Identification of Ethiopian *Triticum aestivum*, *T. turgidum*, and *Eragrostis tef* using morphological, SDS-PAGE and AFLP characterisation

by

Yilma Amenu Hika

Submitted in fulfillment of the requirements for the

Magister Scientiae Agriculturae

Department of Plant Breeding,
Faculty of Agriculture,
University of the Orange Free State

November 2000

Supervisor: Prof. M.T. Labuschagne
Co-Supervisor: Dr. C.D. Viljoen

This work is dedicated to my father,

Rev. Amenu Hika

and my mother,

Mrs. Jale Wakjira

Acknowledgements

I am deeply grateful to the following institutions and persons for their contribution towards the success of this thesis.

The Ethiopian Seed Enterprise (ESE) and National Seed industry Agency (NSIA) for awarding me the Graduate Research Fellowship through which I accomplished the study.

I am very grateful to Prof. Maryke Tine Labuschagne and Dr. Chris Viljoen for their excellent supervision, help, and advice during the study.

My special gratitude also goes to Elizma Koen (a fellow student) and Dr. Hilke Maartens (a staff member at the department of plant breeding) for their wonderful technical support in the laboratory.

I also acknowledge the personnel at the department of plant breeding for their support and above all for their friendship. It is also my pleasure to express my gratitude to my friends who directly or indirectly influenced my studies. Finally, I would like to thank God, who made all this possible.

Contents

Chapter 1

Introduction

Agriculturally important crop species exist as a number of genetically distinct, but related, varieties. These varieties are either locally adapted landraces or, more commonly, cultivated varieties (cultivars), produced by plant breeders (Lee *et al*, 1996). Such cultivars often differ in their quality and other important agronomic characteristics (Cooke, 1984), and thus, the difference between growing one variety and another may be the difference between a profit and a loss. In consequence, it is essential for the buyer to get the variety he asks for.

Present methods of variety identification are based on (1) morphological markers, (2) biochemical markers (such as proteins), and (3) deoxyribonucleic acid (DNA) markers. Each of the three classes of genetic markers has its own merits and drawbacks. Nevertheless, a method for identification depends on the available resources and the level of identification required.

Morphological markers can be monitored visually without specialized biochemical or molecular techniques. However, this method can be a time consuming and expensive process, requiring large area of land and highly skilled personnel making what are often subjective decisions. Also, morphological descriptors can be altered by environmental factors; their alleles interact in a dominant-recessive manner, thereby making it impossible to distinguish the heterozygous individuals from homozygous individuals, and in some species the number of descriptors is limited or no longer sufficient for identification of all varieties (Cooke, 1995b; Kumar, 1999).

Biochemical tests offer significant advantages over morphological methods of variety identification in that they are rapid, relatively cheap, eliminate the need to grow plants to maturity, and are largely unaffected by the growth environment (Ainsworth and Sharp, 1989). Biochemical markers are proteins produced as a result of gene expression, and hence they reveal differences in the gene sequence. They also function

as co-dominant markers. However, their use is limited due to the limited number in some crops and also because they are subjected to posttranslational modifications (Kumar, 1999).

In DNA marker analysis, the natural variation in the DNA sequence is made use of and no expression of a certain gene is required. Thus, in any genome, the number of DNA markers is ubiquitous and numerous compared to the number of morphological and biochemical markers. In addition, DNA analysis can be carried out at any growth stage of the plant from almost any tissues in the plant (Kumar, 1999). This offers significant advantages over the biochemical tests, which may be profoundly influenced by tissue specificity and the developmental stage of the plant (Ainsworth and Sharp, 1989). DNA markers provide a highly discriminating means for the identification of crop varieties, but they are expensive and technically difficult compared to biochemical markers. DNA markers can be dominant or co-dominant depending upon how the polymorphism is revealed.

The composition of wheat storage proteins and DNA markers, and their relation to variety identification has never been studied in Ethiopia. The aims of the study were, therefore:

i) to identify Ethiopian bread wheat and durum wheat cultivars based on morphological, biochemical, and DNA markers,

ii) to calculate the genetic distances among the varieties based on the three types of genetic markers, and

iii) to cluster all the cultivars based on their genetic distances.

Chapter 2

Literature review

2.1 Introduction

This chapter reviews the role of morphological, protein, and DNA markers in fingerprinting crop varieties. First of all, the need to identify between cultivated varieties was considered broadly. Then, the use of morphological characters in the identification of cultivated varieties was discussed, including their discriminational ability and constraints. The importance of protein electrophoresis in the identification of crop varieties was also reviewed extensively, with particular emphasis on gliadins and high molecular weight glutenin subunits (HMW-GS) were researched in this study. Finally, the use of DNA markers to detect differences between crop varieties was dealt with. The review also highlights the advantages and disadvantages of the three classes of genetic markers, with respect to their resolving power, time required, environmental effects, and cost effectiveness.

2.2 The need to identify cultivated varieties

The unambiguous, fast, and cost-effective identification of crop plant populations and varieties is important in many areas of agriculture as well as pure and applied plant research (Morell *et al.* 1995). Within the agricultural community, it is important to be able to distinguish among different cultivars (or varieties) of particular crops. This is partly because cultivars differ in their quality and other agronomic characteristics (Cooke, 1984; Lookhart and Wrigley, 1995). The other circumstances in which it is necessary to be able to assess identity are considered in this section.

First, the farmer must be sure that the appropriate variety is being sown, thus ensuring agronomic and disease resistance advantages are obtained. At harvest, in turn, varietal identity provides an important indicator of processing quality and market value (Wrigley, 1995). Cooke (1995a) stated that the ultimate consumers of

the harvested seed also often need to be certain that they are purchasing the correct variety, particularly if the grain is to be used for large scale processing, mechanised breadmaking, for instance.

In a typical plant breeding program, cultivar identification enables the choice of parental material containing desired traits for crossing and the subsequent selection for progenies caring combination(s) of required traits (Cooke, 1995b). For this purpose, plant breeders are looking for genetic markers, which can identify a specific desired trait(s). Such markers can be morphological characters, but increasingly they are proteins or nucleic acids (DNA).

Cultivar identification is also useful for distinctness testing of new varieties produced by plant breeders prior to registration. In addition, for cultivars to be nationally or internationally registered and for breeders to be granted plant breeders rights of protection, varieties must successfully pass inspection for the criteria of distinctness, uniformity, and stability, the so-called D.U.S. criteria (Bailey, 1983; Law *et al*, 1998). Thus, cultivar identification is important in plant variety protection (PVP) and patents through which intellectual property protection can be enforced on the end product.

As far as seed production is concerned, Cooke (1995b) reported that all the processes involved from the initial breeding of the variety through its official registration, seed multiplication, certification, sale, and ultimate utilisation depend to some degree on the ability to distinguish between and identify varieties. In the production of F1 hybrids, cultivar identification is important to determine the success of the crossing procedure, i.e., to measure hybrid (or genetic) purity. One approach to checking hybrid seed lots for purity is to grow samples under field conditions and quantify the frequency of female selfs. However, field growing-out procedures are expensive, time consuming, and liable to environmental influence. Fortunately, protein analysis by electrophoresis provides an alternative approach which is a much more accurate, fast, and cost effective means of hybrid purity testing compared to field-based morphological observation (Smith and Wych 1986;

Cooke, 1989). The great attraction of electrophoresis for this purpose lies in the fact that protein or isozyme bands are inherited in a Mendelian fashion and expressed co-dominantly. This means that when two inbred lines are crossed for F1 hybrid production, the resulting progeny will contain and express all of the protein bands from both parents. Thus, impurities arising from either self-pollination of the female parent (sibs) or pollination by unintended male parent can be readily identified (Cooke, 1995a).

The use of suitable electrophoretic methods to analyse a polymorphic protein or enzyme can thus easily and rapidly give a measure of the purity of F1 hybrid seed. The only prerequisites, although not always met completely, are that (1) the parents should be fixed at the loci encoding the protein(s) of interest (i.e. not segregating); (2) the parents must differ genetically with respect to at least one pair of homologous proteins or isozymes so that the banding phenotype of the hybrid be distinguishable from that of its parents; (3) the male line should preferably possess protein band(s) not present in the female, otherwise sib detection is impossible (Cooke, 1988). Reports are already available on the use of electrophoresis to assess hybrid purity in crops such as *Brassica oleracea* (Wills *et al*, 1979; Arus *et al*, 1982), tomato (Tanskley and Jones, 1981), and maize (Smith and Weissinger, 1984). Analysis of DNA, in this area, is used where protein or isozyme polymorphisms are lacking or insufficient. Such instances were reported in pepper (*Capsicum annum*) (Livneh *et al*, 1990; Ballester and Carmen de Vicente, 1998), watermelon (*Citrullus lanatus*) and tomato (*Lycopersicum esculentum*) (Hashimuze *et al*, 1993), and *Cichorium intybus* L. (Bellamy *et al*, 1996).

In terms of grain production, an increasingly important and widespread use of variety identification is in quality control checking of grain in trade, primarily milling wheat and malting barley. Since the operation of automated machinery for processing cereals is dependent on the quality of the grain used, varietal identity and purity are critical elements in assessing the grain entering factories. Electrophoresis of seed storage proteins are now routinely used throughout North America, Europe, and Australia to check grain for processing (Cooke, 1995a).

Assessment of varietal identity is also an important element in the cataloguing and documentation of genetic resources in gene banks and similar collections. Conventionally, morphological characters are used as an aid to germplasm collection and conservation strategy (Pecetti *et al*, 1992; Demissie and Bjørnstad, 1996; Bechere *et al*, 1996). Considerable use has also been made of protein electrophoresis (Bretting and Widrlechner, 1995), and there are reports of the electrophoretic characterisation of potatoes (Huaman and Stegemann, 1989) and wheat and barley (Damania *et al*, 1983), for instance. The use of DNA markers, in this area, can be particularly useful where protein or isozyme polymorphisms are limited or non-existent. In addition, since DNA markers show a higher level of variation, it can be used to indicate potential areas where significant genetic variation exists for germplasm collection and *in situ* conservation. It also offers the prospect of being able to reduce unnecessary duplication within and among accessions that need to be stored (Bernatzky Tanksley, 1989; McFerson, 1998).

Furthermore, unlike morphological records of fingerprint, protein and DNA based descriptors can have other applications in addition to the identification task. For instance, they can be used in tests of parentage, in genetic mapping of loci conditioning economic traits, in measurement of genetic diversity, and in discerning patterns of genetic diversity (Smith and Smith, 1992). It is thus clearly important from many points of view to be able to distinguish between and identify crop cultivars.

2.3 Cultivar identification based on morphological characteristics

The use of morphological descriptors is the oldest and traditional way of identification in which visual characters are used to confirm the identity and varietal purity of crop plants. It involves the observation, recording, and analysis of a number of morphological characters of seeds and/or growing plants (McDonald, 1991). In practice, such an approach is said to be extremely successful and largely forms the basis, for instance, of current D.U.S. testing procedures (Cooke, 1995a).

Visual identification of grain samples is the traditional approach and is based on distinguishing characteristic of the grain, such as colour, shape, size, etc. The presence of fragments from the head (e.g., awns for wheat) in grain samples may provide further clues for identification (Wrigley and Batey, 1995). Even though its power of resolution is minimum, it is still an invaluable tool and the only one that can provide an immediate and on-the-spot analysis to settle price and premium wherever cereals are traded (Jarman, 1995). It has also served the seed industry very well (Wrigley, 1992).

Such visual examination of grain samples is a portable skill that requires the minimum of resources. Even though it requires a high degree of skill, the reference handbooks available for many national sets of varieties can hasten the process of acquiring such skill. In some instances, however, problems can be encountered because of natural variations in kernel characteristics or as a result of environmental factors, thus complicating the task of identification (Lookhart *et al*, 1995). Thus, visual identification, as reviewed by Gore and Sutton (1995), should rather be used in cases where this method provides sufficient discrimination, and identification is not considered critical.

The other approach involves a detailed description of the whole plant part. Appropriate experience and reference list of varietal characteristics is also required in this case. Although this approach is slow, taking the whole growing season, it offers distinct advantages in situations where growing facilities are readily available (Wrigley and Batey, 1995).

The physical appearance (phenotype) of a plant is a result of an interaction between its genetic composition (genotype) and the environment in which it is expressed (Brown, 1978; Lin and Binns, 1984). This interaction is particularly relevant to variety identification, since varieties tend to be grown in a wide range of different climatic and soil conditions with varying husbandry practices. Thus, the morphological characters used for variety identification ideally should be those that are consistently expressed in different environmental conditions. Unfortunately,

this ideal is rarely attained in practice, and thus experiments are usually carried out at different environmental situations (Cooke, 1995b).

International union for the protection of new varieties of plants (UPOV) produces a list of morphological characters that can be used for DUS tests. These characteristics are categorised as either essential or supplementary characters. Essential characters are typically assessed for every registration, and supplementary characters are assessed only when necessary (Morell *et al*, 1995). Some of these descriptors involve actual measurements (i.e. they are quantitative or continuous), whereas others can be assessed visually, either on single plants or groups of plants (Cooke, 1995b). Thus there is no shortage of possible morphological characters that could be useful for variety identification purposes.

2.3.1 Discriminational ability of morphological data

Morphological descriptors can provide unique identification of cultivated varieties (Molina-Cano and Elena Rossello, 1978). Continued use of morphological data (Ebba, 1975; Smith and Smith, 1989; Tefera *et al*, 1990; Martiniello, 1992; Jarvie and Barkworth, 1992; Bechere *et al*, 1996; Zewdie and Zeven, 1997; Sharopova *et al*, 1997; Assefa *et al*, 1999) to describe cultivars also indicates that these data retain popularity as descriptors. However, morphological data vary from plant to plant. plot to plot, and year to year and statistical criteria are required to separate genuine varietal differences from chance variation (Patterson and Weatherup, 1984). Increased numbers of genetically related releases by plant breeders have made unique identification more difficult. This problem is especially acute in crops with a limited level of phenotypic diversity, where discrimination between cultivars using morphological criteria is more difficult (Cooke, 1985).

In wheat, the situation has been further exacerbated with the use of exotic germplasm and genotypes of different classes as parents in breeding crosses. Besides, identification by seed type becomes very difficult after breeders have deliberately released multilines or varieties having multiple biotypes, although the

genotypes within varieties generally had uniform agronomic characteristics (Lookhart and Bietz, 1990).

2.3.2 Constraints of morphological data

Methods of varietal identification are most frequently based on assessment of a range of morphological characteristics. While data from these methods are relatively easy to collect, and often useful for other purposes as well (Van Hintum and Elings, 1991), morphological comparisons may have limitations including subjectivity in the analysis of the character; the influence of environmental or management practices on the character; limited diversity among cultivars with highly similar pedigrees; and confining of expression some diagnostic characters to a particular stage of development, such as flowering or fruit ripening (Morell *et al*, 1995).

Morphology-based classification also utilises characters, which are often multigenic and continuously expressed (quantitative), requiring replicated measurements in order to apply statistical means of establishing identity or distinguishing between individuals. The environmental interaction with the characters again necessitates replication of sampling and analysis. This requires time consuming and labour intensive manual methods of measuring, recording, and processing the information. Again, in some species the number of descriptors is limited or is no longer sufficient for identification of all varieties (Cooke, 1995a).

Smith (1986) has also indicated that the genetic control of many morphological characters is assumed to be complex, often involving epistatic interaction, although it has not been elucidated. In addition, many morphological markers are recessive and therefore only expressed in the homozygous condition. Therefore, it is impossible to determine how completely the genome is sampled by morphological descriptions or the extent to which similar phenotypes reflect similar genotypes. Furthermore, descriptions based on morphological data are fundamentally flawed

in their ability to provide reliable information for the calculation of genetic distance or the validation of pedigrees (Smith and Smith, 1992).

2.4 Protein electrophoresis and variety identification

2.4.1 Principle of electrophoresis

Many biologically important molecules such as proteins and nucleic acids possess ionizable groups and can therefore be made to exist in solutions as electrically charged species, either as cations (+) or anions (-). Moreover, molecules with a similar charge will have different charge/mass ratios because of inherent differences in their molecular weight. In combination, these differences form a sufficient basis for a differential migration when the ions in solution are subjected to an electric field. Cations move to the cathode (-) and anions move to the anode (+) at rates which depends upon the balance between the impelling force of the electrical field on the charged sample ions and the retarding forces between the migrating molecules and the surrounding medium which are principally frictional and electrostatic. The sample material must be dissolved or suspended in buffer for electrophoresis to take place and the supporting medium must also be saturated with buffer in order to conduct the current (Davis and Simpkins, 1975). This is the principle of electrophoresis.

Factors affecting the migration rate include the charge, size, and shape of the molecules. The rate of migration increases with an increase in the net charge. The magnitude of the charge is generally pH dependent. On the other hand, the rate of migration decreases for larger molecules, due to the increased frictional and electrostatic forces, which are exerted by the surrounding medium. Molecules of similar size but different shapes such as fibrous and globular proteins also exhibit different migration characteristics because of the differential effect of frictional and electrostatic forces (Davis and Simpkins, 1975).

In addition, the rate of migration is directly proportional to the current, and the distance migrated by the ions will be proportional to the length of time for which the current is supplied. For maximum reproducibility therefore, the current should be kept constant during electrophoresis. Direct current must always be used. On the contrary, the rate of migration is inversely proportional to the resistance, which depends on the type and size of the supporting medium and on the ionic strength of the buffer. Resistance increases with the length of the supporting medium and decreases both with its width and with increasing buffer ion concentration (Davis and Simpkins, 1975).

Furthermore, pH determines the extent of ionisation of organic compounds. For instance, the ionisation of organic acids increases with pH, whereas the reverse applies for organic bases; therefore their degree of migration will be pH dependent. For compounds such as amino acids that have basic and acidic properties both effects can apply. Thus, buffer of a required pH range has to be used to produce the required separations (Davis and Simpkins, 1975).

2.4.2 Value of grain proteins as documents of identity

Identification by protein electrophoresis is possible as proteins are the direct product of gene transcription and translation, and therefore reflect the genotype and history of the organism (Cooke, 1984). Proteins can thus be regarded as markers for the structural genes that encode them, and methods for comparing protein composition provide a measure of the genetic variation between individuals and populations (Cooke, 1995a). Therefore, if information about the identity or even genetic history of a grain sample is sought, it should be possible to read this information from the grain protein, provided that appropriate technique is used (Wrigley, 1992).

