and among-regions diversity. The coefficient of regional diversity differentiation is 0.29 implying that 71% of the total variation was explained by the within region diversity (Table 8.2). Among the thirteen traits, biomass yield, spike length, harvest index, grain filling period and spike length showed high within region variation indicating their relative significance for differentiating accessions within regions. On the other hand, the two phenological traits, days to maturity and days to heading, and plant height contributed more to the between region variation. The partitioning of the between and within altitudinal diversity revealed that 95% and 5% of the total variation was attributed to the within and between altitudinal classes variation, respectively (Table 8.2). The contribution of individual characters to the within altitudinal diversity revealed that spiklets/spike, thousand kernel weight, grain filling period, and biomass yield have slightly more strong effect than others. These results concur with the findings of Baye (2003) and Chowdhury and Slinkard (2000) who reported a more significant contribution of within region diversity than between region diversity. In Ethiopian tetraploid wheat, studies (Bekele 1984; Bechere et al. 1996, Pecetti and Damania 1996) showed greater contributions of the lower (within populations, and among populations within regions and altitude zones) than the higher (among regions and altitude zones) level hierarchies to the total phenotypic variation. Likewise, a consistent and highly significant variation among tef germplasm populations both within regions and altitude zones for all the measured traits was reported (Kebebew et al. 2002).

Phenotypic diversity was also examined using multivariate analyses. To this end, principal component analysis was computed on the diversity index of regions of origin and altitudinal class for the 13 quantitative to examine the regional and altitudinal pattern of variation. The eigenvectors, eigenvalues and percent of total variance of the principal components which is computed using the means of regions and altitude represented by rows and the values of the 13 traits arranged in the columns as variables, is reported in Table 8.3. On regional bases, the first four axes, whose eigenvalues are greater than one, explained about 82 % of the observed phenotypic diversity in the 271 tetraploid wheat

accessions (Table 8.3). The first and second axis accounted for about 29.8% and 20.5% of the total variation, respectively. Following the criteria set by Johnson and Wichern

Table 8.3. Eigenvalues, total variance, cumulative variance, and eigenvectors of the first five principal components of 13 metric traits of 271 Ethiopian tetraploid wheat landraces.

Characters	Regions				Altitude	
	Eigenvectors					
	PC1	PC2	PC3	PC4	PC1	PC2
Grain Yield	-0.44	0.40	0.48	-0.15	-0.84	0.32
Biomass Yield	-0.06	0.13	0.21	-0.30	0.97	0.20
Harvest Index	-0.41	-0.14	0.45	-0.17	-0.02	-0.36
Days to Maturity	-0.39	0.28	-0.53	-0.33	-0.32	0.75
Days to Heading	-0.89	-0.36	-0.20	0.33	-0.14	0.03
Grain Filling Period	-0.18	0.04	0.12	0.61	0.15	0.20
Plant height	-0.10	0.33	-0.54	0.09	-0.48	-0.29
Spiklets per spike	0.43	-0.14	-0.76	-0.21	0.08	0.17
Spiklet length	0.54	0.71	0.12	0.12	0.02	-1.13
Kernels per spike	0.48	-0.83	-0.01	0.15	0.07	-0.14
Spikes per plant	0.41	-0.15	0.40	-0.17	-0.02	0.25
Kernels per plant	0.13	-0.43	0.17	-0.35	-0.21	-0.15
Thousand kernel weight	0.61	0.17	0.09	0.38	0.14	0.14
Eigenvalue	2.85	1.96	1.89	1.13	3.61502	2.38762
Percent of total variance explained	29.78	20.49	19.73	11.78	54.01	35.672
Cumulative percent of total variance	29.78	50.26	69.99	81.78	54.01	89.682

(1988), traits such as days to heading, kernels per spikes, spike length and spikes per plant accounted for much of the variation on these axes. On altitudinal bases, only the

first two principal components, which produced eigenvalues greater than 1, explained 89.7% of the total variation (Table 8.3).

8.5 Discussion

Efficient utilization of the genetic potential held in the germplasm collections requires a better knowledge of the genetic diversity present in the collected material. Understanding the extent of variability for plant traits and association of specific traits with geographic origin not only facilitates efficient synthesis of breeding populations but also will help to define needs and locations for future collection of germplasm. In this paper, Shannon Weaver diversity index was employed to examine the phenotypic diversity in 271 Ethiopian tetraploid wheat accessions in relation to characters, regions of origin and altitude. Results indicated that most traits are polymorphic. The overall diversity index for all traits was 0.74. Because Shannon-Weaver index is sensitive to both the type of phenotypic descriptor and number of descriptor classes used (Grenier et al. 2004), direct comparison of Shannon Weaver diversity indexes from different studies involving different descriptors and descriptor classes need caution. In Ethiopian tetraploid wheats, Jain et al. (1975), Negassa (1986), Bechere et al. (1996), and Eticha et al. (2005) employed the same method to investigate phenotypic diversity and reported a Shannon Weaver diversity index of 0.70, 0.81, 0.87 and 0.71 respectively. As with the findings of phenotypic diversity studies, the presence of appreciable genetic diversity in Ethiopian tetraploid wheats has been reported from variability studies using microsatellites (Messele 2001; Alamerew et al. 2004; Teklu et al. 2006a; 2006b), isozymes (Tsegaye *et* al. 1994; 1996), glutenine and gliadine storage protein and amplified fragment length polymorphisms (Messele 2001). Many microsatellite loci might be significantly linked to agronomically important traits (Teklu et al. 2006a; 2006b). Various authors also confirmed the uniqueness of the Ethiopian tetraploid wheat germplasm for different useful traits. For example, they have valuable features such as early ripening, short culm, long coleoptiles, and low tillering (Porceddu et al. 1973); resistance to powdery mildew and glume blotch (Negassa 1986), Hessian fly (Amri et al. 1990), stripe rust (Belay et al. 1992), and moderate resistance to pH and drought (Porceddu et al. 1973). Vavilov (1951) found Ethiopian tetraploid wheat that had 20% protein. Ethiopian tall type (rht) landraces

of durum wheat (*Triticum durum* Desf.) showed higher root penetration ability than semi-dwarf (Rht) varieties bred in North America (Kubo et al. 2004).

Natural populations harbor rich genetic diversity, which is eco-geographically structured and largely adaptive (Nevo 1988). As a result, landraces provide a valuable resource for plant breeding as well as for the preservation of genetic diversity. Under Ethiopian condition, where wheat landraces cultivation is predominant (Bechere et al. 1996; Tessema et al. 1993; Tessema and Bechere 1998), the first step in breeding should be the utilization of indigenous materials (Tessema 1991). Although landraces are characterized by low yield, they are relatively stable (Tessema 1991). Enhancing their yield, while maintaining an appreciable level of genetic diversity, is crucial to improve their competitiveness with modern varieties and maximize their utilization (Tessema and Bechere 1998). A modification of phenotype mass selection by selecting pure lines from genetically mixed landrace populations through yield testing and then bulking two or more superior pure lines has been suggested to improve the productivity of the landrace cultivars grown by the farmers (Tesfaye 1991). Composites, which are competitive with modern varieties with respect to yield, help to raise productivity while keeping diversity alive (Tessema and Bechere 1998). Experts assembled by FAO in a 1998 workshop on 'Broadening the Genetic Base of Crop Production' recommended the utilization of evolutionary plant breeding on composite (or synthetic) populations derived from a broad germplasm base across diverse environments to ensure wide genetic variation in long-term crop improvement programmes (Ortiz 2002). According to Tessema et al. (1993), the yield potential of landraces can be realized if they are tested at or near their original collection sites. Moreover, they mentioned that the incorporation of the most desirable characteristics from the exotic varieties into adapted and relatively high yielding agrotypes and the selection of segregants that combine the most desirable traits from the two parents is also necessary to improve the yield of landraces. Several authors reported the presence of useful attributes in Ethiopian tetraploid wheat landraces. For instance, Negassa (1986) reported that Ethiopian wheats have hard kernels, a trait which is positively correlated with flour yield (Konzak 1977). It is fairly easy to incorporate kernel hardness into breeding lines as this character is controlled by few major genes (Baker

1977). Resistance of Ethiopian tetraploid wheat to some diseases and insect pests has been reported. Traditional farmers built defense to diseases and pests into the genetic structure of landraces through selection over many generations and it may be necessary to introgress such defense mechanisms into modern cultivars to make them sustainable (Martin et al. 1991). Research and development of germplasm with polygenically conferred multiple disease resistance should be given more emphasis. Hence, hybridization programs should be strengthened. Research at CIMMYT has led to the development of > 600 new synthetic wheats in which many of these crosses have produced rapid improvements in important characteristics, including disease resistance, abiotic stress tolerance, and yield. Work at CIMMYT using synthetic wheat clearly indicates that this strategy is extremely promising (Hoisington et al. 1999). In breeding, identification of crossing materials that would result in improved grain yield and/or yield attribute is important. Under the current Ethiopian conditions, conventional breeding can easily be used to identify new germplasm sources that, when crossed with existing varieties would result in enhanced yields, quality traits and yield components. Theoretically, phenotypic diversity should approximate genetic diversity and thus phenotypic differences may also elucidate genetic differences (Cui et al. 2001). Grafius et al. (1976) and Grafius (1978) were among the first to apply this concept to practical breeding by employing cultivar differences in morphological traits to select genetically diverse breeding pairs (Cui et al. 2001). For the foreseeable future, conventional agriculture will be the primary response to feed the world in the coming years (Hoisington et al. 1999).

There are large amounts of wheat germplasm that have been collected and maintained mainly in the Institute of Biodiversity Conservation, Ethiopia. Although the institute has provided a number of accessions for researchers, the general consensus is the huge diversity have not been exploited effectively in cultivar development. Hoisington et al. (1999) described this phenomenon as valuable genetic resources are essentially ‘‘sitting on the shelf’’ in what have been dismissively termed ‘‘gene morgues.’’ The most attributed reason could be the large numbers of accessions, which make it difficult and time consuming to evaluate for all useful yield traits in the field trials and choose the

most promising ones with which to work. Development of wheat core collections based on species could facilitate utilization of the huge diversity stored in gene banks in Ethiopia. Core collections could provide plant breeders a manageable number of accessions to use in the search of new characters or character combinations and a structured way to evaluate whole collections (Rao and Hodgkin 2002). In addition, germplasm enhancement may be one of the keys for maximizing utilization of germplasm. It has become an important tool for the genetic improvement of breeding populations by gene introgression or incorporation of wild and landrace genetic resources into respective crop breeding pools. The term 'germplasm enhancement' or 'prebreeding' refers to the early component of sustainable plant breeding that deals with identifying a useful character, 'capturing' its genetic diversity, and the transfer or introgression of these genes and gene combinations from non-adapted sources into breeding materials (Peloquin et al. 1989).

Some wheat growing areas of Ethiopia are considered as marginal according to the definition that a region is defined as marginal when wheat production drops to 70% of optimal yield levels (Rajaram 2001). Therefore, genetic improvement in yield of cultivars under marginal environment is also very critical. As mentioned above, Ethiopian durum wheat has high root penetration ability. A deep root system is synonymous with more water uptake from the soil and better performance under drought. This indicates the presence of opportunities for developing drought tolerant varieties, which is an important breeding target. Moreover, the presence of appreciable variability for phenological traits like days to heading and days to maturity (Pecetti and Damania 1996; Teklu et al. 2006a; 2006b) signals the possibility of developing varieties with different growing periods.

Wheat breeding has made good contribution to improve the productivity of the crop in some regions of Ethiopia. Comparisons of the yield potential of wheat varieties that have been released over years have revealed that the yield potential of bread wheat has increased upto 89% during the last 38 years of wheat breeding activities. That of durum wheat has increased by 56% during the last 25 years (Amsal 1994). These gains were attributed to genetic contribution. Comparisons of performance of improved varieties

made with the reported average national wheat yield indicated that, between 1994-95 and 1998-99, model farmers who used improved varieties with their associated package in the central highland and Eastern highland areas had attained yields that were higher than the national average by about 102 to 140% and 38 to 103%, respectively (Eshetu 2002). At a national level, nevertheless; the desired increment in productivity hasn't increased significantly. Apart from the production constraints mentioned earlier, inadequate adoption of technology and concomitant low agricultural productivity is still a major concern and problem that attributed to the low gains in productivity. Jalletta (2004) and Mulatu and Belete (2001) have pointed out that one cause of low adoption appears to be research centers' recommendations that are irrelevant to the small farmers' priorities and resource constraints, as well as inappropriate to the physical, cultural and economic environment. Jalletta (2004) presented this as a more important factor than the frequently alleged farmers' reluctance (conservatism), weak extension services, poor policy and shortage of inputs. Modern cultivars have increased potential to take advantage of favorable environmental conditions and production inputs. However, under unfavorable conditions or if inputs are withdrawn, yield advantage of newer cultivars may be significantly reduced (Jalletta 2004). He mentioned that farmers refrained from the adoption of some 'improved' wheat varieties because the varieties performed poorly under farmers' conditions. Similar reports were made by Mulatu and Belete (2001) in Ethiopian sorghum. While breeding programs have clearly increased yield potential, they might have increased the responsiveness of most modern cultivars to better environments (i.e. decreased yield stability) (Slafer and Kernich 1996). It was largely recognized that most of the improved varieties released so far have been recommended for broad adaptation. The reality, however, shows that each variety is best suited to specific zones (Jalletta 2004). Thus, the inconsistency of the performances of improved varieties over locations could be attributed to high genotype by environment interactions. The Ethiopian peasant farms are characterized by highly varied microenvironments (Tessema 1991). In Ethiopia, wheat is grown under rainfed conditions. Rainfed environments are characterized by unpredictable and highly variable seasonal rainfall and hence highly variable yields (Richards et al. 2002). Hence, introducing improved varieties for a wide adaptation into Ethiopia's wide range of microclimates, which differ sharply from one

village to the next, results in slow genetic advance in breeding programs because genetic variation in yield is masked by large G×E interactions (Calhoun et al. 1994). Thus, it is impossible to ignore the impacts of G×E, which drastically affects the stability of cultivars, in crop breeding programs in Ethiopia. Selection of genotypes under low input cultivation could be important to achieve success in breeding programmes dealing with strong genotype by environment interactions (Ceccarelli 1996). Cecarelli (1997) found that genetic gain for yield under low input cultivation was possible working with adapted barley landraces.

Traditionally, agricultural recommendations are developed by researchers on experiment stations with the primary purpose of maximizing yield per unit area of land, whereas small farmers in Ethiopia seek stability under their along with yield (Jalletta 2004). As a goal of plant breeding, the stability of yield is often considered to be of equal importance to yield itself (Federer and Scully 1993). To get the desired yield gains and stability, breeders should identify appropriate selection and test environments so that the improved varieties will perform as intended when they are grown in the target environment. It is a commonly accepted maxim that new varieties "of any crop must be tested under the conditions in which they will be grown" (Stoskopf et al. 1993). Agro-ecology based breeding strategy could be a potential tool to harmonize test and target environments. Agro-ecological based research characterization is central for targeting research as defined by potential resource base, to respond to climatic change in resource use, identifying research priorities for location specific and selection of appropriate research centers and extrapolating research results among zones by establishing agro-climatic analogies. In fact, although it has not been effectively materialized, EARO has made substantial efforts to develop national research strategy based on agro ecology and three other approaches: stakeholders participation, farming systems, and multidisciplinary and balanced approach. This strategy needs to be fully implemented in all agro ecology zones of the country.

According to Amsal (1994), there was no indication of a yield potential plateau in wheat over the period studied implying the possibilities of additional gains. The presence of

diversity for useful attributes in Ethiopian tetraploid wheat germplasm could make such progress possible. Generally, there exists substantial new challenge and opportunities for Ethiopian tetraploid wheat breeders to improve grain yield potential, quality traits and yield attributes. Ethiopian tetraploid wheats deserve broader characterization both at phenotypic and molecular levels; including mapping of important traits that can be used in future crop improvement programs (Teklu et al. 2006a; 2006c). It is continuing reciprocation between advances in plant breeding and that of agronomy that makes breeding progress possible and creates opportunities for maximizing yield (Teklu and Tefera 2005). Therefore, development of appropriate agro-ecology based agronomic packages is also useful while deploying improved varieties.

In crop improvement, it is not only working with the existing genetic variation that is central but also parallel and periodic assessment of the threat of loss of diversity is necessary. Using the calculation scheme: gene erosion = 100% - gene integrity, i.e., the still extant landraces, a genetic erosion upto 100% was detected in *T. durum*, *T. dicoccon* and *T. turgidum* in some districts of Eastern Ethiopia (Teklu and Hammer 2006d). Other reports (Worede 1983; Hailu 1991; FAO 1996a) also reported the problem of genetic erosion in Ethiopian tetraploid wheats. Therefore, priority should also be placed on collection and conservation of landraces, which are irreplaceable materials, if lost. The best method of conservation is the use of complementary approach of the 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. The guiding principle of 'conservation through use' should be respected because only in use diversity can be appreciated enough to be saved, only in use it can continue to evolve, and thus retain its value (Partap, l996).

Chapter 9 Farmers Perception and genetic erosion of Ethiopian tetraploid wheat landraces

9.1 Abstract

Assessing genetic erosion has been suggested as the first priority in any major effort to arrest loss of genetic diversity. In Ethiopia, although it is generally accepted that significant amount of genetic erosion has occurred and is still occurring, there is little data on its amount and extent. Thus, this study is conducted to quantify the extent of genetic erosion in Ethiopian tetraploid wheat landraces and to identify major causes of genetic erosion. To this end, a field survey of 126 farmers, randomly selected over five districts in eastern, south-eastern and central highlands of Ethiopia during 2001/2002 and 2002/2003 main cropping seasons was undertaken. Questioner was used to collect primary data from farmers who are potentially rich sources of information on genetic erosion at the variety level. Additional data were collected through key informant interviewing. Moreover, resampling was made from Tulo, Chiro and Harar Zuriya districts in eastern Ethiopia. Analysis of history profiles from primary and secondary data indicated a reduction in the use of local varieties over years. *T. polonicum* and *T. turgidum* are becoming very localized, and therefore, they are under greater threat of extinction. Using the calculation scheme: gene erosion=100%-gene integrity, i.e., the still extant landraces, genetic erosion was calculated for the three different areas where resamplings were made. Genetic erosion of 100% was observed both in accessions belonging to the relationship of *T. durum* and *T. dicoccon* in Tulo district. Likewise, genetic erosion of 85.7, 100 and 77.8%, respectively, was calculated for *T. durum*, *T. turgidum* and *T. dicoccon* in Chiro district. In Harar Zuriya, a genetic erosion of 88.9% for *T. durum* and 100% both in *T. turgidum* and *T. dicoccon* was detected. The number of farmers growing landraces of tetraploid wheats drastically decreased in all surveyed areas in the past decades. Displacement of landraces by other crops was the prominent factor for ending landrace cultivation. Farmers' preference to yield potential and cash crops subsequently reduced the chance of maintaining landraces. Institutional factors like access to credit and the extension advice have influenced farmers' decision regarding cultivar choice. In all surveyed areas, the most important initial source of seed of

improved wheat varieties is the seed credit from the Ministry of Agriculture which uses a 'plant now, pay later' scheme to promote the distribution of improved varieties and fertilizers. The problem of genetic erosion through inappropriate maintenance of *ex situ* collections was also recognized and discussed.

