ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

RAPD ANALYSIS FOR GENETIC DIVERSITY AND RELATIONSHIPS IN TETRAPLOID WHEATS (AABB Genome) OF ETHIOPIA

AMEHA YAEKOB GEBRE-IYESUS

Addis Ababa University, Feb., 2005

RAPD ANALYSIS FOR GENETIC DIVERSITY AND RELATIONSHIPS IN TETRAPLOID WHEATS (AABB Genome) OF ETHIOPIA

A Thesis Presented to the
School of Graduate Studies
Addis Ababa University

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology
(Applied Genetics)

By

AMEHA YAEKOB GEBRE-IYESUS
Addis Ababa University

Feb. 2005

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

RAPD ANALYSIS FOR GENETIC DIVERSITY AND RELATIONSHIPS IN TETRAPLOID WHEATS (AABB Genome) OF ETHIOPIA

BY

AMEHA YAEKOB

Approved by:

Examiner: ______________________ ______________

Examiner: ______________________ ______________

Advisor: ______________________ ______________

Advisor: ______________________ ______________

Chairman: ______________________ ______________

DECLARATION

I, the undersigned, declare that the information provided in this work is an original work, and that it has not been presented in other universities or colleges, seeking for similar degree or other purposes.

Name	Date	Signature

This work is presented under supervisions of,

Name	Date	Signature

Name	Date	Signature

ACKNOWLEDGEMENTS

My deepest gratitude goes to my advisors, Prof. Endashaw Bekele and Dr. Getachew Belay for their advice, interest and unreserved support.

I would like to thank the DZARC management in general and Dr. Solomon Assefa in particular for the help in administrative matters and his encouragement. I would like to acknowledge also Bemnet Gashawbeza and all the National Durum Wheat Improvement Project staffs, for providing me the access to use the available facility.

I would like to thank Dr. Kibebew Assefa for providing me statistical softwares and helping me in most of the data analysis.

My appreciation goes to the Institute of Biodiversity Conservation management for allowing me to use their lath house, and generously providing me germplasm for the study. Tessema Tanto, Tamene Yohannis and Nigist are thanked for their moral and technical support.

I would like to thank also my friends and colleagues, Teshome Dagne and Assefa Gizaw who provided me computer facility and were always near to help me in any aspect; and Sewalem Amogne who helped me in literature gathering. I am grateful to Ato Getachew Alemu and W/o Hamelmal Wube for their help in social matters.

Last but not least, I am indebted to my uncle, 'Merigeta' Yohannis Girma who raised me as a father; My mother, Asres Tekeste, who suffered most of her life time in raising kids who lost their father at a very tender age; Fikru Yaekob and Sebledingle Yaekob who were on my side all the way morally and materially.

The study was financed by the Ethiopian Agricultural Research Organization (EARO) – Agricultural Research Training Project (ARTP); and Genetics Lab. of the Department of Biology supplied all lab. facilities, which are thankfully acknowledged.

TABLE OF CONTENTS

Title **Page**

ACKNOWLEDGEMENTS……………………………………………………I

TABLE OF CONTENTS………………………………………………….II

LIST OF TABLES ……………………………………………………V

LIST OF FIGURES ..…………………………………………………..VI

LIST OF APPENDICES …………………………………………………VII

LIST OF ACRONYMS ………………………………………………… VIII

ABSTRACT ………………………………………………………………IX

I. INTRODUCTION ………………………………………………… 1

II. LITERATURE REVIEW …………………………………………… 5

2.1 Wheat Classification, Origin and Evolution …………………… 5

2.1.1 Classification ..…………………………………… 5

2.1.2 Origin and Evolution ..…………………………… 7

2.2 Wheat Genetic Resources in Ethiopia …………………… 10

2.3 Marker Systems Used for Genetic Study ………….………… 13

2.3.1 Morphological and Biochemical Markers ….………… 13

2.3.2 Molecular (DNA) Markers …………………….……….. 14

2.3.2.1 Classification of DNA Markers ..…………….. 14

2.3.2.2 Uses of Nucleic Acid Markers ………..……..…… 15

Title **Page**

2.4 RAPD markers …...……………………………..…..…… 17

2.4.1 Diversity Studies …………………………………… 18

2.4.2 Genetic Relationship Studies …………………………… 19

III MATERIALS AND METHODS …………………………………… 20

3.1 Plant Materials ..…………………………………………… 20

3.2 DNA Extraction …………………………………………… 22

3.3 PCR Amplification and Electrophoresis ….…………………… 24

3.4 Data Scoring and Analysis …………………………………… 25

IV RESULTS ………………………………………………………… 28

4.1 Diversity analysis ..…………………………………………. 28

4.1.1 Regional diversity of landraces ….…………………… 31

4.1.2 Species diversity of landraces …………………… 32

4.1.3 Diversity within improved durum wheat cultivars. 32

4.2 Relationship analysis …………………………………… 34

4.2.1 Cluster analysis ……….…………………………… 34

4.2.2 Discriminant analysis ..…………………………… 39

V DISCUSSION ……………………………………………………… 42

5.1 Diversity in Tetraploid Wheat Landraces ……………………… 42

5.2 Diversity in Improved Durum Wheat Cultivars ……………… 43

5.3 Relationships of Tetraploid Wheat Species ……………… 44

5.4 Correlation of RAPD Cluster Analysis with Pedigrees of Cultivars…… 46

Title	Page
VI SUMMARY …………………………………………………	47
VII CONCLUSION AND RECOMMENDATIONS………………….	49
VIII REFERENCES …………………………………………………	51
IX APPENDICES …………………………………………………	62

List of Tables

Title **Page**

Table 2.1 The group of varieties (cultivars) of the genus *Triticum* (Morris and Sears, 1967) 10

Table 3.1 List of improved durum wheat cultivars, year of release, origin and their pedigrees. 21

Table 3.2 List of the 6 primers tested to generate RAPD markers in 40 wheat genotypes.. 25

Table 4.1 Amplified products of the two primers used to generate RAPD markers in 40 wheat genotypes... 28

Table 4.2. Number and percentage of polymorphic bands for four species of tetraploid wheat and of their three regions of origin. 29

Table 4.3. Estimates of the Shannon-Weaver diversity index (H) for 4 species of tetraploid wheat and of their three regions of origin. 31

Table 4.4. Partitioning of genetic variation into within and between species as well as regions of origin of the landrace collections. 33

Table 4.5. Estimates of the Shannon-Weaver diversity index (H), and number and percentage of polymorphic bands (P) for 15 improved durum wheat cultivars. 33

Table 4.6. Nei's genetic distance among 24 tetraploid wheat landraces 37

Table 4.7. Nei's genetic distance among 15 durum wheat improved cultivars 38

Table 4.8. Nei's genetic disimilarity among 4 species of 24 wheat landraces 39

Table 4.9. Nei's genetic disimilarity among 3 regions of origin of 24 wheat landraces 39

Table 4.10. Classification results of 24 tetraploid wheat landraces on the basis of their species. 41

List of Figures

Title	Page
Fig. 2.1. Proposed origins and relationships of wheat (Kerby & Kuspira 1987)	9
Fig. 2.2. Spike samples of Ethiopian tetraploid wheat species: *T. turgidum, T. aethiopicum, T. durum, T. polonicum*	12
Fig 4.1. Amplification products from genomic DNA of 14 improved durum cultivars amplified with primer OPA-13	30
Fig 4.2. Amplification products from genomic DNA of 8 tetraploid wheat landraces amplified with primer OPA-13	30
Fig. 4.3. Dendrogram for 24 landrace tetraploid wheat genotypes generated from RAPD based on the unweighted pair group method with arithmetic averages (UPGMA) analysis of Jaccard similarity.	36
Fig. 4.4. Dendrogram for 15 improved durum wheat cultivars generated from RAPD based on the UPGMA analysis of Jaccard similarity.........	36
Fig 4.5. Plot of canonical discriminant functions 1 and 2 based on the 31 polymorphic RAPD loci; species classification.	40
Fig 4.6. Plot of canonical discriminant functions 1 and 2 based on the 31 polymorphic RAPD loci; regional grouping.	41

List of Appendices

Title	Page
Appendix 1. List of genotypes included in the experiment with their region of origin, species group, population and entry/accession number, code and source institute	62
Appendix 2. Dendrogram for 40 tetraploid wheat genotypes generated using RAPD based on the unweighted pair group method with arithmetic averages (UPGMA) analysis of Jaccard similarity	63
Appendix 3. Genetic Similarity matrix table for forty genotypes studied …	64
Appendix 4. Number and size of RAPD fragments	66

LIST OF ACRONYMS

AFLP - Amplified Fragment Length Polymorphism

AHRI- Armour Hannson Research Institute

DNA- Deoxyribonucleic Acid

DZARC- Debre Zeit Agricultural Research Center

EtBr- *Etidium Bromide*

IBC- Institute of Biodiversity Conservation

IRAP- Inter- Retrotransposon Amplified Polymorphism

ISSR- Inter-Simple Sequence Repeats

MAS- Marker Assisted Selection

RAPD- Randomly Amplified Polymorphic DNA

REMAP- Retrotransposon-Microsatellite Amplified Polymorphism

RFLP- Restriction Fragment Length Polymorphism

SNP- Single Nucleotide Polymorphism

SSR- Simple Sequence Repeats

STMS- Sequence-Tagged Microsatellite Site

STS- Sequence Tagged Sites

TAE- Tris-Acetate EDTA

ABSTRACT

Twenty-five tetraploid wheat landraces and 15 improved durum cultivars were used to study the diversity and relationships within and among tetraploid species and regions of origin, and improved cultivars. Thirty-one polymorphic bands produced by two RAPD marker primers were used for the analysis. The mean Shannon diversity index (H) for landrace collections and improved cultivars were 0.34 and 0.23, respectively. Among the four species studied, *T. durum* was with the highest (0.32) diversity value whereas *T. polonicum* with the lowest (0.26). Similarly, Wello region collections revealed highest (0.27) tetraploid wheat diversity while Shewa collections, the lowest (0.22). The within species and regions variations were 81.4% and 72.2%, respectively. It suggests the need to collect more wheat germplasm samples within regions. Cluster analysis for all genotypes grouped mainly into landrace collections and improved cultivars. Clustering of landrace genotypes resulted in four groups mainly according to their regions of origin. However, it failed to group on the basis of their species. It suggests the presence of more genetic differentiation among regions than species. The lowest (0.00) and highest (0.73) genetic distances were observed between Wet1224 and Wet1570, and Wet1650 and Bae1117, respectively. The between species genetic distance was lowest (0.016) between *T. polonicum* and *T. turgidum*, but highest (0.051) between *T. aethiopicum* and *T. durum*. Clustering of improved durum cultivars formed four groups at 80% similarity coefficient. The lowest genetic distance (0.032) was observed between cultivars Ld357 and Boohai, Foka and Kilinto, and Tob66 and DZ 1640. The highest distance (0.34) was between Asassa and Bichena, Asassa and Boohai, and Bichena and Ude cultivars. The average distance within improved durum cultivars was 0.157. It indicates that all the 15 improved cultivars are relatively similar. Little correlation was observed between groups formed by cultivars and common parentage.

positive for *M. bovis.* The results indicated that *M. tuberculosis* is the causative species for tuberculous lymphadenitis in Dera.

I. INTRODUCTION

Wheat is one of the most important cereal crops, which provides a staple food for over one-third of the world population (Mori *et al*., 2001) and is grown on 240 million hectares annually (Knott 1987). It evolved from wild grasses growing in the Eastern Mediterranean and the Near East areas. Its domestication is believed to be at least 9000 yrs ago in the Near East area known as the Fertile Cresent, a mountainous hilly region in the upper reaches of Tigris and Euphrates drainage basin (Simmon, 1987).

Ethiopia is the second largest wheat producer in sub-Saharan Africa. It is grown at elevations ranging from 1600 to a little over 3000 masl. According to Central Statistics Authority's (CSA) 2000 report, in 1999/2000 main season the area covered by wheat was estimated to be over one million hectares. The total wheat production and average yield in the season were 1.21 m tones and 1.18 t/ha, respectively. Both tetraploid (mainly *Triticum durum* Desf.) and hexaploid (*T. aestivum* L) species are grown. Tetraploids are indigenous to the country while the hexaploids are recent introductions compared with tetraploids (Tesfaye, 1986; Hailu, 1991). Although almost all regions of the country cultivate wheat, Shewa, Gondar, Gojam, Wello, Arsi and Bale regions are the main producers.

In Ethiopia, about 50 % of the wheat area is devoted to the production of the tetraploid types. It is cultivated by small-scale farmers under rainfed conditions on heavy black clay soils (vertisols) of the highlands between 1800 and 2800 masl. Mostly, the tetraploids are landrace

cultivars, composed of mixtures of several genetic lines and species (Tesemma and Belay, 1991; Eticha *et al*. 2004).

Classification of *Triticum* and its related genera in *Triticeae* was highly debated, although gene-pool classification based on ease of crossability, fertility of hybrids, chromosome pairing and ease of gene transfer is recommended (Mac Key 1966 in Tesfaye and Getachew 1991). Lack of clear-cut morphological discontinuity among species within *Triticum* has also been the major problem in wheat taxonomy. However, most scientists agree on the classification of *Triticum* based on ploidy and 'byphyletic' genome differentiation (B vs. G genome), as they are the only isolating mechanisms offering adequate species border. In this classification, all tetraploid groups with AABB genome are treated as a single species (*T. turgidum* L.) (Kimber and Sears 1987; Mac Key, 1988). However, in other cases each group is treated as different species in tetraploids (Drofeev *et al*. 1979; Gandilyan, 1980).

Evaluation of genetic resources is a prerequisite for proper use and conservation. Knowledge about the extent and pattern of genetic variation in a region is decisive to design efficient conservation as well as collection strategies. Besides, to optimize and accelerate breeding, it is essential to screen, evaluate and classify the genetic variability (Strelchenko *et al*. 1999).

The level of genetic diversity in a population, species or any taxonomic group could be estimated from morphological or agronomic traits, cytogenetic analysis, or by molecular markers at protein (allozyme, isoenzyme, storage protein etc.), and DNA (RFLP, PCR-based, SNP etc.) levels. Even if they have different efficiency to extract the available genetic

variation, for different methods sample genetic variation at different levels, and differ in their power of genetic resolution and quality of information content, the combined use of all/most of the methods is highly recommended (Cao *et al*., 1999; Ayana *et al*., 2000). However, DNA markers are advantageous over conventional markers for they are practically unlimited in number, they are not growth stage specific, nor are they subject to either environment or to epistatic interactions (Koebner *et al*., 2001).

Randomly Amplified Polymorphic DNA (RAPD) is the first PCR-based DNA marker to be used in genetic analysis. RAPD markers represent amplification products from a PCR utilizing arbitrary primers and genomic DNA. Most variation among individuals for RAPDs probably arises from base-pair substitutions or insertions/deletions that modify (or eliminate) the primer site, or from insertions in the genomic sequence that separates the primer sites to a distance that will not permit amplification (Williams *et al*. 1990). In addition to its efficient use in measuring genetic diversity, it is applied for gene mapping/linkage analysis (Fahima *et al*. 1999; Warburton *et al*. 1996; Faure, 1993), marker assisted selection, and species/cultivar identification (Kongkiatngam *et al*. 1996; Cao *et al*.,1999). Moreover, despite its drawbacks of poor levels of reproducibility and dominant inheritance of markers, RAPD is widely used as a reliable, quick, easy, and cost-effective method in genetic relationship study also, particularly at intraspecific level and among closely-related species (Ayana *et al*., 2000; Fahima *et al*. 1999; Millan *et al*., 1996; Hoey *et al*., 1996).