For variety identification, it is necessary to utilise proteins that exist in multiple molecular forms (i.e., are polymorphic), and also preferably that are present in relatively large amounts and are easy to extract. The most commonly used proteins

for cultivar discrimination are thus seed storage proteins, which are known to be polymorphic with respect to either size, charge or both parameters in almost all species investigated (Cooke, 1984).

This includes albumins (water-soluble proteins, mainly enzymes), globulins (the typical salt-soluble storage proteins of legume seed), prolamins (the typical alcohol-soluble storage proteins of cereal seeds), and glutelins (detergent-soluble structural or enzymatic proteins) (Cooke, 1995a). In fact, the classification system of cereal protein still follows fairly closely that proposed by Osborne (1907), as cited in Bietz (1985).

The proportion of each class of protein present in a seed varies from species to species. In cereals, for instance, the major storage protein is usually prolamin, although oats (Peterson *et al*, 1995) and rice (Juliano, 1995) have high levels of globulin and glutelin proteins respectively. In leguminous crops, globulins represent the major part of the seed protein. A large part of the work concerning the application of electrophoresis to characterise cereal crop cultivars has thus been concerned with the analysis of prolamins, which are known by their common names (gliadin in wheat, hordein in barley, zein in maize, avenin in oats etc), although there are methods involving globulins and glutelins (e.g., glutenins in wheat) (Cooke, 1984).

2.4.3 Protein fractionation by electrophoretic methods

Of the various methods of determining protein composition for variety identification, gel electrophoresis has been the traditional technique for obtaining information about identity from plant proteins. The importance and methods of gel electrophoresis for the classification and identification of varieties of agricultural and horticultural species is now firmly established (Wrigley *et al*, 1982; Cooke, 1984, 1988; Gardiner and Forde, 1988). Several investigators have also emphasised the use of seed protein electrophoretic patterns in the identification of cultivars of different crops such as wheat (Shewry *et al*, 1978; Fullington *et al*, 1980), barley

(Nielsen and Johansen, 1986), oat (Lookhart, 1985), maize (Smith and Wych, 1986), soybean (Chauhan *et al*, 1985), beans (Hussain *et al*, 1986), peas (Cooke, 1983), cotton (Rao *et al*, 1990), and sweet potato (Stegemann *et al*, 1992).

Electrophoresis involves four basic steps, which can be summarised as gel preparation, sample preparation (i.e., extraction of proteins to obtain a solution containing either all of the proteins or only a particular class of proteins), sample separation (i.e., fractionation of the proteins in a gel matrix), and gel staining and interpretation (Cooke, 1995b). Variations in each of these four basic steps permit the choice of conditions suited to variety identification of most cereal crops. Review articles are available that provide comprehensive information about the relative advantages of variations of electrophoretic techniques to the specific crop varieties (Wrigley *et al*, 1982; Cooke, 1984,1988; Lookhart, 1991; Bietz and Simpson, 1992).

Various electrophoretic methods are available including starch gel electrophoresis, polyacrylamide gel electrophoresis (PAGE), and sodium dodecyl sulfate (SDS)-PAGE. Unlike the other electrophoretic methods, the SDS-PAGE procedure separates proteins on the basis of their apparent sizes because the surface charge is made uniformly negative by the SDS (Lookhart and Wrigley, 1995).

The way in which electrophoresis is utilised for varietal identification varies from species to species (Cooke, 1988). However, two main approaches have been recognised (Cooke, 1989). The first approach involves the electrophoretic examination of proteins, which are polymorphic and genetically encoded at more than one locus. Cereal seed storage protein provides a good example. They are encoded by multigenic loci and hence the products of a single locus can comprise several protein bands. The criterion for distinctness between varieties is taken as the presence or absence of a particular protein band or set of bands at a defined position or positions on the gel. This is what Cooke (1989) called the "direct, multilocus approach". For self-pollinating cereal crops, this approach has generally been found more useful. The second approach is the "indirect, single locus

approach", involving the utilisation of proteins which, although polymorphic, are derived from a single locus. Such proteins are enzymes, and are known as isozymes or allozymes (Cooke, 1989).

In wheat, albumins and globulins differ little among varieties, and hence they are poorly suited to varietal identification (Wrigley, 1982). However, gliadins and glutenins show considerable heterogeneity in different cultivars, and are hence successfully used for varietal identification.

2.5 The gliadins

Gliadins are defined as the wheat proteins soluble in aqueous in the classic Osborne extraction procedure, as cited in Eliasson and Larsson (1993). An alternative nomenclature has been proposed by Shewry *et al* (1986), in which the gliadins are defined as monomeric proteins. The gliadin conformations are stabilised by hydrogen bonding and hyrophobic interactions, and the disulfide bonds present are all intramolecular (Eliasson and Larsson, 1993).

The gliadin proteins are clearly the best and most often used for cultivar identification. They are readily extracted and fractionated, and the genetic control of their synthesis is well understood. Furthermore, the gliadin electrophoregram is not affected by the growth environment of the grain, by its protein content, by sprouting, dusting or fumigation of the grain, or by heat treatment up to and beyond that required to destroy baking quality (Wrigley, 1982).

For instance, Lookhart and Finney (1984) found that frost affected baking quality of wheat but it did not change the gliadin profiles. Likewise, Clements (1987) found no environmental effects on the gliadin electrophoretic profile of soft wheat. The electrophoretic similarities of profiles from immature and mature seed provided additional evidence of the intransigence of seed protein profiles to factors other than genetic change (Clements, 1987). Similarly, Huebner and Bietz (1988) were unable to find differences in gliadin electrophoretic profiles of seed lots

grown in soils with different sulfur levels. Zillman and Bushuk (1979) have also found that the gliadin electrophoregram is not affected by the area of growth by examining grain of five Canadian wheat cultivars grown at 10 locations in Manitoba and Saskatchewan and grain of five Australian cultivars grown in Canada and Australia.

Significant changes occur in the relative intensities of gliadin bands only when sulfur is severely deficient during growth (Wrigley *et al*, 1980), and this condition rarely, if ever, exists in commercial crops. Lookhart and Pomeranz (1985) also detected slightly different gliadin electrophoretic profiles for two wheat cultivars grown in soils that were severely deficient in sulfur, but no differences could be detected due to various levels of soil nitrogen.

Gliadins can be categorised into four groups, namely the α-, β-, γ-, and ω-gliadins, when fractionated by gel electrophoresis (Mosleth and Uhlen, 1990a). α-gliadins are the fastest moving, and ω-gliadins are the slowest. The proportion of the total protein content has been estimated for different gliadins from the electrophoretic patterns. The ω-gliadins constitute about 8-13% of the protein, whereas the sum of the α-, β-, and γ-gliains constitutes about 34-48%. The ω-gliadins are found in the lowest amount, followed by α-gliains, β-gliadins, and γ-gliadins, respectively, in increasing amounts (Eliasson and Larsson, 1993).

Gliadin is detectable in wheat seed seven to 10 days after pollination, and it accumulates rapidly until about 40 days after pollination (Larkins, 1981). The genes controlling the gliadin proteins are located on the short arms of both the group 1 and group 6 chromosomes. All of the ω-gliadins, most of the γ-gliadins, and few of the β-gliadins are controlled by genes on chromosomes 1A, 1B, and 1D whereas all the α-gliadins, most of the β-gliadins and a few of the γ-gliadins are controlled by chromosomes 6A, 6B, and 6D (Payne *et al*, 1982).

Gliadins are composed of proteins of relatively low molecular weight as compared to the HMW proteins of the glutenin fraction, the dividing line between the two

groups of proteins being an apparent molecular weight of more or less 100kDa (Hamauzu *et al*, 1972). Gliadins comprise molecules of single polypeptide chains, while glutenin is a group of large polymeric molecules composed of both high molecular weight (HMW) and low molecular weight (LMW) subunits linked together by interpolypeptide disulfide bonds (Gao and Bushuk, 1991).

2.6 The high molecular weight glutenin subunits

Glutenins are the remaining endosperm protein after albumins, globulins, and gliadins are extracted. Glutenins are soluble, or rather dispersible, in dilute acid or alkali, in denaturants such as urea, and in surfactants. The low solubility of glutenin is mainly because of their high molecular weight. Molecular weights up to 20 million daltons have been reported (Eliasson and Larsson, 1993).

Glutenin, the least soluble portion of gluten, is less amenable to fractionation than gliadin, but suitable separation techniques have already been devised, including SDS-PAGE. This procedure, which fractionates the glutenin proteins as the reduced polypeptide subunits, provides useful distinction between varieties (Shewry *et al*, 1978). Since the synthesis of glutenin is under different genetic control from that of gliadin (Lawrence and Shepherd, 1980), glutenin analysis provides different information about genotype than gliadin analysis.

Glutenin is a polymeric protein, ranging in molecular weight (M_r) from 80k to several millions, in which many sub-units are cross-linked by disulphide bonds. These subunits have been classified on the basis of their mobilities in SDS-PAGE under reducing conditions into two main groups, the high molecular weight glutenin subunits (M_{rs} of 80-120k) and the low molecular weight glutenin subunits (M_{rs} of 30-50k) (Redaelli *et al*, 1995; Lew *et al*, 1992).

The high molecular weight glutenin subunits (HMW-GS) are coded by genes at the Glu-A1, Glu-B1, and Glu-D1 loci on the long arms of the chromosomes 1A, 1B, and 1D respectively (Payne *et al*, 1982), and allelic variation at each locus (Payne

and Lawrence, 1983) has produced extensive variability in wheat cultivars. There is considerable variation in the pattern of the HMW-GS when the proteins of different wheat varieties are fractionated by SDS-PAGE (Payne and Lawrence, 1983), due to the presence of different alleles at each of the three gene loci. So far, more than 20 allelic forms of these subunits have been found in wheat cultivars (Payne *et al*, 1987). Although the HMW-GS only account for around 10% of the wheat storage proteins (He Zhong-hu *et al*, 1992), they show considerable variation among wheat cultivars upon fractionation by SDS-PAGE, and are hence successfully used for cultivar identification.

The HMW-GS are readily fractionated by SDS-PAGE using total kernel protein extracts. However, the low molecular weight glutenin subunits (LMW-GS) have molecular weights close to that of the monomeric gliadin storage proteins, and, therefore, it is difficult to identify LMW-GS variation using total protein extracts by SDS-PAGE (Sontag-Strohm, 1996). Thus, LMW-GS are identified reliably by two-dimensional electrophoretic techniques (Jackson *et al*, 1983), two step one-dimensional electrophoretic method (Singh and Shepherd, 1988), or by a simple one-step one-dimensional electrophoretic procedure (Singh *et al*, 1991; Gupta and MacRitchie, 1991) using free glutenin subunits separate by sequential extraction.

By and large, the various techniques of protein electrophoresis had an enormous impact in the area of crop variety identification and the use of electrophoresis is both widespread and growing. However, it is by no means complete, and thus there are good reasons for progressing one step beyond proteins and considering the potential of deoxyribonucleic acid (DNA) analysis for variety identification.

2.7 The use of DNA for variety identification

It has been indicated (see section 2.4) that electrophoresis can reveal genetic variability in protein composition. If such variability exists, then it follows that there must be variation in the underlying genetic material (DNA). Indeed, it is virtually certain that there will be far more DNA variability, since not all differences will occur in regions of the DNA which are expressed phenotypically (Cooke, 1995a).

Most proteins that have been used in electrophoretic or chromatographic profiling are coded by genes that are unequally dispersed throughout the genome. This prevents them from providing thorough genomic sampling. Again, mutations that are not translated into conformational, net electric charge, size, or hydrophobicity change in the protein can occur, even though variation revealed by electrophoresis is dependent on one or more of these factors (Smith and Smith, 1992). For instance, as much as 75% of nucleotide substitutions may have no effect on these protein characteristics and will thus remain undetected by protein assay techniques (Marshall and Brown, 1975). Thus, an extremely powerful tool for variety identification would be available if DNA polymorphism could be detected and assessed.

Several DNA-based marker systems are available for variety identification in plant species. These markers can be classified into two categories based on the principle involved: 1) restriction fragment length polymorphism (RFLP) (Botstein *et al*, 1980) which is detected by probing southern genomic blots with labelled clones or DNA fragments, and 2) polymerase chain reaction (PCR)-based markers, such as random amplified polymorphic DNA (RAPD) (Williams *et al*, 1990), microsatellite markers, (Litt and Luty, 1989), and amplified fragment length polymorphism (AFLP) (Vos *et al*, 1995; Zabeau and Vos, 1993).

The PCR relies on the use of specific class of enzymes, DNA polymerases, which all living cells possess and use to copy their own DNA. DNA polymerases copy

single stranded DNA from the 3'OH end of double stranded DNA. In PCR, the sample is first heated to separate the double-stranded DNA into single-stranded molecules. Next, the temperature is lowered to allow short synthetic DNA molecules called primers to anneal to complementary sequences. These double-stranded complexes serve as starting points for the copy of single-stranded DNA by the polymerase. By flanking a region of DNA with specific DNA primers and cycling the temperature to facilitate strand separation, primer annealing, and primer extension, PCR can exponentially amplify a single copy of a DNA molecule to yield sufficient DNA for electrophoretic analysis. The use of heat-stable enzyme from *Thermus aquaticus*, known as *Taq* polymerase, and the development of thermocyclers capable of cycling temperatures quickly and accurately, have facilitated the automation of this process (Morell *et al*, 1995).

The specificity of the PCR essentially depends upon the initial interaction of the primers with the DNA template. Although the primers used in PCRs are typically 20 or more nucleotides long, thus allowing for stringent (high temperature) annealing, any interaction of primer with genomic template may give rise to extension product. What makes the amplification of a defined target feasible is the dependence of the reaction on the specificity of two primers, which must bind to sites on either complementary strand of DNA. A high degree of specificity is only required during the early cycles of the PCR as during the later cycles most of the templates are the perfect targets amplified in previous cycles (Hill and Stewart, 1992).

The most critical component for optimising the specificity of any PCR-based assay is the choice of the annealing temperature (Ruano *et al*, 1991) until they find complementary annealing sites. In later cycles, denaturation temperatures may even be shortened in order to preserve enzymatic activity (Gelfand, 1989). Yu and Pauls (1992) concluded that the best results should be obtained by optimising for the shortest possible denaturing time. Too many cycles may result in primer depletion and subsequent priming by amplification products, which often leads to longer products and smears in the gel (Rolfs *et al*, 1992). However, conventional PCR is

limited in usefulness because of the time and expense required to obtain the DNA sequence information required for the design of primers that will uniquely amplify the desired sequence (Kochert, 1994).

2.8 Amplified fragment length polymorphism (AFLP)

The AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. The technique involves three steps: (i) restriction of the DNA and ligation of oligonucleotide adapters, (ii) selective amplification of sets of restriction fragments, and (iii) gel analysis of the amplified fragments. In this technique, specific double-stranded DNA adapters are ligated to the DNA restriction fragments, so that the sequence of the adapters and the adjacent restriction sites serve as primer-binding sites. The primers are designed to contain the sequences that are complementary to those of adapters and the restriction sites, along with one to three selective bases added at their 3' ends. The use of selective bases allows amplification of only a subset of the restriction fragments, which still generate a large number of bands facilitating the detection of polymorphism (Vos *et al*, 1995).

The method allows the specific co-amplification of high numbers of restriction fragments. The number of fragments that can be analysed simultaneously, however, is dependent on the resolution of the detection system. Typically 50-100 restriction fragments are amplified and detected on denaturing polyacrylamide gel. The AFLP technique is robust and reliable because stringent reaction conditions are used for primer annealing. AFLP detects genomic restriction fragments and resembles in that respect the RFLP technique; with the major difference that PCR amplification instead of southern hybridisation is used for detection of fragments. Thus, the AFLP technique combines the reliability of RFLP technique with the power of PCR technique, and the resemblance with RFLP technique is the basis to choose the name AFLP (Vos *et al*, 1995).

Although microsatellites show a much higher level of polymorphism than any other marker system (Röder *et al*, 1998), the average number of bands per lane or per PCR is by far higher for AFLPs (49.5) compared to (1.0) band per lane or per PCR for SSRs (Russell *et al*, 1997). Thus, even if AFLPs do not offer the highest level of polymorphisms, they have the capacity to reveal many polymorphic bands in single lane.

A comparison of different mapping techniques – RFLP, RAPD, SSR, and AFLP – for their relative efficiency in detecting polymorphism demonstrated that AFLP is the most efficient (Powell *et al*, 1996, Russell *et al*, 1997). Unlike STS (sequence-tagged-site) PCR and microsatellite markers, AFLP markers do not require a prior knowledge of DNA sequences and there is almost no marker development cost. The advantages of the AFLP technique in wheat include the detection of a large number of independent genetic loci in a single reaction and reliability determined by the highly stringent PCR conditions and the highly specific primers (Ma and Lapitan, 1998).

The AFLP technique has been shown to be highly effective for detecting large number of polymorphisms in species including wheat (Law *et al*, 1998; Barrett and Kidwell, 1998; Ma and Lapitan, 1998; Bohn *et al*, 1999), barley (Becker *et al*, 1995; Hayes *et al*, 1997; Qi *et al*, 1998), rice (Mackill *et al*, 1996; Maheswaran *et al*, 1997), tomato (Thomas *et al*, 1995), potato (Van Eck *et al*, 1995), sugar beet (Schondelmaier *et al*, 1996), soybean (Maughan *et al*, 1996; VanToai *et al*, 1997), lentil (Sharma *et al*, 1996), lettuce (Hill *et al*, 1996), common bean (Tohme *et al*, 1996), sunflower (Hongtrakul *et al*, 1997), hop (Hartl and Seefelder, 1998),and tef (Bai *et al*, 1999).

Chapter 3

Morphological characterisation of Ethiopian wheat cultivars

Abstract

Wheat varieties possess distinguishing characters that contribute to their identification. Twenty wheat varieties and advanced lines (11 bread wheat and nine durum wheat) were grown and evaluated for 14 morphological, phenological, and agronomic characters in four replicated randomised complete blocks at the University of Orange Free State during the year 1999. The experiment was conducted under greenhouse and field conditions. The objective of the study was to evaluate the cultivars using phenotypic traits. The distinguishing characters were measured and recorded when appropriate plant stages showed full expression of the characters. The cultivars were distinctly different in some characters and similar in others. The cultivars were grouped using Unweighted Pair Group Mean Arithmetic Analysis (UPGMA) (Sneath and Sokal, 1973) into clusters. A dendrogram obtained from the cluster analysis grouped the cultivars into four main clusters.