9.2 Introduction

Thousands of genetically distinct varieties of our major food crops owe their existence to years of evolution and to careful selection and improvement by our farmer ancestors. Nevertheless, processes that once took hundreds or thousands of years to develop could then be carried out within decades or even years under human influence (Hammer 2004). There has been a significant loss of genetic diversity during the last 100 years and the process of gene-erosion continues (Hammer et al. 2003). In the field of plant genetic resources for food and agriculture the irreversible loss of single genes or combinations of genes in genotypes, the so-called gene-erosion, is of major concern. Diversity is the basic factor of evolution in species. It made it possible for crops to be adapted to the most different environments and uses, and genetic diversity will allow them to respond to the arising challenges (Hammer et al. 1999). As a result, the loss of biodiversity belongs to one of the central problems of mankind, next to other important matters such as climate change and securing an adequate supply of drinking water. In his centers of diversity, Vavilov was able to observe and document the unbroken result of an evolutionary process that had lasted thousands of years. N.I. Vavilov and even Jack Harlan are sometimes proposed as the first researchers that became aware of genetic erosion in the l920s and l930s (Scarascia-Mugnozza and Perrino 2002). In fact, this phenomenon was observed for the first time by Baur (1914), see also Flittner (1995). So far, the American plant breeders H.V. Harlan and M. L. Martini (1938) have been credited with first recognizing the problem of genetic erosion in crops (Brush l999). The concept emerged forcefully between l965 and l970, in a period when crop improvement had clearly demonstrated its power to transform local crop populations in industrialized countries and in certain less developed regions (Brush l999) and the term gene erosion was coined (Bennett l968). Several approaches have then been employed to estimate the degree of genetic erosion that a particular taxon faces in a certain region over a given time. Methods usually rely on

either the analysis of molecular data (Provan et al. 1999) and allozyme analysis (Akimoto et al. 1999), or comparison between the number of species/cultivars still in use by farmers at present time to those found in previous studies (Hammer et al. 1996) or using the genetic assessment model presented by Guarino (1999) or using a checklist of risk factors (Oliveira et al. 2002). The most widely used figures in estimating genetic erosion is indirect, i.e., the diffusion of modern crop varieties released from crop breeding programs. Various authors have estimated genetic erosion in different crops using different approaches. The two case studies conducted by Hammer et al. (1996) to estimate genetic erosion in landraces revealed that genetic erosion was found to be 72.4% in Albania and 72.8% in South Italy. The study of 220 landraces with 147 forms in South Korea (Ahn et al. 1996) showed a medium gene erosion of 74%. Akimoto et al. (1999) evaluated the threat of genetic erosion faced by Asian wild rice in Thailand and reported that the wild rice population was seriously destroyed and fragmented. Stephen et al. (2002) also informed that farmers in the northeastern Philippines had a marked reduction in rice diversity from 1996 to 1998. Gao (2003) reported that the widespread adoption of high-yielding rice varieties has led to biological impoverty of rice germplasm, as local rice varieties are abandoned for modern varieties.

To reverse the unabated gene erosion, conservation of genetic diversity is a fundamental concern in conservation and evolutionary biology, as genetic variation is the raw material for evolutionary change within populations (Frankel and Soulé 1981). Detecting and assessing genetic erosion has been suggested as the first priority in any major effort to arrest loss of genetic diversity. Generally, nevertheless, many national programs have not regarded quantification of genetic erosion as a high priority, as apparent from the paucity of information in the State of the World Report (FAO 1997). Also in Ethiopia, while there is clear evidence for a reduction in the number of tetraploid wheat landraces grown and a decline in the area in which landraces are grown, the extent to which allelic diversity has been lost has not been documented. Hence, this study was conducted to determine the extent of genetic erosion, and to investigate farmers' perception about the causes of genetic erosion. The study also assessed the measures that are being taken to reduce the problem of genetic erosion.

9.3 Materials and methods

Selection of study sites and farmers

A stratified random sampling procedure was used to identify farmers. First, the different wheat producing districts were selected in consultation with IBC and MoA personnel. Accordingly, two districts namely Chiro and Habro from eastern Ethiopia, two districts namely Hetosa and Tiyo from Arsi region of south-eastern Ethiopia, and two other districts known as Ginchi and Gimbichu from central highlands of Ethiopia were identified (Figure 9.1). Within each district, major wheat producing peasant associations (PAs) were selected. Within each PAs, a group of old and experienced farmers were selected in close contact with development agents (extension staff of MoA) and a total of 126 farmers were randomly sampled from the different groups. The household characteristics and major crops grown in surveyed districts are presented in Table 9.1.

Data Collection and Analysis

To examine the extent of genetic erosion occurred in the last decades temporal comparison was used. This was done by re-sampling and through indigenous knowledge surveys as described by Guarino (1999). Re-sampling of landraces of *T. dicoccon*, accessions belonging to the relationships of *T. turgidum* and *T. durum* Desf. was done in three areas namely Harar Zuriya, Tulo and Chiro districts in eastern Ethiopia in 2001/2002 and 2002/2003 main cropping seasons and compared with germplasm accessions collected by IBC from the same areas at different times in the past and conserved *ex situ*. For relocating the sites, where previous collections were made, to undertake re-sampling information on the passport data from earlier years of collection was used. Among others, the passport data of IBC contains information on collection site like administrative region, district name, altitude, latitude, and longitude of collecting site including the distance from the nearby town.

Brown et al. (1997) and Synneväg et al. (1999) provided a useful list of features or indicators that could be measured singly or in combinations on individuals and populations of a given species in a defined area as part of a systematic effort to monitor changes in genetic diversity in the species. Modified lists of such indicators were used in

developing the questioner used to collect primary data on indigenous knowledge and experiences of local farmers that are potentially rich sources of information on genetic erosion at the variety level. The main topics included in the questioners were topics like change in cropping systems and reasons for change, adoption of improved varieties of wheats, assessment of the seed supply systems, change with regard to the use of landraces and reasons for change, trends in the area of cultivation of tetraploid wheats, farmers perception about comparative advantages of landraces. The questionnaires were administered to sample farmers in collaboration with development agents using local languages. Additional data were collected through key informant interviewing. Key informants included MoA staff, wheat researchers, IBC staff, developments agents, and NGOs staff. Data was also collected from secondary sources like scientific publications, reports of extension departments of MoA, IBC, and EARO.

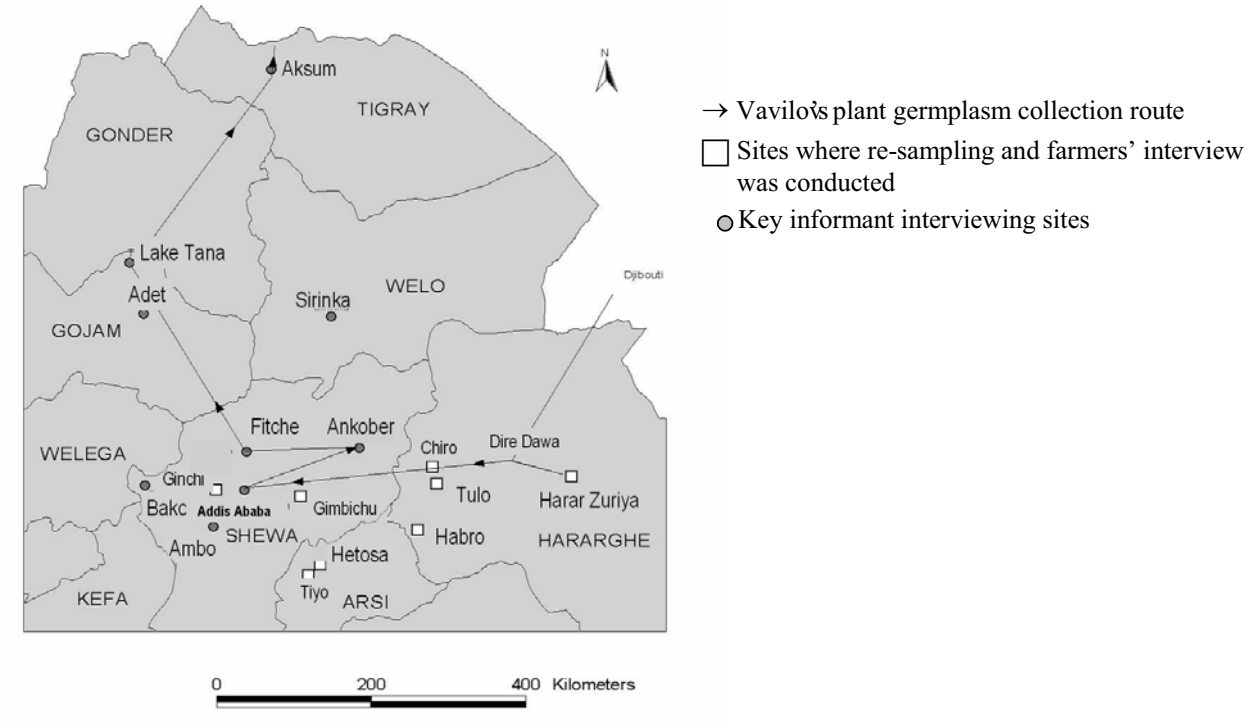


Figure 9.1. Map showing experimental sites.

For each question, the percentage of farmers who gave similar responses was calculated for each district. For re-sampled areas, genetic erosion (GE) was calculated as GE=100%

- GI (Genetic integrity) (see Hammer et al. 1996). This approach is possible because collecting missions have been carried out in 2001/2002 and 2002/2003 covering the same areas, and following the same procedures of IBC germplasm collection method. However, pooled accessions collected between 1963 and 1988 in Harar Zuriya, between 1979 and 1988 in Tulo district and between 1964 and 1987 in Chiro district were used as a base for calculating genetic erosion unlike Hammer et al. (1996) who used comparisons of accessions collected in 1941 and 1993 in Albania and in 1950 and 1983/1986 in South Italy. For this experiment, it was not possible to collect the rate of genetic erosion per year because of the longer time difference in the pooled accessions.

9.4 Results

Assessment of genetic erosion: field studies

Using the calculation scheme: gene erosion = 100% - gene integrity, i.e., the still extant landraces, a genetic erosion was calculated for Harar Zuriya, Tulo and Chiro districts. Genetic erosion of 100% was observed both in *T. durum* and *T. dicoccon* in Tulo. Likewise, genetic erosion of 85.7%, 100% and 77.8%, respectively, was detected in *T. durum*, *T. turgidum* and *T. dicoccon* in Chiro. In Harar Zuriya, a genetic erosion of 88.9% in *T. durum* and 100% in *T. turgidum* and *T. dicoccon* was found (Table 9.2).

All the interviewed farmers in the different districts reported that they were growing tetraploid wheat landraces mainly *T. durum* and *T. dicoccon* in the past. However, the number of farmers growing landraces of tetraploid wheats drastically decreased in the past two decades in all surveyed areas (Table 9.3), implying reduction in the area coverage of landraces as other crops were adopted. The highest displacement of landraces was observed in Hetosa and Tiyo where 87.5% of the sampled farmers grew only improved varieties of bread wheat. Adoption of modern wheat varieties is inversely correlated with landraces diversity mainly in Ginchi, Hetosa and Tiyo, in which farmers have abandoned landraces to a greater extent (Table 9.3).

Table 9.1. Household characteristics of surveyed areas.

Name of district	N	Gender		Average age of farmers (years)	Average family size (no.)	Average farm size (ha)	Major crops grown
		Male	Female				
Chirro and Habro	32	25	7	47.3	7.9	1.72	Sorghum, maize, khat, tef, potato, wheat, haricot bean
Hetosa and Tiyo	40	35	5	54.9	6.0	2.41	Wheat, barley, potato, noug, beans, peas
Ginchi	24	21	3	51.2	7.6	1.96	Wheat, tef, maize, noug, beans, peas
Gimbichu	30	22	8	49.5	6.3	2.27	Tef, wheat, lentil, chickpea

Table 9.2. Estimating genetic erosion in landraces of crops in Harar Zuriya, Tulo and Chiro districts by comparison between collecting missions at various times. GI = genetic integrity, GE = Genetic Erosion.

Crop	Harar Zuriya				Tulo district				Chiro district**			
	1963-1988*	2002	GI%	GE%	1979-1988*	2002	GI%	GE%	1964-1987*	2003	GI%	GE%
	Samples collected	Samples Collected			Samples collected	Samples Collected			Samples collected	Samples Collected		
Durum	18	2	9.1	88.9	15	2	13.3	76.7	14	2	14.3	85.7
Tturgidum	3	0	0	100	-	-	-	-	2	0	0	100
Dicoccon	4	0	25	75	7	0	0	100	18	4	22.2	77.8

*, accessions were collected by the Institute of Plant Genetic Resources Center, Ethiopia.

**, Some parts of Habro district were also included in the analysis.

Assessment of genetic erosion: a review

Worede (1983) reported that the traditional tetraploid wheat varieties have been almost completely replaced by modern, uniform, and advanced cultivars in areas such as Arsi and Bale, major wheat growing areas of the country. There are reports indicating the decline of area cultivation of tetraploid wheats in the country. Hailu (1991) reported that the estimated area of tetraploid and bread wheat to be, respectively, 85 and 15% in 1967 and 60 and 40% in 1991, whereas Aquino et al. (2000) as cited by Eshetu (2002) reported the wheat area covered by tetraploid and bread wheat in Ethiopia to be 40 and 60%, respectively. The native Ethiopian tetraploid wheat is suffering from serious genetic erosion and is being lost (FAO 1996a). Owing to their soaring price, the acreages of tef and khat have increased at the expense of major crops such as sorghum, wheat, maize, and barley (Hailu 1991). According the report of IBCR (2002), populations of *Triticum polonicum* faced extinction problems. The presence of modern varieties in a farming system is taken as prime facie evidence of genetic erosion. Mulugetta (1994) studied the economics of smallholder wheat production and technology adoption in five wheat growing districts of Arsi region during the 1990/1991 cropping season from a sample of 426 wheat farmers and found that all the sample farmers planted improved wheat varieties. Likewise, Setotaw et al. (2001) surveyed a total of 300 farm households in five major wheat-growing areas of the Arsi zone to examine the rate of adoption of wheat production technology and about 95% of the sampled farmers had grown wheat during the 1998 crop season. They revealed that 91.5% of the farmers adopted improved bread wheat varieties. According the their findings, about 73% of wheat growers planted a single variety of wheat. Similar trends were also observed in other major tetraploid wheat growing areas of Ethiopia. Bekele et al. (2000) reported that the total area planted to the most important improved bread wheat varieties from 1992 to 1997 has increased dramatically in Bale province. In Yelmana Densa and Farta District of northern Ethiopia, the rate of adoption of improved wheat varieties increased from less than 1% in 1981 to 72% in 1998 (Tesfaye et al. 2001).

Table 9.3. Comparison of percentage of farmers growing tetraploid wheat landraces or improved wheat varieties in the past and at present. IV = Improved varieties, FV = farmers varieties (landraces).

		In the past (before 10-20 years)			During 2002 and 2003 main cropping seasons		
Name of district	N	Growing FV only	Growing IV only*	Growing both	Growing FV only	Growing IV only	Growing both
Chirro and Habro	32	18.75	31.3	50	0	9.4	3.1
Hetosa and Tiyo	40	22.5	42.5	35	0	87.5	12.5
Ginchi	24	45.8	16.7	37.5	8.3	54.1	16.7
Gimbichu	30	60	10	30	40	13.3	36.7

*, Improved varieties include both improved bread wheat varieties and durum wheat varieties.

The heterogeneity of farming systems in centers of diversity is believed to limit the diffusion of modern varieties and maintain production spaces for indigenous varieties. Contrary to this, improved Kenyan wheat varieties were found by the German H. Kuckuck 'in very remote areas of Ethiopia, accessible only by mules' (Fowler and Mooney 1990). They also reported that the native wheats of the Nile Valley will soon be gone, replaced by modern varieties provided by a government program. Currently, with the aggressive extension package in action and the dynamism of the farmer to farmer seed exchange in Ethiopia, the diffusion of improved varieties is likely to be deeper than expected.

Reasons for the displacement of landraces: survey results

The main reason for the reduction or abandonment of cultivation of landraces is displacement of landraces by other crops (Table 9.4). In Hararghe, only 9.4% of the interviewed farmers grew landraces only and 3.1% farmers grew landraces along with improved wheat varieties (Table 9.3). The remaining 87.5% of the interviewed farmers abandoned landraces and shifted to cultivating khat, maize and sorghum. The major

reason for the expansion of khat production is its high price. In Ginchi, Hetosa and Tiyo districts, emmer and other tetraploid wheats were replaced by bread wheat cultivation because the latter is high yielding and fetches a higher price. Low grain yield of landraces is among the important factors mentioned for the replacement of tetraploid wheat landraces by improved bread wheat varieties (Table 9.4). The erratic and unstable rainfall coupled with the longer growing period of landraces also forced farmers to adopt early maturing modern wheat varieties or other crops that either escape or tolerate droughts. In Habro and Chiro districts, farmers shifted from wheat to sorghum, maize, khat and tef production because these crops are less vulnerable to low moisture stress than wheat. The pro-improved varieties extension advice and credit facilities have influenced farmers' decision regarding cultivar choice (Table 9.4). Farmers were motivated to grow improved varieties so as to benefits from the associated credits. Decrease in soil fertility was also one of the major factors which led farmers to start using inorganic fertilizers consequently the landraces were unable to perform well because of lodging problems (Table 9.4). In most surveyed areas, the main initial source of seed of improved varieties is the seed credit from the MoA which uses a 'plant now, pay later' scheme to promote the distribution and multiplication of improved varieties and fertilizers (Table 9.5). In Gimbichu district, community seed bank is the basic source of seeds of landraces (composites) for farmers (Table 9.5).

Reasons for the displacement of landraces: a review

There have been several catastrophic droughts in the country that caused complete crop failures and subsequently severe genetic erosion has taken place in the landraces that have been maintained through many generations. Farmers have been forced to consume the seeds normally kept for planting. The famine of the mid-1980s seriously threatened Ethiopia's biological resources. In some cases, food grain from relief agencies became the only source of seed for planting after farmers ate their own seed, or sold as food commodity in order to survive (Worede and Mekbib 1993; Worede 1998).

Farm location and the ways in which farmers articulate with formal seed supplies is an important determinant of the impact these have on farm level diversity (Cromwell and

Almekinders 2000). Of the different surveyed areas, landrace replacement by bread wheat was found to be higher in Hetosa and Tiyo districts (Table 9.3), which are near to Kulumsa Agricultural Research Centre and a seed multiplication and processing firm of the Ethiopian Seed Industry Agency. After seed has been released from the formal sources, the local seeds systems like gifts for relatives or friends and exchange of seeds among farmers or bartering were found to be the main roots of improved seed dissemination. Seed aids by NGOs and relief agencies at times of famine were also one of the seed sources in Hararghe. The availability of improved varieties of bread wheat seeds in larger quantities as compared to durum wheats has been responsible for the displacement of tetraploid wheat (Bechere et al. 2000).

Table 9.4. Farmers' reasons given to end cultivation of landraces (% of responses)*.

Reasons	Habro and Chiro (31)**	Hetosa and Tiyo (35)	Ginchi (18)	Gimbichu (7)
Displacement by improved varieties of durum	3.22	-	5.6	14.2
Displacement by other crops	80.6	88.5	61.1	28.57
Decrease in soil fertility	35.4	25.7	33.3	-
Decreased and unstable rainfall	38.7	17.1	27.7	14.28
Low yields	61.3	82.8	61.1	57.14
Weeds, disease and insects	6.4	20	16.7	0
Lodging problems	25.8	40	33.3	14.28
Marketability	19.4	34.3	16.7	28.6
Longer growing period	22.5	25.7	22.2	-
Threshability problems	22.5	22.8	22.2	-
Influence of extension agents	9.6	25.7	16.7	-

*, Sums over 100% are due to multiple answers.

**, Figures in parentheses refers to number of farmers who ended cultivating landraces and interviewed for the identifying reasons to end cultivation of landraces.

In Ethiopia, comparisons of the yield potential of wheat varieties that have been released over years have revealed that the yield potential of bread wheat has increased (Amsal 1994). These changes in bread and durum wheat potential grain yields in the central highlands of Ethiopia were strongly associated with changes in harvest index with no

change in biological yield, which is the characteristic of most modern wheat varieties containing dwarfing genes. This is a welcome development approach, although, paradoxically, it is a threat to genetic diversity on which future crop improvement work is based. Since the start of the high input extension package in 1994–1995, cultivar abandonment and replacement has been taking place especially in the major wheat growing areas of the central rift valley and central highland of Ethiopia by both package and non-package user farmers (Eshetu 2002). The better performance of bread wheat varieties with fertilizers has contributed to their increased adoption (Tesfaye et al. 2001). The two main inputs, fertilizer and improved seeds, have witnessed widespread and increasing rates of adoption, since the 1995/1996 cropping season the period that the aggressive fertilizer and seed credit transfer package was taken up by the MoA in collaboration with the SG-2000 (Eshetu 2002). At present, the package program is fully operational in all regional states and ecological zones of the country. The higher price of tef in the market and its elasticity in terms of ecological distribution attributed to the displacement of tetraploid (Bechere et al. 2000; Eshetu 2002). Institutional factors, principally access to credit, extension contacts, and farmer education level, significantly influenced the adoption of bread wheat production technologies (Setotaw et al. 2001). In Ethiopia, agribusiness sectors like small-scale private farmers, state farms, and producer cooperatives who were active in major wheat growing areas of south eastern Ethiopia were in favor of monoculture cropping and therefore have also played a prominent role in the increased adoption of improved bread wheat varieties at the expense of traditional varieties of tetraploid wheats.