RAPD analysis is being used to determine phylogenetic relationships and genetic diversity in a variety of crops; *Triticum* (Cao *et al*., 1999; Szucs *et al*., 2000; Pujar *et al*., 1999; Castagna *et al*., 1997;), Barley (Strelchenko *et al*., 1999), Sorghum (Ayana *et al*., 2000), *Pisum* (Hoey *et*

al., 1996), *Cicer* (Ahmed, 1999), *Rosa* (Millan *et al*., 1996), *Vicia* (Potokina *et al*., 1999), *Brassica oleracea* (Divaret *et al.*,1999), Red clover (Kingkiatngam, *et al*., 1996), Coffee (Aga, 2002), Enset (Birmeta et al, 2002), and Tef (Bai et al, 2000). However, such information, at DNA level, about the Ethiopian tetraploid wheat species is lacking except an AFLP and microsatellite reports by Tesfaye (2001) and Sentayehu *et al* (2004).

Based on their pedigree record, Ethiopian improved durum wheat cultivars seem to have limited genetic diversity (Table 3.1). However, since pedigree records do not take into account effects of selection and genetic drift, its correlation with various kinds of molecular markers to estimate genetic similarity is low or moderate at least in wheat and barley (Koebner *et al*., 2001). The same authors also indicated the importance of information on correlations between molecular marker profiles and known pedigrees for integrating cultivar identification.

OBJECTIVES

The objectives of this study are therefore to:

1. Identify the genetic relationship among tetraploid wheat landrace species of *T. turgidum, T. durum, T. aethiopicum* and *T. polonicum*.
2. Estimate the extent of genetic variation of landrace collections based on RAPD markers data among and within species and regions of origin.
3. Estimate the genetic variation within improved durum wheat cultivars, and compare the genetic similarity based on RAPD markers with pedigree (common parentage) data.

II. LITERATURE REVIEW

2.1 Wheat Classification, Origin and Evolution

2.1.1 Classification

Taxonomically wheat belongs to the genus *Triticum*, subfamily *Pooideae* of the grass family *Gramineae (Poaceae)* (Briggle and Reitz 1963). Based on the number of somatic cell chromosome count, *Triticum* is classified into three ploidy groups, with 7 basic chromosomes (x=7) (Zohary and Fieldman 1962). The diploid wheat types have 14 chromosomes (2n=2x=14), the tetraploids 28 (2n=4x=28), and the hexaploid types 42 (2n=6x=42). According to Mac Key (1975) quoted in Bekele (1984), six species of wheat are recognized; two species in each ploidy group. In diploids, *T. monococcum* and *T. urartu*, have the same basic (A) genome but differ on genetic, morphological and biochemical bases. The former species has both cultivated and wild types, while the latter only wild type. In tetraploid group, *T. timopheevi* and *T. turgidum* share the A genome but the other genome is designated as G in *T. timopheevi* (AAGG), whereas B in *T. turgidum* (AABB) (Giorgi and Bozzini 1969, in Bechere *et al* 2000). The source of the A genome is *T. urartu* (Breiman and Graura, 1995). Its hybridization with the other genome source, and subsequent chromosome doubling resulted in tetraploid species. However, the origin of the B genome is not confirmed so far. The two species recognized in hexaploid group are *T. zhukovskyi*, in which two A genomes and a G genome are represented (AAAAGG), and *T. aestivum* with an A, B and D genomes (AABBDD). *T. aestivum* is derived from a cross between a tetraploid and *T. tauschii* followed by chromosome doubling (Morris and Sears 1967) (Fig. 2.1).

According to Kerby and Kuspira (1987), diploid species of wheat include *T. monococcum* (A-genome progenitor), *Aegilops* spp. (B-genome donor), and *Ae. Squarossa* (syn. *T. tauschi*i).

However, the A-genome has more recently been considered to originate from *T. urartu* L. based on cytogenetic and molecular analysis (Breiman and Graura, 1995). *T. dicoccoides, T. dicoccum, T. turgidum, T. polonicum, T. carthlicum* etc. are tetraploid species (Droffeve et al, 1979; Gandilyan, 1980). In hexaploid group only two speices (*T. aesticum* L. and *T. zhukovskyi* Men.&Er.) are recognized. However, *T. aestivum* has been divided into the subspecies *compactum, sphaerococcum, vulgare, spelta, macha*, and *vavilovii* (Mac Key, 1966)

Classifying *Triticum* with clear-cut discontinuity at species level has been very difficult. The use of ploidy level and genomic biphyletic differentiation are the only isolating mechanism offering adequate species border (Mac Key 1966 in Tesfaye and Getachew, 1991).

The difficulty of classifying wheat is suggested to be settled by the use of gene-pool classification concept. According to this classification, *Triticum* and its related genera are classified into three gene-pools, based on ease of crossability, fertility of hybrids, chromosome pairing and ease of gene transfer (Harlan and De Wet 1971 in Tesfaye and Getachew, 1991).

2.1.2 Origin and Evolution

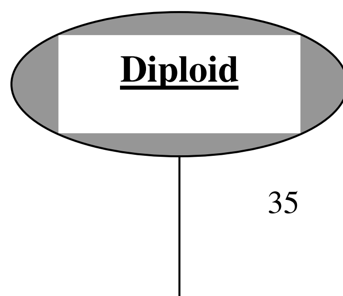
The wild diploid species are presumably monophyletic in origin although they have diverged from each other. This is shown in the seed dispersal units and their ecological requirements and geographical distributions. This taxonomic classification is confirmed by cytogenetic data, which indicated that each diploid has distinct genome (Simmonds 1976).

Of the tetraploid wheats, the primitive groups are *Triticum dicoccoides* and *T. timopheevii*. It is assumed that they were taken into cultivation about 8000 B.C. *T. dicoccoides* subsequently gave rise to cultivated emmer. Durum presumably originated from emmer by an accumulation of mutations that reduced the toughness of the glumes to the point that free-threshing was attained (Morris and Sears, 1967).

Except the *carthlicum* group, all other tetraploid wheats may be regarded as variant types of recent origin. The *turgidum* group differs little from the *durums*. The *polonicums* are mainly distinguished by their long glumes (Morris and Sears, 1967).

At the hexaploid level, the principal differences among groups of cultivated varieties are due to single genes, with the exception of the Vavilovi group. The *aestivum* group arose from *spelta* by mutation of q to Q; the origin of the *compactum* group of varieties from *aestivum* involved only a single mutation from c to C; and to produce the *spaerococcum* wheats, only a single mutation S to s in *aestivum* was required. No variety of *T.tauschii* has been found that carries either C or s. Therefore, neither the *compactums* nor the *sphaerococcums* could have been the first hexaploid to arise. As between the *spelta* and *aestivum* groups, the evidence indicates to the *spelta* as being the older. A *spelta* type, for example, is produced whenever either the wild tetraploid *dicoccoides* or its cultivated derivative, the *dicoccum* group, is crossed with *T. tauschii* and the chromosome number doubled (Morris and Sears, 1967).

Wheat evolution in Ethiopia is restricted to tetraploid wheats, As the wild progenitors and near relatives are absent, the diploid (*T. monococcum*) is not grown and the hexaploid wheat is recently introduced, probably by the Portuguese or the Italians (Hailu, 1991). According to Feldman (1976, cited in Tesfaye and Getachew 1991), the cultivated tetraploid (*T. dicoccum*) is assumed to be introduced by the Hamites 5000 years ago. The first free threshing tetraploid wheat (*T. durum*) is also expected to be cultivated in the country since that time or before.



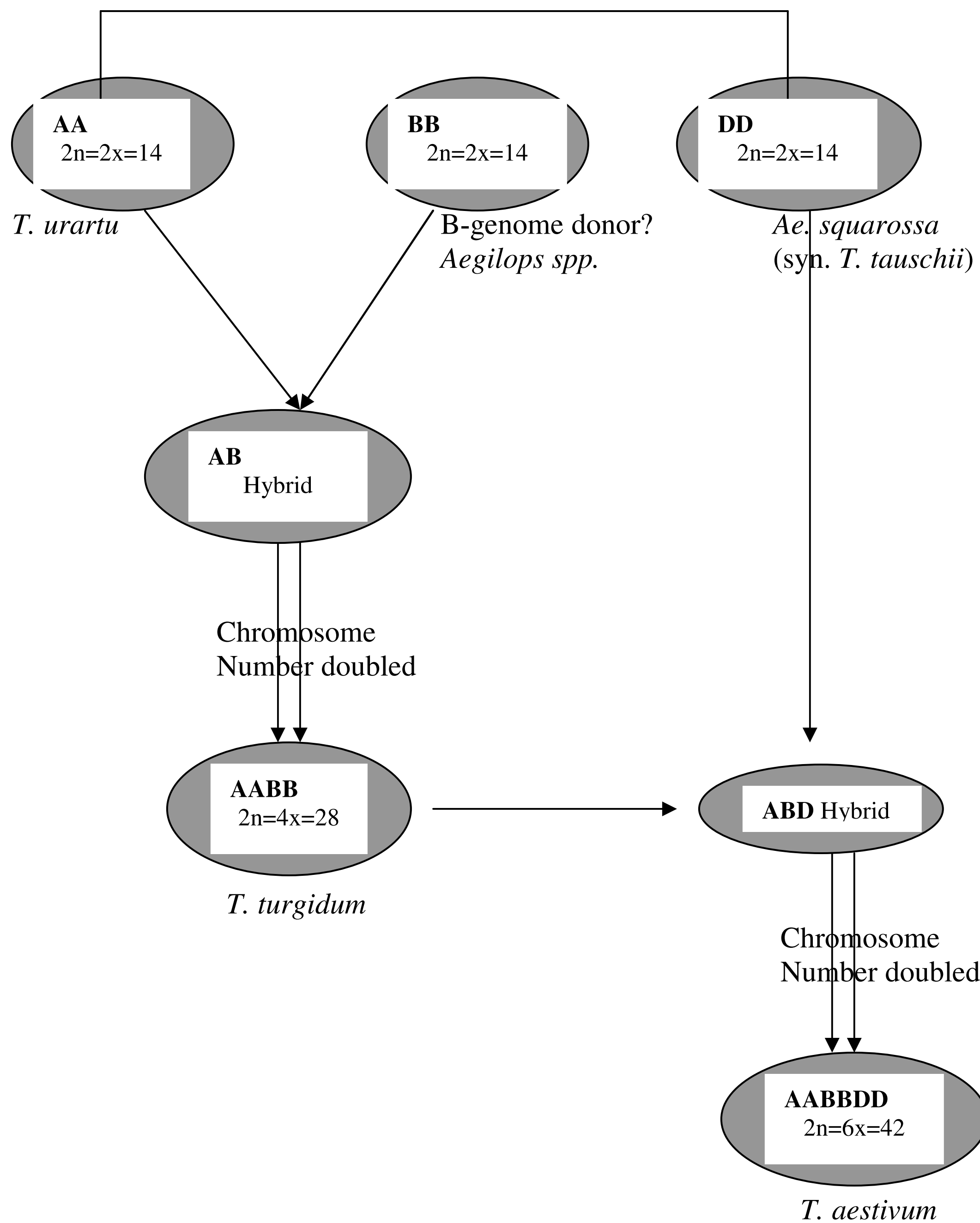


Fig. 2.1. Proposed origins and relationships of wheat. Each capital letter represents a genome composed of seven chromosomes (after Kerby & Kuspira, 1987)

Table 2.1 The group of varieties (cultivars) of the genus *Triticum* (Morris and Sears, 1967)

Species	Varietal Group	Formula (genome)	Common name

Triticum monococcum L.	----	A	Einkorn
T. turgidum L.	*dicoccon*	AB	Emmer
	durum	AB	Durum
	turgidum	AB	Poulard wheat Branched wheat
	polonicum	AB	Polish wheat
	carthlicum	AB	Persian wheat
T. timopheevii (Zhuk;) Zhuk.			
Var. timopheevii	-----	AG	None
Var. Zhukovskyi (Men.&Er.)	-----	AG	None
T.aestivum L. em Thell.	*Spelta*	ABD	Spelt
	Vavilovii	ABD	None
	Aestivum	ABD	Common wheat
	Compactum	ABD	Club wheat
	Sphaerococcum	ABD	Shot wheat

2.2 Wheat Genetic Resources in Ethiopia

Ethiopia is recognized as one of the world's most important centers of crop genetic diversity. It is often referred to as a major Vavilovian gene center. Earlier introduction of some Mediterranean crops and centuries of natural and artificial selections on native crops has resulted in tremendous genetic diversity. The major crops in which valuable genetic diversity has been observed include cereals, pulses, forage and oil crops. Ethiopia is center of origin for crops like anchote [*Coccinia abyssinica* (Lam) Cogn.], Chat (*Cata edulis* Forsk), Coffee (*Coffe arabica* L), endod (*Phytolaca dodecandra* L'Herti) etc. From the cereals, tetraploid wheat is a prominent crop for which the country is recognized as the secondary center of diversity (Abebe and Giorgis, 1991).

From all *Triticum* accessions (12,000) conserved in the Institute of Biodiversity Conservation (IBC), seventy two percent are tetraploid wheat (Abebe and Giorgis, 1991). The first six

species, out of the following eight identified from the collected germplasms, are tetraploid types; *T.durum, T.turgidum, T. dicoccum, T. polonicum, T. pyrimidale, T. aethiopicum, T. aestivum,* and *T. compactum* (Tesfaye and Getachew, 1991).

Besides, the number of species identified in *Triticum*, reports about within species genetic diversity are interesting. Different morphological and agronomic trait studies, on various populations from different regions, documented the extent of variation for a number of traits. Glume color, awn color, beak length, spike density, seed color, seed shape, glume pubescence, awn size, straw strength, stem structure, spike fertility, head width, and spike length are some of the characters with high genetic variation (Bechere *et.al*, 1996; Tsegaye *et.al*, 1996; Tesfaye *et.al*, 1991; Negassa, 1986; Bekele, 1984; Jain *et.al*, 1975). Spike morphological diversity samples in the four tetraploid wheat species included in the study are shown on Fig. 2.2. Similar diversity studies using various protein (Tsegaye *et al*. 1996, Tsegaye and Tessema, 1996; Tsegaye *et al*., 1994; Abebe, 1990; Negassa, 1986b; Dominici *et al*, 1988;) and cytogenetic (Tesfaye 2001; Belay *et al*., 1997) analyses also substantiate the availability of high genetic diversity in Ethiopian tetraploid wheat landraces.

Fig. 2.2 Spike samples of Ethiopian tetraploid wheat species: 1= *T. turgidum,* 2= *T. aethiopicum*,3= *T. durum,* 4= *T. polonicum.*

2.3 Marker Systems Used for Genetic Study

2.3.1 Morphological and Biochemical Markers

Morphological markers are the efficient and accurate markers to study wheat diversity as reported by Tesfaye (2001). They have been successfully used for estimating diversity, classification and cultivar identification.

Assays for protein markers are of different kinds like those that differentiate immunological properties of proteins or measure the rate of migration of protein molecules through a gel (Westman and Kresovich, 1997).

When proteins (biochemicals) are used as genetic markers, the assumption is made that any variation between proteins reflects heritable variation in their amino acid sequences. It is debatable, however, since protein phenotypes can be affected by factors such as post-translational modification, plant phenology, and environmental conditions during plant growth. The use of protein markers is also limited now-a-days for the lack of specific histochemical stains that react with most proteins. Moreover, isozymes have limited genome coverage as compared to nucleic acid markers (Coryell *et al.* 1999)

2.3.2 Molecular (DNA) Markers

Molecular markers range from highly conserved to hyper variable (Westman and Kresovich, 1997). The nucleic acids used as markers could be entire genomes, single chromosomes, fragments of DNA or RNA, and single nucleotides. Molecular markers are more important for genetic analysis than morphological traits for they are unlimited in number, entirely heritable, and not tissue or stage specific. They are, with exponential increase in number and wider application than initially thought, being used successfully.