Introduction

Wheat (*Triticum spp*) is one of the most important cereals cultivated in Ethiopia. Both tetraploid (*T. turgidum L.*) and hexaploid (*T. aestivum L.*) species are grown. The former are indigenous to Ethiopia while the latter are of recent introduction. The durum and bread wheat species grown in Ethiopia are represented by tetraploids (2n = 28) and hexaploids (2n = 42) respectively. Currently durum wheat occupies more than two-thirds of the area under wheat production. Of the tetraploid durum wheat species (*T. turgidum* var durum) is the predominant and the most extensively cultivated (Bechere *et al*, 1994; Tesemma *et al*, 1993).

Traditionally, varietal identification has been carried out by what might be called a classical taxonomic approach. This involves a detailed study of a wide range of morphological,

phenological, and agronomic characters, both of the seed and of the growing and mature plant (Cooke, 1984). Although the use of such phenotypic traits is associated with many limitations (see section 2.3.2), the approach is very successful in practice and should not be decried.

It is commonly known that morphological data can be of dubious taxonomic reliability because of environmental interaction and the largely unknown mechanisms of genetic control of these traits. However, problems associated with the interpretation of morphological descriptions can be minimised by measuring traits in a number of environments or by limiting comparisons to those traits for which the effects of environmental interaction are smallest. For the practical purpose of routinely describing varieties for plant variety protection (PVP) or patenting, it has not been customary to make detailed descriptions of numerous traits in replicated plots over three or more years. Thus it is desirable to identify those traits that show the highest repeatability or least interaction with the environment (Smith and Smith, 1989).

So far, most of the studies on Ethiopian wheat species have been on the diversity of morphological characters that are highly heritable (Bekele, 1984; Tesfaye *et al*, 1991). Several studies have dealt with the variability in the Ethiopian wheat landraces for morphological, agronomic, and quality characters most of which are geared towards quantifying the variation within and between geographic regions and populations (Belay *et al*, 1993). The information generated by such studies could be useful to breeders, particularly when certain agronomic characters are associated with morphological characters of the genotype. Such evaluation has revealed useful variation in wheat landraces from Israel (Poiarkova and Blum, 1983), India (Pathak and Nema, 1985), Iran (Ehdaie and Wains, 1989) and Jordan (Jaradat, 1991).

Thus, wheat varieties possess distinguishing morphological characters that aid in the identification of one variety from the others. In this experiment, 20 wheat varieties and advanced lines were grown and described under greenhouse and field conditions. The aim of

the study was to determine the clustering patterns of all the entries tested on the basis of phenotypic differences.

Materials and Methods

Plant materials

The plant materials used in this study were obtained from Ethiopian Seed Enterprise and Ethiopian Agricultural Research Organisation. A summary of the cultivars (or lines) used, their pedigree information, and year of release is given in Table 3.1 for bread wheat and Table 3.2 for durum wheat.

Experimental layout

i) *Greenhouse experiment.*

The 20 wheat entries were planted in a greenhouse on the 15th of June 1999, at the University of the Orange Free State. A randomised complete block design with four replications was used. Two litre capacity plastic pots were filled with 2.5kg of loam soil, and mixed with 4g fertiliser (N:P:K = 3:2:0). Six seeds were sown, and the seedlings were thinned to three plants per pot at three to four leaf stages. The plants were watered as required. One ml of Chlorpirifos diluted in one litre of water was used to spray the greenhouse before planting and before booting stage to control aphids. At stem elongation, the main plants were tagged to differentiate the tillers, and all observations were recorded for the three plants in the experimental unit (pot). Finally, a grand mean of the four plots was calculated and used for analysis.

ii) *Field experiment*

The field experiment was carried out at the University of the Orange Free State experimental site, 25km west of the University. The entries were planted on the 9th of June 1999. The experiment was arranged in a randomised complete block design with four replicates. Each plot consisted of 20 rows each 5m long and spaced 80cm apart. Before planting, a 3:2:0 ratio of N:P:K mixture was applied at 100Kg ha^{-1} according to the production potential of the site. Seeds were drilled during planting, and the seedlings were thinned to 15cm between plants in

the row at three to four leaf stages. Soil moisture was supplemented when necessary by overhead sprinkler irrigation. Before booting stage, the plots were sprayed with Chlorpirifos at 600ml ha^{-1} to control aphids, and weeds were controlled by hand. The desired phenotypic traits were collected from nine plants at the centre of each row. Finally, a grand mean of the four replications was used for analysis.

Table 3.1 Bread wheat cultivars used for morphological characterisation.

No	Cultivars	Pedigree information	Year of release
1	HAR 1685	ATILA, NDVG9144//KAL/BB/3/YACO"S"/4/VEE"S" : CM85836-50Y-0M-0Y-3M-0Y	1994
2	HAR 1709	BOW28/RBC : ET1297-F5YR20-6/87	1993
3	Pavon 76	VCM//CNO"S"/7C/3/KAL/BB : CM8399-D-4M-3Y-1M-1Y-1M-0Y	1982
4	Dashen	KVZ/BUHO "S"//KAL/BB	1984
5	HAR 710	MRL"S"/BUC"S" : CM61949-13Y-1M1M-1Y-1M-2Y-0Y	1994
6	ET-13-A2	Encoy X UQ 105 SEL : ET13 A.2.L.3.L	1981
7	K-6290-bulk	AF_1 MAYO/GEM/ROMANY	1977
8	HAR 1522	BOW"S"/BUC"S" : CM74005-8M-1Y-03M-5Y-5B-0Y	1995
9	K-6295-4A	ROMANY/GB-GAMENYA : K6295-4A	1980
10	HAR 1407	COOK/VEE"S"//DOVE"S"/SERI : CM69279-C-2Y-1M-5Y-1M-0Y	1997
11	HAR 604	4777(2)//FKN/GB/3/PVN"S" : CM49912-37M-4Y-4Y-1M-1Y-0M	1994

Table 3.2 Durum wheat cultivars used for morphological characterisation.

No	Cltivars/lines	Pedigree information	Year of release
1	Foka	Cocorit 71/Candeal II, CD 3369	1993
2	DZ-393-4	Illumilo-Cocorit 71	1995
3	DZ-1052	Boohai/4/Mexi "S"	Pipe line
4	DZ-1050	Boohai/ULNV	Advanced line
5	Boohai	(Cr"s"/21563/61-130 x Lds) candeal II	1982
6	DZ-04-118	Agrotype/landrace	1966
7	Cocorir 71	RAE/4 TC60//TW6	1976
8	Kilinto	Illumilo/Inrat 69//Boohai/3/Hora/Jorro/4/Cit 71, DZ 918	1994
9	DZ-2023	DZ 04-1169/DZ 04-12 B.B/4//5/Chen "S"I	Advanced line

Morphological parameters

The following morphological, phenological, and agronomic characters were scored for all entries both from the greenhouse and field experiment during the growing season. These characteristics were chosen for their use in cultivar identification worldwide.

1. Days to heading: It is the number of days from sowing date to full exposure of main plant's spike.
2. Days to maturity: The number of days from sowing date to physiological maturity. i.e. when peduncle of the main plant turns from green to yellowish colour.
3. Plant height (cm): Height from the base to the tip of the spike.
4. Number of productive tillers: This is the number of tillers per plant that has produced a seed/seeds.
5. Flag leaf area (cm^2): This is obtained by multiplying the length and maximum width of the flag leaf by 0.835 as proposed by Miralles and Slafer (1991).
6. Last internode length (cm): The length of the main plant's internode immediately below the spike.

7. Peduncle length above the flag leaf area (cm): The length of the main plant's peduncle between the flag leaf and the base of the spike.
8. Spike length (cm): The length from the base to the tip of the main plant's spike.
9. Number of spikelets/main spike: This is the number of spikelets on the main plant's spike.
10. Number of seeds/main spikelets: This is the number of seeds found in the spikelets of the main plant.
11. 1000 seed weight (g): This is the weight of 1000 seeds.
12. Biomass yield (BY) (g): Total weight of plant part above the ground.
13. Seed weight/plant (g): It is a total weight of seeds harvested from the main plant and its productive tillers.
14. Harvest index (HI): This is the percentage of the ratio of seed weight/plant to BY (i.e. seed weight/plant ÷ BY × 100).

Calculation of genetic distances.

Pairwise genetic distance matrices are produced by comparing the vector of a given individual with that of every other individual and calculating a distance value based on a given formula. The process is repeated for all individuals. Various formulae for calculating genetic distances or dissimilarity are available. One of the most widely used is that of Nei and Li (1979).

In this study a grand mean was computed for all the phenotypic traits recorded, and the subsequent calculations were performed using the NCSS 2000 software package (Hintze, 1998). The index of genetic similarity (F) of Nei and Li (1979) was used to calculate the pairwise genetic distance (D) for all entries.

$$F = 2Nxy/(Nx + Ny)$$

$$D = 1-F$$

Where Nxy = the number of shared traits between any two cultivars X and Y and Nx and Ny are the number of traits for cultivars x and y, respectively (Wang and Tanksley, 1989).

Summarising genetic relationships as dendrograms

While the presentation of a pairwise genetic distance matrix may be informative for small sample sets, it is usual to present the results as a dendrogram. Dendrograms are produced by running the distance matrix through a computer program that performs a cluster analysis. Various algorithms for clustering data are available but the UPGMA (Unweighted Pair Group Mean Arithmetic Analysis, Sneath and Sokal, 1973) method is the most commonly used. In this study the UPGMA method was used for cluster analysis of the pairwise distance matrix, which generated a dendrogram representing the genetic distances among the wheat cultivars.

Results and Discussion

Morphological trait analysis

The morphological data scored for all the 14 traits are given in Table 3.3 for the greenhouse experiment and Table 3.4 for the field experiment. The UPGMA analysis provided the genetic distances between all possible pairs of the wheat entries (which is summarised in Table 3.5 for greenhouse experiment and Table 3.6 for field experiment), detail of the cluster section (Table 3.7 for greenhouse experiment and Table 3.8 for field experiment), and a dendrogram (Fig. 3.1 and Fig 3.2 for the greenhouse and field experiment, respectively) representing the genetic distances among the wheat entries.

Table 3.3 Mean values of morphological traits scored from greenhouse experiment.

No	Entries	DH	DM	PH	PT	FLA	LIL	PLF	SL	SI/ MS	S/ MSI	1000 SW	SW/ P	BY	HI
1	HAR1685	48.0	135.3	70.4	18.0	18.3	24.0	9.6	7.7	15.6	2.11	44.5	7.6	16.0	47.5
2	HAR 1709	50.0	135.3	86.0	14.7	28.2	29.7	11.9	9.9	19.2	3.27	38.2	9.3	20.0	24.3
3	Pavon 76	50.3	136.4	77.4	15.0	29.0	31.3	15.9	9.2	17.4	3.25	40.3	11.0	20.0	55.0
4	Foka	49.6	133.9	93.1	13.2	27.0	40.2	22.2	6.4	14.4	2.77	42.3	6.4	14.8	43.2
5	Dashen	57.2	139.1	75.2	10.5	25.5	24.8	8.9	9.7	20.2	2.80	44.2	8.9	17.8	50.0
6	HAR 710	54.6	138.8	76.7	14.0	37.3	31.0	13.5	11.3	18.8	3.15	39.9	8.7	19.0	45.7
7	ET-13-A2	49.2	138.2	90.4	22.0	24.6	33.9	17.2	8.0	17.8	2.11	39.3	7.7	19.6	39.2
8	DZ-2023	46.9	135.8	84.6	17.5	29.4	38.2	19.0	7.2	14.9	3.14	40.5	7.6	17.4	43.6
9	DZ-393-4	48.7	135.4	90.9	13.2	30.6	37.4	18.8	6.3	14.9	2.99	44.3	7.5	17.1	43.8
10	DZ-1052	45.5	131.6	86.8	12.2	24.1	35.0	16.3	7.0	14.9	2.63	42.6	6.6	15.5	42.5
11	K-6290-bulk	52.0	130.7	91.1	17.0	30.8	32.1	13.9	9.1	19.9	2.71	46.9	11.1	21.5	51.6
12	DZ-04-118	46.0	131.3	85.2	28.6	20.5	37.0	21.2	6.2	14.3	1.25	38.2	5.6	18.3	30.6
13	HAR 1522	60.0	141.3	75.5	14.5	39.9	28.9	11.5	11.7	19.5	3.01	43.7	9.9	20.8	47.5
14	K-62954-A	46.7	134.8	87.9	13.2	28.6	38.0	19.6	8.7	16.2	2.84	43.2	11.6	21.2	54.7
15	DZ-1050	44.7	136.2	85.9	12.5	24.7	33.9	15.8	7.0	14.7	3.11	44.7	6.2	14.8	41.8
16	Kilinto	48.7	136.7	91.4	12.0	23.3	39.9	20.2	6.3	14.9	3.02	48.7	7.9	16.3	48.4
17	Cocorit 71	50.0	142.0	70.0	10.2	28.2	32.2	15.0	5.9	13.7	2.93	39.4	5.4	12.0	45.0
18	HAR 1407	50.2	133.8	75.5	15.0	25.9	27.7	12.8	8.0	18.0	3.07	40.5	8.1	16.6	48.7
19	Boohai	49.4	137.2	95.7	14.2	31.7	37.9	18.6	6.7	14.6	3.36	46.0	6.9	17.1	40.3
20	HAR 604	54.0	140.1	80.1	9.7	34.9	29.9	11.9	10.4	19.4	4.15	37.8	9.8	20.0	49.0

DH = days to heading, DM = days to maturity, PH = plant height, PT = number of productive tillers, FLA = flag leaf area, LIL = last internode length, PLF = peduncle length above the flag leaf, SL = spike length, SI/MS = number of spikelets per main spike, S/MSI = number of seeds per main spikelets, 1000 SW = 1000 seed weight, SW/P = seed weight per plant, BY = biomass yield, HI = harvest index.

Table 3.4 Mean values of morphological traits scored from field experiment.

No	Entries	DH*	DM*	PH	PT	FLA	LIL	PLF	SL	SI/MS	S/MSI	1000 SW	SW/P	BY	HI
1	HAR 1685	120.5	159.3	76.3	9.0	14.33	29.4	11.3	10.0	20.7	2.89	37.2	39.0	69.5	56.1
2	HAR 1709	115.2	158.3	84.8	9.4	11.61	31.2	12.2	12.8	22.4	2.20	36.4	35.8	73.7	48.5
3	Pavon 76	117.7	158.6	78.7	7.6	17.02	33.3	14.4	11.9	20.7	2.83	36.7	37.1	72.4	51.2
4	Foka	117.2	158.6	92.0	7.2	19.97	43.2	20.3	8.3	18.3	2.84	48.4	29.8	65.3	45.6
5	Dashen	123.0	161.3	73.3	10.0	15.90	26.5	8.8	12.6	20.2	3.04	41.4	33.1	62.9	52.6
6	HAR 710	120.0	158.6	81.1	8.2	15.94	32.4	13.4	14.6	19.5	2.83	39.4	31.9	63.9	49.9
7	ET-13-A2	125.0	162.6	90.2	9.9	21.52	38.0	15.9	10.4	21.4	2.66	38.0	35.3	73.8	47.8
8	DZ-2023	117.5	159.3	88.4	6.8	23.49	42.0	18.9	9.3	21.4	2.86	48.3	41.9	85.1	49.2
9	DZ-393-4	120.7	159.3	91.2	7.6	23.68	38.6	15.6	8.2	19.5	2.71	53.0	34.7	74.7	46.4
10	DZ-1052	113.2	157.0	90.9	7.8	19.27	39.4	17.7	9.1	19.3	2.68	52.2	35.7	66.7	53.5
11	K-6290-bulk	120.7	156.0	84.6	7.9	17.39	34.1	15.2	11.8	20.0	2.31	39.2	34.2	72.9	46.9
12	DZ-04-118	120.0	157.0	83.8	6.5	16.62	40.2	21.5	8.6	17.3	2.58	39.5	30.1	50.8	59.2
13	HAR 1522	120.2	159.0	79.1	8.3	20.09	30.9	13.2	15.2	20.0	2.92	41.0	38.3	75.9	50.4
14	K-62954-A	122.7	162.3	86.1	11.0	20.97	38.7	18.1	11.3	17.3	3.28	35.7	35.7	68.7	51.9
15	DZ-1050	110.7	156.0	90.7	7.4	18.93	37.0	15.4	9.1	18.9	2.43	51.1	32.9	67.1	49.0
16	Kilinto	120.2	161.0	86.7	6.9	20.07	41.4	19.1	8.1	18.4	2.75	53.3	26.7	53.5	49.9
17	Cocorit 71	113.2	157.3	71.4	6.2	18.66	32.7	12.9	7.6	18.2	3.07	47.5	31.6	60.8	51.9
18	HAR 1407	120.5	158.3	75.2	9.2	12.01	27.5	10.2	10.4	19.8	2.80	37.6	38.3	72.5	52.8
19	Boohai	119.0	159.3	87.4	7.3	20.56	38.3	16.1	8.7	18.6	3.02	49.2	32.3	64.9	49.7
20	HAR 604	125.7	161.6	78.8	8.3	25.09	26.1	6.2	13.5	23.7	3.74	32.7	35.7	87.5	48.8

DH = days to heading, DM = days to maturity, PH = plant height, PT = number of productive tillers, FLA = flag leaf area, LIL = last internode length, PLF = peduncle length above the flag leaf, SL = spike length, SI/MS = number of spikelets per main spike, S/MSI = number of seeds per main spikelets, 1000 SW = 1000 seed weight, SW/P = seed weight per plant, BY = biomass yield, HI = harvest index.

* Days to heading and days to maturity were delayed because of the cold (winter) weather.

Table 3.5 Pairwise distance matrix of the greenhouse experiment based on the F statistic of Nei and Li (1979).