Loss of diversity in *ex situ* gene bank: a review

The discovery, collection, and conservation of potentially valuable but endangered plant genetic resources for food and sustainable agriculture (as well as other plant genetic resources that have potential value for future development) are the primary obligations of all countries and institutions adhering to the FAO international undertaking on plant genetic resources (Hammer et al. 2003). Consequently, the institute of Plant Genetic Resources Center/Ethiopia (PGRC/E), the then IBCR, was established in 1976 to collect, evaluate, document, conserve and promote the utilization of crop plant germplasm in the

country. Despite the big financial problem and lack of trained personnel it had, the institute has made tremendous efforts to collect, evaluate and maintain genetic resources of Ethiopia since its establishment. The institute currently maintains about 12,000 accessions of *Triticum* species in its *ex situ* gene banks.

Table 9.5. Farmers response of wheat seed sources in the different survey areas (% responses)*.

Factor	Habro and Chirro	Hetosa and Tiyo	Ginchi	Gimbichu
Seed aid from NGOs and relief institutions	34.3	0	0	0
Community Seed Bank	0	0	0	80.0
Seed credit from MoA and SG-2000	53.1	80.0	91.7	20.0
Gifts from relatives and friends	12.5	20.0	50.0	30.0
Exchange of seeds (bartering)	21.9	30	25	26.7
Manual labor for seeds	9.4	0	0	16.7
Local Markets	32	24	34	16.7
Saved seeds (self supply)	21.9	52.5	58.3	60.0
Research centers	9.4	15	0	0

*, Sums over 100% are due to multiple answers.

The problem of genetic erosion through inappropriate maintenance of *ex situ* collections is widely recognized. Genetic erosion can occur at many stages in the preparation, sub-sampling, exchange, storage and regeneration of seed (Sackville Hamilton and Chorlton 1997). They also highlighted loss of diversity through genetic shifts and convergent selection during regeneration as a potentially severe and often under-acknowledged problem. In the world collection, beyond the problem of duplication among accessions, the security of *ex situ* conservation as a whole is endangered. About half of all gene bank accessions urgently require rejuvenation, and in several countries the percentage is even higher (Hammer 2004). The germplasm passport data of IBC mainly contains collection data like accession number, species name, and information on collection site (administrative region, altitude, latitude, and longitude), and date of collection. However,

it is common to find accessions that lack one or more of the above data. It was also observed from the passport data and an interview that samples were collected with a low frequency. The stock of seeds of some accessions in the gene bank has been exhausted as a result of frequent requests but not having been replenished appropriately. As a result of financial problems, lack of staff and shortage of farms, rejuvenation of seeds weren't being done as per scheduled. Tsehaye (2002) observed that the tetraploid wheat materials from the gene bank have showed poor germination potential and vigor in the field, which is an indicator of genetic erosion.

Community gene bank activities

The primary solution to the genetic impoverishment of crop germplasm is genetic conservation and utilization in breeding of the vast genetic variation found in natural populations of the wild progenitors and landraces of cultivated plants (Frankel and Bennett 1970; Tanksley and McCouch 1997). The Global Plan of Action (FAO 1996b) calls for strengthened on farm conservation and management to preserve genetic diversity in farmers' fields, and points to the pressing need for more research. Recognizing this, coordinated *in situ* conservation in Ethiopia started in 1988 when Plant Genetic Resource Centre of Ethiopia in collaboration with the Unitarian Service Committee of Canada (USC/C), implemented the Seeds of Survival Program, Ethiopia (SoS/E) and conducted a farmer-based crop genetic resource conservation and utilization program. One aspect of the program seeks to restore the landraces to regions where farmers had once planted them extensively but where they had been replaced by new, exotic or improved (high input) varieties. In the region of Ada, in Central Shewa, the indigenous tetraploid wheat has nearly disappeared because of displacement by introduced bread and tetraploid wheat varieties (Worede 1993). To promote the conservation, enhancement and utilization of indigenous tetraploid wheat in Ada and other areas of Central Shewa, the gene bank, in close collaboration with the wheat breeding team at Debre Zeit Agricultural Research Centre and the SoS program, undertook extensive collection of landraces from which elite landrace selections were developed as composites, multiplied and distributed to farmers (Tessema 1987). Composites, which are competitive with modern varieties with respect to yield, help to raise productivity while keeping diversity alive (Tessema and

Bechere 1998). Consequently, farmers in Gimbichu district re-introduced landrace cultivation.

Table 9.6. Farmers' perception of comparative advantages of landraces of tetraploid over improved varieties of wheat in Gimbichu district.

Factor	**Landrace Materials**	**Improved Varieties**
High plant length	Taller	Shorter
Food value	Nutritious	Less nutritious
Storage period	Longer as they are resistance to storage pests (weevils)	Shorter because they are easily eaten by weevils
Shattering	Non-shattering	Shatter if not quickly harvested
Weed, disease and insect pest resistance	More tolerant	Susceptible
Tillering capacity	High	Low
Kernel weight per unit volume	Heavier	Lighter
Adaptation	Well adapted	Poorly adapted
Chemical Requirements	Don't require fertilizer and other chemical inputs	Don't perform well without chemicals
Planting Seed requirements	Less seed per unit of land	More seed per unit of land
Stability of yield	Fairly stable	Unstable

A biodiversity conservation project of the Global Environmental Facility program of the UN was also undertaken from 1994 to 2002 (Tsehaye 2002). The program worked on institutional strengthening, community-based conservation activities, and identifying incentives for *in situ* conservation. In 2001, the Ethio-Organic Seed Action Programme (EOSA) was established to promote integrated conservation, use and management of plan genetic resources. The report of EOSA indicated that the policy environment is one challenge they have faced. It has been one where government has been more interested in increased production to cater for growing population demand at the expense of maintaining diversity. This has resulted in less support for conservation initiatives and has proved to be a challenge to program implementation. Mburu and Edilegnaw (2003) observed the presence of mistrusts between EOSA and IBC. The latter fears that the

EOSA can eventually take over the bulking activities and start using them to solicit for funds.

The value of diversity is in its use (Gao 2003). Cognizant to this fact, community gene (seed) banks were established to mitigate the unabated genetic erosion of landraces of indigenous crops with a guiding principle of 'conservation through use'. Currently, there are twelve community gene (seed) banks in Ethiopia. A survey was made to beneficiaries of community seed bank association in Gimbichu district. The association was organized by IBC. To become a member of the association, farmers have to pay a membership fee and contribute an agreed amount of seed as membership shares (one share is equal to 50 kg of seed). A farmer can have up to maximum of 10 shares. During the time of seed scarcity, farmers are given seed from the gene bank on credit basis and should pay in kind at the end of the season. In addition, these farmers are free to sell the rest of the seed to the seed bank at the local market prices. Members of the association indicated that they reintroduced landrace cultivation to benefit from the various advantages of composites (landraces) (Table 6). Landraces provided farmers with reasonable yield under natural conditions, without fertilizers and pesticides. As a result, farmers were free from extension credits which farmers were often unable to pay because of low market price at times of good harvest. Moreover, they expressed that landraces are good for making different local foods such as 'genfo' (porridge), 'nifro' (boiled grain), 'kinche' (crushed kernels cooked with milk or water and mixed with spiced butter), and 'kolo' (roasted grain). In other districts, the nutritional value was the most frequent reason mentioned for maintaining landraces of emmer wheat in face of competition from modern bread wheat varieties and other crops. Farmers expressed that none of the improved varieties released so far were comparable with landraces particularly to the farmer's variety that is locally named as 'Tikur sinde', which is named tikur (black) after its seed color, in making local alcoholic beverages. This local variety is also praised for its long straw, good taste, non shattering characteristics, and its resistance to storage pests and moisture variability. Despite its black color, it has high market price. Since most of landraces are tall in height, they compete well with weeds and their straw is good for thatching roofs. As landraces are resistant to weevils, they have longer storage life. Farmers mentioned their time and

labor shortage problems during harvesting when they grow improved varieties as these varieties shatter if not harvested soon after maturity. Landraces, on the other hand, do not shatter and would keep longer before harvest. Landraces have heavier kernels weight per unit volume than modern varieties. Worede (1998) also reported that a sack of 100 kg capacity filled with landraces weighs more (upto 30 kg in excess) than improved wheats. In addition to reintroducing landrace cultivation into the area, which is one the best achievements of the association; it created a good link between farmers, researchers and IBC staff, an important factor to ensure sustainable on farm conservation activities.

9.5 Discussion

In all the study areas, cultivation of landraces has been decreased. In Hararghe, a genetic erosion up to 100% was observed. Hararghe was one of the areas where tetraploid wheat landraces were growing (Bechere et al. 2000). During a germplasm collecting trip to Ethiopia, the plant explorer N.I. Vavilov (1997) has visited Harar in January 1927 and described the huge wheat variability he observed as 'the characteristic wheat, cultivated in an enormous amounts in the Harar region, no doubt belongs to a special kind, different from every thing I had seen and collected in other agricultural areas of the world. The fields display incredible mixture of varieties. It was necessary to collect hundreds of ears to obtain a representation of the botanical composition. I discovered at once endemic types with violet grains, not known anywhere else in the world' (Vavilov 1997). The number of farmers growing landraces of tetraploid wheats and area of cultivation of these crops has declined. Thus, landraces of these species mainly of *T. polonicum*, *T. diccoccon* and *T. turgidum* might face a survival problem as natural selection is often incapable of action with too small population, and random genetic drift will accelerate the loss of genetic polymorphisms. For plants and some animals, area measurements of habitat patch sizes will provide a reasonable basis to estimate population size (Brown et al. 1997), an important factor determining survival of individuals. The size and number of individual populations are related to their ability to cope with both random (stochastic) fluctuations in the environment and steady (systematic) long-term change. Smaller populations are vulnerable to demographic and environmental stochasticity and the decline in fitness

associated with genetic drift and inbreeding (Frankel and Soulé 1981). Hawkes (1983) reported that smaller area in traditional crops reduces diversity.

The frequency distribution of the sizes of individual populations is likely to reflect the way in which genetic variation is partitioned within and among populations, with small populations being at increased risk of loss of alleles, reduced heterozygosity, increased uniformity, enhanced inbreeding or possible extinction. Van Treuren et al. (1990) reported that in some cases the loss of particular crop varieties is not complete, but instead reduces surviving members of a landrace to a few isolated populations. In such cases there is significant risk of the ultimate loss of diversity, because small populations will lead to increased inbreeding principally in cross breeders which reduces the fitness of individual plants and hence may lead to extinction. One of the factors resulting in the loss of genetic variability is reduction in population size through the decline of plant number, so called bottleneck effect (Leberg 1992). Allozyme genetic diversity, inversions and visible mutations all declined more rapidly in smaller than large populations (Montgomery et al. 2000). Most probably, this resulted in a bottleneck effect over this population and consequently genetic diversity might have diminished, as revealed in allozyme variability.

Genetic erosion in farming systems of centers of crop diversity rested on two conjectures. First, it was believed that modern varieties would diffuse throughout in these systems. Second, it was thought that the adoption of modern varieties would lead farmers to stop planting of landraces (Brush and Meng 1998). Landraces replacement particularly in Hetosa and Tiyo were in agreement with these hypotheses. Much of the evidence for genetic erosion presented in the 1970/71 FAO survey (Frankel 1973) is data on the diffusion of modern cultivars rather than on the loss of local material (Kjellqvist 1973). Adoption of high yielding improved bread wheat varieties is the cause for the displacement of tetraploid wheat landraces. Unless tetraploid wheat production is increased either vertically or horizontally, it is likely that shortages will occur as farmers adopt the high yielding bread wheat varieties (Bechere et al. 2000). Heisey and Brennan (1991) reported that most of the studies have come to a conclusion that yield (or yield

potential) is the most important criterion for the choice of a variety by a farmer. As a result of a gradual decline in the amount and distribution of rainfall farmers are forced to shift from wheat to crops like tef and sorghum, which are relatively more drought tolerant. According to Erskine and Muehlbauer (1990), droughts of just a single season could result in people consuming seed stocks, while successive years of drought can prompt changes in cropping patterns and the geographic distribution of crops. These changes in cropping patterns may also include the use of alternative, more drought resistant crops in preference to the traditional landraces. The study of Stephen et al. (2002) showed a marked reduction in rice diversity in the northeastern Philippines from 1996 to 1998 as a result of drought due to the El Niño phenomenon in 1997 and flooding due to two successive typhoons in 1998. Moreover, because of decline in soil fertility the productivity of tetraploid wheat landraces became low and consequently farmers were forced to grow varieties or crops that perform well with fertilizers unlike landraces that lodge when grown with fertilizers. Indigenous crops are adapted to the conditions of less developed agriculture. As these conditions change with improved traction and fertilizer, the existing adaptation of landraces turns from asset to liability (Harlan 1975).

Traditional high-yielding cultivars adapted to optimal local agronomic conditions are probably the ones that are most at risk of future loss from traditional societies through habitat destruction or by replacement by introduced elite germplasm (Brush 1995). Tunstall et al. (2001) described that landraces, which are grown because of their high resistance to pests during seed storage, may become less important if improved storage systems are introduced. Pesticide introduction could therefore erode landraces and farmers' knowledge of landrace pest resistance.

Farmers are interested to reintroduce more emmer wheat cultivation if they obtain high yielding and easily threshable varieties. However, little attention was paid to fulfill farmers' request. Most of the national and regional research institutions that are researching on wheat emphasize on hexaploid wheat. Tetraploid wheat like *T. polonicum*, *T. dicoccon* and *T. turgidum* are not in wheat research priority lists of most research centers. The promotion of bread wheat over tetraploid wheats is greater. The pro-

improved varieties extension advice and credit facilities have motivated farmers to grow improved varieties so as to benefit from the associated credits. Tunstall et al. (2001) pointed out that the modern world is placing a range of pressures on wild areas and on traditional agricultural communities, and external interests (often dominated by economic or political issues) strongly impinge. The major external forces advocate the introduction of high-yield varieties, accompanied by mechanization and major chemical inputs, as the means to increase total production and economic return. These forces change the nature of the decision-making process dramatically; the farmer is encouraged to grow high-yield varieties in monoculture using inputs of fertilizer and pesticides. Similarly, Louette et al. (1997) reported the fact that farmers were given several socio-economic incentives to replace varieties that evolved within their agro-ecosystem with improved/introduced varieties in many regions of the world.

Due to its large demand by the ministry, wheat, particularly bread wheat, ranks first in the priority settings of the Ethiopian seed industry agency seed multiplication and distribution scheme. Of the various crops for which improved seed was multiplied and sold by the agency, wheat remained the first since the last three decades (Eshetu 2002). It was also found the local seed supply systems are vital enough to enable smallholder farmers to access improved seeds once after it was distributed by research centers and seed agencies. In Mexico, Louette et al. (1997) concluded that seed flow is high enough to mean that no farmer is planting seed stock bequeathed from parents. In the *ex situ* gene bank, the problem of insufficient passport data was observed. This affects not only the utilization of accessions but also results in difficulties in planning where, when and which seed stock to rejuvenate and increase. It also affects the planning of effective collecting trips. The stock of seeds of some accessions in the gene bank has been exhausted as a result of frequent requests but not having been replenished appropriately. Therefore, a strong differentiation should be made between the basic collections and the active collections so as to keep enough seeds. Because of financial reasons collections were made with low frequencies. This exposes the surviving tetraploid wheat landraces, which are under greater risk of replacement by improved bread wheat varieties or other crops, to

further extinction particularly in areas where modern agriculture is expanding. As a result of financial problems, lack of staff and shortage of farms, rejuvenation of seeds were not being done as per scheduled. The long-term storage strongly reduces the metabolism and therefore highly limits viability and seed vigor. Considerable evidence indicated that damage to chromosomes, some of it resulting in heritable changes, takes place as seeds loose their viability. Studies in barley and wheat showed that as storage age increases, chromosome aberrations (per cell) increase (Gunhardt et al. 1953). Changes in the properties of DNA associated with loss of viability in rye seeds, namely the loss of DNA template activity (Holden and Williams 1984) and decreases in the molecular size of extractable DNA (Cheah and Osborne 1978), also have been observed. Therefore, priority should be placed on securing and providing financial support to strengthen the inner structure of IBC that guarantees the necessary gene bank functions such as appropriate collection, maintenance, characterization and documentation of plant genetic resources. In this study, one of the main challenges faced while dealing with genetic erosion is lack of reliable time series data, in the different district bureaus of MoA and research centers, about the number and areas of improved varieties being disseminated and adopted, which is very useful to analyze processes that took place over time. The absence of data on effective crop population area covered by tetraploid wheats both at regional and national level in the Central Statistical Authority was also the major problem to examine the trend in area and population size of these wheats. The authority yearly reports a summed figure for all wheats grown in each administrative regions of the country. Therefore, this problem should be solved to effectively monitor the periodic changes in tetraploid wheats genetic resources.

Although all parties in Ethiopia agreed that conservation of biodiversity is important, the predominant opinion among researchers and policymakers was that widespread adoption of green revolution technologies was absolutely necessary to ensure food security. However, introducing uniform cultivars with narrow genetic base into Ethiopia's wide range of microclimates, which can differ sharply from one village to the next, makes agricultural productivity extremely vulnerable to yield-limiting factors. Therefore, national agricultural policies should pay more attention to agricultural stability. A

mechanism to link the different stakeholders should be devised. National food security strategies and policies on conservation should be harmonized. Policy should direct national, research and agricultural academic institutions to give priority to collaborative research on conservation issues. Mburu and Edilegnaw (2003) noted that effective coordination among the different sectors would eliminate the difficulties faced by extension agents trying to pass mixed messages to the farmers and hence enhance their participation in conservation initiatives. NGOs are being contributing a lot to strengthen on farm conservation of landraces and thus special attention should be paid to guarantee the continuity of these activities particularly after the NGOs left.

References

Ahmed, H.M., Khan B.M., Khan S., Kissana N.S. and Laghari S. 2003. Path coefficient analysis in bread wheat. Asian Journal of Plant Sciences 2 (6): 491-494.

Ahn W.S., Kang H.J. and Yoon M.S. 1996. Genetic erosion of crop plants in Korea. In: Y.G. Park and S. Sakamoto (eds), Biodiversity and Conservation of Plant Genetic Resources in South Asia, pp 41-55. Jpn. Sci. Soc. Press, Tokyo.

Akimoto M., Shimamoto Y. and Morishima H. 1999. The extinction of genetic resources of Asian wild rice, *Oryza rufipogon* Griff.: A case study in Thailand. Genet. Resour. Crop Evol. 46: 419–425.

Akio O. L., Carlos F.L., Neto B. and Fernandes F. 2004. Correlation and path analysis of yield and its components and plant traits in wheat. Ciência Rural 34: 1701-1708.

Akkaya M.S., Bhagwat A.A. and Cregan P.B. 1992. Length polymorphisms of simple sequence repeat DNA in soybean. Genetics 132: 1131–1139.

Alamerew S., Chebotar S., Huang X., Röder M. and Börner A. 2004. Genetic diversity in Ethiopian hexaploid and tetraploid wheat germplasm assessed by microsatellite markers. Genet. Resour. Crop Evol. 51: 559–567.

Allard, R.W. 1999. Principles of Plant Breeding, 2nd edn. New York, Wiley.

Amri A., Hatchett J.H., Cox T.S., Bouhassini M. E. and Sears R.G. 1990. Resistance to Hessian fly from North African durum wheat germplasm. Crop Sci 30: 378-381.

Amsal T. 1994. Yield potential of rainfed wheat in the central highlands of Ethiopia. M.Sc. Thesis. Alemeya, Ethiopia.

Anderson J.A., Churchill G.A., Autrique J.E., Sorrells M.E. and Tanksley S.D. 1993. Optimizing parental selection for genetic linkage maps. Genome 36: 181–186.