2.3.2.1 Classification of DNA Markers

Since 1980, the discovery of the first DNA marker (RFLP), a number of marker types and assay techniques have been developed and different authors classify these markers differently. Some of the classifications were based on generation time (Koebner et al. 2001) and experimental strategy (Caetano-Anolles, 2001). According to the classification based on generation time, RFLP and RAPD markers are grouped in the first generation marker systems; markers like Amplified Fragment Length Polymorphism (AFLP), Sequence Tagged Sites (STS), Sequence-Tagged Microsatellite Site (STMS), Inter-Retrotransposone Amplified Polymorphism (IRAP) and Retrotransposone-Microsatellite Amplified Polymorphism (REMAP) are included in the second; and all Single Nucleotide Polymorphism (SNP) markers in the third generation marker systems (Koebner *et al*., 2001). According to experimental strategy markers are classified as hybridization based analysis (eg. RFLP), amplification based nucleic acid scanning (eg. AFLP), amplification based nucleic acid profiling (eg. STMS), and sequence-targeted techniques (eg. Oligonucleotide arrays) (Caetano-Anolles, 2001).

2.3.2.3 Uses of Nucleic Acid Markers

During the advent of RFLP marker, the 'three major areas of potential marker utilization', were defined as; varietal and parentage identification, identification of genetic loci affecting quantitative economic traits (QTL), and genetic improvement programs, including screening and evaluation of germplasm resources, introgression, improvement of commercial hybrids and within population selection (Soller and Beckmann 1983 in Koebner *et al.*, 2001). However, with subsequent introduction of other marker systems, its application widened to plant genome analysis, plant ecological research, and evolutionary biology (Caetano-Anolles, 2001). Apart from these applications in the plant system, Myakishev *et al.* (2001) and Ellis (2000) also discussed the potential uses of markers, particularly the SNPs, in pharmacogenetics and medical diagnostics.

Ever since molecular markers became available in appreciable numbers, a prominent application has been in the assessment of genetic diversity. Different diversity studies to assess ecogeographical distribution (Kalendar *et al.* 1999b), to test whether the efficiency of breeding programs could be increased by using marker genotyping to predict the prospects of individual crosses for line development before producing and testing lines derived from them (Bohn *et al.* 1999) were reported. Besides, it is also applied to test the common assertion that scientific plant breeding has led to a narrowing in crop diversity overtime (Reeves *et al* 1999; Donini *et al* 2000).

Marker validation, a process of evaluating the association of markers with the trait at different genetic backgrounds, is an important study before using any marker to actual work. It includes the selection of potential markers, identification of appropriate genotypes for the program, and evaluation of the effectiveness of the marker/trait linkage. As indicated by Gupta *et al.* (1999), marker index could be used to identify the potential markers from the available research. Based on marker index comparison, different marker systems are recommended for different purposes. Gupta *et al.* (1999) also suggested that the choice should also consider other factors like cost, convenience and technical feasibility.

Studies conducted at CIMMYT (International Wheat and Maize Improvement Center), to compare the cost effectiveness between conventional and marker assisted selection (MAS) breeding showed as the effectiveness depend up on the type of specific breeding objective. An empirical data collected from a conventional and marker (SSRs) assisted selection techniques in selecting individuals with recessive mutant allele, *opaque 2*, responsible for quality maize protein, indicated that MAS is more cost effective than conventional selection (Dreher *et al.* 2003). Another similar experiment to introgress a specific gene from a line to a high yielding cultivar using the same marker and conventional selection has shown that conventional selection is cost-effective but delayed two years to release the improved cultivar than MAS; a tradeoffs between time and money (Morris *et al*., 2003).

2.4 RAPD Markers

RAPD is 'the practice of PCR without a clue' (Wolf and Liston, 1998). It is the amplification of specific DNA region(s) using primers of arbitrary sequence (Williams et al., 1990; Welsh and McClelland' 1990). The primers are usually 10 nucleotide sequences with higher G+C content. In standard cases, only a single primer and a constant annealing temperature (generally 34-37^{0}C) are used. Therefore, the nucleotide region to be amplified is a sequence flanked by 'inward-oriented' sequences complimentary to the primer and not more than 3000bp apart. The resulting PCR products are generally resolved on 1.5-2.0% agarose gels and stained with ethidium bromide (EtBr). Sometimes polyacrylamide and Silver staining are used. The variations in the amplified products are due to nucleotide(s) changes (mutation) on primer binding sites. Also, the fragment length could vary if there are insertion/deletions in the region flanked by the primer sites (Wolf and Liston, 1998).

The advantages of RAPD marker for genetic analysis are that it does not require prior sequence knowledge, small amount of DNA can be used, requires a minimal amount of laboratory supplies and equipment, detection is radioactive free, and technically simple. Moreover, a large number of potential markers can be generated using readily available primers.

Apart from reproducibility problems, the basic limitations of RAPD markers are deviations from the expectations of strict Mendelian inheritance and problems of homology assessment. Artifactual, non-genetic, variation; organellar bands; and epigenetic interactions may cause the

deviations from Mendelian inheritance expectations. After electrophoresis separation, fragments located in a band are assumed results of homologous loci. However, based on a number of studies, fragments of different loci may co-migrate (Pillay and Kenny, 1995). Reproducibility in RAPD is reported to be improved by using pairs of primers, increasing polymerase concentration, slow ramping from annealing to the extension temperature, and careful control of DNA quality and amplification conditions (Parker *et al*, 1998; Wolf and Liston, 1998).

In wheat, the application of RAPDs is limited owing to the low level of polymorphism and reproducibility problem. The nature of the wheat genome may be favoring the monomorphic amplification of repetitive site fragments by single arbitrary primers (Penner, 1996). However, in polyploid species like wheat the low level of polymorphism and reproducibility are reported to be improved by using genomic DNA enriched for low-copy sequences (Gupta et al., 1999).

2.4.1 Diversity Studies

Applications of RAPD markers for genetic diversity analysis has been reported in a number of crop species; eg. *Vicia* (Potokina *et al*., 1999), *Brassica oleracea* L. (Divaret *et al*., 1999), red clover (Kongkiatngam *et al*., 1996), and *Arachis* L. (Santos *et al*., 2003), where it was reported as an efficient and reliable marker. It has been also employed in the past successfully for genetic diversity analysis in wheat (Castagna *et al.,* 1997; Pujar *et al.,* 1999; Joshi and Nguyen, 1993; Vierling and Nguyen, 1992). There is no genetic diversity analysis report about Ethiopian wheats from RAPD data, but microsatellite and AFLP markers study indicated their

reliability in estimating diversity, and the presence of relatively high genetic variation in tetraploid landraces (Tesfaye, 2001; Sintayehu *et al.,* 2004).

2.4.2 Genetic Relationship Studies

Even if different researchers consider RAPD markers as unsuitable for relationship study, a lot of successful applications have been reported (Landry *et al.*, 1994 in Wolf and Liston, 1998). Parker *et al.* (1998), for example, indicated "RAPD markers application to phylogeny is not as constrained by the occasional artifactual bands as is their application to studies of parentage". However, they stressed the need to sample both more individuals (2-10 times) and more loci to accommodate for the lack of complete genotypic information caused by dominance. Fahima *et al* (1999), Millan *et al* (1996) and Hoey *et al* (1996) also noted the relationship study to focus on intraspecific and among closely related species level, since an increase in genetic distances decreases the probability of equating fragments size with homology.

Scoring of codominant alleles, heteroduplex bands, or repetitive markers as independent loci can result in an overestimate of relatedness (Smith and Devey, 1994). Codominant inheritance results when RAPD polymorphism resulted from insertion/deletions in the amplified sequences, and both bands are resolved. Heteroduplex bands or repetitive markers, on the other hand, are formed when multiple priming sites within a locus produce several non-independent markers (Smith *et al.*, 1994; Hilu and Stalker, 1995).

Besides other species/genus like *Arachis* L. (Santos *et al*, 2003), *Cicer* (Ahmed, 1999), *Pisum* (Hoey *et al.*, 1996), *Rosa* (Millan *et al.*, 1996), RAPD markers have been successfully used for phylogenetic relationship studies in wheat. Nagaoka and Ogihara (1997) found that genetic

relationships estimated by Inter Simple Sequence Repeat (ISSR) markers were identical with those inferred by RFLP and RAPD markers.

III MATERIALS AND METHODS

3.1 Plant Materials

A total of 40 tetraploid wheat genotypes were used in the study (Appendix 1). Fifteen of them were improved durum wheat (*T. durum*) cultivars released in the country until 2003. The remaining (25) genotypes were landraces collected from Wello, Shewa and Bale regions. From the Wello collections three durum, three turgidum (*T. turgidum*) and one- polonicum (*T. polonicum*) species were represented. Whereas from Bale, three genotypes each of aethiopicum (*T. aethiopicum*) and durum species were included. The remaining 12 genotypes were from Shewa representing the four wheat species; durum, aethiopicum, turgidum and polonicum, three genotypes from each species. A hexaploid species (*T. aestivum*, cv. 'Simba') was also included in the study as a control.

Except the four *T. polonicum* genotypes, all the landraces were collected in 2000 by the Debre-Zeit Agricultural Research Center (DZARC), National Durum Wheat Improvement Project staff. Polonicum genotypes (one from Wello and three from Shewa) were obtained from Institute of Biodiversity Conservation (IBC). The seeds of these landraces were rejuvenated in 2000/01 main-season at Akaki substation of DZARC.

All improved cultivars, except DZ-04-118 (Arendeto), are introductions from abroad. They are derived from either crosses, made in the country or directly selected from introduced

germplasm. DZ-04-118 is a cultivar developed by mass-selection from landrace collections (Table 3.1). Each genotype of landraces represents a population in the collection. Populations from the total collection and a genotype within the selected population were selected randomly. A maximum of three genotypes were represented from each wheat species available in each region. Five to seven seeds from spike samples of each landrace genotypes and improved cultivars were grown in a pot. Seedlings were grown in a lathhouse at IBC.

Table 3.1 List of Improved Durum Wheat cultivars, year of release, origin and their Pedigrees.

No.	Variety Name	Year of release	Origin	Pedigree
1	Arendeto	1966	Ethiopia	DZ-04-118
2	Gerardo	1976	CIMMYT	VZ 466/61-130xGII" s", CM9605
3	Cocorit 71	1976	CIMMYT	RAE/4* TC 60// STW 63/3/ AA"S" , DZ 27617-18M-64-OM
4	Ld 357	1979	USA	Ld 357/CI 8155 No. 58-40
5	Boohai	1982	CIMMYT	Coo"s" / CII, CD 3062-BS-1BS-OGR
6	Foka	1993	Ethiopia	Cit 71/CII, CD 3369
7	Kilinto	1994	Ethiopia	Illumillo/ Inrat 69 // BHA /3/ Hora/4/ Cit 71/ Joro, DZ 918
8	Bichana	1995	Ethiopia	Illumilo/ cocorit 71, DZ 393-2
9	Arsi Robe (Tob 66)	1996	CIMMYT	Reichenbacci /Ld 357 // 357/3/ duck"s" /yel
10	Quamy	1996	Ethiopia	?, CD 75533-A
11	Asassa	1997	Ethiopia	Cho"s"/Tarus// yav"s" /3/Fg"s"/4/ Fg"s" /cr"s" /5/Fg"s"Dom"s" /6/ Huis, DZ 2085
12	Robe (DZ 1640)	1999	Ethiopia	Hora/cit"s"//Jo"s"/Gs"s"/3/some" s"/4/hora/Respinegro//CM19908/ 3/Rahum
13	Ginchi (DZ1050)	2000	Ethiopia	Boohai /ULNU
14	Yerer (CD 94026-4Y)	2002	CIMMYT	Chen / Tez /3/ Guil // cII
15	Ude (CD 9524-2Y)	2002	CIMMYT	Chen / ALTAR 84// Ald

3.2 DNA EXTRACTION

Young leaves from 30 to 45-days old seedlings were used for genomic DNA extraction. Five to seven of the youngest leaves from each pot, depending up on the number of seeds germinated, one-leaf from each plant, were pooled to represent each genotype. Leaf samples, collected and labeled in sealed plastic bags, were stored at -20^{0}C until grinding.

The extraction procedure was a Miniprep DNA-isolation (modified as multiple-extraction) developed by ‘Botanisches Instistute, Universitat Bonn, Molekularsystematik-Labor'. The brief description of the protocol is as follows.

0.2 vol% Mercaptoethanol added to CTAB solution (3x700µl per sample) heat up to 65^{0}C in water bath (Clifton unstirred bath). In a 100mg fresh leaf material per sample pulverized thoroughly in mortar with liquid nitrogen, 700 µl warm CTAB solution was added and incubated for 30min. at 65^{0}C. The supernatant then aspirate in a new eppendorf after 5min centrifugation at 15000rpm. New CTAB solution (700µl) then added to tissue pellet in original extraction cap and re-incubated for 30 min at 65^{0}C. Similar centrifugation and supernatant aspiration steps repeated from the same leaf material for the 2nd and 3rd extraction. 600µl chloroform then added to the supernatant, carefully shacked for at least 15 min, centrifuged for 5 min at 15000 rpm and the supernatant pipeted in a new eppendorf-cap. This chloroform extraction repeated once. Half the volume (of the supernatant) cold isopropanol (4^{0}C) added, shacked carefully upside-down and freeze for more than 2h at -20^{0}C. Then after 10min centrifugation at 15000 rpm, the liquid poured out leaving the solid pellet in the tube. 200µl of 70% ethanol then added to the pellet centrifuge for 10min at 15000rpm in a cooled (4^{0}C) centrifuge. The DNA- pellet dried at room temperature, after aspirating the ethanol.

Further to clean the extract, the pellet resuspended in 100µl TE(1x), stored at 4^0C for sometime, half volume cooled (4^0C) 7.5M NH_4Ac-solution and two volumes of cooled 100% ethanol were added and carefully mixed in each step, then the mixture allowed to freeze for more than 2h at -20^0C. After 30min centrifugation at 15000 rpm in cold centrifuge and aspirate the liquid, 200µl of 70% ethanol was added and rinsed the inner cap surface of the cap. Separating the liquid after 10min centrifuge at 15000 rpm, the pellet dried at room temp and resuspended in 100µl TE (1x). The cleaning steps repeated by replacing the 7.5M NH_4Ac-solution with 3M NaAc-solution. Finally the room-temperature-dried pellet, recovered after 10min centrifugation at 15000rpm and aspirating the liquid, resuspended in 50µl TE, and quality tested both on agarose-gel and spectrophotometer.

The quality and concentration of the extracted genomic DNA were determined by agarose-gel and spectrophotometer reading. The DNA sample separated by a 1.5% agarose-gel containing Ethidium Bromide- EtBr (0.5mg/µl) run for 20 min at 90 volt. The quality of the extract was determined by the intensity of the separated bands observed under the UV-transilluminator. Furthermore, the stock DNA concentration was determined by measuring samples in spectrophotometer. For further analysis, working solutions of the DNA were prepared based on the spectrophotometer reading. Of the three DNA extracts from each leaf sample (genotype), the one with better quality was used for further work.

3.3 PCR AMPLIFICATION AND ELECTROPHORESIS

A total of six oligonucleotide primers from Operon Technologies (Almeda, California, USA) were used for PCR amplification (Table 3.1). Genomic DNA samples were amplified using eppendorf Mastercycler gradient. A total of 25µl reaction mix composed of 6.2 µl of 10ng/µl Primer, 25ng of genomic DNA, 10µl of 80nM total dNTPs, 1U of Taq polymerase, and 1x reaction buffer (75mM Tris-HCl, pH 8.8, 20mM $(NH_4)_2SO_4$, 0.1%(v/v)Tween). The master cycler programmed first for 3 min to a denaturing temperature of 94^0C to ensure sufficient genomic DNA denaturation, and last for 10 min to 72^0C a final extension to bring as large a proportion of the amplified products to full length as possible. In between 45 cycles at 94^0C for 1min, 37^0C for 1 min and at 72^0C for 2mins were programmed followed by 4^0C to cool down the reaction products until electrophoresis or cold storage.