No	Cultivar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	HAR 1685	0																			
2	HAR 1709	1.57	0																		
3	Pavon 76	1.33	1.21	0																	
4	Foka	1.65	1.63	1.47	0																
5	Dashen	1.20	1.36	1.09	1.92	0															
6	HAR 710	1.59	1.31	0.76	1.74	0.97	0														
7	ET-13-A2	1.33	1.11	1.16	1.19	1.51	1.38	0													
8	DZ-2023	1.41	1.26	1.00	0.66	1.64	1.31	0.87	0												
9	DZ-393-4	1.47	1.39	1.15	0.52	1.59	1.41	1.06	0.51	0											
10	DZ-1052	1.18	1.38	1.29	0.62	1.62	1.60	1.13	0.73	0.66	0										
11	K-6290-bulk	1.57	1.38	1.01	1.62	1.33	1.27	1.33	1.38	1.28	1.42	0									
12	DZ-04-118	1.73	1.88	2.01	1.45	2.42	2.31	1.16	1.40	1.61	1.42	2.06	0								
13	HAR 1522	1.84	1.47	1.19	2.15	0.99	0.69	1.66	1.74	1.75	2.02	1.41	2.62	0							
14	K-62954-A	1.63	1.47	0.75	1.21	1.59	1.28	1.20	0.94	0.95	1.18	0.95	1.91	1.64	0						
15	DZ-1050	1.17	1.41	1.30	0.73	1.54	1.55	1.18	0.73	0.63	0.50	1.54	1.65	1.93	1.27	0					
16	Kilinto	1.55	1.75	1.37	0.74	1.69	1.71	1.34	0.92	0.60	0.90	1.43	1.84	1.99	1.07	0.76	0				
17	Cocorit 71	1.38	1.78	1.54	1.35	1.57	1.56	1.57	1.21	1.27	1.27	2.15	2.04	1.92	1.82	1.05	1.46	0			
18	HAR 1407	0.86	1.09	0.75	1.32	0.94	1.01	1.10	0.97	1.09	0.94	1.18	1.78	1.43	1.22	0.98	1.35	1.22	0		
19	Boohai	1.66	1.45	1.34	0.71	1.69	1.51	1.18	0.71	0.37	0.91	1.41	1.77	1.78	1.15	0.73	0.66	1.39	1.30	0	
20	HAR 604	1.84	1.18	0.91	1.90	1.10	0.68	1.60	1.46	1.55	1.76	1.49	2.59	0.96	1.43	1.66	1.84	1.68	1.16	1.16	0

Table 3.6 Pairwise distance matrix of the field experiment based on the F statistic of Nei and Li (1979).

No	Cultivar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	HAR 1685	0																			
2	HAR 1709	1.12	0																		
3	Pavon 76	0.70	0.91	0																	
4	Foka	1.80	1.70	1.36	0																
5	Dashen	0.82	1.33	1.05	1.86	0															
6	HAR 710	1.01	1.01	0.66	1.32	0.88	0														
7	ET-13-A2	1.35	1.35	1.16	1.36	1.33	1.23	0													
8	DZ-2023	1.55	1.62	1.15	1.27	1.91	1.57	1.26	0												
9	DZ-393-4	1.57	1.58	1.21	0.77	1.66	1.32	1.04	0.87	0											
10	DZ-1052	1.38	1.43	1.09	0.94	1.71	1.25	1.49	1.07	0.94	0										
11	K-6290-bulk	1.24	0.90	0.78	1.20	1.43	0.83	1.21	1.28	1.07	1.14	0									
12	DZ-04-118	1.57	1.91	1.46	1.37	1.84	1.42	1.88	1.88	1.67	1.21	1.54	0								
13	HAR-1522	0.94	1.13	0.58	1.57	0.99	0.69	1.17	1.25	1.28	1.33	0.94	1.77	0							
14	K-6295-4A	1.34	1.74	1.29	1.47	1.29	1.25	0.98	1.58	1.41	1.52	1.54	1.69	1.24	0						
15	DZ-1050	1.59	1.35	1.20	0.90	1.82	1.27	1.64	1.31	1.00	0.56	1.03	1.42	1.44	1.78	0					
16	Kilinto	1.78	1.90	1.51	0.74	1.70	1.36	1.46	1.62	1.06	1.16	1.51	1.18	1.70	1.53	1.24	0				
17	Cocorit 71	1.33	1.70	1.12	1.32	1.46	1.23	1.88	1.64	1.40	1.15	1.40	1.31	1.42	1.77	1.12	1.27	0			
18	HAR 1407	0.42	0.99	0.74	1.80	0.83	0.94	1.44	1.66	1.59	1.46	1.10	1.70	0.94	1.45	1.56	1.85	1.33	0		
19	Boohai	1.33	1.54	1.01	0.61	1.40	1.01	1.17	1.11	0.62	0.76	1.09	1.21	1.18	1.20	0.90	0.71	0.97	1.39	0	
20	HAR 604	1.56	2.02	1.58	2.40	1.48	1.73	1.62	1.95	1.98	2.35	1.95	2.63	1.39	1.86	2.49	2.49	2.22	1.73	2.00	0

Table 3.7 Cluster detail section formed by UPGMA analysis of morphological data collected from the greenhouse.

Row	Cluster	Name
3	1	Pavon 76
18	1	HAR 1407
4	2	Foka
8	2	DZ-2023
9	2	DZ-393-4
10	2	DZ-1052
15	2	DZ-1050
16	2	Kilinto
19	2	Boohai
6	3	HAR 710
13	3	HAR 1522
20	3	HAR 604
11	4	K-6290-bulk
14	4	K-6295-4A
1		HAR 1685
2		HAR 1709
5		Dashen
7		ET-13-A2
12		DZ-04-118
17		Cocorit

Table 3.8 Cluster detail section formed by UPGMA analysis of morphological data collected from field experiment.

Row	Cluster	Name
1	1	HAR 1685
5	1	Dashen
18	1	HAR 1407
4	2	Foka
9	2	DZ-393-4
10	2	DZ-1052
15	2	DZ-1050
16	2	Kilinto
19	2	Boohai
7	3	ET-13-A2
14	3	K-6295-4A
3	4	Pavon 76
6	4	HAR 710
11	4	K-6290-bulk
13	4	HAR 1522
2		HAR 1709
8		DZ-2023
12		DZ-04-118
17		Cocorit
20		HAR 604

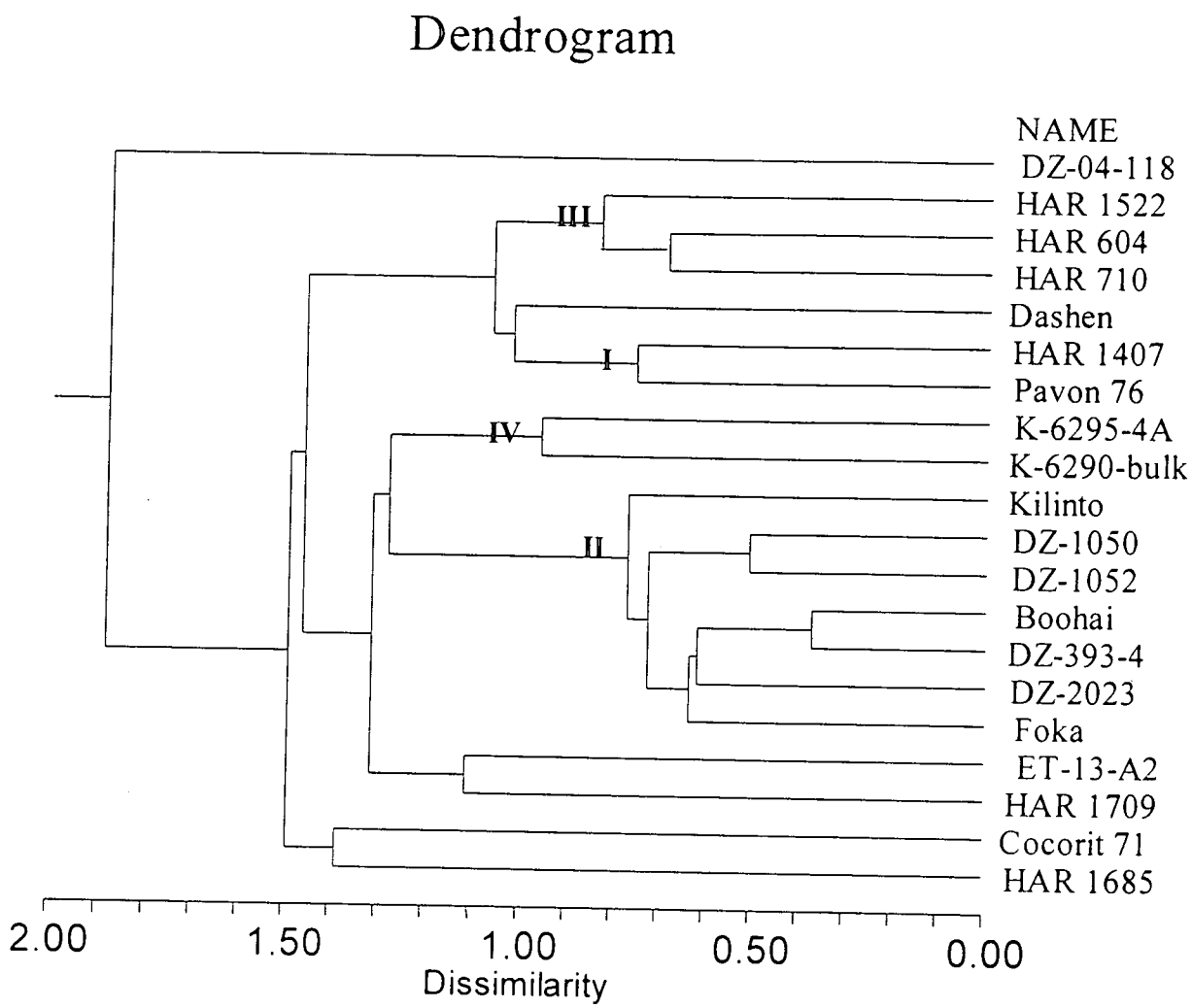

Fig. 3.1 Dendrogram generated by UPGMA analysis of morphological data collected from the greenhouse.

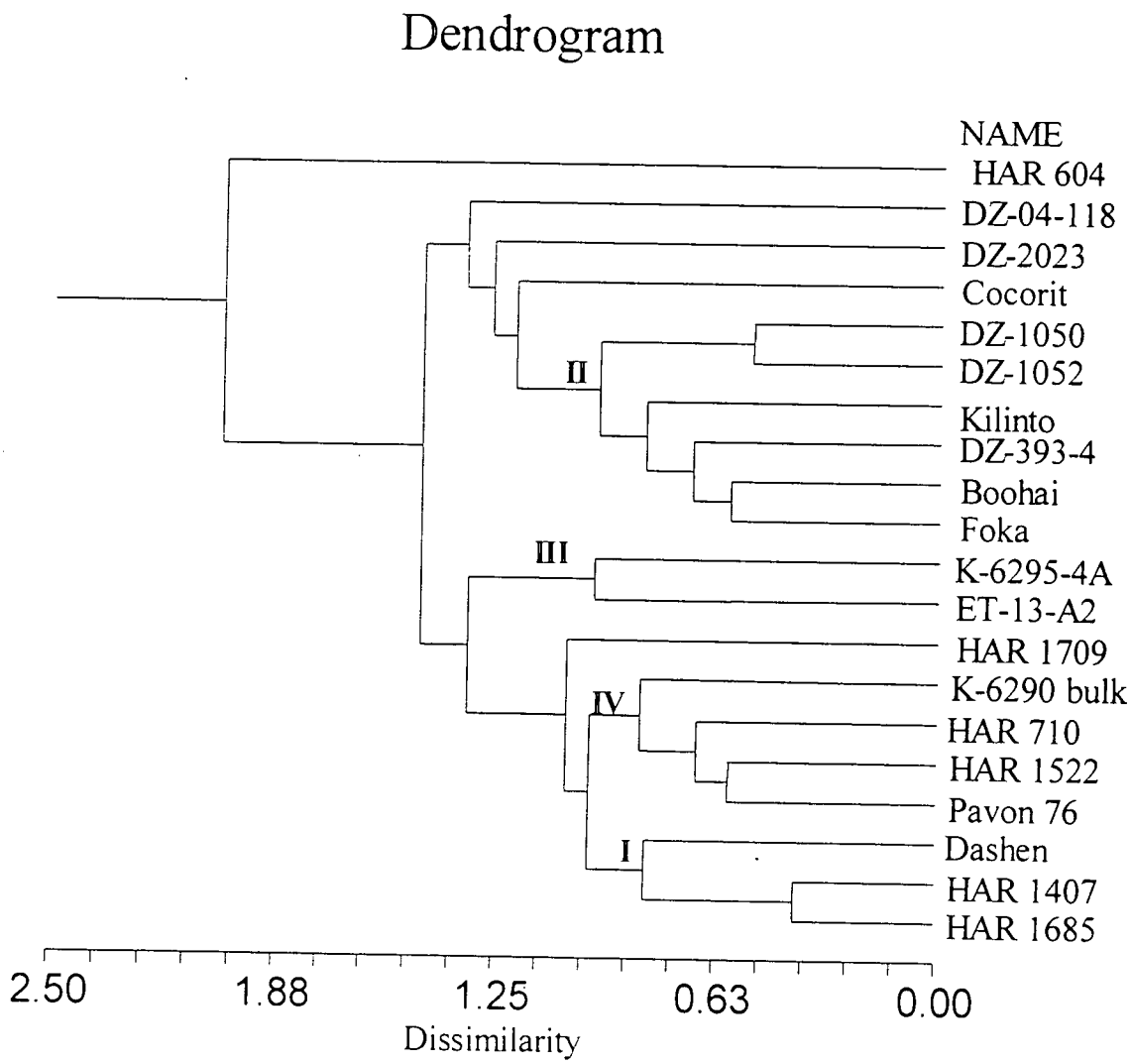

Fig. 3.2 Dendrogram generated by UPGMA analysis of morphological data collected from the field experiment.

Genetic distance analysis

Greenhouse experiment

Pairwise genetic distance (Table 3.5) based on the F statisic of Nei and Li (1979) revealed an average distance of 1.01 among the durum wheat cultivars ranging from 0.37 between DZ-393-4 and Boohai to 2.04 between DZ-04-118 and Cocorit 71. The genetic distances among the bread wheat cultivars ranged from 0.68 between HAR 710 and HAR 604 to 1.84 between (HAR 1685 and HAR 1522; HAR 1685 and HAR 604), with an average genetic distance of 1.24

Field experiment

The genetic distances among the durum wheat entries (Table 3.6) based on the F static of Nei and Li (1979) ranged from 0.56 between DZ-1052 and DZ-1050 to 1.88 between DZ-2023 and DZ-04-118, with an average genetic distance of 1.11. The result also indicated an average distance of 1.16 among the bread wheat cultivars, ranging from 0.42 between HAR 1685 and HAR 1407 to 2.02 between HAR 1709 and HAR 604.

Dendrograms

Greenhouse experiment

A dendrogram constructed using phenotypic traits collected from the greenhouse experiment was given in Fig. 3.1. In this dendrogram, the range of dissimilarity of all the wheat entries was from 0.00-2.00, and the different clusters were formed within this range. Results from the cluster section (Table 3.7) indicated four clusters among which most of the wheat cultivars were divided. Some of the wheat cultivars didn't fall in any of the four clusters mentioned, but stood individually as a separate cluster, and this indicates that they were morphologically dissimilar from the other entries.

Cluster I consisted of two cultivars, HAR 1407 and Pavon 76. Both are bread wheat cultivars. The cultivars in this cluster possessed equal number of productive tillers per plant. In addition, both cultivars had nearly the same days to heading, days to maturity, plant height, number of seeds per main spikelets, and 1000 seed eight.

Cluster II had seven durum wheat cultivars/lines, Kilinto, DZ-1050, DZ-1052, Boohai, DZ-393-4, DZ-2023, and Foka. The cultivars of this cluster had common traits like days to heading, days to maturity, and harvest index. Within this cluster (as seen from Table 3.5) Kilinto and DZ-2023 were distantly related, but Boohai and DZ-393-4 were close to one another.

Cluster III comprised of three bead wheat cultivars, HAR 1522, HAR 604, and HAR 710. These cultivars possessed some common features such as last internode length, peduncle length above the flag leaf, spike length, seed weight per plant, biomass yield, and harvest index. The bread wheat cultivars in this cluster had longer days to heading and maturity, wider flag leaf area, and longer spikes compared to the bread wheat cultivars in cluster I. However, both clusters were closely related in the dendrogram.

Cluster IV consisted of two bread wheat cultivars, K-6295-4A and K-6290 bulk. Common features that were observed within this cluster were plant height, spike length, seed weight per plan, and biomass yield. In the dendrogram, this cluster was not closely related to cluster I and III.

The position of durum wheat cultivars (such as DZ-04-118 and Cocorit 71) and bread wheat cultivars (such as Dashen, ET-13-A2, HAR 1709, and HAR 1685) was placed out of any cluster. This indicates the absence of enough relationship within the cultivars mentioned, or between the cultivars mentioned and the other clusters, based on the 14 phenotypic traits recorded.

Field experiment

A dendrogram constructed using phenotypic traits collected from the field experiment was given in Fig. 3.2. In this dendrogram, the range of dissimilarity of all the wheat entries was from 0.00-2.50, and the different clusters were formed within this range. Results from the cluster section (Table 3.8) indicated four clusters, although some of the wheat entries were not included in any of the clusters, but stood individually as a separate cluster.

In cluster I there were three bread wheat cultivars, Dashen, HAR 1407, and HAR 1685. This cluster had resemblance in some characters such as number of productive tillers per plant, number of spikelets per main spike, and number of seeds per main spikelets. These three bread wheat cultivars were not included in any of the clusters in the greenhouse experiment as noted above.

Cluster II had six durum wheat cultivars, DZ-1050, DZ-1052, Kilinto, DZ-393-4, Boohai, and Foka. In general the cultivars of this cluster had common traits such as plant height, productive number of tillers per plant, last internode length, spike length, and number of spikelets per main spike. Within this cluster (as seen from Table 3.6) DZ-1050 and DZ-1052 were closely related, but Kilinto and DZ-393-4 were distantly related.

Cluster III consisted of two bread wheat cultivars, K-6295-4A and ET-13-A2. Both cultivars had resemblance in characters like days to maturity, flag leaf area, last internode length, and seed weight per plant. In greenhouse experiment K-6295-4A was not clustered with ET-13-A2, but with K-6290 bulk. This indicates that clustering patterns can be altered by the growing conditions. Such differences in clustering position have also been observed in other cultivars grown under greenhouse and field conditions.

Cluster IV consisted of four bread wheat cultivars, K-6290 bulk, HAR 710, HAR 1522, and Pavon 76. The common features observed from the cluster include days to

heading, days to maturity, productive number of tillers per plant, and number of spikelets per main spike.

The durum wheat cultivars (such as DZ-04-118, DZ-2023, and Cocorit 71) and bread wheat cultivars (HAR 604 and HAR 1709) were not included in any of the clusters. This indicates the absence of enough relationship within the cultivars mentioned, or between the cultivars mentioned and the other clusters, based on the 14 phenotypic traits recorded.