Araujo, M.R.A. DE and Coulman B. 2004. Genetic variation and correlation of agronomic traits in meadow bromegrass (*Bromus riparius* Rehm) clones. Ciência Rural 34: 505-510.

Ashraf M., Ghafoor A. Khan N.A. and Yousaf M. 2002. Path coefficient in wheat under rain fed conditions. Pakistan Journal Agricultural Research 17: 1-6.

Asins M.J. and Carbonnel E.A. 1989. Distribution of genetic variability in a durum wheat world collection. Theor Appl Genet. 77: 79–86.

Auricchio S., De Ritis G., De Vincenzi M., Occorsio P. and Silano V. 1982. Effects of gliadin-derived peptides from bread and durum wheat on small-intestine culture from rat fetus and celiac children. Pediatr Res. 16: 1004–1010.

Autrique, E, Nachit M.M., Monneveux P., Tanksley S.D. and Sorrells M.E. 1996. Genetic diversity in durum wheat based on RFLPs, morphophysiological traits, and coefficient of parentage. Crop Sci 36: 735–742.

Ayana A. and Bekele E. 1998. Geographical patterns of morphological variation in sorghum (*Sorghum bicolor* (L.) Moench) germplasm from Ethiopia and Eritrea: qualitative characters. Hereditas 129 (3): 195-205.

Ayana A. and Bekele E. 1999. Multivariate analysis of morphological variation in sorghum [*Sorghum bicolor* (L.) Moench] germplasm from Ethiopia and Eritrea. Genet. Resour. Crop Evol. 46: 273–284.

Ayele M., Tefera H., Assefa K., and Nguyen H.T. 1999. Genetic characterization of two *Eragrostis* species using AFLP and morphological traits. Hereditas 130: 33–40.

Baker R.J. 1977. Inheritance of kernel hardness in spring wheat. Crop Sci 17: 60-962.

Barcaccia G., Molinari L., Porfiri O. and Veronesi F. 2002. Molecular characterization of emmer (*Triticum dicoccon* Schrank) Italian landraces. Genet. Resour. Crop Evol. 49: 415–426.

Bar-hen A., Charcosset A., Bourgoin M, and Guiard J. 1995. Relationship between genetic markers and morphological traits in a maize inbreed lines collection. Euphytica 84: 145-154.

Baur E. 1914. Die Bedeutung der primitiven Kulturrassen und der wilden Verwandten unserer Kulturpflanzen für die Pflanzenzüchtung. Jahrbuch DLG (Saatzuchtabteilung), pp. 104-109.

Baye T. 2003. Exploration, Genetic Diversity and Seed Quality Analysis in Ethiopian Populations of *Vernonia galamensis*. PhD Dissertation, George August University of Goettingen, Germany.

Baye T. and Becker H.C. 2005. Genetic variability and interrelationship of traits in the industrial oil crop *Vernonia galamensis.* Euphytica 142: 119–129.

Bechere E, Belay G., Mitiku D. and Merker A. 1996. Phenotypic diversity of tetraploid wheat landraces from Northern and North-Central regions of Ethiopia. Hereditas 124: 165-172.

Bechere E., Kebede H. and Belay G. 2000. Durum wheat in Ethiopia. An old crop in an ancient land. IBCR, Addis Ababa, Ethiopia.

Bekele E. 1984. Analysis of regional patterns of phenotypic diversity in the Ethiopian tetraploid and hexaploid wheats. Hereditas 124: 165-172.

Bekele H.K., Verkuijl H., Mwangi W. and Tanner D. 2000. Adoption of Improved Wheat Technologies in Adaba and Dodola Woredas of the Bale Highlands, Ethiopia. Mexico, D.F.: International Maize and Wheat Improvement Center (CIMMYT) and Ethiopian Agricultural Research Organization (EARO).

Belay G., Tesemma T., Becker H.C. and Merker A. 1993. Variation and interrelationships of agronomic traits in Ethiopian tetraploid wheat landraces. Euphytica 71: 181–188.

Belay G., Tesemma T., Mitikua D. and Badebo A. 1992. Variability in durum wheat genotypes for leaf and head infections of yellow rust. In: D.G. Tanner and W. Mwangi, (Eds.), Proceedings of the 7th Regional Wheat Workshop for Eastern, Central and Southern Africa, Sept. 16-19 1991. Nakuru, Kenya. Pp 135-141.

Belay, G. Bechere E., Mitiku D., Merker A. and Tsegaye S. 1996. Patterns of morphological diversity in tetraploid wheat (*Triticum turgidum* L.) landraces from Ethiopia. Acta Agriculturae Scandinavicae, Section B., Soil and Plant Sci 47: 221-228.

Bell C.J. and Ecker J.R. 1994. Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis.* Genomics 19: 137–144.

Ben Amer I. M., Börner A. and Röder M. S. 2001. Detection of genetic diversity in Libyan wheat genotypes using wheat microsatellite markers. Genet. Resour. Crop Evol. 48: 579–585.

Bennett E. 1968. Record of the FAO/IBP Technical Conference on the Exploration, Utilization and Conservation of Plant Genetic Resources. FAO, Rome.

Bennett E. 1970. Adaptation in wild and cultivated plant populations. In: Frankel O. H. and E. Bennett (eds.), Genetic Resources in Plants - Their Exploration and Conservation. Oxford: International Biological Programme Handbook No 11. Blackwell Scientific Publications. Pp. 115-129.

Bennett M.D. and Smith J.B. 1976. Nuclear DNA amounts in angiosperms. Phil Trans Roy Soc Lond. B. 274: 227–274.

Bertin P., Gregoire D., Massart S. and de Froidmont D. 2001. Genetic diversity among European cultivated spelt revealed by microsatellites. Theor Appl Genet. 102: 148–156.

Bhatt G.M. 1973. Significance of path coefficient analysis in determining the nature of character association. Euphytica 2: 338-343.

Bohn M., Friedrich H. and Melchinger A.E. 1999. Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs and SSRs and their use for predicting progeny variance. Crop Sci 39: 228–237.

Bretting P.K. and Widrlechner M.P. 1995. Genetic markers and plant genetic resources. Plant Breeding Reviews 13: 11-86.

Brown A., Young A., Burdon J., Christides L., Clarke G., Coates D. and Sherwin, W. 1997. Genetic indicators for state of the environment reporting. Australia: State of the Environment Technical Paper Series (Environmental Indicators), Department of Environment, Sport and Territories, Canberra.

Brush S.B. 1995. *In situ* conservation of landraces in centers of crop diversity. Crop Sci 35: 346–354.

Brush S.B. and Meng E. 1998. Farmers' valuation and conservation of crop genetic resources. Genet. Resour. Crop Evol 45: 139–150.

Bryan G.J., Collins A.J., Stephenson P., Orry A., and Smith J.B. 1997 Isolation and characterisation of microsatellites from hexaploid bread wheat. Theor Appl Genet. 94: 557–563.

Burstin J. and Charcosset A. 1997. Relationships between phenotypic and marker distance: theoretical and experimental investigations. Heredity 78: 477–483.

Burton G.W. and De Vane, E.H. 1953. Estimating heredity in tall fescue (*Festuca arundinacea*) from replicated clonal material. Agronomy J 45: 478–481

Calhoun D.S., Gebeyehu G., Miranda A., Rajaram S. and van Ginkel M. 1994. Choosing evaluation environments to increase wheat grain yield under drought conditions. Crop Sci 34: 673–678.

Cavalli-Sforza L.L., Menozzi P. and Piazza P. 1994. History and Geography of Human Genes. Princeton University Press, Princeton, NY.

Ceccarelli S. 1996. Positive interpretation of genotype by environment interactions in relation to sustainability and biodiversity. In: M. Cooper and G.L. Hammer (Eds.). Plant adaptation and crop improvement, pp. 467–486. Oxon, UK: CAB International.

Ceccarelli S. 1997. Adaptation to low/high input cultivation. In: P.M.A. Tigerstedt (ed.), Adaptation in plant breeding, pp 225-236.

Chauhan J.S. and Nanda J.S. 1983. Inheritance of amylose content and its association with grain yield and yield contributing characters in rice. Oryza 20: 81-85.

Cheah K.S.E. and Osborne D.J. 1978. DNA lesions occur with loss of viability in embryos of ageing rye seed. Nature 272: 593-599.

Choudhry A.R., Shah A.H., Ali L. and Bashir M. 1986. Path coefficient analysis of yield and yield components in wheat. Pakistan Journal Agricultural Researc*h* 7(2): 71-75.

Chowdhury M.A. and Slinkard A.E. 2000. Genetic diversity in grasspea (*Lathyrus sativus* L.) Genet. Resour. Crop Evol. 47: 163-169.

Condit R. and Hubbell S. 1991. Abundance and DNA sequence of two-base repeat regions in tropical tree genomes. Genome 34: 66–71.

Corazza L., Pasquini M. and Perrino P. 1986. Resistance to rusts and powdery mildew in some strains of *Triticum monococcum* L. and *Triticum dicoccum* Schubler cultivated in Italy. Genet Agrar. 40: 243–254.

Cromwell E. and Almekinders C. 2000. The impact of seed supply interventions on the use of crop genetic diversity. In W. de Boef and C. Almekinders (eds.), Encouraging Diversity: The Conservation and Development of Plant Genetic Resources. London: Intermediate Technology Publications. pp. 223–227.

Crouch, H.K., Crouch J.H., Madsen S., Vuylsteke, D.R. and Ortiz, R. 2000. Comparative analysis of phenotypic and genotypic diversity among plantain landraces (*Musa* spp., AAB group). Theor Appl Genet. 101: 1056–1065.

Cuadrado A. and T. Schwarzacher. 1998. The chromosomal organization of simple sequence repeats in wheat and rye genomes. Chromosoma 107: 587–594.

Cui Z., Carter T.E., Burton J.W. and Wells R. 2001. Phenotypic diversity of modern Chinese and North American soybean cultivars. Crop Sci 41: 1954-1967

D'Antuono LF. 1989. Il farro; areali di coltivaziones, caratteristiche agronomiche, utilizzazione e prospettive colturali. L'Informatore Agrario 45: 49–57.

Dai X. and Q. Zhang. 1989. Genetic diversity of six isozyme loci in cultivated barley of Tibet. Theor Appl Genet 78: 281–286.

Damania A.B., Hakim S. and Moualla M.Y. 1992. Evaluation of variation in *Triticum dicoccum* for wheat improvement in stress environments. Hereditas 116: 163–166.

Damania, A.B., Pecetti L. Qualset C.O. and Humeid B.O. 1996. Diversity and geographic distribution of adaptive traits in *Triticum turgidum* L. (durum group) wheat landraces from Turkey. Genet. Resour. Crop Evol. 43: 409–422.

de Langhe E. 1961. La taxonomie du bananier plantain en Afrique Equatoriale. J Agric Trop Bot Appl 8: 419–449.

del Rio A. H. and Bamberg J.B. 2002. Lack of association between genetic and geographic origin characteristics for the wild potato *Solanum sucrense* Hawkes. Am J Potato Res. 79: 335-338.

del Rio A.H., Bamberg J.B., Huaman Z., Salas A. and Vega S.E. 2001. Association of ecogeographical variables and RAPD marker variation in wild potato populations of the USA. Crop Sci 41: 870–878.

Demissie A. and Bjørnstad A. 1997. Geographical, altitude and agro-ecological differentiation of isozyme and hordein genotypes of landrace barleys from Ethiopia: implications to germplasm conservation. Genet. Resour. Crop Evol. 44: 43–55.

Devos K.M., Bryan G.J., Collins A.J. and Gale M.D. 1995. Application of two microsatellite sequences in wheat storage proteins as molecular markers. Theor Appl Genet. 90: 247–252.

Dewey D.R. and Lu K.H. 1959. A correlation and path coefficient analysis of components of crested wheat grass seed production. Agronomy J 51: 515-518.

Dice L.R. 1945. Measures of the amount of ecologic association between species. Ecology 26: 297–302.

Diederichsen A. and Hammer K. 2003. The infraspecific taxa of coriander (*Coriandrum sativum* L.). Genetic Resour Crop Evol 50 (1): 33-63.

Dillmann C., Bar-Hen A., Guérin D., Charcosset A. and Murigneux, A. 1997. Comparison of RFLP and morphological distances between maize *Zea mays* L. inbred lines. Consequences for germplasm protection purposes. Theor Appl Genet. 95: 92–102.

Doebley J.F., Goodman M.M. and Stueber C.W. 1987. Patterns of isozyme variation between maize and Mexican annual teosinte. - Econ. Bot. **41**, 234-246.

Donini P., Stephenson P., Bryan G.J. and Koebner R.M.D. 1998. The potential of microsatellites for high throughput genetic diversity assessment in wheat and barley. Genet. Resour. Crop Evol. 45: 415–421.

Dorofeev V.F., Filatenko A.A., Migušova E.F., Udaĉin R.A. and Jakubciner M.M. 1979. Pšenica. Kul'turnaja Flora SSSR, vol. 1, Leningrad, Russia, 347 pp.

Dudnikov A.J.U. 1998. Allozyme variation in Transcaucasian populations of *Aegilops squarrosa*. Heredity 80: 248–258.

Duvick D. N. 1984. Genetic diversity in major farm crops on the farm and in reserve. Economic Botany 38: 161–178.

Dvorak J. 1998. Genome analysis in the *Triticum-Aegilops* alliance. In: A.E. Slinkard, (ed.). *Proc*. 9th Int. Wheat Genetics Symp., vol. 1, p. 8-11. University of Saskatchewan, Canada, University Extension Press.

Dvorak J. and Zhang H.B. 1990. Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. Proc Natl Acad Sci 87: 9640-9644.

Dvorak J., Luo M.C., Yang Z.L. and Zhang H.B. 1998. The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat. Theor Appl Genet. 97: 657–670.

Dvorak J., McGuire P.E. and Cassidy B. 1988. Apparent sources of the A genomes of wheat inferred from polymorphism in abundance and restriction fragment length of repeated nucleotide sequences. Genome 30: 680-689.

Dvorak, J., di Terlizzi P., Zhang H.B. and Resta P. 1993. The evolution of polyploid wheats: identification of the A genome donor species. Genome 36: 21-31.

EARO. 2000. National Wheat Research Strategy. Kulumsa Research Center, Kulumsa.

Edwards S. 1991. Crops with wild relatives found in Ethiopia. In: Engels, J.M.M., Hawkes, J.G. and Melaku Worede (eds.), Plant Genetic Resources of Ethiopia, Cambridge University Press, Cambridge.

Ehdaie B. and Waines J.G. 1989. Genetic variation, heritability, and path-analysis in landraces of bread wheat from southwestern Iran. Euphytica 41: 183–190.

Elings A. 1991. Durum wheat landraces from Syria. II. Patterns of variation. Euphytica 54: 231–243.

Engelborghs I., Swennen R. and van Campenhout S. 1999. The potential of AFLP to detect genetic differences and somaclonal variants in *Musa* spp. InfoMusa 7: 3–5.

Engels, J. M. M. 1994. Genetic diversity in Ethiopian barley in relation to altitude. Genet. Resour. Crop Evol. 41: 67–73.

Erskine W. and Muehlbauer F. J. 1990. Effects of climatic variations on crop genetic resources and plant breeding aims in West Asia and North Africa. In: Jackson M. T., Ford-Lloyd B. V. and Parry M. L. (eds.), Climatic Change and Plant Genetic Resources, London, Belhaven Press.

Eshetu M. 2002. Seed systems and small-scale farmers: a case study of Ethopia and South Africa, PhD Thesis, University of the Free States, South Africa.

Eticha F, Bekele E., Belay G. and Börner A. 2005. Phenotypic diversity in tetraploid wheats collected from Bale and Wello regions of Ethiopia. Plant Genetic Resources 3: 35-43.

Eujayl I., Sorrells M., Baum M., Wolters P. and Powell W. 2001. Assessment of genotypic variation among cultivated durum wheat based on EST-SSRS and genomic SSRS. Euphytica 119: 39–43.

Evans L.T. 1998. Feeding the Ten Billion: Plants and Population Growth. Cambridge, UK: Cambridge University Press.

Fahima T., Röder M.S., Grama A. and Nevo E. 1998. Microsatellite DNA polymorphism divergence in *Triticum dicoccoides* accessions highly resistant to yellow rust. Theor Appl Genet 96: 187–195.

Fahima T., Röder M.S., Wendehake K., Kirzhner V.M. and Nevo E. 2002. Microsatellite polymorphism in natural populations of wild emmer wheat, *Triticum dicoccoides*, in Israel. Theor Appl Genet 104: 17–29.

Fahima T., Sun G.L., Beharav A., Krugman T., Beiles A. and Nevo E. 1999. RAPD polymorphism of wild emmer wheat populations, *Triticum dicoccoides*, in Israel. Theor Appl Genet. 98: 434–447.

Falconer D.S. 1989. Introduction to Quantitative Genetics. 3rd edn., Longman, London.

FAO 2005 Special Report: FAO/WFP crop and food supply assessment mission to Ethiopia. Rome: FAO, 28 January 2005.

FAO 1996a. First state of the world's plant genetic resources: erosion of biodiversity and loss of genes continues; many gene banks threatened. Rome, Italy.

FAO. 1996b. Global Plan of Action for the Conservation and Sustainable Utilisation of Plant Genetic Resources for Food and Agriculture. FAO, Rome.

FAO. 1997. State of the World's Plant Genetic Resources for Food and Agriculture. Food and Agriculture Organisation of the United Nations, Rome.

Farnsworth N.R. and Soejarto D.D. 1985. Potential consequence of plant extinction in the United States on the current and future availability of prescription drugs. Econ Bot. 39: 231-240.

Federer WT and Scully BT (1993) A parsimonious statistical design and breeding procedure for evaluating and selecting desirable characteristics over environments. Theor Appl Genet. 86: 612–620.

Fehr W.R. 1987. Principles of Cultivar Development, Vol. I., MacMillan, New York.

Feldman M. 1979. Wheats (Triticum spp.). In: Simmonds N.W (ed.), Evolution of Crop Plants. Longman Group Limited, London.

Ferguson, M.E., B.V. Ford-Lloyd, L.D. Robertson, N. Maxted, and H.J. Newbury 1998: Mapping the geographical distribution of genetic variation in the genus *Lens* for the enhanced conservation of plant genetic diversity. Mol Ecol 7: 1743–1755.

Figliuolo G.and Perrino P. 2004. Genetic diversity and intra-specific phylogeny of *Triticum turgidum* L. subsp. *dicoccon* (Schrank) Thell. revealed by RFLPs and SSRs. Genet. Resour. Crop Evol. 51: 519-527.

Filatenko A.A., Grau M., Knüpffer H. and Hammer K. 2001. Wheat Classification – John Percival's contribution and the approach of the Russian School. The Linnean Society of London, special issue no. 3: 165–184.

Flittner M. 1995. Sammler, Räuber und Gelehrte. Die politischen Interessen an pflanzengenetischen Ressourcen 1895-1995. Campus, Frankfurt, New York.

Forster B.P., Ellis R.P., Thomas W.T.B., Newton A.C., Tuberosa R, T.D., El-Enein R.A., Bahri M.H., Ben Salem M. 2000. The development and application of molecular markers for abiotic stress tolerance in barley. Journal Experimental Biology 51: 19–27.

Fowler C. and Mooney P. 1990. Shattering: Food Politics, and the Loss of Genetic Diversity. University of Arizona Press, Tucson, AZ.

Franco, J., Crossa, J., Ribaut, J.M., Betran, J., Warburton, M.L. and Khairallah, M. 2001. A method for combining molecular markers and phenotypic attributes for classifying plant genotypes. Theor Appl Genet 103: 944–952.

Frankel O. H. 1973. Survey of Crop Genetic Resources in Their Centers of Diversity, First Report. Rome: Food and Agricultural Organization of the U. N.

Frankel O.H. and Bennett E. 1970. Genetic Resources in Plants Their Exploration and Conservation. Blackwell, Oxford.

Frankel O.H. and Soulé M.E. 1981. Conservation and Evolution. Cambridge University Press, Cambridge.

Frankel O.H., Brown A.H.D. and Burdon J.J. 1995. The Conservation of Plant Biodiversity. Cambridge Univ. Press, Cambridge.