Amplified products were electrophoresed in a 0.8% agarose gel containing EtBr (0.5mg/ml). However, initially 1.5% agarose was used that resulted poor separation. The entire reaction product in an eppendorf plus 5µl of loading buffer (0.12%BfB, 30% glycerol and water) was loaded in each well. In a 1x TAE running buffer, the electrophoresis run for 2 hrs in 90 volt. Each gel was used to separate 15 genotypes sparing one well for marker. The marker was a 100bp ladder.

Table 3.2 List of the six primers tested to generate RAPD marker in 40 wheat genotypes

No.	Name of the primer	Sequence 5' to 3'
1	OPA-13	CAGCACCCAC
2	OPC-02	GTGAGGCGTC
3	OPA-17	GACCGCTTGT
4	OPA-08	GTGACGTAGG
5	OPA-09	GGGTAACGCC
6	OPA- 20	GTTGCGATCC

3.4 DATA SCORING AND ANALYSIS

The data were scored as present -“1” or absent -“0” of a band at a specific position in a gel with reference to base-pair ladder. Each band position is considered as a particular locus. All detectable bands were scored. The length (base pair) of each fragment in the amplified product was determined with reference to the marker ladder. The bands in a gel were scored at least two times independently, and those consistently scored bands were used for further analysis.

To estimate the variation within and between species, and within and between regions of origin Shannon-Weaver diversity index (H) was calculated as:

$$H = -\sum_{i=1}^{n} pi \log pi$$

$$H' = -\sum_{i=1}^{n} pi \log pi / \log n$$

Where *pi* is the proportion of the total number of entries in the ith region of origin or i^{th} species and *n* is the number of phenotypic classes for a particular character. H' was calculated for each locus and for all genotypes, by dividing H by the logarithm of the number of locus classes.

Pair-wise genetic similarity/distance matrices were generated among the 40 genotypes, regions of origin, species, and durum cultivars using Nei's (1978) unbiased measures of genetic identity and genetic distances using PopGen Statistical software, or Jaccard Similarity Coefficient.

Nei's genetic distance was calculated as:

$$D_{ij} = -\ln\left[G_{xy} / \sqrt{G_x G_y} \right]$$

Where G_x, G_y, and G_{xy} are the means of $\sum p_i^2$, $\sum q_i^2$ and $\sum p_i q_i$ overall loci in the genome, respectively.

Jaccard Similarity coefficient was calculated as:

$$S_{ij} = \frac{a}{a+b+c}$$

Where *a* is the total number of bands shared between accessions *i* and *j*; *b* is the total number of bands present in accession *i* but not in accession *j*; and *c* is the total number of bands present in accession *j* but not in accession *i*. The main feature of this similarity coefficient is

that it does not consider negative matches. Since the absence of a particular RAPD band may have several causes, it is reasonable not to consider similarity arising from mutual absence of bands.

Phenogram was produced using unweighted pair-group method arithmetic average (UPGMA) clustering. The program NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System),version 1.8 (Rohlf, 1993) was used for generation of the distance matrix, and UPGMA clustering employing the SAHN (Sequential, Agglomerative, Hierarchical and Nested clustering) for the whole genotype, landrace genotypes and improved durum cultivars.

IV RESULTS

4.1. Diversity analysis

Only two of the six primers (Table 4.1) showed polymorphism and were used in the analysis. The first primer (OPA-13) produced 17 bands of which 16 (84.12%) were polymorphic. All of the 14 bands scored from OPC-02 primer were polymorphic. The molecular weight of the fragments amplified by these OPA-13 and OPC-02 primers ranged 290-1640, and 100-1500 bp, respectively (Table 4.1). The number of bands amplified in each species ranged from 15 (in *T. polonicum* and *T. turgidum*) to 19 (in *T. durum*) with a mean of 16.25 and total number of bands 31. Similarly, the number of bands per collection region also ranged from 12 (Shewa) to 17 (Wello) with a mean of 15 (Table 4.2). Samples of RAPD gels obtained during the study in the improved cultivars and landraces are shown in Figs. 4.1, and 4.2.

Table 4.1 Amplified products of the two primers used to generate RAPD markers in 40 wheat genotypes

No.	Name of the primer	No. of amplified products	Nucleotide fragment size (bp)
1	OPA-13	17	290-1640
2	OPC-02	14	100-1500

Table 4.2. Number and percentage of polymorphic bands for four species of tetraploid

wheat and of their three regions of origin.

Category	OPA-13	OPC-02	Total (%)
Species:			
T. aethiopicum	6 (35.3)	10 (71.4)	16 (51.6)
T. durum	11 (64.7)	8 (57.1)	19 (61.3)
T. polonicum	9 (53)	6 (42.9)	15 (48.4)
T. turgidum	8 (47.1)	7 (50.0)	15 (48.4)
Mean	8.5	7.8	16.3 (52.4)
Entire Bands	17	14	31
Region:			
Bale	6 (35.3)	10 (71.4)	16 (51.6)
Wello	11 (64.7)	6 (42.9)	17 (54.8)
Shewa	6 (35.3)	6 (42.9)	12 (38.7)
Mean	7.7	7.3	15 (48.4)

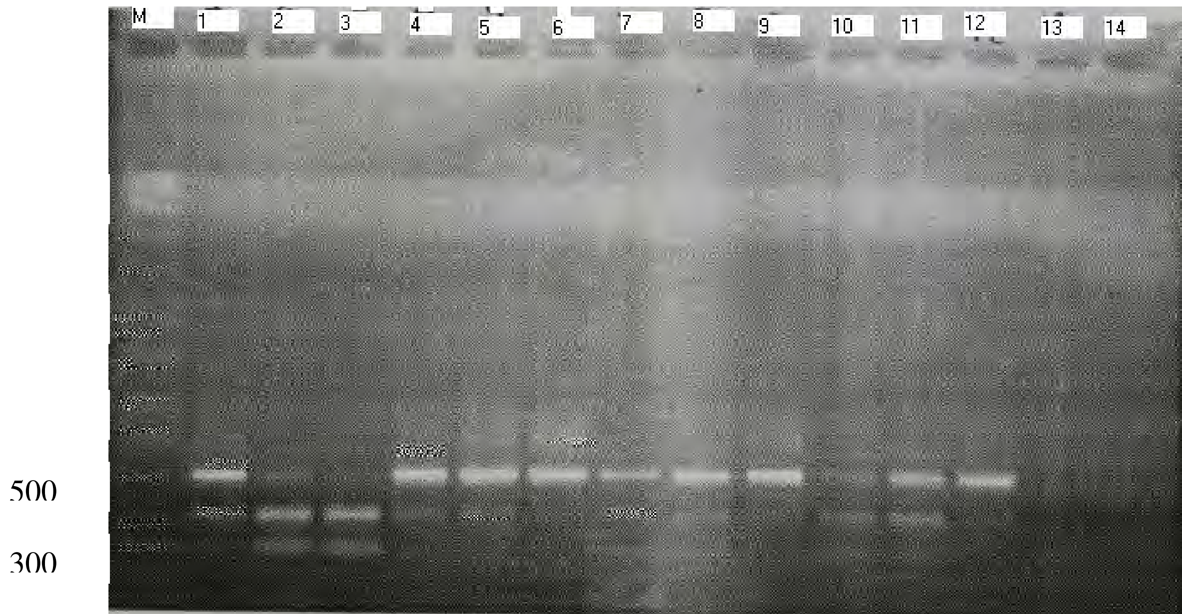


Fig 4.1 Amplification products from genomic DNA of 14 improved durum cultivars amplified with primer OPA-13. The molecular weight marker (100 bp ladder) is shown in the left lane (M). *1= Tob 66, 2= Yerer, 3= DZ 1640, 4= Asassa, 5= Boohai, 6= Quamy, 7= Ude, 8= Cit 71,9= DZ 04-118, 10= Bichena, 11= Gerardo, 12= Foka, 13= Kilinto, 14= negative control*

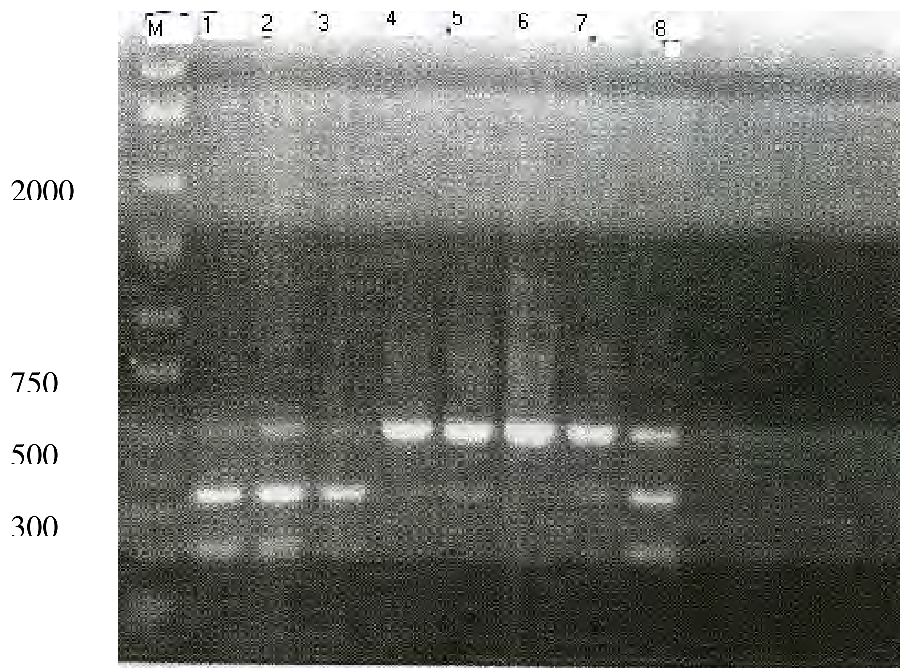


Fig 4.2 Amplification products from genomic DNA of 8 tetraploid wheat landraces amplified with primer OPA-13. The molecular weight marker (100 bp ladder) is shown in the left lane (M). *1= Bae1117, 2= Wed919, 3= Simba, 4= Shd7618, 5= Bad27, 6= She 52110, 7= Wet1570, 8= Shp214370*

4.1.1 Regional Diversity of Landraces

The estimate of diversity of regions for each primer is shown in Table 4.3. The Shannon information index (H) within regions of origin varied from 0.22 for Shewa to 0.27 for Wello (Table 4.3). The extent of mean diversity of the regions pooled over the two primers was 0.26.

Partitioning of the total variation into the within and between regions indicated that a large portion of the total variation 72.2% was found within regions of origin (Table 4.4). The remaining (27.8%) of the variation was between regions.

Table 4.3 Estimates of the Shannon-Weaver diversity index (H) for four species of tetraploid wheat and of their three regions of origin.

Category	OPA-13	OPC-02	Mean ± SE
Species:			
T. aethiopicum	0.18	0.35	0.27 ± 0.08
T. durum	0.37	0.27	0.32 ± 0.05
T. polonicum	0.30	0.23	0.26 ± 0.03
T. turgidum	0.27	0.28	0.27 ± 0.01
Mean	0.28	0.29	0.29 ± 0.01
Entire data	0.35	0.33	0.34 ± 0.05
Region:			
Bale	0.20	0.33	0.26 ± 0.06
Wello	0.33	0.22	0.27 ± 0.05
Shewa	0.22	0.23	0.22 ± 0.01
Mean	0.25	0.26	0.26 ± 0.04

4.1.2 Species Diversity of Landraces

The extent of genetic variation within and among species was determined using Shannon-Weaver diversity index (Table 4.3). The diversity index ranged from 0.18 (*T. aethiopicum*) to 0.37 (*T. durum*), for OPA-13 primer. Likewise, for OPC-02 primer it ranged from 0.23 in *T. polonicum* to 0.35 in *T.aethiopicum*. The respective mean values of the species for the two primers were 0.28 and 0.29. The overall mean diversity index pooled over the two primers for species was 0.29.

Partitioning of the variation into within and among species revealed that 81.4% of the total variation was found within species and the remaining 19.6% among species, indicating limited differentiation among the species. The within species variation measured by the two primers, OPA-13 and OPC-02, was 75.6% and 87.3%, respectively (Table 4.4).

4.1.3 Diversity within improved durum wheat cultivars

In the improved cultivars OPA-13 and OPC-02 primers separately showed 4 (23.5%) and 11 (78.5%) polymorphic loci, respectively. Therefore, the two primers in total amplified 15 (48.39%) polymorphic bands (Table 4.5).

The Shannon-Weaver diversity index for the improved durum cultivars varied from 0.13 to 0.33 for OPA-13 and OPC-02, respectively. The mean diversity estimate showed limited (0.23) diversity within improved varieties (Table 4.5).

Table 4.4 Partitioning of genetic variation into within and between species as well as within and between regions of origin of the landrace collections.

Primer	Species				Region		
	H_{TOTAL}	$H_{SPECIES}$	H_{SPE}/H_{TOT}	$(H_{TOT}-H_{SPE})/H_{TOT}$	H_{REGION}	H_{REG}/H_{TOT}	$(H_{TOT}-H_{REG})/H_{TOT}$
OPA-13	0.372	0.281	0.756	0.245	0.251	0.673	0.327
OPC-02	0.333	0.291	0.873	0.127	0.259	0.777	0.223
Mean	0.353	0.286	0.814	0.186	0.255	0.722	0.278

Table 4.5 Estimates of the Shannon-Weaver diversity index (H), and number and percentage of polymorphic bands (P) for 15 improved durum wheat cultivars.

H			P		
OPA-13	OPC-02	Mean ± SE	OPA-13	OPC-02	Total
0.1274	0.3263	0.2268 ± 0.09	4 (23.53)	11 (78.57)	15 (48.39)

4.2 Relationship Analysis

4.2.1 Cluster analysis

Cluster analysis of the 40 tetraploid genotypes group individuals mainly into improved cultivars and landraces (Appendices 2 and 3). Pair-wise genetic distances among the landraces and the improved cultivars are presented separately in Tables 4.6 and 4.7.

The lowest genetic distance (0.032) for improved cultivars was found between Ld357 and Boohai, Foka and Kilinto, and Tob-66 and DZ1640 cultivars. The highest coefficient of genetic distance (0.343), on the other hand, was observed between Asassa and Bichena, Asassa and Boohai, and Bichena and Ude. The average genetic distance was 0.157, which may signify the presence of little diversity.

The lowest genetic distance (0.00) within tetraploid wheat landrace collections was found between Wet1224 and Wet1570 (both Wello turgidum entries), while the highest distance (0.73) between Wet 1650 and Bae 1117 (Wello-turgidum and Bale-aethiopicum entries, respectively). The mean genetic distance for germplasm collections was 0.26.

Cluster analysis of the 24 wheat landraces failed to group the individuals on species basis. However, region wise, groups and subgroups of the same origin were clearly observed. At about 70% similarity, four major groups were formed leaving four genotypes ungrouped (Fig 4.3). The first group was formed by all Wello genotypes. The second cluster grouped all (three) Bale genotypes. Both the third and fourth clusters contained all Shewa collections except a Bale genotype (Bad 27) grouped in the third cluster. The four ungrouped genotypes were Bae 664, Bae 1117, Wed 156 and Wed 919.

Pair-wise genetic distances among the four species revealed the lowest distance (0.016) between *T. polonicum* and *T. turgidum,* where as *T. aethiopicum* and *T. durum* species were the most distant (0.051) (Table 4.8).

The genetic distance between collections of the different regions indicated that collections from Bale and Shewa were with the least coefficient (0.073) where as those from Shewa and Wello were with the highest (0.18) (Table 4.9).

A dendrogram constructed for the improved cultivars based on Jaccard genetic similarity matrix and UPGMA method formed four clusters at about 80% coefficient. In these cluster groups cultivars, Ld-357, Boohai, Quamy and Bichena grouped in the first; and Ude and Yerer in the second. The third cluster contained Gerardo, Ginchi, Tob-66 and DZ1640 cultivars. DZ04-118, Foka, Kilinto and Cocorit-71 formed their own group. Asassa was the only durum cultivar found ungrouped at the indicated similarity coefficient (Fig. 4.6).