Conclusions

It was found that morphological data could provide unique identification of cultivars. This is evident from the dendrograms constructed using morphological data collected from greenhouse (Fig. 3.1) and field experiment (Fig. 3.2), in which all of the accessions were distinctly separated from each other. In other words, none of the accessions were clustered at 100% similarity. However, there was a significant mismatch between the dendrograms. This indicates that morphological characters cannot accurately describe or portray relationships among different cultivars.

In the greenhouse experiment, for instance, HAR 1407 and Pavon 76 were clustered together; whereas in the field experiment they fell into separate clusters. In the same way, seven durum wheat cultivars, Foka, DZ-2023, DZ-393-4, Boohai, DZ-1050, DZ-1052, and Kilinto were clustered together in the greenhouse experiment, but in the field experiment, DZ-2023 fell apart as a separate cluster. Again, three bread wheat cultivars, HAR 710, HAR 604, and HAR 1522 were grouped into the same cluster in the greenhouse experiment; whereas in the field experiment, four bread wheat cultivars, HAR 710, HAR 1522, Pavon 76, and K-6290-bulk were grouped together, but HAR 604 fell apart as a different cluster. Furthermore, K-6295-4A and K6295-bulk fell in to the same cluster, but in the field experiment K-6295-4A and ET-13-A2 were clustered together. This suggests that environmental influence significantly alters the expression of morphological traits. Thus, morphological traits cannot efficiently

detect the genetic distance values among different cultivars. This is consistent with the findinge of Schut *et al* (1997). Smith and Smith (1992) also concluded that morphological differences cannot be interpreted to provide accurate estimates of genetic differences.

Generally, there was a tendency of durum wheat cultivars to cluster together and bread wheat cultivars to cluster together. However, morphological data collected from field experiment yielded a dendrogram that was more consistent with the known genetic relationships of the cultivars in the study as compared to the morphological data collected from greenhouse experiment. In conclusion, the result of this study revealed that morphological traits could be used for cultivar identification although it cannot provide accurate estimates of genetic distances. However, since the expression of morphological markers is not reproducible over a range of environments, the results of morphological data should be supplemented with biochemical or DNA markers.

Chapter 4

Morphological characterisation of Ethiopian tef [*Eragrostis tef* (Zucc.) Trotter] cultivars

Abstract

Tef, Eragrostis tef (Zucc.) Trottr, is one of the most important cereal crops in Ethiopia. An experiment was conducted to investigate phenotypic diversity of 10 Ethiopian commercial tef cultivates based on 16 morphological, phenological, and agronomic characters in four replicated randomised complete blocks at the University of Orange Free State during 1999. All accessions were grown under similar conditions in a greenhouse represented by three individuals. The objectives were to assess phenotypic differences between the cultivar, and to estimate the genetic distances among the cultivars based on the phenotypic data. The distinguishing characters were collected when appropriate plant stage reached full expression of the characters. Genetic distances among the cultivars were calculated using NCSS 2000 software package according to the formula of Nei and Li (1979). A dendogram from the UPGMA cluster analysis of genetic distances based on phenotypic traits grouped the cultivars into three clusters.

Introduction

Tef is the staple cereal of Ethiopia, the only country in the world where the grain is used for human consumption (Tefera *et al*, 1990). It constitutes about 32% of the total acreage and 21% of the gross grain production of all cereals cultivated in the country (Central Statistical Authority, 1996). It is small grained (0.2-0.3mg/kernel), self-pollinated, allotetraploid (2n = 40), C_4 metabolism cereal plant of medium stature and short life cycle (2-4 monthes) (Ayele *et al*, 1996; Ketema, 1997). It has a wide adaptation and grows from sea level up to 3000 meters above sea level and on a range of soil types. It is also well adapted to drought and waterlogged conditions. Due to its adaptability it has also been introduced as a forage crop in India, South

Africa, and Australia. Post-harvest losses are minimal since the grains are not attacked by insect pests and diseases. The grains can be stored for a long period of time even under traditional farmers' storage conditions, thus, indicating the potential of the crop as a strategic food reserve (Tatham *et al*, 1996; Assefa *et al*, 1999).

The tef plant originated in Ethiopia and its centre of diversity is also the same country (Vavilov, 1951). It is believed that it might have been domesticated in the highlands of Ethiopia by the Pre-Semitic inhabitants before the introduction of wheat and barley, although it is not certain for how long it was under their culture (Tefera *et al*, 1995). Tef belongs to the family Poaceae, subfamily Eragrostoideae, tribe Eragrosteae, and genus Eragrostis. The genus contains about 300 species (Ketema, 1997). To the best of the authors' knowledge, tef is the only member of the genus, which is being used as a cereal crop. It was initially domesticated in Ethiopia from E. *pilosa*, which is still collected as food by people in many parts of Africa, other than Ethiopia, in times of famine. This suggests that the domestication of tef started in time of food scarcity and implies that tef has the potential to withstand various environmental stresses (Bekele *et al*, 1995).

Tef grain makes the best kind of 'injera' (a flat, circular and very soft bread), which comprises the major component of the staple dish of most Ethiopians. It also gives high returns in flour, which is 99% compared to 60-80% from wheat (Ebba, 1969). This clearly indicates that tef is an economical crop because so little of the grain is wasted. Like other cereals, the seeds of tef contain albumin, globulin, prolamin, and glutelin. However, tef is different from other cereals in that it has a very little content of alcohol soluble prolamins but a high content of albumin. Compared to most other cereals like wheat, rye, barley and oats, tef grains are high in methionine, isoleucine, leucine and valine, but lower in lysine, arginine and threonine contents (Bekele, 1995). Tef is also rich in certain minerals, especially iron. In Ethiopia, the absence of iron deficiency, anemia, seems to be correlated with tef eating (Costanza *et al*, 1980). In addition, tef straw is used as building material and as cattle feed especially during the long dry season of the year, indicating high utilisation of the by-product (Tefera *et al*, 1990). Thus, tef serves the farmers as a cash crop because both its grains and straw fetch high market prices than that of other cereals.

Despite its great importance for Ethiopia, the national average grain yield of tef is relatively low, approximately one ton per hectare (Ayele *et al*, 1999). Its poor productivity has, among others, been due to low yield potential of the landraces under cultivation, susceptibility to lodging, unimproved traditional cultural practices, and drought (Assefa *et al*, 1999). In order to increase its productivity, however, some attempts have already been made to measure genetic diversity in tef on the basis of morphological and agronomic characters (Ebba, 1975; Costanza *et al*, 1979; Tefera *et al*, 1990; Tadesse, 1993). The objectives of the present study were to determine the genetic variability clustering patterns of 10 Ethiopian commercial tef cultivars using phenotypic traits.

Materials and methods

Plant materials

The plant materials were obtained from the Ethiopian Seed Enterprise and Ethiopian Agricultural Research Organization. The genotypes are listed in Table 4.1 along with their pedigree information and year of release.

Experimental layout

The experiment was conducted in a greenhouse at the University of Orange Free State. Before planting, the greenhouse was sprayed with one ml of chlorpirifos diluted in one litre of water to control aphids. The cultivars were grown in a two litre capacity pot containing 2.5 kg loam soil mixed with 2g fertilizer (N:P:K = 3:2:0). A randomised complete block design of four replications was used. An experimental unit consisted of three plants grown per pot. Watering was done as required. At stem elongation, the main plants were tagged to differentiate them from tiller plants. Data were collected from the three plants in the experimental unit, and a grand mean of the four replications was calculated and used for analysis. The trial was also planted in the field, but failed due to the tef cultivars not being adapted to South African conditions.

Table 4.1 List of tef cultivars used for morphological characterization.

No	Cultivars	Pedigree information	Year of release
1	DZ-01-354	Mass selection from a landrace	1970
2	DZ-01-196	Mass selection from a landrace	1970
3	DZ-01-787	Mass selection from a landrace	1978
4	DZ-01-974	Mass selection from a landrace	1995
5	DZ-01-99	Mass selection from a landrace	1970
6	DZ-Cr-37	(186 x 566) T-4-TB'75-37-4LS-OBS	1984
7	DZ-Cr-255	{[(186 x 566) 196][(186 x T-4) 155]} {[(T-140 x 566)(T-140 x 186][(186 x 418) 354]}-TB'76-255	1993
8	DZ-Cr-82	(186 x 566) 354-TB'75-82-14LS-OBS	1982
9	DZ-Cr-44	(566 x 186) 354-TB'75-44-3LS-OBS	1982
10	DZ-Cr-358	(42 x 566) HT'83-358	1995

Morphological parameters

Data were collected for the following characters, when the plant stage showed full expression of the characters.

1. Days to heading: It is the number of days from sowing date to full exposure of main plant's panicle.
2. Days to maturity: The number of days from sowing date to physiological maturity. i.e. when peduncle of the main plant turns from green to yellowish colour.
3. Plant height (cm): Height from the base to the tip of the panicle.
4. Number of productive tillers: The number of tillers per plant that has produced a seed/seeds.
5. First internode length (cm): Length of the 1st internode from the base.
6. Second internode length (cm): Length of the 2nd internode from the base.
7. Diameter of the 1st internode (mm): Diameter of the 1st internode from the base.

8. Diameter of the 2nd internode (mm): Diameter of the 2nd internode from the base.
9. Panicle length (cm): Length from the base to the tip of the main plant's panicle.
10. Number of panicle branches: The number of branches on the main plant's panicle.
11. Kernel weight/main panicle (g): The weight of kernels obtained from panicles of the main plant.
12. Kernel weight/tillers' panicle (g): The weight of kernels obtained from panicles of the tiller plant.
13. Kernel weight/plant (g): It is the total weight of seeds harvested from the main plant and its productive tillers.
14. 1000 seed weight (g): This is the weight of 1000 seeds.
15. Biomass yield (BY) (g): Total weight of plant part above the ground.
16. Harvest index (HI) (g): This is the percentage of the ratio of seed weight/plant to BY (i.e. seed weight/plant ÷ BY x 100).

Calculation of genetic distances

Pairwise genetic distance among the tef cultivars were calculated based on the formula of Nei and Li (1979) as described in Chapter 3. The UPGMA method was used for cluster analysis of the pairwise distance matrix, which generated a dendrogram representing the genetic distances among the tef cultivars. Calculation of the distance matrix and cluster analysis was carried out using NCSS 2000 software package (Hintze, 1998).

Results and Discussion

Morphological trait analysis

Mean values of the morphological data scored for the 16 traits are given in Table 4.2.

The genetic distances between all possible pairs of tef entries obtained from the UPGMA analysis is provided in Table 4.3. In addition, results of the UPGMA analysis provided detail of the cluster section (Table 4.4), and summary of the genetic distance as a dendrogram (Fig 4.1).

Table 4.2 Mean values of the morphological traits scored for the 16 traits.

No	Entries	DH	DM	PH	NPT	1st IL	2nd IL	D1st I	D2nd I	PL	NPB	KE/ MP	KW/ TP	KE/P	1000 SW	BY	HI
1	DZ-01-354	85	148	145	8.83	6.07	17.37	2.03	2.18	46.60	35.50	1.27	7.20	8.47	0.3262	23.05	36.94
2	DZ-01-196	82	147	156	8.91	11.25	14.62	2.52	2.36	53.60	30.25	1.80	6.62	8.45	0.4084	22.72	37.31
3	DZ-01-787	87	145	145	10.50	9.12	16.00	2.32	2.18	41.50	35.00	1.17	8.34	9.52	0.3244	29.90	32.10
4	DZ-01-974	87	139	147	9.75	10.12	19.62	2.39	2.27	43.00	35.25	1.60	7.70	9.30	0.3856	25.25	37.00
5	DZ-01-99	78	150	122	16.58	7.37	14.37	1.88	1.94	40.75	32.25	1.15	6.26	7.37	0.3558	25.37	29.15
6	DZ-Cr-37	73	138	121	16.91	13.00	15.50	1.89	2.12	39.50	26.00	1.25	7.03	8.28	0.3500	22.25	37.15
7	DZ-Cr-255	85	146	149	8.58	9.75	19.00	2.33	2.32	41.00	34.75	1.45	4.82	6.09	0.3500	19.67	30.95
8	DZ-Cr-82	81	145	147	8.91	9.12	20.00	1.94	2.01	40.50	35.75	1.55	5.45	7.00	0.3160	20.52	34.11
9	DZ-Cr-44	86	147	148	12.08	12.50	14.68	2.44	2.52	39.75	34.00	1.85	7.34	9.20	0.3960	28.10	32.75
10	DZ-Cr-358	82	147	149	11.08	12.50	19.62	2.10	2.00	38.25	37.75	1.67	5.89	7.40	0.4018	23.77	32.87

DH = days to heading, DM = days to maturity, PH = plant height, NPT = number of productive tillers, 1st IL = first internode length, 2nd IL = second internode length, D 1st I = diameter of the first internode, D 2nd I = diameter of the 2nd internode, PL = panicle length, NPB = number of panicle branches, KW/MP = kernel weight per main panicle, KW/TP = kernel weight per tiller panicle, KW/P = kernel weight per plant, 1000 SW = 1000 seed weight, BY = biomass yield, HI = harvest index.

Table 4.3 Pairwise distance matrixes of the tef cultivars based on the F statistic of Nei and Le, 1979.

No	Cultivar	1	2	3	4	5	6	7	8	9	10
1	DZ-01-354	0									
2	DZ-01-196	1.07	0								
3	DZ-01-787	1.13	1.58	0							
4	DZ-01-974	0.94	1.24	1.05	0						
5	DZ-01-99	1.41	1.85	1.48	1.84	0					
6	DZ-Cr-37	1.60	1.84	1.88	1.69	1.36	0				
7	DZ-Cr-255	1.20	1.46	1.78	1.47	1.58	1.92	0			
8	DZ-Cr-82	0.93	1.43	1.68	1.25	1.47	1.62	0.67	0		
9	DZ-Cr-44	1.20	1.24	0.84	1.02	1.48	1.82	1.58	1.50	0	
10	DZ-Cr-358	0.96	1.43	1.37	1.11	1.34	1.72	0.86	0.57	1.11	0

Table 4.4 Cluster detail section provided by UPGMA analysis of the tef morphological traits.

Row	Cluster	Name
7	1	DZ-Cr-255
8	1	DZ-Cr-82
10	1	DZ-Cr-358
3	2	DZ-01-787
9	2	DZ-Cr-44
1	3	DZ-01-354
4	3	DZ-01-974
2		DZ-01-196
5		DZ-01-99
6		DZ-Cr-37

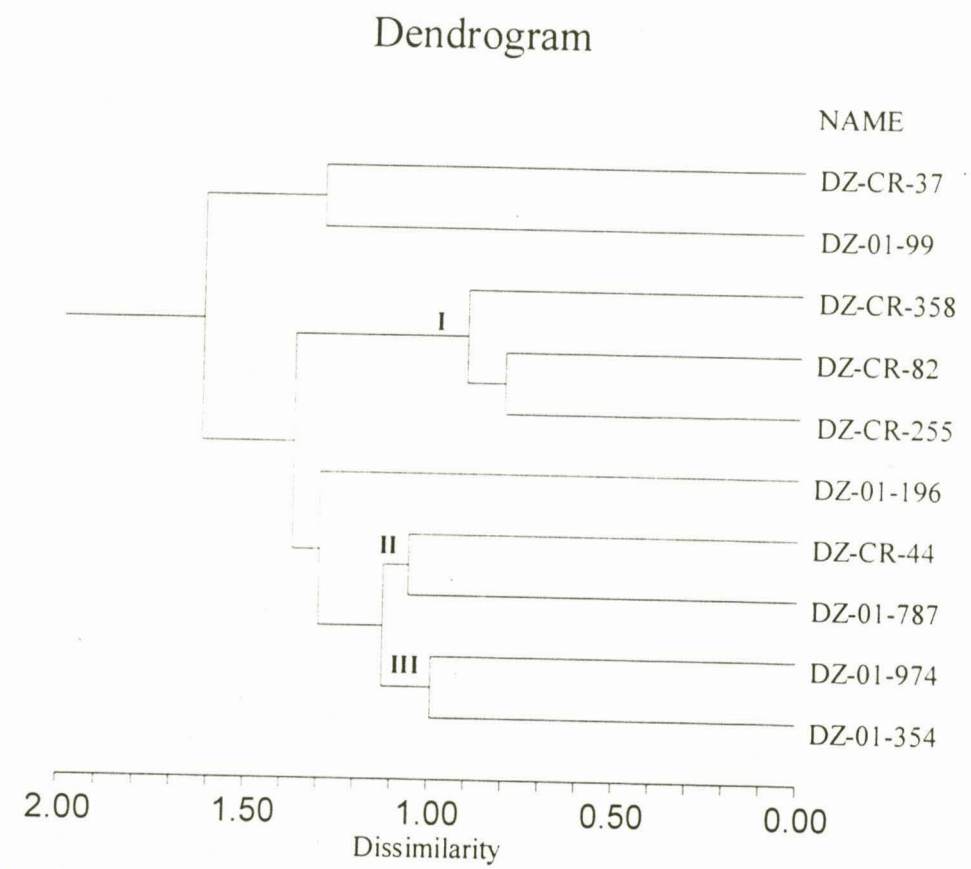

Fig. 4.1 Dendrogram generated by UPGMA analysis of morphological data.

Genetic distance analysis

Pairwise genetic distance (Table 4.3) based on the F statistic of Nei and Li (1979) revealed an average distance of 1.38 among the tef cultivars. The genetic distances among the tef cultivars ranged from 0.57 between DZ-Cr-82 and DZ-Cr-358 to 1.92 between DZ-C-37 and DZ-Cr-255.

Dendrograms

A dendrogram constructed using the 14 phenotypic traits was given in Fig. 4.1. The range of dissimilarity of the dendrogram was from 0.00 – 2.00, and different clusters were formed within this range. Results from the cluster section (Table 4.4) indicated three clusters along which most of the tef cultivars were divided. However, some of the tef cultivars were not included in any of the three clusters.

Cluster I consisted of three cultivars, DZ-Cr-255, DZ-Cr-82, and DZ-Cr-358. This cluster had resemblance in some characters like days to maturity, plant height, second internode length, diameter of the first internode, and kernel weight per plant. Within this cluster, DZ-Cr-82 and DZ-Cr-255 are closely related.

Cluster II had two cultivars, DZ-01-787 and DZ-Cr-44. The cluster had resemblance in days to heading, diameter of the first internode, diameter of the second internode, panicle length, number of panicle branches, and kernel weight per plant.

Cluster III consisted of two cultivars, DZ-01-354 and DZ-01-974. The common features observed from the cluster include diameter of the first internode,diameter of the second internode, number of panicle branches, kernel weight per tiller branches, and harvest index.