Frison E. and Hammer K. 1992. Report, joint FAO/IBPGR mission to survey plant genetic resources programmes in eastern and central Europe. FAO, IBPGR, Rome, pp. 61.

Fulton T.M., Chunwongse J. and Tanksley D. 1995. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Mol Biol Rep 13: 207–209.

Gao L. Z. 2003. The conservation of Chinese rice biodiversity: genetic erosion, ethnobotany and prospects. Genet. Resour. Crop Evol. 50: 17–32.

Gao L.Z., Ge S. and Hong D.Y. 2000. A preliminary study on ecological differentiation within common wild rice *Oryza rufipogon* Griff. Acta Agronomica Sinica 26 (in Chinese with English abstract): 210–216.

Gao L.Z., Zhang S.Z., Zhou Y., Ge S. and Hong D.Y. 1996. A survey of current status of wild rice in China. Chinese Biodiversity 4 (in Chinese with English abstract): 162–166.

Ghaderi A., Adams M.W. and Nassib A.M. 1984. Relationship between distance and heterosis for yield and morphological traits in dry edible bean and faba bean. Crop Sci 24: 37-42.

Gökgöl M. 1955. Bugdaylarin Tansif Anahtari. Ziraat Vekaleti Yayin. No. 76, Istambul; Turkey.

Gomez K.A. and Gomez A.A. 1984. Statistical Procedures for Agricultural Research. 2nd ed. International Rice Research Institute (IRRI). John Wiley and Sons, Inc.

Gomez O.J. Blair M.W., Frankow B.E. and Gullberg U. 2004. Molecular and phenotypic diversity of common bean landraces from Nicaragua. Crop Sci 44: 1412-1418.

Grafius JE, Thomas RL and Bernard J. 1976. Effect of parental component complementation on yield and components of yield in barley. Crop Sci 16: 673–677.

Grafius, JE 1978. Multiple characters and correlated response. Crop Sci 18: 931–934.

Grenier C., Bramel P.J., Dahlberg J.A., El-Ahmadi A., Mahmoud M., Peterso G.C., Rosenow D.T. and Ejeta G. 2004. Sorghums of the Sudan: analysis of regional diversity and distribution. Genet. Resour. Crop Evol. 51: 489 – 500.

Guarino, L. 1999. Approaches to measuring genetic erosion. In: Serwinski, J. and Faberová I. (eds.), Proceedings of the Technical Meeting on the Methodology of the FAO World Information and Early Warning System on Plant Genetic Resources, Research Institute of Crop Production, Prague, Czech Republic 21 - 23 June 1999. pp. 26-28.

Gunhardt H., Smith L., Haferkamp M.E. and Nilan R.A. 1953. Studies on aged seeds. II. Relation of age of seeds to cytogenic effects. Agron J. 45: 438-441.

Gupta P.K. and Varshney R.K. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113: 163–185.

Gupta P.K., Balyan H.S., Sharma P.C. and Ramesh B. 1996. Microsatellites in plants: a new class of molecular markers. Curr Sci 70: 45–54.

Gupta, A.K., Mittal, R.K. and Ziauddin A. 1999. Association and factor analysis in spring wheat. Annals of Agricultural Research 20: 481-485.

Hailu G.M. 1991. Wheat production and research in Ethiopia. In: Hailu Gebremariam, D.G. Tanner, and M. Hulluka (eds.). Wheat Research in Ethiopia: A Historical Perspective. Addis Ababa, Ethiopia: IAR/CIMMYT. Pp. 1-16.

Hammer K. 2004. Resolving the challenge posed by agrobiodiversity and plant genetic resources – an attempt. J of Agr and Rural Dev in the Tropics and Subtropics, Beiheft 76, 184 pp.

Hammer K. and Perrino P. 1984. Further information on farro (*Triticum monococcum* L. and *Triticum dicoccon* Schrank) in South Italy. Kulturpflanze 32: 143–151.

Hammer K., A.A. Filatenko, S. Al-Khanjari, A.Y. Al-Maskri and A. Buerkert. 2004. Emmer (*Triticum dicoccon* Schrank) in Oman. Genet. Resour. Crop Evol. 51: 111-113.

Hammer K., Arrowsmith N. and Gladis T. 2003. Agrobiodiversity with emphasis on plant genetic resources. Naturwissenschaften 90: 241-250.

Hammer K., Diederichsen A. and Spahillari M. 1999. Basic studies toward strategies for conservation of plant genetic resources. In: Serwinski, J. and Faberová I. (eds.), Proceedings of the Technical Meeting on the Methodology of the FAO World

Information and Early Warning System on Plant Genetic Resources, Research Institute of Crop Production, Prague, Czech Republic, 21 - 23 June 1999. pp. 29-33.

Hammer K., Filatenko A.A. and Korzun V. 2000. Microsatellite markers: a new tool for distinguishing diploid wheat species. Genet. Resour. Crop Evol. 47: 497–505.

Hammer K., Knüpffer H., Xhuveli L. and Perrino P. 1996. Estimating genetic erosion in landraces – two case studies. Genet. Resour. Crop Evol. 43: 329–336.

Hamza S., Hamida W.B., Rebai A. and Harrabi M. 2004. SSR-based genetic diversity assessment among Tunisian winter barley and relationship with morphological traits. Euphytica 135: 107–118.

Hanelt, P. 2001.Mansfeld's Encyclopedia of Agricultural and Horticultural Crops. Springer, Berlin, Germany.

Harlan H.V. and Martini M.L. 1938. The effect of natural selection in a mixture of barley varieties. Journal of Agricultural Research 57: 189-199.

Harlan J.R. 1955. The Great Plains Region (Part 4). *Agric.* Food Chem. 3: 29-31.

Harlan J.R. 1971. Agricultural origins: Centers and noncenters. Science 174: 468-474.

Harlan J.R. 1975. Our vanishing genetic resources. Science 188: 618–621.

Harlan J.R. and D. Zohary. 1966. Distribution of wild wheats and barley. Science 153: 1074-1080.

Harlan, J.R. 1971. Agricultural origins: centers and non centers. Science 174: 468-473.

Harlan, J.R. 1992: Crops and Man, 2nd edition. American Society of Agronomy, Crop Science Society of America. Madison, Wisconsin.

Hawkes J. G. 1983. The Diversity of Crop Plants. Harvard University Press, Cambridge, MA.

Hayward, M.D., and E.L. Breese. 1993. Population structure and variability. In: M.D. Hayward, N.O. Bosemark and I. Romagosa (eds.), Plant Breeding: Principles and Prospects, Chapman and Hall, London, UK. pp. 16–29.

Heisey P.W. and Brennan J. 1991. An analytical model of farmers demand for replacement of seed. American Journal of Agricultural Economy 73: 44-52.

Helbaek, H. 1959. Domestication of food plants in the old world. Science 130: 365-372.

Hill J., Becker H.C. and Tigerstedt P.M.A. 1998. Quantitative and Ecological Aspects of Plant Breeding. Chapman and Hall, London.

Hoisington D., Khairallah M., Reeves T., Ribaut J.M., Skovmand B., Taba S. and Warburton M.L. 1999. Plant genetic resources: What can they contribute toward increased crop productivity? Proc Nat Aca Sci 96: 5937–5943.

Holden J.H.W. and Williams J.T. 1984. Crop Genetic Resources: Conservation and Evaluation. George Allen and Unwin Publishers' Ltd., UK.

Holland, J.B., Nyquist W.E. and Cervantes-Martinez C.T. 2002. Estimation and interpreting heritability for plant breeding, an update. Plant Breeding Reviews 22: 9–112.

Huang X. Q., Börner A., Röder M. S. and Ganal M.W. 2002. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor Appl Genet 105: 699–707.

Huang X.Q, Hsam S.L.K., Zeller F.J., Wenzel G. and Mohler V. 2000. Molecular mapping of the wheat powdery mildew resistance gene *Pm24* and marker validation for molecular breeding. Theor Appl Genet 101: 407–414.

Huang X.Q., Cöster H., Ganal M.W. and Röder M.S. 2003. Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (*Triticum aestivum* L.). Theor Appl Genet 106: 1379-1389.

Huang X.Q., Hsam S.L.K. and Zeller F.J. 1997. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em. Thell.) .4. Gene Pm24 in Chinese landrace Chiyacao. Theor Appl Genet 95: 950–953.

Hundera F. 2004. Improvement trends, phenotypic and molecular diversity among hard red winter wheat cultivars in Nebraska. PhD dissertation, The University of Nebraska, Lincoln, USA.

IBCR. 2002. IBCR Newsletter. October 2003, Addis Ababa, Ethiopia.

Jain K.S., Qualset C.O., Bhatt G.M. and Wu K.K. 1975. Geographical patterns of phenotypic diversity in a world collection of durum wheats. Crop Sci 15: 700-704

Jalletta T. 2004. Participatory evaluation of the performance of some improved bread wheat (*Triticum aestivum*) varieties in the Jijiga plains of eastern Ethiopia. Expl Agric. 40: 89–97.

Jaradat A.A. 1991. Phenotypic divergence for morphological and yield related traits among landrace genotypes of durum wheat from Jordan. Euphytica 52: 155-164.

Johnson H.W., Robinson H.F. and Comstock, R.E. 1956. Estimation of genetic and environmental variability in soybean. Agronomy J. 47: 314-318.

Johnson R.A. and Wichern D.W. 1988 Applied Multivariate Statistical Analysis. Prentice-Hall, Englewood Cliffs, NJ.

Kam-Morgan L.N.W., Gill B.S. and Muthukrishnan S. 1989. DNA restriction fragment length polymorphisms: a strategy for genetic mapping of D genome of wheat. Genome 32: 724 – 732.

Kebebew A, Tefera H. and Merker A. 2002. Variation and inter-relationships of quantitative traits in tef (*Eragrostis tef* (Zucc.) Trotter) germplasm from western and southern Ethiopia. Hereditas 136: 116 – 125.

Kebebew A., Merker A. and H. Tefera. 2003. Multivariate analysis of diversity of tef (*Eragrostis tef* (Zucc.) Trotter) germplasm from western and southern Ethiopia. Hereditas 138: 228–236.

Kent M. and Coker P. 1992. Vegetation description and analysis: a practical approach. Wiley, New York.

Khan H.A., Shaik M. and Mohammad D.S. 1999. Characters association and path coefficient analysis of grain yield and yield components in wheat. Deptt. Genetics and Pl. Br; Agri. College, Rajendranagar, Hyderabad 500030, AP, India. Crop Res; Hisar. 1999, 17, 229-233.

Khan N.I. 1990. Variability and character association in wheat. Journal Agricultural Research 28: 193-200.

Khlestkina, E.K., Röder M.S., Efremova T.T., Börner A. and Shumny V.K. 2004. The genetic diversity of old and modern Siberian varieties of common spring wheat determined by microsatellite markers. Plant Breeding 123: 122-127.

Kihara H. 1919. Ueber cytologische Studien bei einigen Getreidearten. 1. Spezies-Bastarde des Weizens und Weizenroggen-Bastard. Bot. Mag. 32: 17-38.

Kihara H. 1944. Discovery of the DD-analyser, one of the ancestors of *Triticum vulgare* (Japanese). Agric Hort 19: 13-14.

Kjellqvist E. 1973. Near East – Cereals, Turkey, Syria, Iraq, Iran, Pakistan. *In:* Frankel O. H. (ed.), Survey of Crop Genetic Resources in Their Centers of Diversity, First Report. Rome: Food and Agricultural Organization of the U. N. Pp. 9-21.

Knapp E.E. and Rice D.K.J. 1998. Comparison of isozymes and quantitative traits for evaluating patterns of genetic variation in purple needlegrass (*Nassella pulchra*). Conservation Biology 12: 1031–1041.

Koenig R. and Gepts P. 1989 Allozyme diversity in wild *Phaseolus vulgaris*: further evidence for two major centers of genetic diversity. Theor Appl Genet 78: 809-817

Konzak C.F. 1977. Genetic control of the content, amino acid composition, and processing properties of proteins in wheat. Advances in Genetics 19: 407-582.

Kresovich S., Williams J.G.K., McFerson J.R., Routman E.J. and Schaal B.A. 1992. Characterization of genetic identities and relationships of *Brassica oleracea* L. via a random amplified polymorphic DNA assay. Theor Appl Genet 85: 190–196.

Krivchenko V.I., Yamaleyev A.M., Isayev R.F. and Gorbunova V.Y. 1979. Role of wheat seed huskiness in bunt resistance. Mycology and Phytopathology 13 (4): 330-333.

Kubo K., Jitsuyama Y., Iwama K., Hasegawa T. and Watanabe N. 2004. Genotypic difference in root penetration ability by durum wheat (*Triticum turgidum* L. var. *durum*) evaluated by a pot with paraffin-Vaseline disc. Plant and Soil 262: 169-177.

Lagercrantz U., Ellegren H. and Andersson L. 1993. The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Res 21: 1111–1115.

Laghetti G., Piergiovanni A.R., Volpe N. and Perrino P. 1999. Agronomic performance of *Triticum dicoccon* Schrank and *T. spelta* L. accessions under Southern Italian conditions. Agr Med 129: 199-211.

Lakew B., Semeane Y., Alemayehu F., Genre H., Grando S., van Leur J.A.G. and Ceccarelli S. 1997. Exploiting the diversity of barley landraces in Ethiopia. Genet. Resour. Crop Evol. 44: 109–116.

Leberg P. L. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. Evolution 46: 477–494.

Lefebvre V., Goffinet B., Chauvet J.C., Caromel B., Signoret P., Brand R. and Palloix A. 2001. Evaluation of genetic distances between pepper inbred lines for cultivar protection purposes: comparison of AFLP, RAPD and phenotypic data. Theor Appl Genet 102: 741-750.

Lester, R.N. 1989. Evolution under domestication involving disturbance of genic balance. Euphytica 44, 125-132.

Lester R.N. and Daunay M.C. 2002. Diversity of African vegetable *Solanum* species and its implications for a better understanding of plant domestication. In: Knüpffer, H. & Ochsmann, J. (eds.), Rudolf Mansfeld and Plant Genetic Resources: Proceedings of a symposium dedicated to the 100th birthday of Rudolf Mansfeld which was held 8-9 October 2001, Gatersleben, Germany. Pp 13-152.

Lewontin R.C. 1984. Detecting population differences in quantitative characters as opposed to gene frequencies. Am Nat 123: 115–124.

Li C.D., Rossnagel B.G. and Scoles G.J. 2000. The development of oat microsatellite markers and their use in identifying among *Avena* species and oat cultivars. Theor Appl Genet 101: 1259-1268.

Lilienfield F.A. 1951. H. Kihara: genomeanalysis in *Triticum* and *Aegilops*. Concluding review. Cytologia 16: 101-123.

Litt M. and Luty J.A. 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44: 397–401.

Louette D., Charrier A. and Berthaud J. 1997. *In situ* conservation of maize in Mexico: Genetic diversity and maize seed management in a traditional community. Econ Bot 51: 20-38.

Löve A. 1982. Genetic evolution of the wheat grasses. Biol. Zentralbl 101: 199–212.

Lu X.M., Smith C.M., Gill B.S. and Tolmay V. 2001. Microsatellite markers linked to six Russian wheat aphid resistance genes in wheat. Theor Appl Genet 102: 504–510.

Lynch, M. 1996. A quantitative-genetic perspective on conservation issues. *In*: J. C. AVISE and J. L. HAMRICK, eds. Conservation Genetics: Case Histories from Nature. Chapman and Hall, New York., pp. 471–501.

Mac Key J. 1966. Species relationship in *Triticum*. Hereditas Suppl. 2: 237-276.

Mahalanobis P.C., 1936 On the generalized distance in statistics. Proc Natl Inst Sci India 2: 49-55.

Manifesto M.M., Schlatter A.R., Hop H.E., Suarez E.Y. and Dubcovsky J. 2001. Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. Crop Sci 41: 682–690.

Mansfield D.C., Brown A.F., Green D.K., Carothers A.D., and Morris S.W. 1994. Automation of genetic linkage analysis using fluorescent microsatellite markers. Genomics 24: 225–233.

Mantel N.A. 1967. The detection of disease clustering and a generalised regression approach. Cancer Res 27: 209–220.

Martin J.M., Blake T.K. and Hockett E.A. 1991. Diversity among North American spring barley cultivars based on coefficients of parentage. Crop Sci 31: 1131–1137.

Mburu J. and Edilegnaw W. 2003. Local organizations involved conserving crop genetic resources in Ethiopia and Kenya: What role for on farm conservation? A paper presented at CAPRI-IPGRI international workshop on property rights collective action and conservation of genetic resources September 29th to October 2, Rome Italy.

McFadden E.S. and Sears E.R. 1944. The artificial synthesis of *Triticum spelta*. Rec Soc Genet Am 13: 26-27.

McFadden E.S. and Sears E.R. 1946. The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J Hered 37: 81-89, 107-116.

McLachlan G.J. 1992 Discriminant Analysis and Statistical Pattern Recognition. Wiley, New York.

Messele T. 2001. Multidisciplinary approach in estimating genetic diversity of Ethiopian tetraploid wheat (*Triticum turgidum* L.) landraces. PhD thesis, Wageningen University, The Netherlands.

Minitab 2000 MINITAB™ Statistical Software. MINITAB Release 13.33. Minitab Inc.

Moghaddam M., Ehdaie B. and Waines J.G. 1997. Genetic variation and interrelationships of agronomic characters in landraces of bread wheat from southeastern Iran. Euphytica 95: 361–369.

Moghaddam M., Ehdaie B. and Waines J.G. 2000. Genetic diversity in populations of wild diploid wheat *Triticum urartu* Tum. ex Gandil. revealed by isozyme markers. Genet. Resour. Crop Evol. 47: 323–334.

Montgomery M.E., Woodworth L. M., Nurthen R. K., Gilligan D. M., Briscoe D. A. and Frankham R. 2000. Relationships between population size and loss of genetic diversity: comparisons of experimental results with theoretical predictions. Conservation Genetics 1: 33–43.

Moser H. and Lee M. 1994. RFLP variation and genealogical distance, multivariate distance, heterosis, and genetic variance in oats. Theor Appl Genet 87: 947-956.

Mulatu E. 2002. Seed systems and small-scale farmers: a case study of Ethopia and South Africa, PhD Thesis, University of the Free States, South Africa.

Mulatu E. and Belete K. 2001. Participatory varietal selection in lowlands sorghum in Eastern Ethiopia: Impact on adaptation and genetic diversity. Experimental Agriculture 37: 211–229.

Mulugetta M. 1994. An economic analysis of smallholder wheat production and technology adoption in the Southeastern highlands of Ethiopia. Ph.D. Dissertation, Michigan State University, Michigan, USA.

Myers N. 1994. Protected Areas - protected from a Greater What? Biodiversity and Conservation 3: 411-418.

Negassa M. 1986a. Estimates of phenotypic diversity and breeding potential of Ethiopian wheats. Hereditas 104: 41-48.

Negassa M. 1986b. Patterns of diversity of Ethiopian wheats (*Triticum* spp.) and a gene center for quality breeding. Plant Breeding 97: 147-162.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci 70: 3321–3323.

Nei M. and Chesser R.K. 1983. Estimation of fixation and gene diversities. Ann Hum Genet 47: 253–259.

Nei M. and Li W.H. 1979. Mathematical model for studying genetic variation in terms of endonucleases. Proc Natl Acad Sci 76: 5269–5273.

Nesbitt M. and Samuel D. 1995. From staple crop to extinction? The archaeology and history of the hulled wheats. In: Padulosi S., Hammer K. and Heller J. (eds), Hulled Wheats. IPGRI, Rome.

Nevo E. 1988. Genetic resources of wild emmer wheat revisited: genetic evolution, conservation and utilization. In: Miller TE and Koebner RMD (eds), Proc. 7 International Wheat genetics Symposium. Cambridge, UK, pp. 121–126.

Nevo E. 1998. Genetic diversity of wild cereals: regional and local studies and their bearing on conservation *ex situ* and *in situ*. Genet. Resour. Crop Evol. 45: 355–370.

Nevo E. BenShlomo R., Beiles A., Ronin Y., Blum S. and Hillel J. 1996. DNA fingerprinting reveals ecological correlations and genetic parallelism of allozyme and mitochondrial DNA diversities in the actively speciating blind mole rats in Israel. In: S. Roger, S. Holmes and H.A. Lim (Eds), Gene Families: Structure, Function, Genetics and Evolution, pp. 55–70.