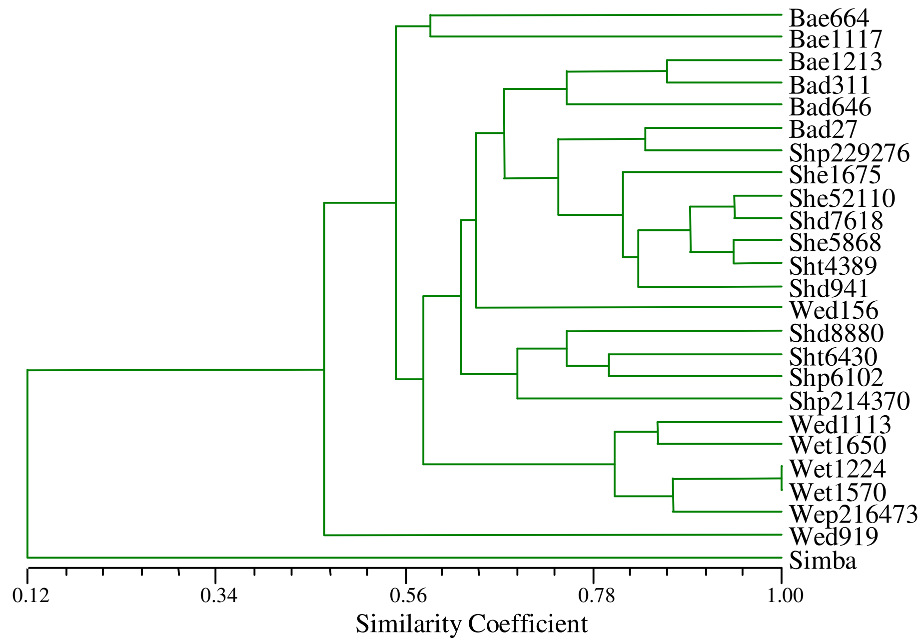


Fig. 4.3 Dendrogram for 24 landrace tetraploid wheat genotypes generated based on the unweighted pair group method with arithmetic averages (UPGMA) analysis of Jaccard similarity.

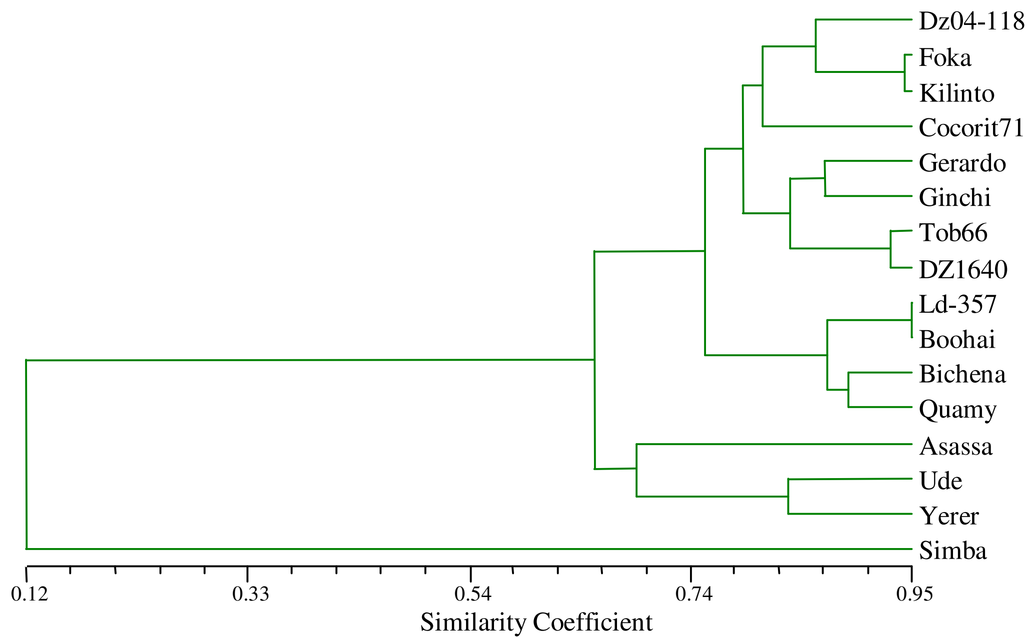


Fig. 4.4 Dendrogram for 15 improved durum wheat cultivars generated based on the unweighted pair group method with arithmetic averages (UPGMA) analysis of Jaccard similarity.

Table 4.6 Nei's genetic distance among 24 tetraploid wheat landraces

pop ID	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Bae664	**																							
Bae1117	0.34	**																						
Bae1213	0.29	0.43	**																					
Bad311	0.29	0.34	0.06	**																				
Bad27	0.38	0.34	0.13	0.21	**																			
Bad646	0.34	0.60	0.10	0.17	0.17	**																		
Wed156	0.34	0.48	0.25	0.17	0.25	0.13	**																	
Wed919	0.60	0.43	0.38	0.48	0.21	0.43	0.54	**																
Wed1113	0.25	0.60	0.10	0.17	0.25	0.13	0.29	0.43	**															
Wet1224	0.25	0.48	0.17	0.25	0.17	0.21	0.29	0.34	0.06	**														
Wet1570	0.25	0.48	0.17	0.25	0.17	0.21	0.29	0.34	0.06	0.00	**													
Wet1650	0.34	0.72	0.17	0.25	0.34	0.13	0.29	0.54	0.06	0.13	0.13	**												
Wep216473	0.34	0.60	0.25	0.34	0.25	0.21	0.29	0.43	0.13	0.06	0.06	0.06	**											
She1675	0.25	0.38	0.17	0.17	0.17	0.21	0.21	0.43	0.21	0.21	0.21	0.21	0.21	**										
She52110	0.29	0.34	0.21	0.21	0.21	0.25	0.25	0.48	0.34	0.34	0.34	0.34	0.34	0.10	**									
She5868	0.29	0.43	0.21	0.21	0.21	0.17	0.17	0.48	0.25	0.25	0.25	0.25	0.25	0.10	0.06	**								
Shd941	0.21	0.43	0.21	0.21	0.21	0.25	0.25	0.48	0.17	0.17	0.17	0.25	0.25	0.10	0.13	0.06	**							
Shd7618	0.25	0.29	0.17	0.17	0.17	0.29	0.29	0.43	0.29	0.29	0.29	0.38	0.38	0.13	0.03	0.10	0.10	**						
Shd8880	0.38	0.43	0.29	0.21	0.21	0.25	0.17	0.38	0.34	0.34	0.34	0.43	0.43	0.25	0.29	0.21	0.21	0.25	**					
Sht4389	0.25	0.38	0.25	0.25	0.25	0.21	0.21	0.54	0.29	0.29	0.29	0.29	0.29	0.13	0.03	0.03	0.10	0.06	0.25	**				
Sht6430	0.38	0.34	0.38	0.29	0.38	0.43	0.34	0.38	0.43	0.43	0.43	0.54	0.54	0.34	0.29	0.21	0.21	0.25	0.13	0.25	**			
Shp6102	0.34	0.38	0.25	0.17	0.25	0.29	0.21	0.54	0.29	0.29	0.29	0.38	0.38	0.21	0.17	0.10	0.10	0.13	0.10	0.13	0.10	**		
Shp214370	0.29	0.25	0.29	0.21	0.29	0.25	0.17	0.38	0.34	0.34	0.34	0.34	0.34	0.17	0.21	0.13	0.21	0.25	0.21	0.17	0.13	0.17	**	
Shp229276	0.34	0.29	0.25	0.25	0.10	0.21	0.21	0.34	0.38	0.29	0.29	0.38	0.29	0.13	0.10	0.17	0.25	0.13	0.25	0.13	0.43	0.29	0.25	**
Simba	0.60	0.79	0.72	0.60	0.72	0.66	0.43	0.48	0.54	0.54	0.54	0.66	0.66	0.66	0.86	0.72	0.60	0.79	0.38	0.79	0.38	0.54	0.48	0.79

**N.B. 1= Bae664, 2= Bae1117, 3= Bae1213, 4= Bad211, 5= Bad27, 6= Bad646, 7= Wed156, 8= Wed919, 9= Wed1113, 10= Wet1224, 11= Wet1570, 12= Wet1650, 13= Wep216473, 14= She1675, 15=She52110, 16= She5868, 17= Shd941, 18= Shd7618, 19= Shd8880, 20= Sht4389, 21= Sht6430, 22= Shp6102, 23= Shp214370, 24= Shp229276*

Table 4.7 Nei's genetic distance among 15 durum wheat improved cultivars

Variety	DZ04118	Cit71	Gerard	Ld-357	Boohai	Foka	Kilinto	Bichena	Quamy	Asassa	Tob 66	Ginchi	DZ1640	Ude	Yerer
DZ04-118	--														
Cit71	0.1018	--													
Gerardo	0.1759	0.2151	--												
Ld-357	0.1759	0.2151	0.2151	--											
Boohai	0.1382	0.1759	0.2559	0.0328	--										
Foka	0.0667	0.1018	0.1018	0.1018	0.1382	--									
Kilinto	0.1018	0.1382	0.0667	0.1382	0.1759	0.0328	--								
Bichena	0.1382	0.1759	0.2559	0.1018	0.0667	0.1382	0.1759	--							
Quamy	0.0667	0.1018	0.1759	0.1018	0.0667	0.0667	0.1018	0.0667	--						
Asassa	0.2559	0.2151	0.1382	0.2985	0.3429	0.1759	0.2151	0.3429	0.2559	--					
Tob 66	0.0667	0.1018	0.1018	0.1759	0.1382	0.0667	0.1018	0.1382	0.0667	0.1759	--				
Ginchi	0.1759	0.1382	0.0667	0.2151	0.2559	0.1018	0.1382	0.2559	0.1759	0.0667	0.1018	--			
DZ 1640	0.1018	0.1382	0.0667	0.2151	0.1759	0.1018	0.1382	0.1759	0.1018	0.1382	0.0328	0.0667	--		
Ude	0.2559	0.2985	0.1382	0.2985	0.3429	0.1759	0.2151	0.3429	0.2559	0.1382	0.1759	0.1382	0.1382	--	
Yerer	0.1759	0.2151	0.1382	0.2985	0.2559	0.1759	0.2151	0.2559	0.1759	0.1382	0.1018	0.1382	0.0667	0.0667	--
Simba	0.6614	0.6008	0.6008	0.8690	0.7949	0.6614	0.7259	0.7949	0.6614	0.4895	0.5436	0.4895	0.4895	0.3895	0.3895

Table 4.8 Nei's genetic disimilarity among four species of 24 wheat landraces

Species	*T.aethiopicum*	*T.durum*	*T.turgidum*	*T.polonicum*
T. aethiopcum	-			
T. durum	0.0514	-		
T. turgidum	0.0379	0.0271	-	
T. polonium	0.0264	0.0238	0.0161	-
T. aestivum	0.4971	0.2947	0.3508	0.3514

Table 4.9 Nei's genetic disimilarity among three regions of origin of 24 wheat landraces

Region	Bale	Wello
Bale	-	
Wello	0.1102	-
Shewa	0.0732	0.1802

4.2.2 Discriminant analysis

The discriminant analysis succeeded in differentiating significantly, on the basis of allele presence or absence, between almost all of the 24 genotypes. The summary of the results is presented in Table 4.10 and Fig 4.7. Locus-31 (OPC-02, bp. length 1500) was chosen as the best differentiating factor since it was the only variable with the largest absolute correlation in discriminant function 1. The correct classification of individual genotypes into their respective species was 95.8%. A graphical illustration of the first and second canonical discriminant functions, which represent 74.7% and 25.3% of the sum of the eigenvalues is displayed in Fig. 4.7. To classify according to their region, the following six loci numbers were also chosen as the best differentiating factors; 25, 22, 17, 26, 11 and 27 (Appendix 4). The correct classification of individual genotypes into their respective regions of collection was 100% (Fig 4.8). The first two cannonical discriminant functions were used in the analysis, which represent 77.4% and 22.6%.

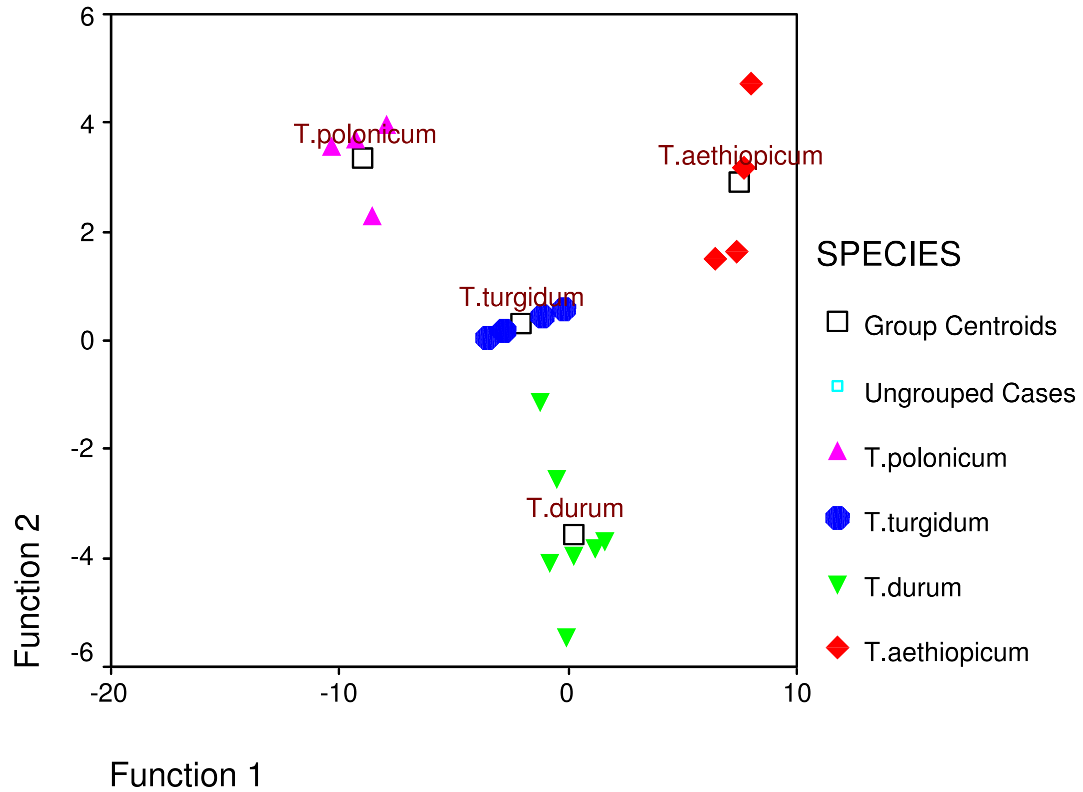


Fig 4.5 Plot of canonical discriminant functions 1 and 2 based on the 31 polymorphic RAPD loci; species classification.