Three of the tef cultivars, DZ-01-196, DZ-01-99, and DZ-Cr-37 were not included in any of the three clusters, and hence they are morphologically dissimilar from the others based on the 14 phenotypic traits recorded.

Conclusion

In this study, all the tef accessions were distinctly separated from each other. Thus all the accessions are morphologically variable and hence phenotypic traits can be used to distinguish one variety from the other. However, since morphological markers are altered by environmental influences, the results of morphological markers should be supplemented with a more reliable biochemical or DNA markers.

Chapter 5

Identification and genetic distance analysis of Ethiopian wheat cultivars using biochemical markers

Abstract

Gliadins and high molecular weight glutenin subunits (HMW-GS) of 20 Ethiopian wheat cultivars and advanced lines (11 bread wheat and nine durum wheat) were separated using sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE), with a discontinuous gel system. The gliadin electrophoregrams were analysed by a computer-aided procedure, whereas the HMW-GS were read visually. The gliadin banding patterns from the 20 wheat varieties were sufficiently unique for the identification of each variety. However, the HMW-GS banding paterns were found to be identical in some of the wheat varieties. The gliadin and HMW-GS data were scored into a binary matrix as present (1) or absent (0) across all the genotypes, and the necessary computations were performed using NCSS program (Hintze, 1998). Estimation of genetic distances among the wheat varieties was calculated based on the pairwise comparisons of Nei and Li (1979). UPGMA clustering of the varieties produced four major groups. In this chapter, biochemical assessment of cultivar identity as well as the estimation of comparative levels of genetic diversity and investigation of relationships among the cultivars are reported.

Introduction

The most important decision of farmers in buying seed is selection of the appropriate cultivar. The decision is based largely on economic factors. The return the farmer receives on his investment is a function of yield, maturity, disease resistance, lodging, and other characteristics. Cultivar purity is also important. To ensure purity within a lot, certification programs have been established to maintain and make available high-quality, genetically pure

seed. Morphological characteristics such as seed size, colour, etc, were first used to differentiate wheat cultivars. However, morphological traits are influenced by environment, and many new cultivars are phenotypically similar and closely related.

Thus, limitations associated with morphological traits have led to the development of sophisticated technologies for wheat cultivar identification. Laboratory tests utilizing polyacrylamide gel electrophoresis (PAGE) is one of such technologies. Since it was first shown that cereal prolamin patterns are genotypical, PAGE has become established as a practical means for identifying cereal varieties. Later, many variations, in apparatus, formulations, and procedures were described (Clements, 1987; Marchylo *et al*, 1989).

The importance of gel electrophoresis for the classification and identification of varieties of agricultural species is now firmly established. The electrophoretic analysis of proteins has been widely applied, ranging from the breeding of new varieties, through seed testing, production and certification, to the selling of harvested grain and its commercial processing (Cooke, 1989).

Gliadins and glutenins are the two major groups of seed storage proteins in wheat. Their expression is nearly invariant under different environmental and agronomic conditions, and hence they serve as sensitive genotypic indicators (Bietz *et al*, 1984; Lookhart *et al*, 1984). Due to their inheritance being well characterized and their high level of polymorphism, they have proved of immense value in cultivar identification, breeding, and genetical research (Morgunov *et al*, 1990). Classification of the wheat storage proteins into these two groups (gliadins and glutenins) is preferably carried out in accordance with the state of aggregation in dissociating media of these proteins. With this classification, gliadins occur as a complex mixture of single polypeptides, whereas glutenin consists of polypeptides or subunits that are linked together mainly by disulfide bonds (Mosleth and Uhlen, 1990b).

Gliadins are heterogeneous group of alcohol-soluble wheat endosperm storage proteins whose composition varies among wheat genotype. Because of their heterogeneity, ready extractability, and relatively low (approximately 30000 daltons) molecular weights, gliadin

proteins are routinely analyzed to identify wheat varieties (Bietz *et al*, 1984). Gel electrophoresis has shown that the gliadin fraction contains at least 40 different protein species, which frequently show extensive variation between cultivars (Lawrence and Shepherd, 1980). Such information may indicate lots of wheat or flour suitable or unsuitable for specific applications, and may also be used in marketing, certifying, registration or breeding wheats.

It is evident from literature that different solvents can be used to extract gliadins from wheat for electrophoretic separations. These include 70% ethanol, 55% isopropanol (IPA), 55% IPA + 1% β-mercaptoethanol (β-ME), 6% urea, 6% urea + 1% β-ME, 18% urea, 18% urea + 1% β-ME, 18% urea + 1% β-ME + 20% 2-chloroethanol (2-CE), 18% urea + 20% 2-CE, and 20% 2-CE (De Villiers and Bosman, 1993).

The glutenin subunits are further divided into two groups based on their molecular weights; the HMW-GS, and low molecular weight glutenin subunits (LMW-GS). This can be achieved by treating glutenin with an agent which breaks disulfide bonds, such as 2-mercaptoethanol (2-ME), and an agent which disrupts, either directly or indirectly, hydrophobic interactions and hydrogen bonds, such as the anionic detergent sodium dodecyl sulfate (SDS) (Lawrence and Shepherd, 1980).

The HMW-GS are easily resolved in one-dimensional SDS-PAGE separation of reduced total protein extracts, but it is difficult to obtain a reliable scoring of the LMW-GS in this separation due to their mobilities overlapping with those of gliadins and certain albumins and globulins (Singh *et al*, 1991). So far, about 20 different HMW-GS were distinguished using this technique, although a single variety usually contains between three and five subunits (Payne *et al*, 1981).

In this study, gliadins and HMW-GS were used for identification and evaluation of genetic relationships among 20 Ethiopian bread and durum wheat cultivars and lines. The objectives were to assess the potential of gliadins and HMW-GS in cultivar identification, and in the investigation of genetic relationships among the cultivars stated.

Materials and Methods

Plant materials

The 20 Ethiopian wheat cultivars and lines listed in Table 3.1 and Table 3.2 were used in this study. In addition, the wheat cultivar Chinese Spring was included in all gels as a standard for the analysis of gliadin bands. The mobilities of the gliadin bands in the 20 wheat cultivars were scored relative to the mobilities of the Chinese Spring bands. In the same way, the cultivars Zaragosa and Karee were included as a standard for the analysis of HMW-GS. This procedure made it unnecessary to compare patterns between gels, thereby avoiding any difficulties associated with slight differences between gels in mobility of bands (Lawrence and Shepherd, 1980).

Extraction of gliadins

The method was adapted from that of De Villiers and Bosman (1993). Single kernel extractions were employed throughout the study. For each variety, six replications of gliadin extracts were prepared. Wheat kernels were crushed and ground to fine powders with a pestle in a mortar. The meal was then transferred to a 1.5-ml eppendorf tube and extracted with an extraction buffer. The extraction procedure was:

Stock solutions for extraction:

1) Extraction buffer:
   1.8g Urea
   0.1 ml β-Mercapto-ethanol
   10 ml H_2O

2) Sample buffer:
   1.0 g Tris (hydroxymethyl aminomethane)
   90ml 50% n-propanol
   Titrate to pH 8 with N HCl. Make up to 100 ml with 50% n-propanol.

Add 40g glycerol, 2g SDS, and 0.02g bromophenol blue.

Extraction procedure:

1) Crush wheat seed into fine powder and transfer to a 1.5-ml eppendorf tube.
2) Add 500µl of extraction buffer to the ependorf tube and put in 60°C waterbath for 1 hour. Vortex at 20 and 40 minutes.
3) Centrifuge tubes for 2 min at 10 000 rpm.
4) Transfer 80µl of the supernatant into a new tube containing 80µl sample buffer. Mix well and centrifuge as before. Samples are now ready for loading. 40µl of the sample will be loaded if a whole-wheat kernel is used. Out of the six replication of gliadin extracts, three were loaded on one gel, and the remaining three on other gel.

Extraction of HMW-GS

The method was adapted from Singh *et al* (1991). As in the case of gliadins, single kernel extractions were employed, and for each variety six replications of HMW-GS were prepared. The extraction procedure is as follows.

Stock solutions for extraction:

1) 50% n-propanol:
   50 ml n-propanol
   50 ml d H_2O

2) Extraction buffer:
   80 mM Tris HCl (pH 8.0) made in 50% n-propanol
   1.0g Tris
   90 ml 50% n-propanol
   Titrate to pH 8 with N HCl. Make up to 100 ml with 50% n-propanol.

3) Sample buffer: Same as above.

Extraction procedure:

1) Crush wheat seed in to fine powder and transfer to a 1.5-ml eppendorf tube.
2) Add 1 ml of 50% n-propanol, shake tube and put in waterbath at 60°C for 30 minutes.
3) Centrifuge for 2 minutes at 10 000 rpm. Remove the 50% n-propanol.
4) Add 85µl extraction buffer containing 1.25% DTT to tubes. Loosen seed material and leave for 1 hour in waterbath at 60°C. [Add 0.1g dithiothreito (DTT) to 8 ml extraction buffer].
5) Add to tubes 85µl of extraction buffer containing 16.8µl/ml vinyl pyridine. That is 168µl vinyl pyridine to 10 ml extraction buffer. Leave in the waterbath at 60°C for 1 hour.
6) Add 160µl-sample buffer to the tubes and put in the waterbath for 15 minutes. Centrifuge for 5 minutes at 10 000 rpm. Samples are now ready for loading. 40µl of the sample is loaded if a whole wheat seed is used.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

The method was adapted from Singh *et al* (1991).

Gel preparation

In this study, a discontinous-pH, two-gel systems, which first concentrate the proteins into a narrow starting zone, were used. Such a discontinous-pH, two-gel systems generally give better resolution of protein bands than a single, continous-pH gel systems (Lawrence and Shepherd, 1980). The discontinuous system of SDS-PAGE is made up of separating gel and stacking gel.

Stock solutions for the discontinuous two gel system:

1) Separating buffer (2x) pH 8.88:

Dissolve 45.412g Tris in 460 ml d H_2O

Titrate to PH 8.88 then add 1.0g SDS

Makes total of 500 ml. Store at 4°C.

2) Separating acrylamide (30% Ac/ 1% crosslinker):

Dissolve 75g acrylamide and 0.75g Bisacrylamide in 181 ml d H_2O

Makes total 250 ml. Store in dark at 4°C.

3) Stacking buffer (2x) pH 6.8:

Dissolve 6.06g Tris in 190 ml d H_2O

Titrate to pH 6.8 then add 0.4g SDS

Maks total 200 ml. Store at 4°C.

4) Stacking acrylamide (35% Ac/ 1.5% crosslinker):

Dissolve 87.5g acrylamide and 1.32g bisacrylamide in 181 ml d H_2O

Makes total 250 ml. Store in dark at 4°C.

Preparation of separating gel (10% unifom):

	For 2 gels	For 4 gels
Separating buffer	38 ml	76 ml
Separating acrylamide	28.1 ml	56.2 ml
D H_2O	14 ml	28 ml
Temed	165µl	330µl
Ammonium persulphate (APS) (10%)	190µl	380µl

The amounts of chemicals mentioned above were mixed and APS was added just before casting. Then, the gel was poured as vertical slaps between two glass plates clamped to the side of a stand, and 3 ml n-buthanol was immediately added on top of the gel for levelling and left to set. After the gel was set, the n-buthanol was removed by washing.

Preparation of stacking gel:

	For 2 gels	For 4 gels
Stacking buffer	10 ml	20 ml
Stacking acrylamide	2.6 ml	5.2 ml
D H_2O	7.4 ml	14.8 ml
Temed	40µl	80µl
APS (10%)	100µl	200µl

The above chemicals were mixed and APS was added just before casting the gel. Then, the stacking gel was poured on top of the separating gel, and the slot-forming comb was inserted to form wells into which the protein samples are loaded for electrophoresis.

Electrode buffers:

1) Cathode buffer:
   Mix 30.28g Tris, 144g glysine, 10g SDS, and make up to 1 litre with d H_2O. Dilute 10x before use.
2) Anode buffer:
   Mix 30.28g Tris with 800 ml d H_2O. Then titrate to pH 8.4, and make up to 1 litre with d H_2O. Dilute 10x before use.

Running of the gel:

Prior to electrophoresis, the sample tubes were briefly centrifuged, and 40µl of the clear supernatant was loaded into each well. The standard wheat cultivar, Chinese Spring, was loaded at the centre as well as both sides of each gel. The unknown samples were loaded in the remaining wells. This ensured that no unknown samples were run in the slots adjacent to the edge of the gel where "edge effects" are sometimes noticed. Electrophoresis was carried out using a vertical slab gel electrophoresis unit, Model SE 600 System (Hoefer Scientific Instruments, San Francisco, CA), with the negative terminal (cathode buffer) in the upper tank

and the positive terminal (anode buffer) in the bottom tank at a constant current (66 mA) for about 3 hours. During electrophoresis, the temperature was controlled at 15°C by circulating water using Multitemp II Thermostatic Cicular. The run was terminated when the marker dye reached the bottom of the gel.

Staining and destaining of the gel:

The staining method of Wrigley (1992) was used. Gels were immersed in a fixing solution consisting of methanol, glacial acetic acid, and d H_2O (4:1:5, by volume) for about 1 hour. Then, the gels were stained overnight in a staining solution consisting of 30g trichloroacetic acid made up to 200 ml with d H_2O, and 0.1g Coomassie Blue made up to 10 ml in methanol. In both steps, the gels were briefly shaked using a belly dancer in order to get optimum results. Finally, the stained gels were rinsed in d H_2O until clear bands were observed.

Gel analysis

In the case of gliadins, the gels were analysed with the help of "Molecular Analyst Fingerprinting" software of Bioard. The gliadin banding patterns of Chinese Spring was used as a standard. Gels were scanned with the help of Gel Doc 1000 using a UV-gel camera and VGA graphics in 256 colours as recommended. Band pattern migration distances were directly acquired from a gel and put into a computer file, thus obviating the need to manually transcribe data. The analysis procedure consisted of three steps: 1) the conversion of the gel, 2) the normalisation of the tracks and 3) the analysis of the tracks.

The resolution was set at 200 points. A densitometeric curve of every replication of the cultivars was drawn and from this the migration distances were determined. Only bands with an intensity of more than 15% were accepted. An average value was calculated for the six replications of each cultivar, and these values were used to compare the cultivars with each other.

In the case of HMW-GS, the banding patterns of the unknown samples (i.e. the 20 Ethiopian wheat entries) were scored manually relative to the mobilities of the standard cultivars, Zaragosa (2*, 17+18, 2+12) and Karee (1, 7+9, 5+10), bands. Unlike gliadins, the HMW-GS contain only few bands (between three and five) in a single cultivar, and hence can easily be scored manually. The bands of unknown samples (i.e. the 20 Ethiopian wheat entries) were scored according to the numbering system of Payne *et al* (1981).

Calculation of genetic distances

Estimation of similarity among the 20 wheat cultivars was calculated based on the pairwise comparisons of the SDS-PAGE data of gliadins and HMW-GS. For the genetic distance analysis, a presence (1) or absence (0) was scored for the SDS-PAGE data of gliadins and HMW-GS (Appendix, Table 10.1). The gliadin and HMW-GS data were then combined and used for genetic distance analysis. The index of genetic similarity (F) of Nei and Li (1979) was used to calculate the pairwise genetic distance (D) for all entries.

$$F = 2Nxy/(Nx + Ny)$$

$$D = 1 - F$$

Where Nxy = the number of shared bands between any two cultivars X and Y and Nx and Ny are the number of bands for cultivars X and Y, respectively (Wang and Tankslay, 1989). Cutivars were clustered using the UPGMA method calculated by NCSS cluster analysis procedure (Hintze, 1998).

i) The gliadins

The results of the gliadin band migration distances of each cultivar is given in the following tables (see Table 5.1-5.21). Six replications of the gliadin band patterns with their intensity were shown. Only bands that appeared in at least four of the six replications were accepted. The intensity of the bands was grouped into five classes, from one (very light) to five (very dark). Accordingly, a class of one (very light) was given to a band with an intensity ranging from 1-50, two (light) 51-100, three (medium) 101-150, four (dark) 151-200, and five (very dark) more than 200. In some replications, the same bands had very light and very dark intensity. It was thus impossible to give an average intensity class. Thus, a score of six was given in such instances. The last column indicates average values of the six replications that were accepted as a specific peak positions for each entry.

Chinese Spring

Table 5.1 The migration distance and intensity of gliadin subunits of Chinese Spring.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
10	5	7	5	5	5	9	5	8	5	11	5	8.33	5
25	5	22	5	21	5	25	5	23	5	26	5	23.66	5
39	5	36	5	34	5	38	5	37	4	39	5	37.16	5
49	1	51	3	50	3	53	4	52	3	55	4	51.66	3
55	3	57	2	55	2	58	3	57	2	59	2	56.83	2
66	4	62	5	61	5	64	5	62	4	65	5	63.33	5
-	-	-	-	71	2	72	2	73	2	73	2	72.25	2
93	3	94	2	-	-	91	3	89	2	91	3	91.60	3
105	3	101	2	100	4	102	2	101	3	103	3	102.00	3
125	2	120	3	120	3	122	3	120	2	122	3	121.50	3
-	-	135	2	134	2	137	3	133	2	137	2	135.20	2
155	1	-	-	154	1	-	-	153	1	155	1	154.25	1
-	-	175	1	174	1	175	1	174	1	175	1	174.60	1

Rep. = Replication.
MD = Migration distances.
INT = Intensity.
The symbol "-" was given wherever the band patterns were absent.