Ntundu W.H., Bach I.C., Christiansen J.L. and Andersen S.B. 2004. Analysis of genetic diversity in bambara groundnut [*Vigna subterranea* (L.) Verdc.] landraces using amplified fragment length polymorphism (AFLP) markers. African Journal of Biotechnology 3 (4): 220-225.

Nyquist W.E. 1991. Estimation of heritability and prediction of selection response in plant populations. Critical Reviews Plant Science 10: 235–322.

Oliveira L.O. and Martins E.R. 2002. A quantitative assessment of genetic erosion in ipecac (*Psychotria ipecacuanha*). Genet. Resour. Crop Evol. 49: 607–617.

Ortiz R. 2002. Germplasm enhancement to sustain genetic gains in crop improvement. In: Engels J.M.M., Ramanatha V.R., Brown A.H.D. and Jackson M. (eds), Managing Plant Genetic Diversity. CABI publishing, Wallingford, UK. p 275-290.

Owuor E.D., Fahima T., Beiles A., Korol A. and Nevo E. 1997. Population genetics response to microsite ecological stress in wild barley, *Hordeum spontaneum*. Molec Ecol 6: 1177–1187.

Page R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12: 357–358.

Panse V.G. 1957. Genetics of quantitative characters in relation to plant breeding. Indian Journal Genetics Plant Breeding17: 318-328.

Partap P.S., Dhankhar B.S., Pandita M.L. and Dudi B.S. 1980. Genetic divergence in parents and their hybrids in okra (*Albelmoschus esculentus* (L) Moench. Genetic Agriculture 34: 323-330.

Partap T. 1996. Mountain Agriculture Biodiversity Perspectives and Framework for Management. Discussion Paper, Kathmandu, Nepal: ICIMOD. In: International Symposium of Agriculture Biodiversity in the HK-Himalayan Region: Status and Management Issues in China/ India /Nepal/ Pakistan. June 1996, Chengdu, China.

Paul S., Wachira F.N., Powell W. and Waugh R. 1997. Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L.) 0. Kuntze) revealed by AFLP markers. Theor Appl Genet 94: 255–263.

Pecetti L. and Damania A.B. 1996. Geographic variation in tetraploid wheat (*Triticum turgidum* ssp. *turgidum* convar.*durum*) landraces from two provinces in Ethiopia. Genet. Resour. Crop Evol. 43: 395–407.

Pecetti L., Annicchiarico P. and Damania A.B. 1992. Biodiversity in a germplasm collection of durum wheat. Euphytica 60: 229–238.

Peeters J.P. and Martinelli J.A. 1989. Hierarchical cluster analysis as a tool to manage variation in germplasm collections. Theor Appl Genet 78: 42–48.

Peloquin S.J., Yerk G.L., Werne J.E. and Darmo E. 1989. Potato breeding with haploids and 2n gametes. Genome 31: 1000–1004.

Peña-Chocarro L. 1996. *In situ* conservation of hulled wheat species: the case of Spain. In: Padulosi S., Hammer K. and Heller J. (eds), Hulled Wheats. Promoting the conservation and use of underutilized and neglected crops. Proc. of 1st Int. Workshop on Hulled Wheats, July, 21–22 1995, Castelvecchio Pascoli, Lucca, Italy, pp. 129-146.

Peng J.H., Fahima T., Röder M.S., Li Y.C., Dahan A., Grama A., Ronin Y.I., Korol A.B. and Nevo E. 1999. Microsatellite tagging of the stripe-rust resistance gene *YrH52* derived from wild emmer wheat, *Triticum dicoccoides*, and suggestive negative crossover interference on chromosome 1B. Theor Appl Genet 98: 862–872.

Perera L., Russell J.R., Provan J. and Powell W. 2003. Studying genetic relationships among coconut varieties/populations using microsatellites. Euphytica 132: 121-128.

Perrino P., Laghetti G., D'Antuono L.F., Al Ajlouni M., Kanbertay M., Szabo´ A.T. and Hammer K. 1996. Ecogeographical distribution of hulled wheat species.In: Padulosi S., Hammer K. and Heller J. (eds), Hulled wheats, Proc. Int. Workshop, Castelvecchio Pascoli, Italy, pp. 101–119.

Pflüger L.A., Martín L.M. and Alvarez J.B. 2001. Variation in the HMW and LMW glutenin subunits from Spanish accessions of emmer wheat (*Triticum turgidum* ssp. *dicoccum* Schrank). Theor Appl Genet 102: 767–772.

Phillips S. 1995. Poaceae (Gramineae). In: Hedberg I. and Edwards S. (eds), Flora of Ethiopia and Eritrea, Volume 7, Uppsala, Sweden.

Piergiovanni A.R. and Blanco A. 1999. Variation of HMW glutenin and γ-gliadin subunits in selected accessions of *Triticum dicoccum* (Schrank) and *T. spelta* (L.) Cereal Res Commun 27: 205–211.

Plaschke J., Ganal M.W. and Röder M.S. 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91: 1001–1007.

Porceddu E., Perrino P. and Olita G. 1973. Preliminary information on an Ethiopian wheat germplasm collection mission. In: G.T.Scaracia Mugnozza (ed.), Genetics and Breeding of durum wheat, University of Bari, Italy, pp. 181-200.

Prasad M., Varshney R.K. Roy J.K., Balyan H.S. and Gupta P.K. 2000. The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor Appl Genet 100: 584–592.

Provan J., Russell J.R., Booth A. and Powell W. 1999. Polymorphic chloroplast simple sequence repeat primers for systematic and population studies in the genus Hordeum. Molecular Ecology 8: 505–511.

Qualset, C.O., Damania, A.B., Zanatta, A.C.A. and Brush, S.B. 1997. Locally based crop plant conservation, In: Maxted, N., Ford-Lloyd, B.V. and Hawkes, J.G., (eds.) Plant genetic conservation: The *in situ* approach. Chapman and Hall, London. pp. 160-175.

Rajaram S. 2001. Prospects and promise of wheat breeding in the 21st century. Euphytica 119: 3–15.

Ramakrishnan, A.P., Meyer, S.E., Waters, J. and Stevens M.R. 2004. Correlation between molecular markers and adaptively significant genetic variation in *Bromus tectorum* (Poaceae), an inbreeding annual grass. Am J Bot 91: 797-803.

Rangaswamy R. 1995. A text book of agricultural statistics. Wiley eastern limited, New Delhi, India.

Rao V.R. and Hodgkin T. 2002. Genetic diversity and conservation and utilization of plant genetic resources. Plant Cell, Tissue and Organ Culture 6: 1-19.

Rasmuson M. 2002. The genotype-phenotype link. Hereditas 136: 1–6.

Reed, D. H., and Frankham, R. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55: 1095–1103.

Richards R.A., Rebetzke G.J., Condon A.G. and van Herwaarden A.F. 2001. Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. Crop Sci 42: 111–121.

Ripley B.D. 1996. Pattern Recognition and Neural Networks. Cambridge University Press, Cambridge.

Röder M.S., Korzun V., Wendehake K., Plaschke J., Tixier M.H., Leroy P. and Ganal M.W. 1998. A microsatellite map of wheat. Genetics 149: 2007–2023.

Röder M.S., Wendehake K., Korzun V., Bredemeijer G., Loborie D., Bertranel L., Isaac P., Rendell S., Jackson J., Cooke R.J., Vosman B. and Ganal M.W. 2002. Construction and analysis of a microsatellite-based database of European wheat varieties. Theor Appl Genet 47: 163 – 169.

Rohlf F.J. 1998. NTSYS-pc: numerical taxonomy and multivariate analysis system, vers. 2.0. Applied Biostatistics Inc. New York.

Roldan-Ruiz I., van Eeuwijk F.A., Gilliland T.J., Dubreuil P., Dillmann C., Lallemand J., de Loose M. and Baril C.P. 2001. A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (*Lolium perenne* L.) varieties. Theor Appl Genet 103: 1138–1150.

Rongwen J., Akkaya M.S., A.A. Bhagwat, Lavi U. and Cregan P.B. 1995. The use of microsatellite DNA markers for soybean genotype identification. Theor Appl Genet 90: 43–48.

Rotondi A., Magli M., Ricciolini C. and Baldoni L. 2003. Morphological and molecular analyses for the characterization of a group of Italian olive cultivars. Euphytica 132: 129–137.

Roussel V., Koenig J., Beckert M. and Balfourier F. 2004. Molecular diversity in French bread wheat accessions related to temporal trends and breeding programmes. Theor Appl Genet 108: 920–930.

Sackville Hamilton N.R. and Chorlton K.H. 1997. Regeneration of accessions in seed collections: a decision guide. IPGRI / FAO Handbooks for Genebanks 5. IPGRI, Rome, Italy.

Sakamura T. 1918. Kurze Mitteilung über die Chromosomenzahlen und die Verwandtschaftsverhaltnisse der Triticum-Arten. Bot Mag 32: 151-154.

Scarascia-Mugnozza G.T. and Perrino P. 2002. The history of ex situ conservation and use of plant genetic resources. In: Engels J. M. M., Ramanatha R.V., Brown A. H. D., and Jackson M. T. (eds.), Managing Plant Genetic Diversity. pp 1-22.

Scheiner S.M., Mitchell R.J. and Callahan H.S. 2000. Using analysis to measure natural selection. Journal Evolution Biology 13: 423–433.

Schut J.W., QI X. and Stam P. 1997. Association between relationship measures based on AFLP markers, pedigree data and morphological traits in barley. Theor. Appl. Genet., 95: 1161–1168.

Senior M.L., Murphy, J.P., Goodman M.M. and Stuber, C.W. 1998. Utility of SSRs for determining genetic similarities and relationships in maize using an agarose-gel system. Crop Sci 38: 1088–1098.

Serret M.D., Udupa S.M. and Weigand F. 1997. Assessment of genetic diversity of cultivated chickpea using microsatellite-derived RFLP markers: implications for origin. Plant Breeding 116(6): 573-578.

Setotaw F., Tanner D.G., Verkuijl H. and Takele G. 2001. The adoption of bread wheat production technologies in the Arsi Zone. In: Raupp J. (ed.) Annual Wheat Newsletter, Kansas State University, Manhattan, USA. Vol. 47 p. 44.

Shannon C. E. and Weaver W. 1949. The mathematical theory of communication. Urbana IL: University of Illinois Press.

Sharma H.C., Waines J.C. and Foster K.W. 1981. Variability in primitive and wild wheats for useful genetic characters. Crop Sci 21: 555–559.

Sidwell R.J., Smith E.L., and McNew R.W. 1973. Inheritance and interrelationships of grain yield and selected yield related traits in a hard red winter wheat cross. Crop Sci 16: 650-654.

Singh R.K. and Chaudhry B.D. 1979. Biometrical Methods in Quantitative Genetic Analysis. Kalyani publishers, Ludhiana, New Delhi, India, pp, 303.

Singh S.P. and Ramanujan S. 1981. Genetic divergence and hybrid performance in *Cicer arietinum* L. Indian J. Genet., 41: 268-276.

Skovmand B., Reynolds M.P. and Delacy I.H. 2001. Mining wheat germplasm collections for yield enhancing traits. Euphytica 119: 25-32.

Slafer G.A. and Kernich G.C. 1996. Have changes in yield (1900–1992) been accompanied by a decrease yield stability in Australia cereal production? Australian Journal of Agricultural Research 47: 323–334.

Smale M., Bellon M.R., Jarvis D. and Sthapit B. 2004. Economic concepts for designing policies to conserve crop genetic resources on farms. Genet. Resour. Crop Evol. 51: 121–135.

Smith J.S.C. and Smith O.S. 1989 The description and assessment of distances between inbred lines of maize. II. The utility of morphological, biochemical and genetic descriptors and a scheme for the testing of distinctiveness between inbred lines. Maydica 34: 151-161.

Sneath P.H.A. and Sokal R.R. 1973. Numerical Taxonomy. W. M. Freeman and Company, San Francisco.

Sneller C.H., Nelson R.L., Carter T.E. and Cui Z. 2005. Genetic Diversity in Crop Improvement: The Soybean Experience. Journal of Crop Improvement 14: 103-144.

Solcri D., and Smith S.E. 1995. Morphological and phenological comparisons of two hopi maize varieties conserved *in situ* and *ex situ*. Econ Bot 49: 56-77.

SPSS. 2003. SPSS 12 for Windows and Smart viewer. SPSS Inc., Chicago.

Srivastava J.P. and Damania A.B. 1989. Use of collections for cereal improvement in semi-arid areas. In: Brown A.H.D. Frankel O.H., Marshall D.R., Williams J.T. (eds), The Use of Plant Genetic Resources. Cambridge University Press, UK, pp 88–104.

Stachel M., Lelley T., Grausgruber H. and Vollmann J. 2000. Application of microsatellites in wheat (*Triticum aestivum* L.) for studying genetic differentiation caused by selection for adaptation and use. Theor Appl Genet 100: 242–248

Stallknecht G.F., Gilbertson K.M. and Romey J.E. 1997. Alternative wheat cereals as good grains: Einkorn, emmer, spelt, kamut, and triticale. In: J. Janick (ed). Progress in new crops. ASHS Press, Alexandria VA. Pages 156-170.

Stephen R. M., Calibo M., Garcia-Belen M., Pham J.L. and Palis F. 2002. Natural hazards and genetic diversity in rice. Agriculture and Human Values 19: 133–149.

Stoskopf N.C., Tomes D.T. and Christie B.R. 1993. Plant Breeding Theory and Practice. Boulder, Colorado: Westview Press.

Struss D. and Plieske J. 1998. The use of microsatellite markers for detection of genetic diversity in barley populations. Theor Appl Genet 97: 308–315.

Synnevag G., Huvio T., Sidibe´ Y. and Kanoute´ A. 1999. Farmers' indicators for decline and loss of local varieties from traditional farming systems. A case study from northern Mali. In: Serwinski J. and Faberová I. (eds.): Proceedings of the Technical Meeting on the Methodology of the FAO World Information and Early Warning System on Plant Genetic Resources. FAO, Rome

Szabó A.T. and Hammer K. 1996. Notes on the taxonomy of faro: *Triticum monococcum*, *T. dicoccon* and *T. spelta.* In: S. Paduloiv (ed.), Hulled Wheats, Proc. Int. Symp. Castelvecchio Pascoli, Italy, pp. 2-40.

Tang S. and Knapp S.J. 2003. Microsatellites uncover extraordinary diversity in native American land races and wild populations of cultivated sunflower. Theor. Appl. Genet. 106: 990–1003.

Tanksley S.D. and McCouch S.R. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277: 1063–1066.

Tarekegn A. 1994. Yield potential of rainfed wheat in the central highlands of Ethiopia. M.Sc. Thesis. Alemeya, Ethiopia.

Tautz D. and Renz M. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucl Acids Res 23: 249–255.

Tautz D., M. Trick and Dover G.A. 1986. Cryptic simplicity in DNA is a major source of genetic variation. Nature 322: 652–656.

Teklu Y. 1997. Highlights of wheat improvement program. Proceedings of the 14th Annual Research and Extension Review Meeting, Alemaya University of Agriculture, 132–146.

Teklu Y. and Hammer K. 2006a. Multivariate analysis of quanatiative trait variation in tetraploid wheat landraces. Euphytica (under review).

Teklu Y. and Hammer K. 2006b. Genetic variation and association of metric traits in Ethiopian tetraploid wheat germplasm. Journal of Agricultural Sciences (Cambridge) (under review).

Teklu Y. and Hammer K. 2006c. Diversity of Ethiopian tetraploid wheat germplasm: Breeding opportunities for improving grain yield potentials and quality traits. Plant Genetic Resources (Cambridge) (Submitted).

Teklu Y. and Hammer K. 2006d. Farmers Perception and genetic erosion of Ethiopian tetraploid wheat landraces. Genet. Resour. Crop Evol. (in press).

Teklu Y. Hammer K., Huang X.Q. and Röder M.S. 2006a. Analysis of microsatellite diversity in Ethiopian tetraploid wheats. Genet. Resour. Crop Evol. (in press).

Teklu Y., Hammer K., Huang X.Q. and Röder M.S. 2006b. Regional patterns of microsatellite diversity in Ethiopian tetraploid wheat landraces. Plant Breeding (in press).

Teklu Y., Hammer K. and Röder M.S. 2006c. Simple sequence repeats marker polymorphism in emmer wheat (*Triticum dicoccon* Schrank): Analysis of genetic diversity and differentiation. Genet. Resour. Crop Evol. (in press).

Teklu Y., Hammer K. and Röder M.S. 2006d. Comparative analysis of diversity indices based on morphological and microsatellite data in tetraploid wheats. Journal of Genetics and Breeding (in press).

Teklu Y and Tefera H. 2005. Genetic Gain in Grain Yield Potential and Associated Agronomic Traits of Tef (*Eragrostis tef*). Euphytica 141: 247-254.

Tesfaye Z., Girma T., Tanner D.G., Verkuijl H., Aklilu A. and Mwangi W. 2001. Adoption of Improved Bread Wheat Varieties and Inorganic Fertilizer by Small-Scale Farmers in Yelmana Densa and Farta Districts of Northwestern Ethiopia. Mexico, D.F.: Ethiopia Agricultural Research Organization (EARO) and International Maize and Wheat Improvement Center (CIMMYT).

Teshome A., Baum B.R., Fahrig L., Torrance J.K., Arnason T.J. and Lambert J.D. 1997. Sorghum (*Sorghum bicolor* (L.) Moench) landrace variation and classification in North Shewa and South Welo, Ethiopia. Euphytica 97: 255–263.

Tessema T. 1987. Improvement of indigenous wheat landraces in Ethiopia. In: Engels J.M.M. (ed.) Proceedings of the International Symposium on Conservation and Utilization of Ethiopian Germplasm, 13-16 October 1986, pp. 232-238.

Tessema T. 1991. Improvement of indigenous durum wheat landraces in Ethiopia. In: Engels J.M.M., Hawks J.G. and Worede M. (Eds.). Plant Genetic Resources of Ethiopia. Cambridge University Press.

Tessema T. and Bechere E. 1998. Developing elite durum wheat landrace selections (composites) for Ethiopian peasant farm use: Raising productivity while keeping diversity alive. Euphytica 102: 323–328.

Tessema T, Becker H.C., Belay G., Mitiku D., Bechere E. and Tsegaye S. 1993. Performance of Ethiopian tetraploid wheat landraces at their collection sites. Euphytica 71: 221–230.

Tessema T., Belay G. and Worede M. 1991. Morphological diversity in tetraploid wheat landrace populations from central highlands of Ethiopia. Hereditas 114: 171–176.

Tsegaye S., Becker H.C., and Tesemma T. 1994. Isozyme variation in Ethiopian tetraploid wheat (*Triticum turgidum*) landrace genotypes of different color groups. Euphytica 75: 143-147.

Tsegaye S., Tesemma T. and Belay G. 1996. Relationships among tetraploid wheat (*Triticum turgidum* L.) landraces revealed by isozyme markers and agronomic traits. Theor Appl Genet 93: 600-605.

Tsehaye Y. 2002. The significance of *in situ* conservation: a comparative analysis of durum wheat diversity between *in situ* (Bale and eastern Shewa Zones) and *ex situ* (Ethiopia) conservation sites. In: Raupp J. (ed.) Annual Wheat Newsletter, Kansas State University, Manhattan, USA, vol 48, pp. 54-59.

Tunstall V. Teshome A. and Torrance J.K. 2001. Distribution, abundance and risk of loss of sorghum landraces in four communities in North Shewa and South Welo, Ethiopia. Genet. Resour. Crop Evol. 48: 131–142.

Van Treuren R., Bijlsma R., Van Delden W. and Ouborg N. J. 1990. The significance of genetic erosion in the process of extinction. Heredity 66: 181-189.

Vanhala T.K., van Rijn C.P.E., Buntjer J., Stam P., Nevo E., Poorter H. and van Eeuwijk F.A. 2004. Environmental, phenotypic and genetic variation of wild barley (*Hordeum spontaneum*) from Israel. Euphytica 137: 297–309.

Vavilov N.I. 1931. The Linnaean species as a system. Tr. po Prikl. Bot. Genet. Sel. [Bull Appl Bot and Genet Sel.] 26(3): 109-134.