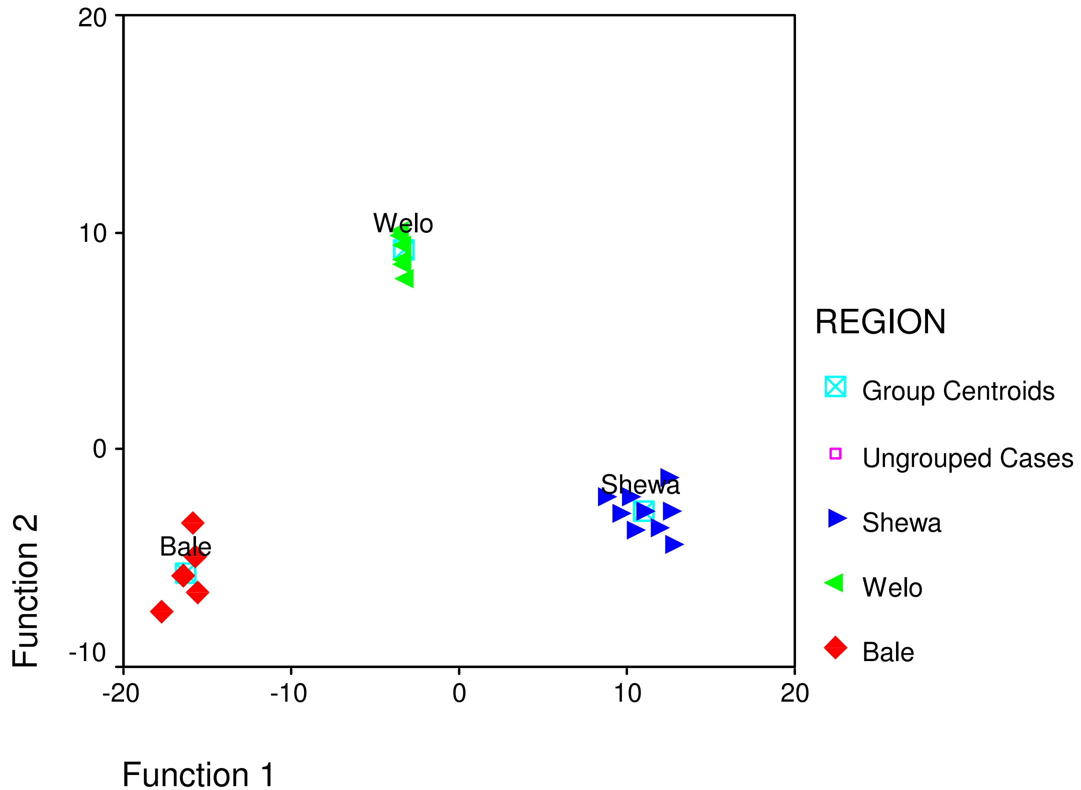


Fig 4.6 Plot of canonical discriminant functions 1 and 2 based on the 31 polymorphic RAPD loci; regional grouping.

Table 4.10 Classification results of 24 tetraploid wheat landraces on the basis of their species.

			Predicted Group Membership				Total
	Species		*T. ae*	*T. du*	*T. tur*	*T. pol*	
Original	Count (%)	**T. ae**	6 (100)	0	0	0	6 (100)
		T. du	0	8 (88.9)	1 (11.1)	0	9 (100)
		T.tur	0	0	5 (100)	0	5 (100)
		T. pol	0	0	0	4 (100)	4 (100)
A 95.8% of original grouped cases correctly classified							

* *T.ae= T. aethiopicum, T.du= T. durum, T. tur= T. turgidum, T. pol= T. polonicum*

V DISCUSSION

5.1 DIVERSITY OF TETRAPLOID WHEAT LANDRACES

The diversity estimates of tetraploid wheat landrace collections from the three regions of Ethiopia using RAPD markers in the present study were low. Sentayehu et al (2004) reported the presence of good genetic diversity in two of the Ethiopian tetraploid wheat landrace species (*T. durum* and *T. aethiopicum*) using wheat microsatellite markers. Similar studies at DNA level (AFLP and Microsattelite markers) as well as morphological markers by Tesfaye (2001) reported the availability of appreciable genetic variation (Ht= 0.50-AFLP, 0.55-Microsatellite, 0.80 morphology, and 0.77 storage proteins) from tetraploid wheat landrace collection samples. The low genetic variation observed in this study could be due mainly to the limited number of oligonuclotide primers used for analysis. Besides, it could be owing to the nature of the wheat genome which may favour the monomorphic amplification of repetitive site fragments (Penner, 1996). This result may be in agreement with tef [*Eragrostis tef* (Zucc.) Trotter], an allotetraploid crop, where a low level of polymorphism in tef genotypes using RAPD markers (Bai *et al*. 2000) but relatively higher genetic variation using ISSR markers (Assefa *et al*. 2003) are documented.

Liu *et al*. (1999) described the possibility to effectively separate even genetically and morphologically similar wheat genotypes using RAPD marker and denaturing gradient gel electrophoresis. However, Joshi and Nugyen (1993) and Maric et al. (2004), using the same marker, reported a high level of polymorphism and a high number of clearly amplified bands in wheat.

Genetic variation within species and regions was found higher, 81.4% and 72.2% respectively, than the variation observed among species and regions of origin. This result is in agreement with findings from wheat germplasm morphological marker analysis that reported 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 variation (Bekele 1983, 1984; Bechere et al. 1996). Similar results in other crop germplasm collected from Ethiopia like tef (Assefa et al. 2001) and Barley (Bekele 1983) are also documented. Hence the present data suggest the need to collect more wheat germplasm samples within regions with reasonable coverage of different regions either for conservation or breeding purposes.

5.2 DIVERSITY OF IMPROVED DURUM WHEAT CULTIVARS

The level of genetic diversity observed in improved durum wheat varieties was very low (H=0.23). This could probably be due to the common parents used to develop most of the cultivars. However, with the exception of a few pairs, cultivars sharing a common parent did not reveal relatively higher similarity coefficient. The similarity coefficient among all the cultivars varied from 62% to 95%. Similarly higher similarity coefficient among studied tef genotypes, ranged between 84% and 96%, using RAPD markers was reported by Bai *et al.* (2000). This higher similarity coefficient results could also be due to the possibility of overestimation of genetic similarity because fragments with the same size could have different origins, which could be the case particularly for wheat because of the complexity of its genome (Maric, 2004).

5.3 GENETIC RELATIONSHIP ANALYSIS

Clustering of the 40 genotypes formed two groups: improved cultivars and landrace collections at 57% similarity coefficient, although Bae 664 and Bae 1117 are grouped to improved cultivars and Wed 919 remain ungrouped. A similar analysis result on Indian wheat germplasms, by Pujar *et al*. (1999), succeeded to separate wild and cultivated genotypes, and among cultivated wheats distinct groups were formed by the durum cultivars, durum landraces and *T. dicoccum* cultivars. This analysis result generally indicates that all the 15 improved durum cultivars are relatively similar. DZ-04-118, an improved durum cultivar developed by mass selection from landrace collections, was found grouped to exotic improved cultivars, rather than to the landraces. Probably it could be due to the effect of gene flow since the cultivar has been cultivated with exotic genotypes/cultivars for nearly 40 years and gene flow is frequent even among tetraploid wheat species (Belay and Furuta, 2001).

The lowest genetic distance (0.00) in tetraploid wheat landraces was observed between two Wello genotypes of *T. turgidum* (Wet 1224 and Wet 1570) while the highest distance (0.73) between a Bale *T. aethiopicum* and Wello *T. turgidum* entries (Bae 1117 and Wet 1650). The mean genetic distance among germplam collections was 0.26. As expected, it is relatively higher than the mean distance value observed in improved durum cultivars.

In contrast to a report by Pujar et al. (1999), in which grouping of durum landraces was independent of their geographic distribution, clustering in the present study of tetraploid landraces was generally successful to group in to their regions. The presence of clustering

pattern showing trends of grouping based on origin is in agreement with the finding of Ayana and Bekele (1998) with Ethiopian sorghum. This result suggests the presence of genetic differentiation among regions.

Pair-wise genetic distances among species revealed the lowest value (0.016) between *T. polonicum* and *T. turgidum,* while the highest value (0.051) between *T. aethiopicum* and *T. durum* species. Similarly, the genetic distance between collections from Bale and Shewa were the lowest (0.073) whereas between those from Shewa and Wello with the highest (0.18). Since only three regions were assessed and the highest and lowest distance values were observed between two neighboring regions, it was difficult to conclude from the genetic distance analysis whether germplasm exchange between regions exist.

The discriminant analysis using the RAPD data succeeded in differentiating significantly between almost all the 24 individuals. The correct classification of individual genotypes into their respective species and regions was 95.8% and 100%, respectively. These results indicate that RAPD markers can be used to classify tetraploid wheats according to their respective species and regions based on multilocus analysis. In this result, classifying genotypes according to their region of origin was relatively better than to their respective species.

5.4 CORRELATION OF CLUSTER ANALYSIS WITH PEDIGREE OF CULTIVARS

An examination of the pedigree of the 15 durum cultivars used in this study revealed little correlation between the groups formed by the cultivars in the dendrogram and their parentage.

Five of the cultivars (Boohai, Foka, Kilinto, Ginchi and Yerer), for example, share at least one common parent (CII and/or Boohai) but only Foka and Kilinto were grouped in one cluster. Similarly, although Ld-357 is a parent for Tob-66, the two cultivars grouped in separate clusters. Such weak correlation between RAPD data and pedigree records in wheat were reported by several authors (Barrett *et al*. 1998; Maric *et al*. 2004). One of the several possible explanations for such weak correlation is that a parent contributes 50% of its genetic base to the offspring, however as a result of selection pressure or random genetic drift this ratio can considerably be different. Besides, sensitivity of the working conditions and equipments used can influence the results of RAPD analysis. However, three out of the four cultivars (Cocorit 71, Foka, Kilinto and Bichena), sharing a common parent (Cit71), were found in a group. Several authors also reported that cultivars form subgroups based on common parentage (Pujar *et al*. 1999).

VI Summary

Ethiopian tetraploid wheat landraces have high genetic variation; based on morphological, cytogenetic and protein analyses. On the other hand, improved durum wheat cultivars of the country released until 2003, seem to have limited diversity (from their pedigree records). The present study was conducted with the objectives to estimate the genetic variation within and among tetraploid wheat landraces collected from Wello, Shewa and Bale regions using RAPD data, and estimate the genetic relationships among tetraploid wheat species of *T. aethiopicum, T. durum, T. turgidum* and *T. polonicum*. Besides, the study had the aim to evaluate the genetic diversity in improved durum wheat cultivars using RAPD and compares the genetic similarity based on RAPD marker with pedigree (common parentage) data.

The Shannon diversity index (H) of the 25 tetraploid wheat landrace genotype samples was 0.34. The mean regional and species diversity indices were 0.29 and 0.26 respectively. The maximum value among species was observed in *T. durum* (0.32) whereas the minimum in T. polonicum species (0.26). Similarly, the maximum value among regions of origin assessed was recorded in Wello (0.28). Partitioning the total variation into within and among species and regions revealed that 81.4% and 72.2% of the variations were the within species and regions, respectively.

Cluster analyses to estimate the genetic relationships among genotypes were computed for the whole genotypes, landraces, improved durum wheat cultivars, tetraploid species and regions of collection. Clustering of the whole genotypes revealed two groups of landraces and improved cultivars, which implied the similarity of improved cultivars to one another. Similar

analysis for landraces alone formed four groups at about 70% similarity mainly according to their regions of origin. The genetic distance between *T. turgidum* and *T. polonicum* species was the lowest (0.016) where as it was highest (0.051) between *T. durum* and *T. aethiopicum*. Among regions of origin, the lowest genetic distance (0.073) was observed between Bale and Shewa, while the highest between Shewa and Wello (0.18). Discriminant analyses, using species and regions of origin as classifying variables, revealed 95.8 % and 100% correct original classification, respectively.

The magnitude of genetic diversity within improved durum wheat cultivars was low (0.23), and the mean genetic distance among them was 0.16. At 0.75 similarity coefficient more than 80% of the cultivars were clustered in one of the groups. This high similarity of cultivars could be due to the use of common parents to develop most of the cultivars. The lowest genetic distance (0.032) was observed between Foka and Kilinto, Tob66 and DZ1640, and Ld357 and Boohai. The highest coefficient of genetic distance (0.345) was also observed between Asassa and Bichena, Asassa and Boohai, and Bichena and Ude cultivars. The cluster grouping of cultivars formed, however, did not follow common parentage in their pedigree. It could be due to effects of selection pressure and genetic drift, and sensitivity of the working conditions and equipments in RAPD analysis.

VII CONCLUSION AND RECOMMENDATIONS

The diversity estimates within tetraploid wheat landraces based on RAPD marker were low (H=0.34). However, based on microsatellite and AFLP markers data, these estimates were H=0.55 and 0.50, from different samples of the tetraploid landraces of the country (Tesfaye, 2001). The reduced diversity estimates of the present study could be due to the limited number of primers, and hence loci, used in the analysis. Besides, the numbers of genotypes sampled were small (25). Therefore, in such analysis it is advisable to consider more number of loci. Nei (1978) indicated the advantages of using at least 50 loci. Parker *et al*. (1998) also indicated that, in such dominant marker study, 3-10 times more genotype samples are required for reliable estimate.

The genetic diversity within the 15 improved durum wheat cultivars was (H) 0.23. The mean genetic distance among these varieties was 0.16. As it may be expected these estimates are low, because in their pedigree most of the cultivars share common parents. Such information about genetic similarity/distance between cultivars would be helpful for plant breeders to choose diverse parents for crossing. Since almost all of the cultivars were derived from genotypes introduced from CIMMYT, tetraploid wheat improvement programs of the country may need to consider other sources for future cultivar development.

Partitioning the total diversity estimates of tetraploid wheat landraces into within and among species and regions of origin, the highest proportion was observed within species (81.4%) and regions (72.2%). These result suggest the need to collect more germplasm samples within regions with reasonable coverage of different regions for conservation or breeding purposes.

Cluster analysis of all the tetraploid wheat genotypes form groups of improved durum cultivars and tetraploid landraces, and within landrace group, sub groups of genotypes derived from the same region of origin were formed. The present result, i.e clustering of genotypes in their regions, revealed existence of more genetic differentiation among regions. Discriminant analysis using species and regions of origin as a classifying variable revealed a 95.8% and 100% correct original classification according to their species and regions respectively. From these results it can be concluded that in multilocus analysis RAPD markers can be used to classify tetraploid wheat genotypes according to their respective species and regions.

Small cluster groups formed (at about 0.80 similarity coefficient) within improved durum cultivars were not according to common parentage. Besides, effects of genetic drift and selection pressures; sensitivity of the working conditions and equipments of the RAPD technique are reported to influence the results. Therefore, for such analysis it may be appropriate to use other (more) powerful marker systems.

VII. REFERENCES

Abebe Demissie (1990). Ethiopian wheat germplasm collection, conservation, characterization/evaluation and utilization. In: (pp. 327-334), D.G. Tanner, M Van Ginkel, and W. Mwange (eds)., Sixth Regional Wheat Workshop for Eastern, Central and Southern Africa, CIMMYT, D.F.: Mexico

Abebe, D. and Giorgis, H. (1991). Wheat genetic resources in Ethiopia. In: (pages 33-46). Hailu Gebre Mariam, D. G. Tanner and Mengistu Hulluka, (eds): Wheat Research in Ethiopia: A Historical Perspective. Addis Ababa: IAR/CIMMYT

Aga, E. (2002). Genetic diversity of forest Arabica Coffee (*Coffea arabica* L.) in Ethiopia as revealed by Random Amplified Polymorphic DNA (RAPD) analysis; Manuscript on RAPD data. *Hereditas* **138**: 36-46.

Ahmed F. (1999). Random Amplified Polymorphic DNA (RAPD) analysis reveals genetic relationships among annual *Cicer* species. *Theor. Appl. Genet.* **98**:657-663

Assefa, K., Tefera, H., A. Merker, Kefyalew T. and Hundera F. (2001). Variability, heritability and genetic advance in pheno-morphic and agronomic traits of tef [*Eragrostis tef* (Zucc.) Totter] germplasm from eight regions of Ethiopia. *Hereditas* **113**: 103-113.

Assefa, K., A. Merker and Tefera, H. (2003). Multivariate analysis of diversity of tef [*Eragrostis tef* (Zucc.) Totter] germplasm from western and southern Ethiopia. *Hereditas* **138**: 228-236

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

Ayana, A., T. Bryngelsson and Bekele, E. (2000). Genetic variation of Ethiopian and Eritrean Sorghum (*Sorghum bicolor* (L.) Moench) germplasm assessed by random amplified polymorphic DNA (RAPD). *Gene Resour Crop Evol.* **00**:1-12

Bai G., Ayele, M., Tefera, H. and H.T. Nguyen (2000). Genetic diversity in tef [*Eragrostis tef* (Zucc.) Totter] and its relatives as revealed by randomly amplified polymorphic DNAs. *Euphytica* **112**:15-22

Barrett, B.A. and K.K. Kedwell (1998). AFLP based genetic diversity assessment among wheat cultivars from the Pacific Northwest. *Crop Sci.* **38**: 1261-1271.