HAR 1685

Table 5.2 The migration distance and intensity of gliadin subunits of HAR 1685.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
8	3	8	5	7	5	6	5	6	5	5	4	6.66	5
21	4	21	4	21	5	20	5	19	5	19	5	20.16	5
33	5	33	5	32	5	31	5	30	5	30	5	31.50	5
39	2	41	1	42	1	41	1	41	1	-	-	40.80	1
57	4	55	2	56	2	53	2	53	2	56	3	55.00	3
61	3	60	2	59	2	58	2	-	-	64	2	60.40	2
66	1	68	1	65	1	65	1	68	1	67	2	66.50	1
72	1	73	1	69	1	69	2	71	1	70	1	70.60	1
88	2	88	2	-	-	85	1	85	2	-	-	86.50	2
101	3	101	3	99	2	100	4	97	3	-	-	99.60	3
112	5	110	3	111	4	109	4	108	4	107	4	109.50	4
123	3	122	2	121	2	120	3	119	3	118	3	120.50	3
129	3	129	2	129	2	126	3	126	2	125	2	127.33	2
-	-	141	3	143	2	139	5	139	4	139	4	140.20	4
149	5	148	2	147	3	145	3	145	4	144	5	146.33	6
189	1	185	1	-	-	189	2	188	2	189	1	188.00	1

HAR 1709

Table 5.3 The migration distance and intensity of gliadin subunits of HAR 1709

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
6	5	6	5	5	5	3	5	3	5	3	5	4.33	5
22	5	21	4	20	4	19	4	18	5	18	5	19.66	5
34	5	34	4	33	5	31	5	30	5	30	5	32.00	5
56	1	-	-	54	5	-	-	53	1	51	1	53.50	6
67	3	67	1	66	3	64	3	63	1	63	1	65.00	2
74	2	73	1	72	2	71	2	-	-	69	1	71.80	2
95	1	94	2	93	1	92	1	89	1	-	-	92.60	1
101	2	100	3	99	1	98	2	98	2	-	-	99.20	2
110	3	109	3	109	3	107	3	106	3	106	3	107.83	3
122	3	121	3	120	3	119	3	118	3	118	2	119.66	4
129	2	129	2	131	2	125	2	-	-	-	-	128.50	2
187	2	185	2	185	2	189	2	-	-	187	2	186.60	2

Pavon 76

Table 5.4 The migration distance and intensity of gliadin subunits of Pavon 76.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
6	5	6	5	5	5	5	5	6	4	5	4	5.50	5
26	5	26	5	25	5	24	5	25	5	24	5	25.00	5
31	5	31	5	30	5	29	5	30	5	30	5	30.16	5
53	2	53	1	54	1	51	1	53	2	-	-	53.33	1
57	2	57	1	56	1	55	1	-	-	56	2	56.20	1
65	2	64	1	64	2	63	1	63	2	63	2	63.66	2
71	1	72	1	71	2	70	1	70	1	69	1	70.50	1
98	3	99	3	97	4	-	-	97	3	96	3	97.40	3
106	4	106	4	106	4	103	5	104	4	104	4	104.83	4
120	2	119	3	119	3	117	3	118	2	118	2	118.50	3
-	-	184	1	-	-	181	2	181	1	181	1	182.00	1

Foka

Table 5.5 The migration distance and intensity of gliadin subunits of Foka.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
23	5	23	5	24	5	23	5	22	5	22	5	22.83	5
50	2	51	2	51	2	50	2	49	1	49	1	50.00	2
71	3	71	2	72	3	69	3	70	4	70	2	70.50	3
98	3	99	4	99	3	96	3	97	4	97	4	97.66	4
112	4	113	4	113	4	110	3	110	4	111	4	111.50	4
123	1	124	1	124	2	120	1	122	2	122	1	122.50	1
131	2	132	2	133	2	129	2	130	2	130	2	130.83	2
137	2	137	2	138	3	135	2	135	3	135	2	136.16	2
147	3	148	3	148	3	145	3	145	4	145	3	146.33	3
159	1	160	1	159	1	156	1	157	2	157	1	158.00	1
182	2	184	2	182	2	181	2	180	2	183	1	182.00	2

Dashen

Table 5.6 The migration distance and intensity of gliadin subunits of Dashen.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
8	5	8	5	8	5	4	5	3	4	3	4	5.66	5
14	5	14	5	14	5	10	5	10	5	10	5	12.00	5
28	5	27	5	27	5	24	5	24	5	23	5	25.50	5
38	5	38	5	38	5	36	5	35	5	35	5	36.66	5
72	2	71	3	72	3	71	3	71	3	70	4	71.16	3
78	2	78	2	77	2	78	1	77	2	77	2	75.50	2
83	1	82	1	82	1	83	1	82	1	-	-	82.40	1
101	3	102	4	-	-	102	5	103	5	101	5	101.80	4
111	2	111	4	-	-	117	3	116	3	116	2	114.20	2
125	3	125	2	124	2	129	4	128	3	127	3	126.33	3
-	-	137	2	-	-	140	2	139	2	138	2	138.50	2
151	2	152	3	152	4	153	3	150	3	149	3	151.16	3

HAR 710

Table 5.7 The migration distance and intensity of gliadin subunits of HAR 710.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
12	5	14	4	14	5	8	4	6	5	6	4	10.00	5
33	5	33	5	33	5	34	4	35	3	33	4	33.50	4
58	2	60	3	58	2	56	2	55	3	56	3	57.16	3
64	2	64	3	64	2	60	3	60	3	60	3	62.00	3
71	2	73	3	73	3	71	4	68	2	69	4	70.83	3
-	-	83	1	83	1	80	1	80	1	80	2	81.20	1
104	2	105	3	105	3	103	4	103	2	103	3	103.83	3
112	2	110	2	-	-	-	-	111	3	114	2	111.75	2
125	2	126	3	126	2	126	3	125	3	125	3	125.50	3
134	2	133	2	133	2	134	2	133	3	133	2	133.33	2
176	3	177	1	-	-	179	1	179	1	178	2	177.80	2
-	-	183	1	183	1	184	1	184	1	183	2	183.40	1

ET-13

Table 5.8 The migration distance and intensity of gliadin subunits of ET-13.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
12	5	12	5	13	5	5	5	5	5	4	4	8.50	5
28	5	29	5	30	5	22	5	22	5	21	5	25.33	5
54	1	-	-	-	-	53	2	53	3	50	5	52.50	6
59	2	59	4	60	2	59	2	59	3	-	-	59.20	3
64	3	65	2	65	2	68	3	69	3	68	2	66.50	3
73	3	74	2	75	3	76	2	75	1	76	2	74.83	2
101	3	100	2	102	2	103	3	102	3	100	3	101.33	3
106	3	106	2	108	3	111	3	111	4	111	3	108.83	3
114	4	114	2	115	3	-	-	-	-	117	2	115.00	3
126	2	127	2	128	2	124	2	125	3	125	3	125.83	2
138	2	138	2	138	2	137	2	137	2	137	2	137.50	2
-	-	152	2	153	3	150	2	151	4	151	2	151.40	3
171	2	170	1	-	-	172	1	169	2	170	2	170.40	2

DZ-2023

Table 5.9 The migration distance and intensity of gliadin subunits of DZ-2023.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
28	5	29	5	28	5	21	5	21	5	20	5	24.50	5
38	5	38	5	37	5	30	5	30	5	30	5	33.83	5
43	3	43	3	42	3	35	3	36	4	35	5	39.00	4
48	1	54	1	53	1	47	2	47	1	47	1	49.33	1
62	4	62	4	60	3	55	4	56	5	56	4	58.50	4
79	3	80	3	79	3	73	5	73	2	74	3	76.33	3
93	2	93	2	-	-	88	2	92	3	89	2	91.00	2
107	3	107	3	106	2	103	4	103	3	103	3	104.83	3
120	2	119	1	117	1	115	2	-	-	-	-	117.75	2
131	2	131	2	129	1	128	2	-	-	-	-	129.75	2
139	2	139	2	138	2	137	3	136	4	136	3	137.50	3
156	2	156	2	155	1	155	1	158	2	154	2	155.66	2
169	3	-	-	-	-	165	2	165	1	170	4	167.25	6

DZ-393-4

Table 5.10 The migration distance and intensity of gliadin subunits of DZ-393-4.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
31	5	30	5	29	5	30	5	30	5	31	5	30.16	5
43	1	42	2	42	1	44	1	44	1	44	1	43.16	1
52	1	51	2	51	1	54	1	53	1	53	2	52.33	1
59	3	58	3	58	2	62	3	61	3	61	3	59.83	3
75	1	74	3	74	2	79	3	78	2	78	2	76.33	2
97	1	96	2	96	2	-	-	-	-	96	2	96.25	2
104	3	103	3	102	2	-	-	-	-	103	2	103.00	3
136	1	135	2	-	-	139	3	138	2	138	3	137.20	2
164	1	164	1	-	-	163	2	162	1	162	2	163.00	1
192	1	192	1	192	1	196	1	-	-	195	1	193.40	1

DZ-1052

Table 5.11 The migration distance and intensity of gliadin subunits of DZ-1052.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
28	5	29	5	30	5	33	5	32	5	32	5	30.66	5
57	2	57	3	58	4	62	4	62	4	61	4	59.50	4
98	2	97	2	97	2	95	2	96	1	92	1	95.83	2
102	2	102	2	103	2	104	2	103	2	102	2	102.66	2
-	-	114	1	114	1	110	2	109	2	108	2	111.00	3
129	2	129	3	129	3	123	1	-	-	-	-	127.50	2
133	1	134	2	134	2	138	1	138	3	137	3	135.66	2

K-6290-bulk

Table 5.12 The migration distance and intensity of gliadin subunits of K-6290-bulk.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
11	5	11	5	11	5	12	5	13	5	13	5	11.83	5
24	5	24	5	25	5	26	5	27	5	27	5	25.50	5
34	4	34	5	35	5	37	4	38	5	38	4	36.00	5
57	2	58	2	58	2	61	1	-	-	-	-	58.50	2
69	2	68	2	70	2	73	2	73	2	72	2	70.83	2
88	2	85	1	90	2	85	1	86	1	90	1	87.33	1
97	2	98	2	98	1	95	2	95	1	95	1	96.00	2
-	-	107	1	-	-	107	1	112	2	113	3	109.75	2
-	-	131	1	-	-	128	2	128	2	129	1	129.00	2
136	3	135	2	136	2	139	2	-	-	140	2	137.20	2

DZ-04-118

Table 5.13 The migration distance and intensity of gliadin subunits of DZ-04-118.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
25	5	26	5	27	5	28	5	28	5	29	5	27.16	5
32	3	33	3	34	3	35	3	35	3	36	3	34.16	3
61	4	62	3	63	5	61	4	62	3	62	4	61.83	4
68	1	70	1	70	1	66	5	66	4	68	5	68.00	6
95	2	90	1	-	-	92	1	-	-	96	1	93.25	1
107	2	107	2	108	2	107	2	108	2	108	2	107.50	2
114	2	-	-	-	-	113	2	115	2	114	2	114.00	2
134	2	132	3	136	2	131	1	132	1	132	1	132.83	2
141	2	-	-	-	-	142	2	143	1	143	2	142.25	2

HAR 1522

Table 5.14 The migration distance and intensity of gliadin subunits of HAR 1522.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
14	5	14	5	13	5	15	5	14	5	13	5	13.83	5
33	5	33	5	32	5	35	5	34	5	33	5	33.33	5
38	5	38	5	36	4	40	5	38	5	-	-	38.00	5
66	2	65	2	63	1	64	2	-	-	-	-	64.50	2
73	3	73	3	70	2	69	2	75	2	74	3	72.33	3
98	2	97	2	96	2	98	2	-	-	-	-	97.25	2
-	-	105	2	-	-	103	2	102	2	102	3	103.00	2
132	2	132	1	-	-	131	2	130	1	130	2	131.00	2
139	1	-	-	137	1	139	1	142	2	139	2	139.20	1

K-6295-4A

Table 5.15 The migration distance and intensity of gliadin subunits of K-6295-4A.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
10	5	9	5	9	5	10	5	10	5	9	5	9.50	5
24	5	24	5	24	5	25	5	25	5	24	5	24.33	5
32	2	32	1	32	2	32	2	32	3	32	2	32.00	2
37	3	37	4	38	4	37	3	37	5	37	4	37.16	4
60	1	60	1	60	2	58	2	59	3	60	1	59.10	2
72	2	72	2	71	2	72	2	71	3	71	2	71.50	2
-	-	-	-	93	2	97	2	92	2	97	2	94.75	2
104	2	104	2	105	2	-	-	103	2	103	2	103.80	2
122	1	122	1	122	2	121	1	121	2	120	1	121.33	1
134	3	135	1	131	2	133	3	-	-	133	2	133.20	2

DZ-1050

Table 5.16 The migration distance and intensity of gliadin subunits of DZ-1050.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
29	5	28	5	27	5	29	5	30	5	29	5	28.66	5
59	4	58	4	58	3	59	4	60	4	60	4	59.00	4
92	2	91	1	91	1	91	2	-	-	92	1	91.40	1
-	-	98	2	97	2	97	3	97	2	-	-	97.25	2
-	-	131	3	130	2	130	3	130	3	-	-	130.25	3
136	2	-	-	135	2	-	-	135	2	134	2	135.00	2
154	1	156	1	-	-	152	1	154	1	153	1	153.80	1

Kilinto

Table 5.17 The migration distance and intensity of gliadin subunits of kilinto.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
19	5	20	5	20	5	22	5	22	5	22	5	20.83	5
29	5	30	4	30	4	32	4	32	3	32	3	30.83	4
35	2	35	2	36	2	37	2	37	1	-	-	36.00	2
48	2	49	1	49	1	49	2	50	2	50	2	49.16	2
56	3	57	2	58	4	58	3	58	2	57	1	57.33	3
71	2	71	2	72	2	72	2	72	2	73	3	71.83	2
89	2	-	-	91	1	90	2	92	2	91	1	90.60	2
100	4	100	3	101	3	100	2	100	3	101	2	100.33	3
113	4	-	-	-	-	113	1	114	2	114	4	113.50	2
123	1	123	1	124	1	123	1	122	1	-	-	123.00	1
133	2	133	2	134	2	133	2	134	1	134	1	133.50	2
152	2	152	2	154	1	151	2	154	2	151	2	152.33	2

Cocorit 71

Table 5.18 The migration distance and intensity of gliadin subunits of Cocorit 71.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
22	5	22	5	20	5	23	5	23	5	24	5	22.33	5
29	3	30	2	29	4	33	4	33	4	33	4	31.16	4
47	2	48	2	48	2	50	3	50	2	51	2	49.00	2
57	4	57	4	57	2	59	3	59	3	60	3	58.16	3
64	2	65	2	65	3	67	2	67	2	68	2	66.00	2
71	3	72	3	72	4	73	3	74	3	74	3	72.66	3
95	3	95	3	95	4	97	3	97	3	97	3	96.00	3
101	3	101	4	101	4	102	4	102	4	102	4	101.50	4
123	2	123	1	123	1	123	2	124	1	125	1	123.50	1
134	3	133	3	134	2	134	3	134	2	135	3	134.00	3
143	1	143	2	144	2	143	2	144	1	143	1	143.33	2
154	1	155	1	155	2	154	2	156	1	155	1	154.83	1
169	1	-	-	164	1	168	1	-	-	163	1	166.00	1
194	2	194	1	194	2	193	2	-	-	194	1	193.80	1

HAR 1407

Table 5.19 The migration distance and intensity of gliadin subunits of HAR 1407.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
8	5	8	5	6	5	14	5	13	5	12	5	10.16	5
21	5	21	5	20	5	26	5	26	5	25	5	23.16	5
32	5	32	5	31	5	30	3	-	-	-	-	31.25	5
71	1	70	1	-	-	71	2	-	-	73	1	71.00	1
99	1	96	2	-	-	97	2	98	2	95	2	97.00	2
119	2	118	1	117	2	122	2	122	1	120	1	119.66	2
131	1	131	1	132	1	134	1	135	1	-	-	132.60	1

Boohai

Table 5.20 The migration distance and intensity of gliadin subunits of Boohai.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
34	5	33	5	31	5	32	5	32	5	30	5	32.00	5
47	2	46	2	45	1	45	2	45	1	45	1	45.50	2
57	2	56	1	57	1	55	1	55	1	54	1	55.66	1
67	3	66	3	65	2	65	3	64	3	64	2	65.16	3
83	3	82	2	80	2	80	3	80	4	80	2	80.83	3
103	2	102	1	100	1	99	1	100	2	101	2	100.83	2
116	3	114	3	114	1	112	3	111	4	109	3	112.66	3
130	1	129	1	128	1	126	1	-	-	126	1	127.80	1
-	-	146	2	145	2	144	2	147	2	145	2	145.40	2
172	1	170	2	171	2	167	2	166	2	167	2	168.83	2

HAR 604

Table 5.21 The migration distance and intensity of gliadin subunits of HAR 604.

Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 5		Rep. 6		Average	
MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT	MD	INT
10	5	9	5	10	5	12	5	12	5	12	5	10.83	5
25	5	24	4	24	5	26	5	26	5	26	5	25.16	5
37	5	36	5	37	5	38	5	38	5	38	5	37.33	5
75	4	79	2	78	3	79	2	79	2	79	2	78.16	3
112	2	112	2	111	3	108	2	110	2	111	2	110.66	2
134	1	133	1	132	3	130	2	130	2	130	2	131.50	2
148	1	146	1	147	1	144	2	144	1	145	1	145.66	1
168	1	165	1	167	1	165	1	164	1	163	1	165.33	1

ii) The HMW-GS

The results of the HMW-GS are given in Table 4.22.

Table 5.22 The HMW-GS data of the Ethiopian bread durum wheat cultivars tested.

No	Cultivars	A genome	B genome	D genome
	Bread wheats			
1	HAR 1685	2*	7	5+10
2	HAR 1709	0/2*	7+9	2+12
3	Pavon 76	2*	17+18	5+10
4	Dashen	1	7+9	5+10
5	HAR 710	2*	17+18	5+10
6	ET-13-A2	0/2*	7+8	2+12
7	HAR 1522	2*	17+18	5+10
8	K-6295-4A	0/2*	7+8	2+12
9	HAR 604	2*	7+9	5+10
10	K-6290-bulk	2*	7+8	5+10
11	HAR 1407	2*	7+9	5+10
	Durum wheats			
1	Foka	0	14+15	
2	DZ-393-4	0	14+15	
3	DZ-1052	0	14+15	
4	DZ-1050	0	14+15	
5	Boohai	0	14+15	
6	DZ-04-118	0	7+8	
7	Cocorit 71	0	7+8	
8	Kilinto	0	6+8	
9	DZ-2023	0	6+8	

Concerning bands coded by the A genome (Table 5.22), one bread wheat cultivars had band 1, and seven had band 2*. It was not possible to clearly distinguish between band 2* and the null (0) band. In such instances a 0/2* combination was given, and three of the bread wheat cultivars had this combination. None of the durum wheats had bands coded by the A genome. Concerning B genome bands, one bread wheat cultivar had band 7, three had 7+8 and four had 7+9. For the durum wheats, five had bands 14+15, tow had bands 7+8 and two had bands 6+8. Concerning bands coded by the D genome, eight bread wheat cultivars had bands 5+10 and three had bands 2+12. The durum wheats have no D genome, and, hence, no band was coded at this genome.

iii) Detail of the cluster, genetic distances, and dendrogram.