Vavilov N.I. 1951. The origin, variation, immunity and breeding of cultivated plants. Chron Bot 13: 1-36.

Vavilov N.I. 1964. World resources of cereals, legumes, flax cultivars and their utilization in breeding. Wheat. (Nauka, Moskow and Leningrad).

Vavilov N.I. 1997. Five continents. IPGRI, Rome, Italy.

Wallace D.H., Baudoin J.P., Beaver J., Coyne D.P., Halseth D.E., Masaya P.N., Munger H.M., Meyers J.R., Silbernagel M., Yourstone K.S. and Zobel R.W. 1993. Improving efficiency of breeding for higher crop yield. Theor Appl Genet 86: 27–40.

Wang Z., Weber J.L., Zhong G. and Tanksley S.D. 1994. Survey of plant short tandem DNA repeats. Theor Appl Genet 88: 1–6.

Worede M. 1983. Crop Genetic Resources in Ethiopia. In: J.C. Holmes and W.M. Tahir (eds.), More Food from Better Technology. FAO, Rome. pp. 143-147.

Worede M. 1997. Ethiopian *in situ* conservation. *In:* N. Maxted, Ford-Lloyd B.V. and Hawkes J.G. (eds.), Plant Genetic Conservation. The *in situ* approach, pp. 290-301.

Worede M. 1998. SOS/E: Promoting Farmers' Seed - its conservation, enhancement and effective utilization. A paper presented at the SSC- Africa Project Workshop, Harare, Sept. 27th - Oct.1st 1998.

Worede M. and Mekbib H. 1993. Linking Genetic Resource Conservation to Farmers in Ethiopia. In: Boef W. de, Amanour K., Wellard K. and Bebbington A. (eds.), Cultivars Knowledge. London: Intermediate Technology Publications. pp. 78-84.

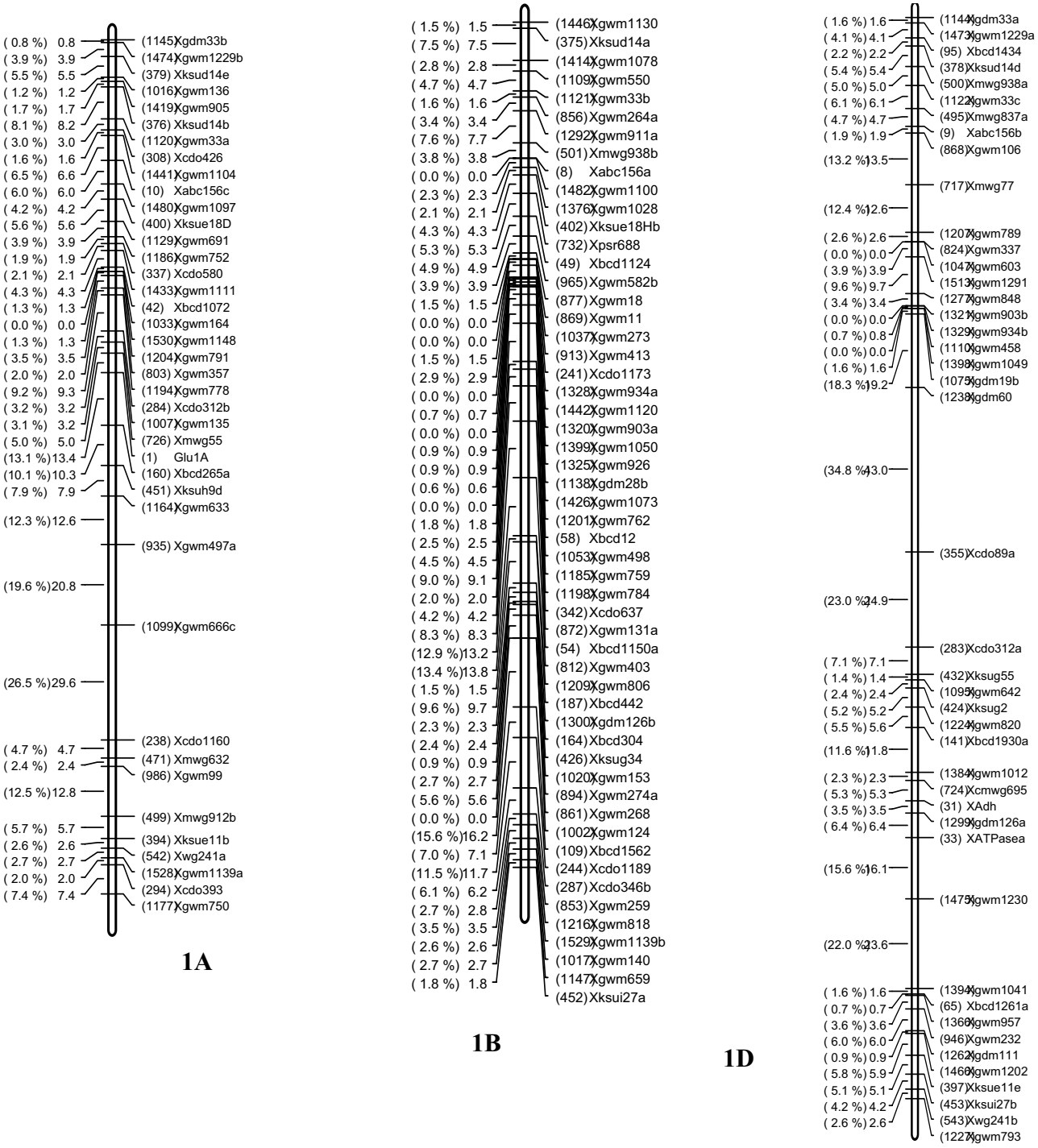
Worede, M. 1993. The role of Ethiopian farmers in the conservation and utilization of crop genetic resources. In: D. R. Buxton et al. (ed.). International Crop Science Society of America, Madison, USA.

Yu P., Li Z.C., Zhang H.L., Li D.Y., Wang M.X., Sun J.L. and Wang X.K. 2004. Genetic diversity of common wild rice (*Oryza rufipogon Griff.*) by using SSR markers and phenotypic traits in Guangxi Province. Yi Chuan Xue Bao 31(9): 934-40.

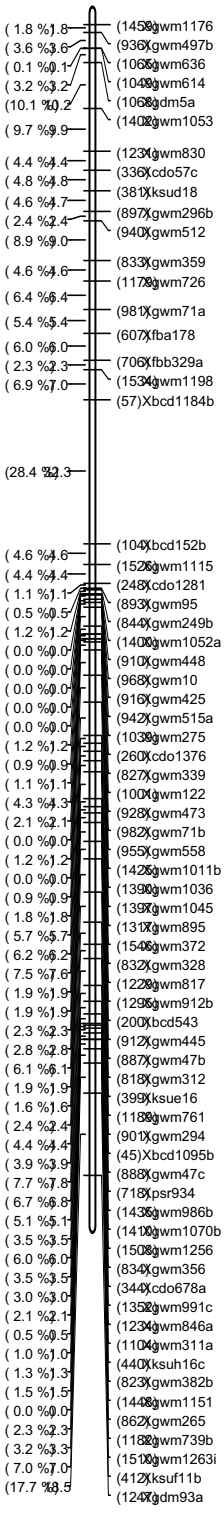
Zaharieva M., Dimov A., Stankova P., David J. and Monneveux P. 2003. Morphological diversity and potential interest for wheat improvement of three *Aegilops* L. species from Bulgaria. Genet. Resour. Crop Evol. 50: 507–51.

Zhang, X.Y., C.W. Li, L.F. Wang, H.M. Wang, G.X. You, and Y.S. Dong 2002: An estimation of the minimum number of SSR alleles needed to reveal genetic relationships in wheat varieties. Information from large-scale planted varieties and cornerstone breeding parents in Chinese wheat improvement and production. Theor Appl Genet 106: 112–117.

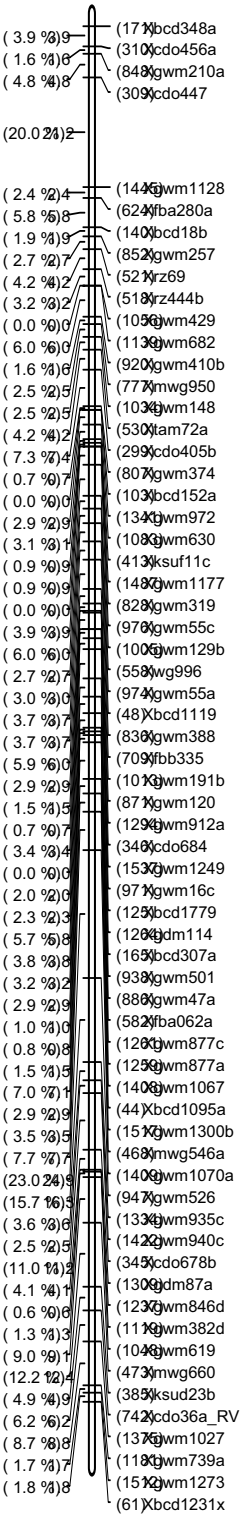
List of Appendices



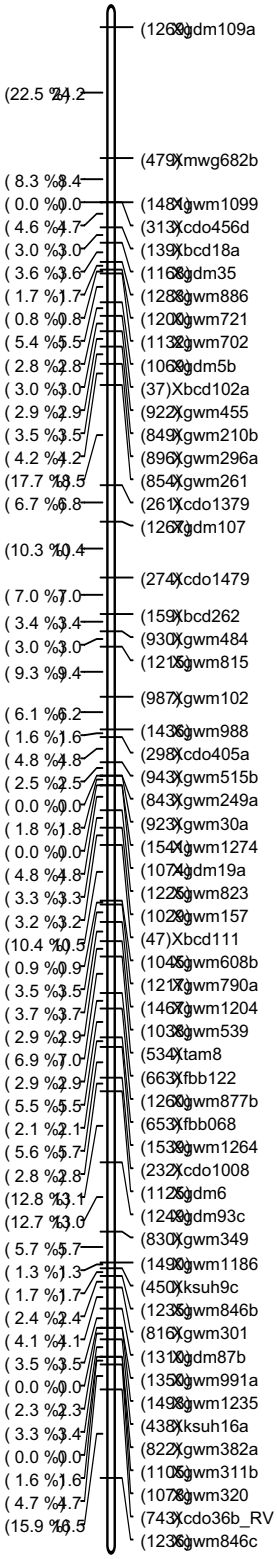
Appendix 1.Molecular linkage map of wheat. Short arms of chromosomes are at the top. The microsatellite loci are indicated in bold and carry the lab designator "*gwm*" (*G*atersleben *w*heat *m*icrosatellite). The centromeres are indicated in black. Primer sets that amplify more than one locus are marked by an asterisk. Dashed lines connect orthologous loci amplified by one microsatellite primer set.