Bechere, E., Belay, G., Mitiku, D., and A. Merker (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. Institute of Biodiversity Conservation and Research (IBCR), Ethiopia.

Beckmann J.S. and M. Soller (1990) Review toward a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellite sites. *Biotech.* **8**:930-932.

Bekele, E. (1983). Some measures of genetic diversity analysis on landrace populations of Ethiopian barley. *Hereditas* **98**:127-143.

Bekele, E. (1984). Analysis of regional patterns of phenotypic diversity in Ethiopian tetraploid wheats. *Hereditas* **100**: 131-154.

Belay, G. and Furuta, Y. (2001). Zymogram patterns of a-amylase isozymes in Ethiopian

tetraploid wheat landraces: insight into their evolutionary history and evidence for gene flow. *Gene. Resour. Crop Evol.* **48**: 507-512.

Belay, G., Merker A. and Tessema T. (1994). Cytogenetic studies in Ethiopia landraces of tetraploid wheat (*Triticum turgidum* L.). Spike morphology vs ploidy level and karyomorphology. *Hereditas* **121**: 45-52

Belay, G., Bechere, E., Mitiku, D., Merker A., and Tsegaye, S. (1997). Patterns of morphological diversity in tetraploid wheat (*Triticum turgidum* L.) landraces from Ethiopia. *Acta Agric. Scand. Sect. B. Soil and Plant Sci*. **47**:221-228.

Birmeta, G., Hilde Nybom and Bekele, E. (2002). RAPD analysis of genetic diversity among clones of Ethiopian crop plant *Ensete ventricosum. Euphytica* **124**: 315-325.

Bohn, M., H.F. Utz, and A.E. Melchinger (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.

Botstein D., R.L. White, M. Skolnick and R.W. Davis (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet*. **32**:314-331.

Breiman, A. and D. Graur (1995). Wheat evolution. Israel J. Plant Sci. 43: 85-98

Briggle, L.W. and L.P. Reitz (1963). Classification of *Triticum* species and of wheat varieties grown in the United States. U.S. Dept.Agri. Bulletin 1278.

Caetano-Anolles, G. (2001). Plant Genotyping using Arbitrarily Amplified DNA. In: Plant Genotyping the DNA Fingerprinting of Plants (ed. R.J. Henry) pp 29-41. CAB inter

Cao, W., G. Scoles, P. Hucl and R.N. Chibbar (1999). The use of RAPD analysis to classify *Triticum* accessions. *Theor. Appl. G*enet. **98**:602-607

Castagna, R., S. Gnocchi, M. Perenzin, and M. Heun (1997). Genetic variability of the wild diploid wheat *Triticum urartu* revealed by RFLP and RAPD markers. *Theor. Appl. Genet.* **94**: 424-430

Central Statistical Authority (2000). Agricultural sample survey 1999/2000 Vol.1.

Report on area and production of major crops (private peasant holdings, meher season). Statistical Bulletin 227, Addis Ababa, Ethiopia.

Coryell, V.H., H. Jessen, J.M. Schupp, D.Webb and P. Keim (1999). Allele-specific hybridization markers for soybean. *Theo. Appl. Genet* **98**: 690-696.

Divaret, I., E. Margale, and G. Thomas (1999). RAPD markers on seed bulks efficiently assess the genetic diversity of *Brassica oleracea* L. collection. *Theor.Appl. Genet.* **98**: 1029-1035

Dominici, L., C. Grottanelli, D. Tomassini, E. Lafiandra, E. Porcedu and A.B. Damania (1988). Electrophoretic variability in landraces of durum wheat from Ethiopia. *Rachis* **7**: 34-36.

Donini, P., JR. Law, R.M.D. Koebner, J.C. Reeves, and R.J. Cooke (2000). Temporal trends in the diversity of UK wheat. *Theor. Appl. Genet***. 100**:912-917.

Dreher, K., M. Khairallah, J.M. Rebaut and M. Morris (2003). Money Matters (I): Costs of field and laboratory procedures associalted with conventional and marker assisted maize breeding at CIMMYT. *Mol .Breeding* **11**: 221-234.

Dorofeev, V.F., A.A Filatenko, E.F. Migushova, R.A. Udaczin, and M.M. Jakubziner

(1979). Wheat Vol. 1. In: Flora of Cultivated Plants (Dorofeev, V.F. and Korovina, O.N. eds) Leningrad (St. Petersburg), Russia. 346 pp.

Ellis, M. D. (2000). 'Spot-on' SNP genotyping. *Genome Res.* **10**: 895-897.

Eticha, F., Belay, G. and Bekele, E. (2004). Species diversity of tetraploid wheat germplasm from two regions of Ethiopia. *Gene. Resour. Crop Evol.* (in press).

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

Faure S., J. L. Noyer, J. P. Horry, F. Bakry, C. L. anaud, and D. Gonzalez de Leon (1993). A molecular marker-based linkage map of diploid banana (*Musa acuminata*). *Theor Appl Genet* **87**: 517-526

Gandilyan, P.A. (1980). Key to wheat, Aegilops, rye and barley. Academy of Science. Armenian SSR, Erevan 190.

Gupta, P.K., R.K. Varshney, P.K. Sharma and B. Ramesh (1999). Molecular markers and their applications in wheat breeding: Review. *Plant Breeding* **118**:369-390.

Hilu, K. W., and H. T. Stalker (1995). Genetic relationships between peanut and wild species of Arachis sect. Arachis (Fabaceae): evidence from RAPDs. *Plant Sys. Evol.* **198**:167-178.

Hoey, K.B., R.K. Crowe, V.M. Jones and N.O. Polans (1996). A phylogenetic analysis of *Pisum* based on morophological characters, and allozyme and RAPD markers. *Theor. Appl. Genet* **92**:92-100

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

Joshi, C. P., and Nguyen, H. T. (1993). Application of the random amplified polymorphic DNA technique for the detection of polymorphism among wild and cultivated tetrapoid wheats. *Genome* **36**: 602-609.

Kalendar, R., E. Nevo, and A.H. Schulman. (1999b). Genome diversification in wild barley (*Hordeum spontaneum*) populations revealed with retrotransposons REMAP markers p. 187. In: Proc. Plant and Animal Genome VII conference. San Diego. CA. USA.

Kerby, K. and J. Kuspira (1987). The phylogeny of the polyploid wheats *Triticum aestivum* (bread wheat) and *Triticum turgidum* (macaroni wheat). *Genome* **29**: 722-737.

Kimber G. and E.R. Sears (1987). Evolution in the genus *Triticum* and the origin of cultivated wheat. In: Wheat and Wheat Improvement, 2nd Ed. (Heyne, E.G. ed). American Society of Agronomy. Madison, WI pp. 154-164.

Knott, R. (1987). Novel approaches to Wheat Breeding. In: Genetic Improvement in Yield of Wheat. Edward Smith (ed.) CSSA Publication No.13. Crop Science Society of America, Madison, WI. Pp. 25-40.

Koebner, R.M.D., W. Powell and P. Donini (2001). Contributin of DNA molecular marker technologies to the genetics and breeding of wheat and barley. *Plant Breed. Rev.* **21**: 181-220.

Kongkiatngam P., M. J. Waterway, B. E. Coulman and M. G. Fortin (1996). Genetic variation among cultivars of red clover (*Trifolium pratense* L.) detected by RAPD markers amplified from bulk genomic DNA. *Euphytica* **89**:355-361

Landergen, U., M. Nilsson, and P.Y. Kwok (1998). Reading bits of genetic information

:Methods for single nucleotide polymorphism analysis. *Genome Res*. **8**: 769-776.

Liu Q. Z., Y. Pei and Z.J. Pu (1999). Relationship between hybrid performance and genetic diversity based on RAPD markers in wheat, *Triticum aestivum* L. *Plant Breed.* **118**:119-

Mac Key, J. (1988). A plant breeders perispective on taxonomy of cultivated plants. *Biologisces Zeatralblatt* **107**: 369-379.

Maric, S., Bolaric, S., Martincic, J., Pejic, I. and V. Kozumplik (2004). Genetic diversity of hexaploid wheat cultivars estimaed by RAPD markers, morphological traits and coefficient of parentage. *Plant Breeding* **123**: 366-369.

Millan T., F. Osuna, S. Cobos, A.M.Torres, and J.I. Cubero (1996). Using RAPDs to study phylogenetic relationships in *Rosa*. *Theor. Appl. Genet.* **92**:273-277

Mori, N., T. Ishii, G. Belay, S. Takumi, T. Kawahara, Y. Ogihara, and C. Nakamura (2001). Variation in chloroplast microsatellite loci among wild and cultivated species of Emmer wheat. In: Hernandez, P., Moreno, M. T., Cubero, J.I., and Martin, A. (eds.), "Proceedings of the 4th international *Triticeae* symposium". September 10-12, 2001, Cordoba, Spain. Pp. 75-81.

Morris, R and E.R. Sears (1967). The cytogenetics of wheat and its relatives. In: Wheat and Wheat Improvement. Quisnberry and Reitz (eds) Madison. Pp. 19-87.

Morris, M., K. Dreher, J.M. Rebaut and M. Khairallah (2003). Money Matters (II): Costs of maize inbred line conversion schemes at CIMMYT using conventional and marker assisted selection. *Mol.Breeding* **11**:235-247.

Myakishev, M.V., Y. Khripin, S. Hu and D.H. Hamer (2001). High-throughput SNP Genotyping by allele-specific PCR with universal energy-transfer labeled primers. *Genome Res***.** **11**: 163-169.

Negassa M. (1986). 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. (1978). Genetic Distance between populations. *Am. Nat.* **106**: 283-292.

Parker, G. P., A. A. Snow, M. D. Schug., G. C. Booton, and P. A. Fuerst (1998). What molecules can tell us about populations: choosing and using a molecular marker. *Ecol.* **79(2)**: 361-382.

Penner A.G. (1996). RAPD analysis of plant genomes. In: Methods of genome analysis

in plants (Jauhar P.P. ed). CRC press Inc. Boca Raton. Pp. 251-268.

Pillay, M., and S. T. Kenny. 1995. Anomalies in direct pair-wise comparisons of RAPD fragments for genetic analysis. *BioTechniques* **19**: 694-698.

Potokina, E., N. Tomooka, D. A. Vaughan, T. Alexandrova and R. Su (1999). Phylogeny of *Vicia* subgenus *Vicia* (*Fabaceae*) based on analysis of RAPDs and RFLP of PCR-amplified chloroplast genes. *Genet Resou. Crop Evol.* **46**: 149-161

Pujar, S., S. A. Tamhankar, V. S. Rao, V. S. Gupta, S. Naik and P. K. Ranjekar (1999). Arbitrarily primed-PCR based diversity assessment reflects hierarchical groupings of Indian tetraploid wheat genotypes. *Theor. Appl. Genet.* **99**:868-876

Reeves, J.C., J.R. Law, P.Donini, R.M.D. Keobner, and R. Cooke (1999). Changes over time in the genetic diversity of UK cereal crops. P. 84-89. In: J. Serwinski and I. Faberova (eds.), proc. Technical Meeting on the methodology of the FAO World Information and Early Warning System on Plant Genetic Resources. FAO. Prague.

Rolf, FG. (1993). NTSYS-pc Numerical Taxonomy and Multivariate Analysis System. Version 1.8. Applied Biostatistics Inc., New York.

Santos, V.S, M.A Gimenes, J.F. Valls and C.R. Lopes (2003). Genetic variation within and among species of five sections of the genus *Arachis* L. (Leguminoseae) using RAPDs. *Genet. Resou. Crop Evol*. **50**:841-848.

Sentayehu A., S. Chebotar, X. Huang, M. Roder and A. Borner (2004). Genetic diversity in Ethiopian hexaploid and tetraploid wheat germplasm assessed by microsatellite markers. *Genet. Resour. Crop Evol*. **51**:559-567.

Smith, D. N., and M. E. Devey (1994). Occurrence and inheritance of microsatellites in Pinus radiata. *Genome* **37**: 977-983.

Simmonds, N.W. (1976). Evolution of Crop plants. Longman, London.

Simmons, R. S. (1987). Growth, Development and Physiology. In Wheat and Wheat Improvement. E.G.Heyne (ed). Wisconsin pp 77-114.

Strelchenko P., O. Kovalyova and K. Okuno (1999). Genetic differentiation and geographical distribution of baley germplasm based on RAPD markers. *Genet Resou. Crop Evolu*. **46**: 193-205

Szucs, P., A. Juhasz, I. Karsai, L. Lang, O. veisz, and Z. Bedo (2000). Use of molecular markers for studying genetic diversity in durum wheat (*Triticum durum* Desf.) *J. Genet and Breed* **54**: 25-33

Tesfaye, M. (2001). Multidisciplinary approach in estimating genetic diversity of Ethiopian tetraploid wheat (*Triticum turgidum* L.) landraces. PhD Thesis Wageningen University.

Tessema, T. and Belay, G. (1991). Aspects of Ethiopian Tetraploid wheats with Emphasis on

Durum Wheat Breeding and Genetics Research. In pages 47-72. Hailu Gebre Mariam, D. G. Tanner and Mengistu Hulluka, (eds): wheat Research in Ethiopia: A Historical Perspective. Addis Ababa: IAR/CIMMYT

Tesfaye, T., Belay, G. and Worede, M. (1991). Morphological diversity in tetraploid wheat landrace populations from the central highlands of Ethiopia. *Hereditas* **114**: 171-176.

Tesfaye, T. (1986). Improvement of indigenous durum wheat landraces in Ethiopia. Pp. 232-238. In: Engles, J.M.J., ed. The conservation and utilization of Ethiopian Germplasm. Proc.Int.Symp. Addis Ababa. Ethiopia: PGRC/E.

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

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

Tsegaye, S. and Tessema, T. (1996). Variation of leaf esterases in some Ethiopian tetraploid wheat landraces. *Genet Resour Crop Evol.* **43**: 119-123

Verling, R., and H.T. Nguyen (1992). Use of RAPD markers to determine the genetic relationships of diploid wheat genotypes. *Theor. Appl. Genet*. **84**: 835-838.

Warburton M. L., V. L. Becerra-velasquez, J. C. Goffereda, and F. A. Bliss (1996). Utility of RAPD markers in identitying genetic linkages to genes of economic interest in Peach. *Theor Appl Genet* **93**: 920-925

Welsh, J. and M. McClelland (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* **18**:7213-7218.

Westman, A.L. and S. Kresovich (1997). Use of Molecular Marker Techniques for Description of Plant Genetic Variation. In: Biotechnology and Plant Genetic Resources: Conservation and Use (eds. Callow, J.A., Ford-Lioyd, B.B, and H.J. Newbury). Biotechnology in Agri. Series, No.19 CAB Inter. Wallignford. pp9-47.

Williams J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Research* **18**:6531-6535.

Wolf, D.A. and A. Liston (1998). Contributions of PCR-Based Methods to Plant Systematics and Evolutionary Biology. In D.E. Soltis, P.S. Soltis, and J.J. Doyle, eds. Plant molecular systematics II. Chapman and Hall, New York, USA.Pp 43-86.