2A

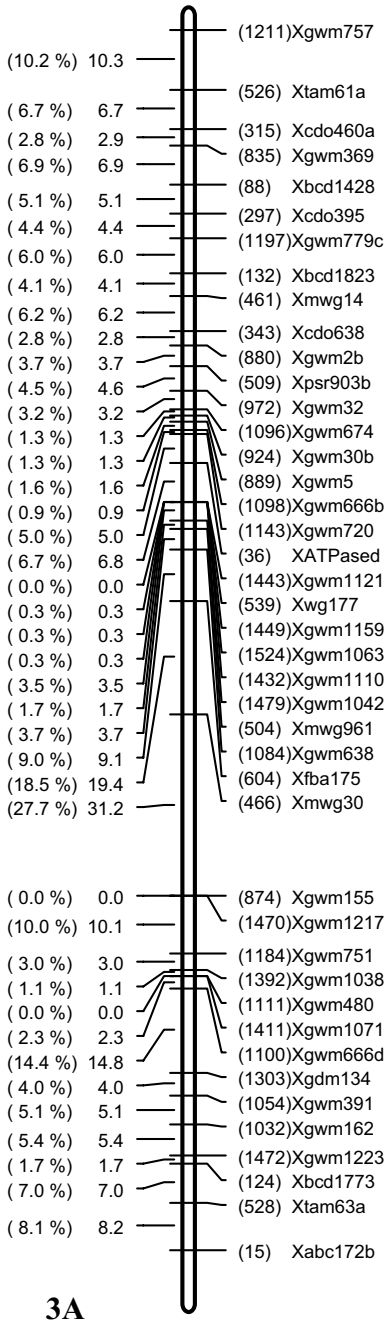


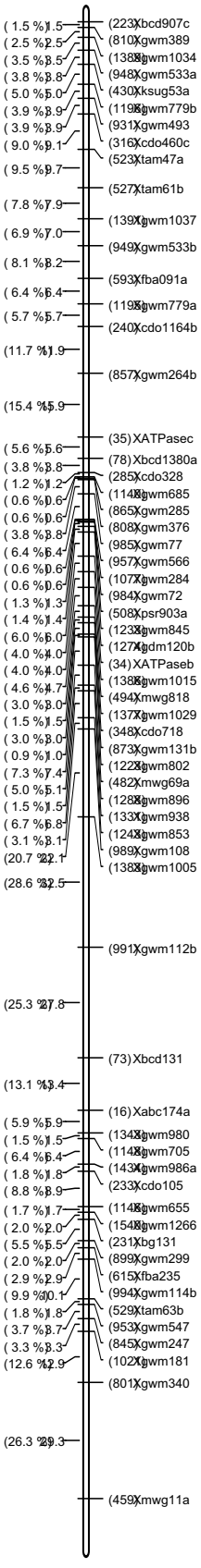
2B

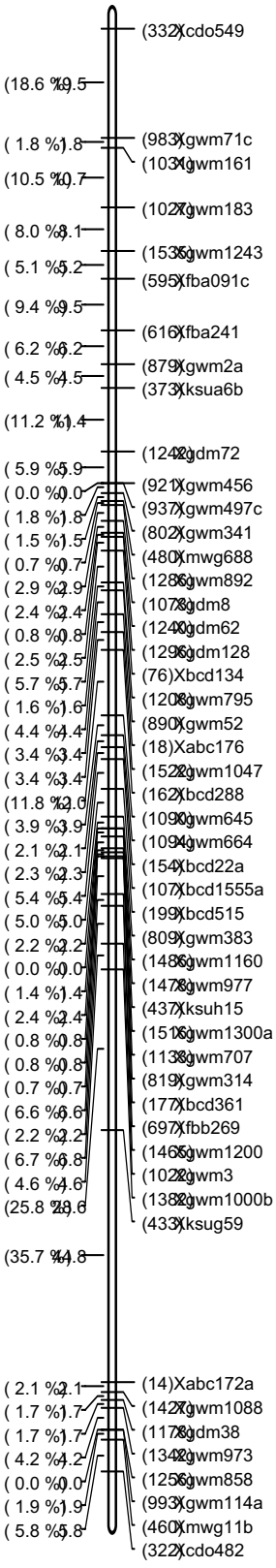


2D

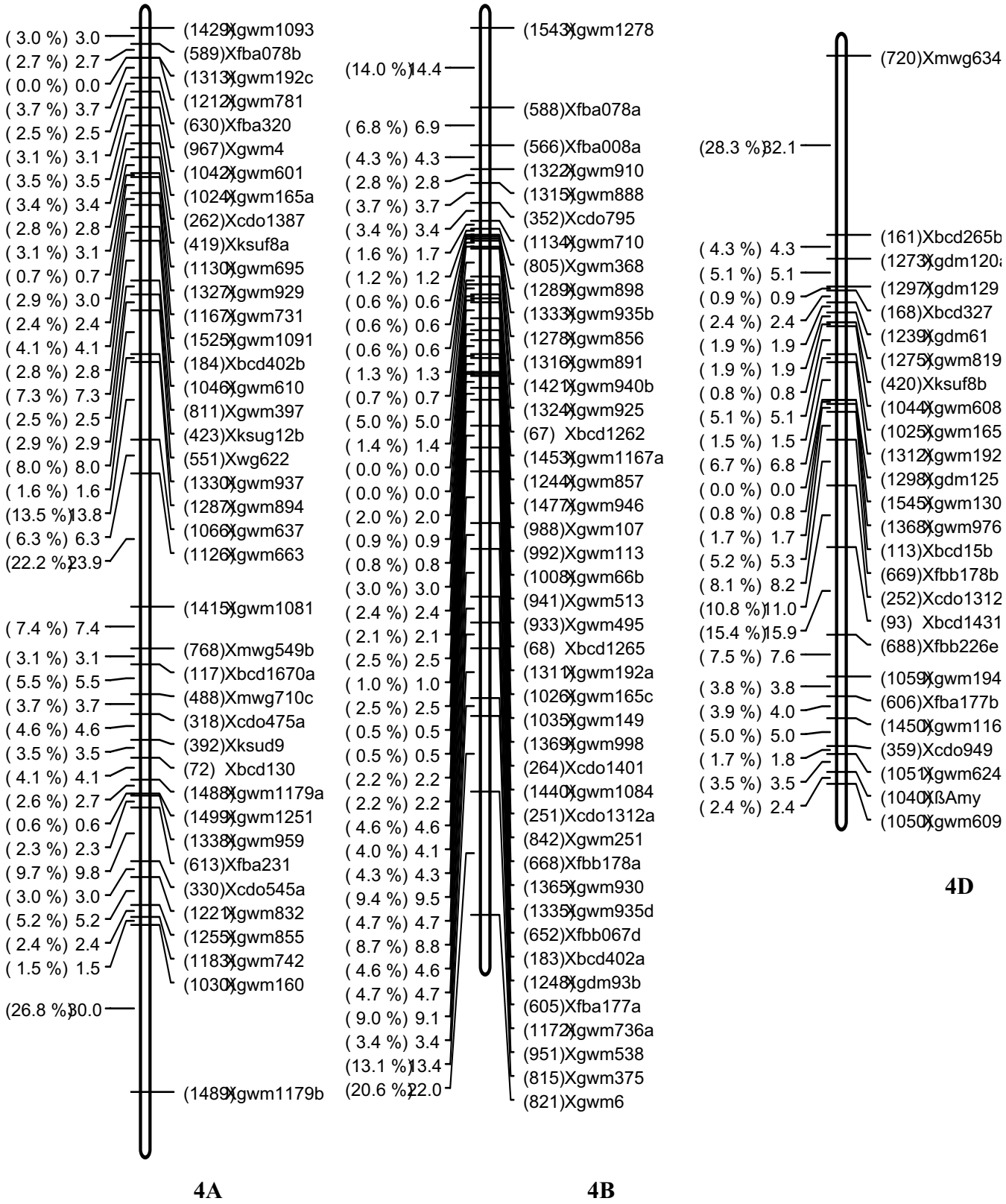
Appendix 1. Continued



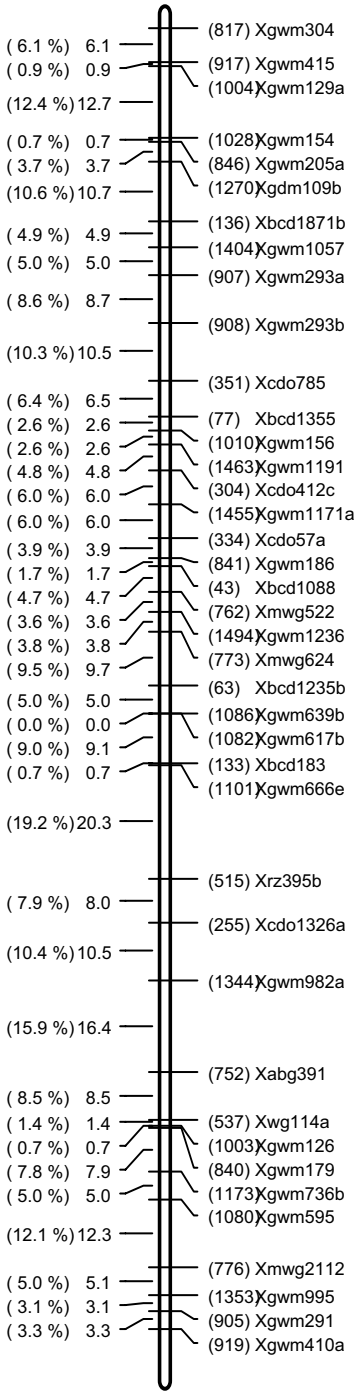




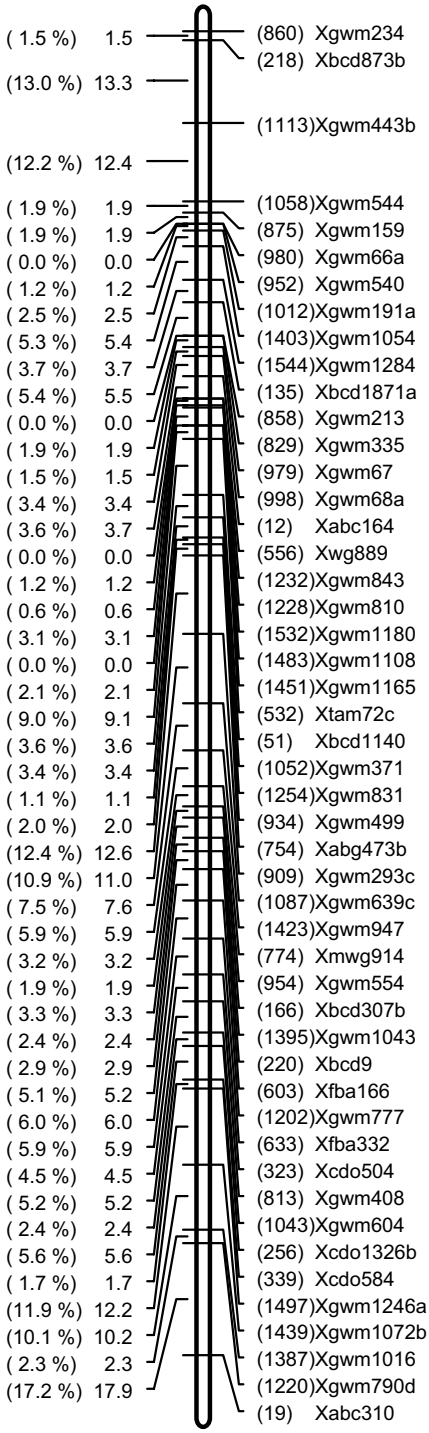
Appendix 1. Continued



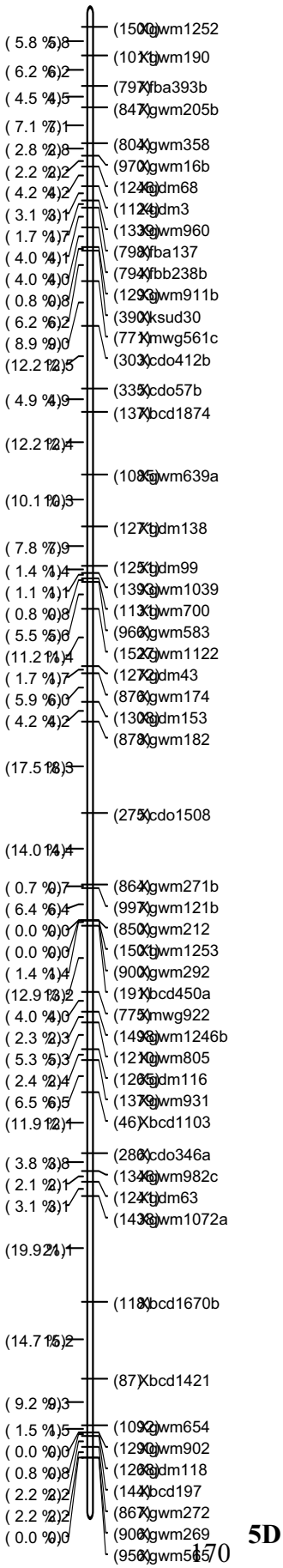
Appendix 1 Continued



5A

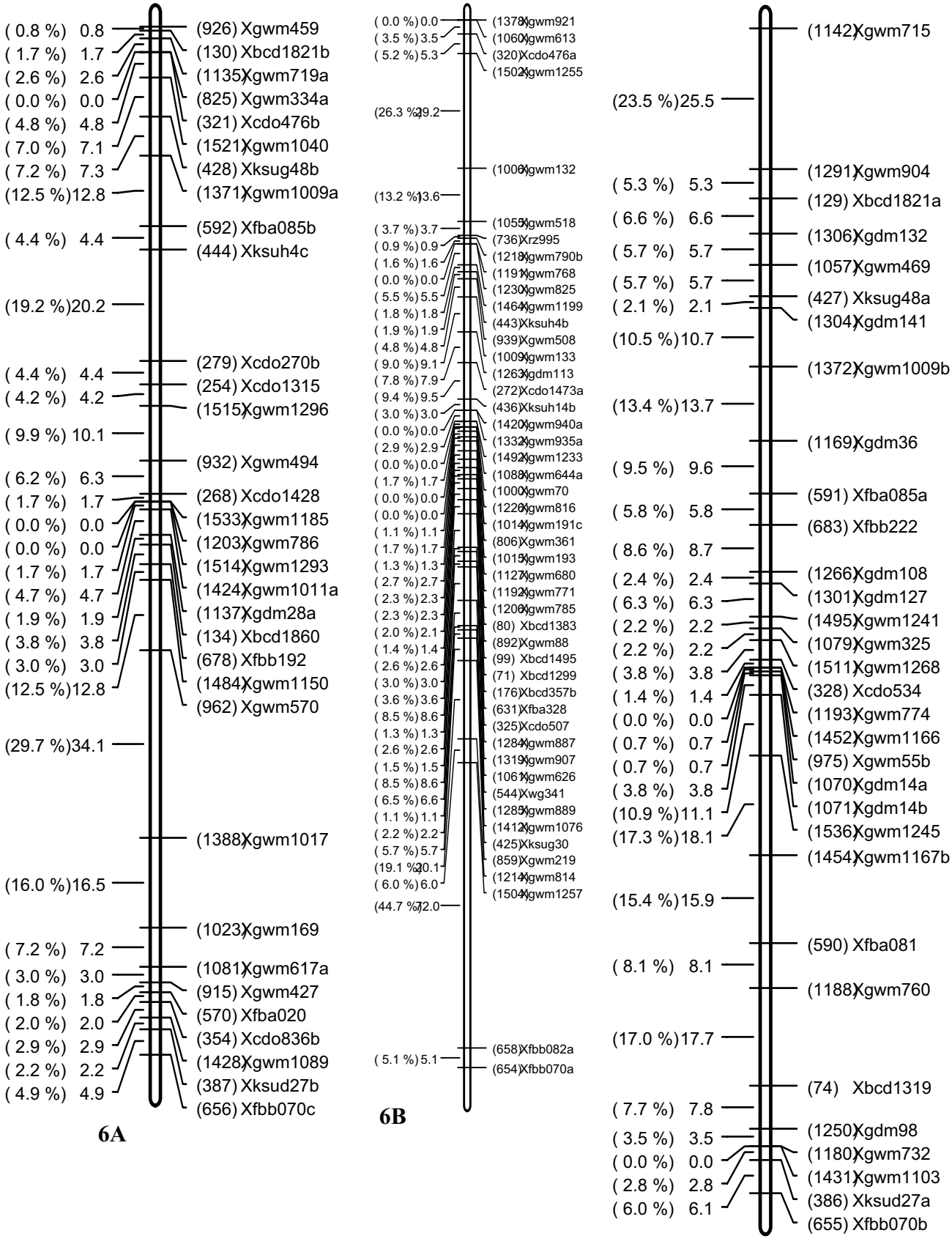


5B

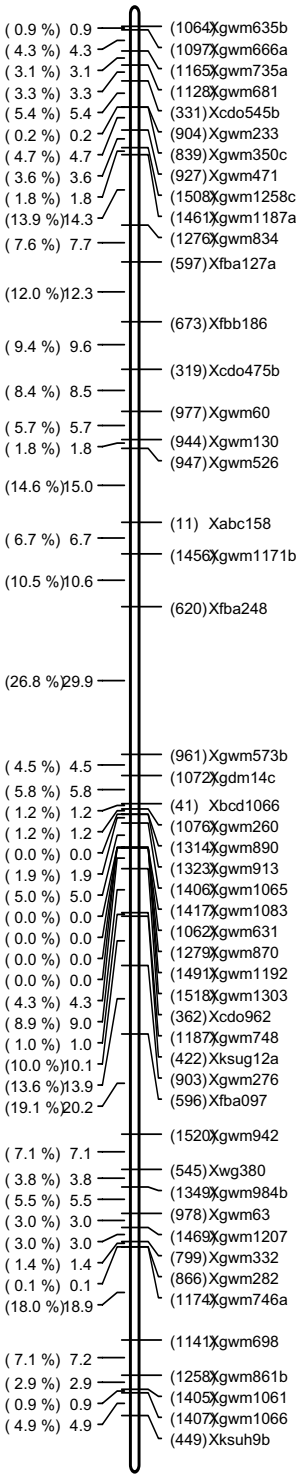


5D

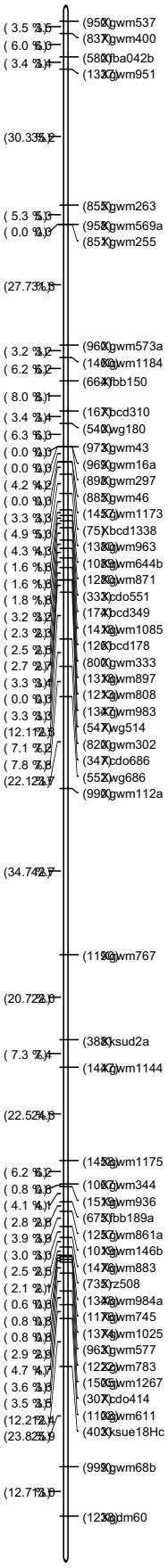
Appendix 1. Continued



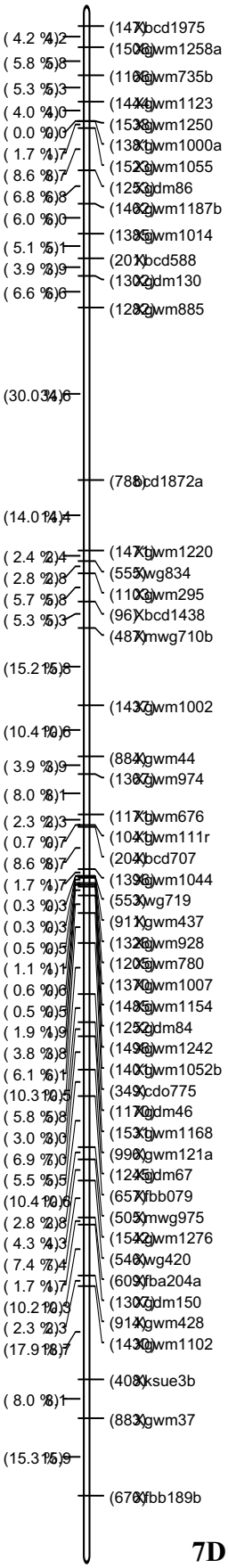
Appendix 1. Continued



7A



7B



7D

Appendix 1. Continued

Appendix 2. Mahalanobis distance (D^2) of the ten clusters of 271 tetraploid wheat accessions

Clusters	I	II	III	IV	V	VI	VII	VII	IX	X
I	0.00									
II	3.18	0.00								
III	3.91	3.82	0.00							
IV	3.59	2.16	3.39	0.00						
V	2.71	2.77	3.37	2.65	0.00					
VI	3.58	3.79	6.01	4.17	4.47	0.00				
VII	3.58	4.52	5.74	5.43	3.82	4.08	0.00			
VII	3.31	3.72	3.84	4.13	3.34	5.18	3.53	0.00		
IX	2.83	3.18	3.09	2.84	3.96	4.34	5.69	4.10	0.00	
X	2.62	3.55	2.67	3.63	3.02	4.43	3.88	2.71	3.07	0.00

Appendix 3 Altitudinal distributions of the 271 tetraploid wheat landraces over ten clusters.

	Accessions from altitude class				
Cluster	I	II	III	IV	Total
I	13	44	30	7	94
II	13	26	25	2	66
III	9	17	15	1	42
IV	6	22	11	0	39
V	0	5	3	1	9
VI	2	6	4	0	12
VII	1	2	0	0	3
VII	0	0	1	0	1
IX	3	1	0	0	3
X	0	0	0	1	1
Sum	47	123	89	12	271

Appendix 4. Number of Alleles (NA) and Genetic diversity (GD) in different genomes, chromosomes, and motifs across 29 loci

Chromosome	*T. durum*		*T. dicoccon*		*T. turgidum*	
	NA	GD	NA	GD	NA	GD
1	12	0.78	8	0.63	12	0.79
2	14	0.6525	6.75	0.55	6	0.54
3	13	0.82	7.5	0.66	9	0.63
4	7.75	0.575	5.25	0.638	12.75	0.78
5	7.5	0.5725	6	0.505	7.25	0.59
6	11.5	0.715	8.5	0.74	7.75	0.68
7	11.25	0.65	6.5	0.588	10	0.79

Appendix 5. Number of species specific alleles and shared alleles

	No of species specific alleles in			No of Shared alleles between		
Microsatellites	Durum	Turgidum	Dicoccon	Durum and Turgidum	Durum and Dicoccon	Dicoccon and Turgidum
Xgwm752	4	0	0	6	5	4
Xgwm357	1	0	1	7	4	3
Xgwm95	1	1	0	6	4	3
Xgwm312	7	4	3	17	13	7
Xgwm720	2	2	0	12	10	7
Xgwm155	3	1	0	8	6	6
Xgwm601	3	1	0	7	7	5
Xgwm160	1	0	0	4	4	4
Xgwm415	0	2	0	1	1	1
Xgwm186	0	3	1	7	6.5	6
Xgwm459	4	2	2	11	8	9
Xgwm1089	4	0	1	8	8	6
Xgwm631	0	0	0	4	1	1
Xgwm698	2	6	1	5	9	3
Xgwm18	1	0	0	6	6	6
Xtaglgap	7	2	0	10	6	7
Xgwm268	3	6	4	12	10	12
Xgwm148	3	0	1	7	4	4
Xgwm619	5	0	0	2	1	1
Xgwm389	0	1	0	9	8	6
Xgwm655	5	2	0	8	6	5
Xgwm898	1	0	1	6	5	4
Xgwm513	0	1	0	6	4	4
Xgwm540	5	1	1	5	3.5	4.5
Xgwm408	2	2	3	8	6	5
Xgwm680	0	1	5	7	6	5
Xgwm219	3	2	0	6	3	3
Xgwm46	4	4	0	8	7	6
Xgwm577	2	2	0	10	8	5
Total	73	46	24	213	171	142

Appendix 6. Genetic similarity coefficients of between 55 pairs of 11 geographical regions of Ethiopia.

	Bale	Shewa	Hararghe	Welo	Gamugofa	Gondar	Arsi	Gojam	IKS	Welega
Bale										
Shewa	0.37									
Hararghe	0.38	0.41								
Welo	0.24	0.37	0.32							
Gamugofa	0.21	0.20	0.29	0.38						
Gondar	0.31	0.27	0.22	0.17	0.24					
Arsi	0.30	0.16	0.42	0.23	0.39	0.19				
Gojam	0.24	0.20	0.41	0.27	0.47	0.20	0.52			
IKS	0.32	0.41	0.29	0.35	0.14	0.21	0.20	0.14		
Welega	0.23	0.29	0.51	0.35	0.29	0.19	0.37	0.41	0.20	
Tigray	0.27	0.45	0.37	0.27	0.21	0.27	0.32	0.33	0.25	0.31

Curriculum Vitae

Personal

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Place of Birth	Langie	Hararghe	Ethiopia
	Tawn	Province	Country
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Education

Period of study (MM/YY)	**Name of the University**	**Major**	**Degree obtained**
O9/1989 to 08/ 1993	Alemaya University, Ethiopia	Plant Sciences	B Sc
10/1996 to 03/ 1998	Alemaya University, Ethiopia	Agronomy (Plant breeding)	MSc
April 2002 –Feb 2006	University of Kassel, Germany	Agro-biodiversity	PhD Degree (with Summa Cumma Laude)

M.Sc. Thesis title: "Genetic Gain in Grain Yield Potential and Associated Agronomic Traits of Tef (*Eragrostis tef*)".

PhD dissertation topic: "Genetic Erosion and Morphological and Molecular Markers Diversity in Ethiopian Tetraploid Wheat Landraces: Implications for Conservation and Utilization".

Work experiences

January 1994-August 1995	Graduate Assistant in the Department of Plant Sciences of Alemaya University of Agriculture, Ethiopia.
April 1998 - September 2001	Lecturer in the Department of Plant Sciences of Alemaya University of Agriculture, Ethiopia.

Publications

Journal Articles

Teklu Y. and Tefera H. 2005. Genetic Gain in Grain Yield Potential and Associated Agronomic Traits of Tef (*Eragrostis tef*). Euphytica 141: 247-254.

Teklu Y. and Hammer K. 2006a. Multivariate analysis of quantitative trait variation in tetraploid wheat landraces. Euphytica (under review).

Teklu Y. and Hammer K. 2006b. Genetic variation and association of metric traits in Ethiopian tetraploid wheat germplasm. Journal of Agricultural Sciences (Cambridge) (under review).

Teklu Y. and Hammer K. 2006c. Diversity of Ethiopian tetraploid wheat germplasm: Breeding opportunities for improving grain yield potentials and quality traits. Plant Genetic Resources (Cambridge) (under review).

Teklu Y. and Hammer K. 2006d. Farmers Perception and genetic erosion of Ethiopian tetraploid wheat landraces. Genetic Resources and Crop Evolution (***in press***).

Teklu Y., Hammer K., Huang X.Q. and Röder M.S. 2006a. Analysis of microsatellite diversity in Ethiopian tetraploid wheats. Genetic Resources and Crop Evolution (***in press***).

Teklu Y., Hammer K., Huang X.Q. and Röder M.S. 2006b. Regional patterns of microsatellite diversity in Ethiopian tetraploid wheat landraces. Plant breeding (**i*n press***).

Teklu Y., Hammer K. and Röder M.S. 2006c. Simple sequence repeats marker polymorphism in emmer wheat (*Triticum dicoccon* Schrank): Analysis of genetic diversity and differentiation. Genetic Resources and Crop Evolution (***in press***).

Teklu Y., Hammer K. and Röder M.S. 2006d. Comparative analysis of diversity indices based on morphological and microsatellite data in tetraploid wheats. Journal of Genetics and Breeding (***in press***).

Hammer K. and **Teklu Y**. 2006. Erhaltungsstrategien pflanzengenetischer Ressourcen – die PGR-Bewegung, und was dann? Vorträge für Pflanzenzüchtung (***in press***).

International Newsletter

Singh H., Dhaliwal H.S, and **Teklu Y**. 2001. Germplasm enhancement through wide hybridization. In: Raupp J. (ed.) Annual Wheat Newsletter, Kansas State University, Manhattan, USA. 47: 56-63.

Book Chapter Contributions

Hammer K. and **Teklu** Y. 2005. Plant Genetic Resources: Gene Pools and land productivity, loss of diversity, and GMO issues. In: Zoebisch M. (ed.), Agrobiodiversity and Land productivity. Science Publishers, New Hampshire, USA (under edition).

Hammer, K., **Teklu**, Y., Khoshbakht, K. and Heuser, F. 2006. Plant genetic resources for breeding. In: Acquaah G (ed.), Principles of plant genetics and breeding; with highlights by industry professionals (***in press*)**.

International meetings, conferences and trainings attended

International Workshop on Tef Genetics and Improvement, which was held from16 -19 October 2000, Addis Ababa. EARO, Addis Ababa, Ethiopia.

International Training Course on Organization and Management of Formal and Informal Seed Programs, which was held from 14 June – 14 July 2000 in Germany.

Gene Flow Meeting which was held on Dec 13 and 14th, 2005, Kansas City, Missouri, USA.

Language proficiency

English, German, and Ethiopian languages such as Amharic and Oromifa.

Statistical software and computer skills

MS office, Minitab, MSTATc, SPSS, NTSYC-pc, Genstat and Statistica.

Declaration

I, the undersigned, declare that the work contained in this dissertation is original and it has not been previously submitted to any university for a degree.

_____ 20 February 2006, Witzenhausen, Germany

Signature Date and Place