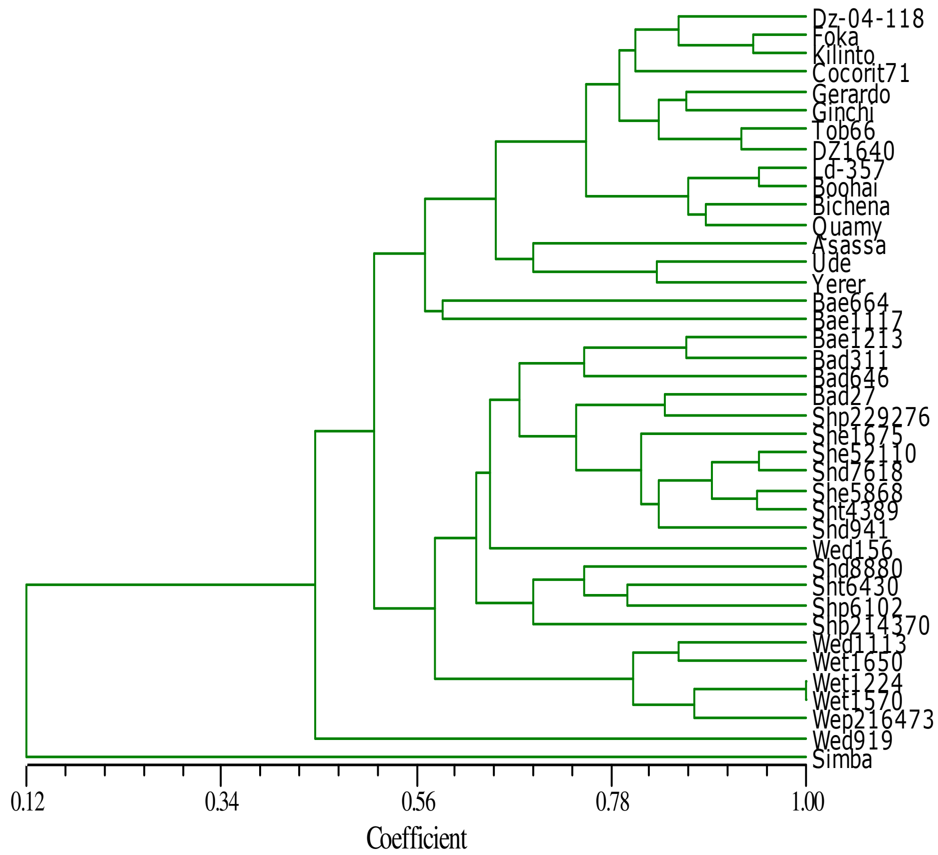
Zabeau M. and P. Vos. (1992). European Patent Application. Publication no. 0 543 858 A1.

Zohary, D. and M. Feldman (1962). Hybridization between amphidiploids and the evolution of polyploids in the wheat (Aegilops-Triticum) group. *Evolution* **16**: 44-61.

IX APPENDICES

Appendix 1. List of genotypes included in the experiment with their region of origin, species group, population and entry/accession number, code and source institute.

No	Region	*Species*	Pop.no	Entry/acce. No.	Code	Source
1	Bale	*T. eathiopicum*	6	64	Bae 664	DZARC
2	Bale	*T. eathiopicum*	11	17	Bae 1117	DZARC
3	Bale	*T. eathiopicum*	12	13	Bae 1213	DZARC
4	Bale	*T. durum*	3	11	Bae 311	DZARC
5	Bale	*T. durum*	2	7	Bad 27	DZARC
6	Bale	*T. durum*	6	46	Bad 646	DZARC
7	Wello	*T. durum*	15	6	Wed 156	DZARC
8	Wello	*T. durum*	9	19	Wed 919	DZARC
9	Wello	*T. durum*	11	13	Wed 1113	DZARC
10	Wello	*T. turgidum*	12	24	Wet 1224	DZARC
11	Wello	*T. turgidum*	15	70	Wet 1570	DZARC
12	Wello	*T. turgidum*	16	50	Wet 1650	DZARC
13	Shewa	*T. eathiopicum*	16	75	She 1675	DZARC
14	Shewa	*T. eathiopicum*	52	110	She 52110	DZARC
15	Shewa	*T. eathiopicum*	58	68	She 5868	DZARC
16	Shewa	*T. durum*	9	41	Shd 941	DZARC
17	Shewa	*T. durum*	76	18	Shd 7618	DZARC
18	Shewa	*T. durum*	88	80	Shd 8880	DZARC
19	Shewa	*T. turgidum*	43	89	Sht 4389	DZARC
20	Shewa	*T. turgidum*	64	30	Sht 6430	DZARC
21	Shewa	*T. turgidum*	91	51	Sht 9151	DZARC
22	Wello/S.Gondar	*T. polonicum*	--	216473	Wep 216473	IBC
23	Shewa	*T. polonicum*	--	6102	Shp 6102	IBC
24	Shewa	*T. polonicum*	--	214370	Shp 214370	IBC
25	Shewa	*T. polonicum*	--	229276	Shp 229276	IBC
26	--	*T. durum*	--	--	DZ-04-118	DZARC
27	--	*T. durum*	--	--	Cocorit 71	DZARC
28	--	*T. durum*	--	--	Gerardo	DZARC
29	--	*T. durum*	--	--	Ld 357	DZARC
30	--	*T. durum*	--	--	Boohai	DZARC
31	--	*T. durum*	--	--	Foka	DZARC
32	--	*T. durum*	--	--	Kilinto	DZARC
33	--	*T. durum*	--	--	Bichena	DZARC
34	--	*T. durum*	--	--	Quamy	DZARC
35	--	*T. durum*	--	--	Asassa	DZARC
36	--	*T. durum*	--	--	Tob 66	DZARC
37	--	*T. durum*	--	--	Ginchi	DZARC
38	--	*T. durum*	--	--	DZ 1640	DZARC
39	--	*T. durum*	--	--	Ude	DZARC
40	--	*T. durum*	--	--	Yerer	DZARC



Appendix 2. Dendrogram for 40 tetraploid wheat genotypes generated based on the unweighted pair group method with arithmetic averages (UPGMA) analysis of Jaccard similarity.

Appendix 3

Genetic Similarity matrix table for fourty genotypes studied.

	Dz 04-118	Cocorit 71	Gerardo	Ld-357	Boohai	Foka	Kilinto	Bichena	Quamy	Asassa	Tob 66	Ginchi	DZ 1640	Ude	Yerer
Dz 04-118	1														
Cocorit 71	0.824	1													
Gerardo	0.722	0.667	1												
Ld-357	0.75	0.7	0.7	1											
Boohai	0.789	0.737	0.65	0.947	1										
Foka	0.882	0.824	0.824	0.842	0.789	1									
Kilinto	0.833	0.778	0.882	0.8	0.75	0.941	1								
Bichena	0.789	0.737	0.65	0.85	0.895	0.789	0.75	1							
Quamy	0.882	0.824	0.722	0.842	0.889	0.882	0.833	0.889	1						
Asassa	0.588	0.625	0.733	0.579	0.526	0.688	0.647	0.526	0.588	1					
Tob 66	0.875	0.813	0.813	0.737	0.778	0.875	0.824	0.778	0.875	0.667	1				
Ginchi	0.706	0.75	0.867	0.684	0.632	0.813	0.765	0.632	0.706	0.846	0.8	1			
DZ 1640	0.813	0.75	0.867	0.684	0.722	0.813	0.765	0.722	0.813	0.714	0.929	0.857	1		
Ude	0.588	0.529	0.733	0.579	0.526	0.688	0.647	0.526	0.588	0.692	0.667	0.714	0.714	1	
Yerer	0.688	0.625	0.733	0.579	0.611	0.688	0.647	0.611	0.688	0.692	0.786	0.714	0.846	0.833	1
Bae 664	0.55	0.579	0.579	0.619	0.65	0.55	0.6	0.65	0.632	0.529	0.611	0.556	0.556	0.444	0.529
Bae 1117	0.565	0.591	0.522	0.694	0.727	0.565	0.609	0.652	0.636	0.409	0.545	0.435	0.5	0.476	0.55
Bae 1213	0.476	0.5	0.5	0.478	0.5	0.476	0.524	0.5	0.476	0.529	0.526	0.474	0.474	0.368	0.444
Bad 311	0.45	0.474	0.474	0.455	0.476	0.45	0.5	0.476	0.45	0.5	0.5	0.444	0.444	0.412	0.5
Bad 27	0.65	0.6	0.6	0.565	0.591	0.65	0.7	0.591	0.65	0.474	0.632	0.5	0.479	0.474	0.556
Bad 646	0.5	0.526	0.526	0.435	0.455	0.5	0.55	0.524	0.5	0.563	0.556	0.5	0.5	0.389	0.471
Wed 156	0.474	0.5	0.5	0.409	0.429	0.474	0.526	0.5	0.474	0.438	0.529	0.471	0.471	0.533	0.533
Wed 919	0.476	0.429	0.429	0.417	0.435	0.476	0.524	0.435	0.476	0.3	0.45	0.333	0.4	0.3	0.368
Wed 1113	0.4	0.421	0.421	0.409	0.429	0.4	0.45	0.429	0.4	0.438	0.444	0.389	0.389	0.278	0.353
Wet 1224	0.5	0.526	0.45	0.5	0.524	0.5	0.55	0.524	0.5	0.389	0.474	0.421	0.421	0.316	0.389
Wet 1570	0.5	0.526	0.45	0.5	0.524	0.5	0.55	0.524	0.5	0.389	0.474	0.421	0.421	0.316	0.389
Wet 1650	0.364	0.381	0.381	0.375	0.391	0.364	0.409	0.455	0.364	0.389	0.4	0.35	0.35	0.25	0.316
Wep 216473	0.455	0.476	0.409	0.458	0.478	0.455	0.5	0.545	0.455	0.35	0.429	0.381	0.381	0.286	0.35
She 1675	0.24	0.476	0.55	0.522	0.545	0.524	0.571	0.619	0.524	0.421	0.579	0.45	0.526	0.421	0.5
She 52110	0.522	0.478	0.545	0.583	0.609	0.522	0.565	0.682	0.591	0.429	0.571	0.455	0.524	0.429	0.5
She 5868	0.571	0.524	0.6	0.565	0.591	0.571	0.619	0.667	0.571	0.474	0.632	0.5	0.579	0.474	0.556
Shd 941	0.55	0.5	0.579	0.545	0.571	0.55	0.6	0.571	0.55	0.444	0.611	0.474	0.556	0.444	0.529
Shd 7618	0.545	0.5	0.571	0.609	0.636	0.545	0.591	0.636	0.619	0.45	0.6	0.476	0.55	0.45	0.526
Shd 8880	0.588	0.529	0.529	0.5	0.526	0.588	0.556	0.526	0.588	0.467	0.667	0.5	0.6	0.571	0.692
Sht 4389	0.545	0.5	0.571	0.609	0.636	0.545	0.591	0.714	0.619	0.45	0.6	0.476	0.55	0.45	0.526
Sht 6430	0.526	0.474	0.474	0.524	0.55	0.526	0.5	0.55	0.526	0.412	0.588	0.444	0.529	0.5	0.6
Shp 6102	0.579	0.526	0.526	0.571	0.6	0.579	0.55	0.6	0.579	0.471	0.647	0.5	0.588	0.563	0.667
Shp 214370	0.55	0.5	0.579	0.545	0.571	0.55	0.6	0.65	0.55	0.444	0.611	0.474	0.556	0.529	0.625
Shp 229276	0.619	0.571	0.571	0.609	0.636	0.619	0.667	0.714	0.7	0.45	0.6	0.476	0.55	0.45	0.526
Simba	0.118	0.125	0.125	0.1	0.105	0.118	0.111	0.105	0.118	0.077	0.133	0.143	0.143	0.167	0.167

Table contd.

	Bae 664	Bae 1117	Bae 1213	Bad 311	Bad 27	Bad 646	Wed 156	Wed 919	Wed 1113	Wet 1224	Wet 1570	Wet 1650	Wep 216473	She 1675
Yerer														
Bae 664	1													
Bae 1117	0.591	1												
Bae 1213	0.579	0.522	1											
Bad 311	0.556	0.571	0.867	1										
Bad 27	0.524	0.609	0.778	667	1									
Bad 646	0.526	0.417	0.813	0.688	0.722	1								
Wed 156	0.5	0.455	0.588	0.667	0.611	733	1							
Wed 919	0.364	0.522	0.5	0.4	0.684	0.45	0.35	1						
Wed 1113	0.588	0.391	0.8	0.667	0.611	0.733	0.5	0.421	1					
Wet 1224	0.611	0.478	0.706	0.588	0.722	0.647	0.529	0.526	0.857	1				
Wet 1570	0.611	0.478	0.706	0.588	0.722	0.647	0.529	0.526	0.857	1	1			
Wet 1650	0.526	0.36	0.706	0.588	0.55	0.75	0.529	0.381	0.857	0.75	0.75	1		
Wep 216473	0.55	0.44	0.632	0.526	0.65	0.667	0.556	0.476	0.75	0.875	0.875	0.875	1	
She 1675	0.632	0.565	0.722	0.706	0.737	0.667	0.647	0.476	0.647	0.667	0.667	0.667	0.684	1
She 52110	0.619	0.625	0.7	0.684	0.714	0.65	0.632	0.478	0.55	0.571	0.571	0.571	0.591	0.842
She 5868	0.6	0.542	0.684	0.667	0.7	0.722	0.706	0.455	0.611	0.632	0.632	0.632	0.65	0.833
Shd 941	0.667	0.522	0.667	0.647	0.684	0.611	0.588	0.429	0.688	0.706	0.706	0.611	0.632	0.824
Shd 7618	0.65	0.652	0.737	0.722	0.75	0.6	0.579	0.5	0.579	0.6	0.6	0.524	0.545	0.789
Shd 8880	0.444	0.476	0.529	0.6	0.647	0.563	0.643	0.444	0.438	0.471	0.471	0.389	0.421	0.588
Sht 4389	0.65	0.583	0.65	0.632	0.667	0.684	0.667	0.435	0.579	0.6	0.6	0.6	0.619	0.789
Sht 6430	0.474	0.571	0.474	0.529	0.5	0.421	0.471	0.474	0.389	0.421	0.421	0.35	0.381	0.526
Shp 6102	0.526	0.545	0.611	0.688	0.632	0.556	0.625	0.381	0.529	0.556	0.556	0.474	0.5	0.667
Shp 214370	0.579	0.667	0.579	0.644	0.6	0.611	0.688	0.5	0.5	0.526	0.526	0.526	0.55	0.722
Shp 229276	0.571	0.652	0.65	0.632	0.842	0.684	0.667	0.571	0.5	0.6	0.6	0.524	0.619	0.789
Simba	0.125	0.15	0.059	0.067	0.111	0.063	0.154	0.2	0.071	0.133	0.133	0.063	0.118	0.118

Table contd.

	She 52110	She 5868	Shd 941	Shd 7618	Shd 8880	Sht 4389	Sht 6430	Shp 6102	Shp 214370	Shp 229276
She 1675										
She 52110	1									
She 5868	0.895	1								
Shd 941	0.789	0.882	1							
Shd 7618	0.947	0.842	0.833	1						
Shd 8880	0.579	0.647	0.625	0.611	1					
Sht 4389	0.947	0.944	0.833	0.895	0.611	1				
Sht 6430	0.6	0.667	0.647	0.632	0.714	0.632	1			
Shp 6102	0.737	0.824	0.813	0.778	0.786	0.778	0.8	1		
Shp 214370	0.7	0.778	0.667	0.65	0.625	0.737	0.75	0.706	1	
Shp 229276	0.85	0.75	0.65	0.8	0.611	0.8	0.476	0.6	0.65	1
Simba	0.1	0.111	0.125	0.105	0.167	0.105	0.231	0.133	0.2	0.105

Appendix 4. Number and size of RAPD fragments

Number	Primer	Estimated fragment length (bp)
1	OPA-13	290
2	>>	300
3	>>	400
4	>>	450
5	>>	500
6	>>	525
7	>>	550
8	>>	600
9	>>	650
10	>>	725
11	>>	750
12	>>	970
13	>>	985
14	>>	1000
15	>>	1200
16	>>	1500
17	>>	1640
18	OPC-02	100
19	>>	200
20	>>	250
21	>>	350
22	>>	500
23	>>	525
24	>>	625
25	>>	700
26	>>	725
27	>>	875
28	>>	925
29	>>	975
30	>>	1200
31	>>	1500