The UPGMA analysis of gliadin and HMW-GS data provided detail of the cluster section (Table 5.23), the genetic distances between all possible pairs of the wheat entries (Table 5.24), and a dendrogram representing the genetic distances among the wheat entries (Fig. 5.1).

Table 5.23 Pairwise distance matrix of the gliadin and HMW-GS data based on the F statistic of Nei and Li (1979).

No	Cultivar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	HAR 1685	0																				
2	HAR 1709	0.69	0																			
3	Pavon 76	0.59	0.56	0																		
4	Foka	0.63	0.57	0.68	0																	
5	Dashen	0.68	0.68	0.72	0.62	0																
6	HAR 710	0.68	0.62	0.63	0.65	0.63	0															
7	ET-13-A2	0.66	0.66	0.75	0.47	0.62	0.62	0														
8	DZ-2023	0.47	0.66	0.59	0.66	0.62	0.56	0.69	0													
9	DZ-393-4	0.62	0.62	0.66	0.68	0.57	0.57	0.70	0.56	0												
10	DZ-1052	0.63	0.63	0.65	0.57	0.56	0.65	0.69	0.60	0.59	0											
11	K-6290-bulk	0.62	0.65	0.63	0.59	0.50	0.57	0.62	0.59	0.54	0.40	0										
12	DZ-04-118	0.65	0.45	0.66	0.56	0.60	0.54	0.65	0.59	0.57	0.62	0.57	0									
13	HAR 1522	0.59	0.65	0.57	0.62	0.57	0.60	0.59	0.59	0.60	0.62	0.50	0.57	0								
14	K-62954-A	0.63	0.57	0.65	0.50	0.62	0.56	0.50	0.69	0.59	0.57	0.59	0.52	0.59	0							
15	DZ-1050	0.30	0.65	0.57	0.65	0.63	0.63	0.62	0.49	0.57	0.62	0.57	0.60	0.54	0.59	0						
16	Kilinto	0.59	0.65	0.60	0.59	0.50	0.63	0.65	0.65	0.63	0.45	0.33	0.57	0.50	0.59	0.57	0					
17	Cocorit 71	0.57	0.60	0.65	0.69	0.56	0.65	0.63	0.60	0.49	0.60	0.59	0.59	0.56	0.57	0.52	0.52	0				
18	HAR 1407	0.54	0.66	0.68	0.63	0.56	0.59	0.63	0.50	0.56	0.57	0.62	0.59	0.52	0.60	0.49	0.56	0.47	0			
19	Boohai	0.59	0.52	0.57	0.49	0.57	0.54	0.59	0.65	0.63	0.59	0.60	0.50	0.57	0.49	0.54	0.54	0.59	0.56	0		
20	HAR 604	0.66	0.66	0.59	0.63	0.56	0.68	0.63	0.66	0.59	0.50	0.52	0.65	0.56	0.60	0.65	0.49	0.60	0.57	0.62	0	
21	Chiness Spring	0.62	0.59	0.66	0.59	0.63	0.60	0.59	0.68	0.66	0.65	0.63	0.50	0.54	0.56	0.57	0.60	0.65	0.62	.038	0.62	0

Table 5.24 Detail of the cluster section formed by UPGMA analysis of the gliadin and HMW-GS data.

Row	Cluster	Cultivars
19	1	HAR 1407
21	1	HAR 604
10	2	DZ-393-4
11	2	DZ-1052
16	2	DZ-1050
2	3	HAR 1685
12	3	K-6290-bulk
4	4	Pavon 76
7	4	HAR 710
17	5	Kilinto
18	5	Cocorit 71
1	6	Chinese Spring
8	6	ET-13-A2
15	6	K-6295-4A
3		HAR 1709
5		Foka
6		Dashen
9		DZ-2023
13		DZ-04-118
14		HAR 1522
20		Boohai

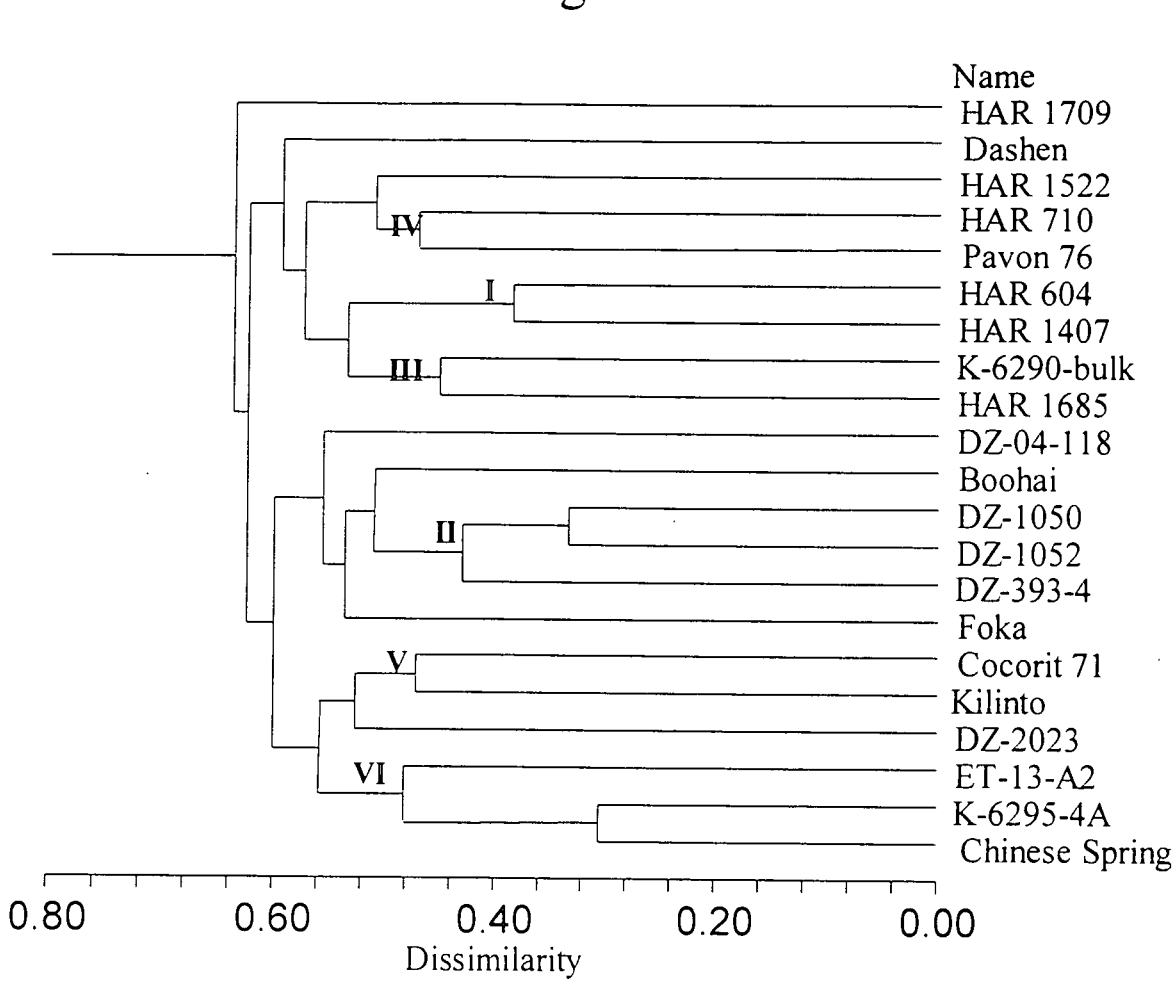

Fig. 5.1 A dendrogram generated by UPGMA analysis of the gliadin and HMW-GS data.

Genetic distance analysis

Pairwise distance matrix of the gliadin and HMW-GS data (Table 5.23) based on the F statistic of Nei and Li (1979) revealed an average genetic distance of 0.58 (58%) among the durum wheat cultivars ranging from 0.45 (45%) between DZ-1052 and Kilinto to 0.69 (69%) between Foka and Cocorit 71. The result also indicated an average genetic distance of 0.61 (61%) among the bread wheat cultivars, with a range from 0.50 (50%) between (Dashen and K-6295-4A; ET-13-A2 and K-6295-4A; K-6290-bulk and HAR 1522) to 0.75 (75%) between Pavon 76 and ET-13-A2.

Dendrograms

A dendrogram constructed using the gliadin and HMW-GS data was given in Fig 5.1. In this dendrogram, the range of dissimilarity was from 0.00 to 0.80, and all the different clusters were formed within this range. Results from the cluster section (Table 5.24) indicated six clusters among which most of the wheat cultivars were divided. Some of the wheat entries were not included in any of the six clusters, but stood individually as a different cluster.

Cluster I consisted of two bread wheat cultivars, HAR 604 and HAR 1407. Cluster II had three durum wheat cultivars. DZ-1050, DZ-1052, and DZ-393-4. In cluster III, two bread wheat cultivars K-6290-bulk and HAR 1685 were included, and cluster IV consisted of two bread wheat cultivars HAR 710 and Pavon 76. Cluster V included two durum wheat cultivars. Cocorit 71 and Kilinto. Cluster VI comprised of three bread wheat cultivars, ET-13-A2. K-6295-4A, and the standard bread wheat cultivar Chinese Spring.

Four durum wheat cultivars (such as DZ-2023, Foka, Boohai, and DZ-04-118) and three bread wheat cultivars (such as HAR 1522, Dashen, and HAR 1709) were not included in any of the six clusters mentioned above, but they fell apart individually as different clusters. This indicated that the four durum wheats and the three bread wheats are distantly related to the above six clusters and are also not closely related to one another based on the gliadin and HMW-GS data.

Conclusion

The gliadin and HMW-GS data effectively distinguished the wheat cultivars from one another. In addition, the gliadin and HMW-GS data yielded a dendrogram that was consistent with the known genetic relationships of the cultivars. In this study, the gliadin banding patterns were sufficiently unique to differentiate the wheat varieties. None of the wheat varieties tested had identical gliadin banding patterns. In the case of HMW-GS, however, three of the bread wheat cultivars had identical bands (2*, 17+18, 5+10) (Table 5.22). In the same way, two of the bread wheat cultivars had identical bands (0/2*, 7+8, 2+12) and another two bread wheat cultivars were also found to have the same bands (2*, 7+9, 5+10). Similarly, five durum wheat cultivars had bands (0,14+15), two durum wheat cultivars had bands (0,7+8), and the other two durum wheat cultivars had bands (0,6+8). This indicates that, unlike the HMW-GS, gliadins can be successfully used to differentiate wheat cultivars. In this study, however, both the gliadins and the HMW-GS were used in order to have further insight into the genome, since the synthesis of the two proteins are under different genetic control. The use of protein markers is also relatively cheap and technically straightforward. Thus, for identification purposes, protein markers can effectively be used.

Chapter 6

Identification and genetic distance analysis of Ethiopian wheat and tef cultivars using AFLP markers

Abstract

The amplified fragment length polymorphism (AFLP) technique was used to assay 20 Ethiopian wheat cultivars and advanced lines. AFLP markers are genomic restriction fragments detected after selective amplification using the polymerase chain reaction (PCR). Digestion with two restriction enzymes and amplification with primers having one- and three-selective bases at their 3' end produced distinct fragment lengths among the genotypes, which were detected using an automatic sequencer. Considerable variation was detected among the wheat entries using two primer combinations. The AFLP data were scored into binary matrix as present (1) or absent (0) across all the genotypes. Calculation of the distance matrix and cluster analysis was carried out using NCSS program (Hintze, 1998). Genetic distances between all possible pairs of the wheat entries were calculated based on the pairwise comparisons of Nei and Li (1979). UPGMA clustering of the AFLP data produced three groups, consisting of two cultivars, with the majority of the cultivars clustered individually, due to the larger number of variability detected among the individual wheat entries.

Introduction

AFLP is a novel molecular fingerprinting technique that can be applied to the DNA of any origin and complexity. The AFLP technique is based on the selective amplification of restriction fragments from a digest of total genomic DNA using PCR. Genomic DNA is first digested by two restriction enzymes. Double stranded oligo nucleotide adapters, homologous to one 5'- or 3'-end generated during restriction digestion, are ligated to the DNA fragments. The ligated DNA fragments are amplified by PCR using primers

complimentary to the adapter and restriction site sequence with additional selective nucleotides at their 3'-end (Vos *et al*, 1995).

Restriction fragments are generated using two restriction endonucleases, a 'rare'-cutting enzyme with 6-base recognition (such as *Eco*RI), in combination with a'frequent'-cutting enzyme of 4-base recognition (such as *Mse*I). Three types of restriction fragments are generated following digestion: (i) fragments cut by the rare cutting enzyme on both ends; (ii) fragments cut with the frequent cutting enzyme on both ends; and (iii) fragments that have been cut by both the rare-cutter and frequent-cutter. Using *Eco*RI and *Mse*I restriction enzymes as example, *Eco*RI-*Eco*RI, *Mse*I–*Mse*I and *Eco*RI-*Mse*I fragments would be produced during restriction enzyme digestion. More than 90% of the fragments are expected to have a frequent-cutter site on both ends (eg. *Mse*I–*Mse*I fragments). However, the AFLP procedure predominantly amplifies the *Eco*RI- *Mse*I fragments, thereby reducing the complexity of the reaction mixture. In addition, the use of selective primers reduces the complexity of the mixture, as only those fragments with complementary nucleotides extending beyond the restriction site will be amplified by the selective primers under stringent annealing conditions. Polymorphisms are revealed by resolving the amplified fragments on an automatic sequencer, and subsequent comparison of the fingerprints generated for each sample (Blears *et al*, 1998; Vos *et al*, 1995).

The AFLP technique has several advantages over other DNA fingerprinting systems including the capacity to analyse an entire genome, larger number of loci detected, as well as its reproducibility (Blears *et al*, 1998). Despite the low level of genetic variability in wheat (Gupta *et al*, 1999), the availability of large number of primer combinations and the high speed of the assay makes the AFLP technique an efficient marker technology for genetic diversity study of wheat. The objective of this study was to determine genetic relationships among the Ethiopian wheat cultivars using AFLP data.

Materials and methods

Plant materials

Twenty Ethiopian wheat entries were used in this study. A list of the 20 wheat genotypes was given in Table 3.1 and Table 3.2. Leaf material collected from young (4 weeks old) plants grown in the greenhouse was used for the DNA extraction.

DNA preparation

Total genomic DNA was isolated from fresh leaf material by a modification of the method described by Edwards *et al* (1991). Approximately 1.5g of fresh leaf was collected from one plant representing each variety. The leaves were homogenised in liquid nitrogen using a mortar and pestle. The powder was then transferred to a 50ml polypropilene tube containing 10ml extraction buffer of pH 7.8-8.0 [5M NaCl, 1M Tris-HCl (pH 8), 0.25M EDTA (ethylenediaminetetraacetic acid) (pH 8), 1.25% (w/v) SDS, 0.2M $Na_2S_2O_5$], mixed well, and incubated at 65°C for 30 minutes, with periodic shaking every 10 minutes. The homogenate was further incubated at 65°C for 1 hour with 1ml of 1% CTAB (cetyltrimethylammonium bromide) solution and 2ml of 5M NaCl. Chloroform-isoamylalcohol (24:1) extractions were performed at 10 000 rpm for 15 minutes. The nucleic acids were precipitated with 100% ethanol. The precipitated DNA was spooled and washed in 70% ethanol. The DNA was dissolved in 1ml of sterile (distilled) water. The DNA concentration was determined spectrophotometrically.

AFLP reaction

Restriction-ligation

The AFLP analysis system from Life Technologies, Inc (GIBCO BRL) was used. The AFLP analysis followed the manufacturer's protocol. Approximately 250ng of genomic DNA was double digested with 2µl (1.25 units/ul) of *Mse*I and *Eco*RI (1.25 units/ul) at 37°C for 2 hours. Following heat inactivation of the restriction endonucleases at 70°C for 15 minutes, the digested DNA fragments were ligated to *Eco*RI and *Mse*I adapters (Table 6.1) using T4 DNA ligase (10 units) for 2 hours at 20°C. Following ligation, the

reaction mixtures were diluted 10-fold in TE buffer and used as templates for subsequent PCR amplification.

PCR amplification

Amplification was performed in two consecutive reactions. In the first reaction, preamplification, adapter ligated genomic DNA fragments were amplified with two AFLP primers each having one selective nucleotide (Table 6.1). Preamplification was performed for 20 cycles with the following cycle profile: a 30 second DNA denaturation step at 94°C, a 1 minute annealing step at 56°C, and a 2 minutes extension step at 72°C. In the preamplification step, 5µl of the diluted ligation reaction was used with 40µl pre-amp primer mix, 5µl of 10X PCR buffer plus Mg, and 1µl of *Taq* DNA polymerase (1 unit/µl), as recommended. Preamplification was confirmed by running 12µl of the reaction mixture on a 1% agarose gel stained with ethidium bromide. The gel was run at a constant current (80mA), and amplification products were visualized under UV light. The unused portion of the reaction mixture was stored at -20°C.

The second amplification reaction was performed using two AFLP primer combinations, each containing three selective nucleotides (Table 6.1). The *Eco*RI primers were fluorescently labelled "Ned" (black) and "Fam" (blue). PCR for selective amplification was as follows: 5 µl of the 50 times diluted preamplification product, 5.5µl of "mix 1", containing1µl labelled *Eco*RI primer, 4.5µl *Mse*I primer which includes dNTPs, and 9.5µl of "mix 2", containing 7.4µl AFLP grade water, 2µl 10X PCR buffer plus Mg, 0.1µl *Taq* DNA polymerase (5 units/µl), in a total volume of 20µl. PCR was carried out in three different cycles. The first cycle of PCR was performed at 94°C for 30 seconds; 65°C for 30 seconds; and 72°C for 60 seconds. In the following nine cycles, the annealing temperature was reduced by 0.7°C in each cycle. The PCR cycle was ended with 23 cycles at 94°C for 30 seconds; 56°C for 30 seconds; and 72°C for 60 seconds. All amplification reactions were performed in a "Hybaid, Touch-down, Hot lid" thermocycler.

Table 6.1 Oligonucleotide adaptors and primers used for AFLP analysis

Name of adapters/primers	Sequences (5'-3')
*Eco*RI adaptors	CTCGTAGACTGCGTACC
	CTGACGCATGGTTAA
*Mse*I adaptors	GACGATGAGTCCTGAG
	TACTCAGGACTCAT
AFLP primers[a]:	
*Eco*RI-A	AGACTGCGTACCAATTCA
*Mse*I-C	GACGATGAGTCCTGAGTAAC
*Eco*RI-AAC-Ned	GACTGCGTACCAATTCAAC
*Eco*RI-ACA-Fam	GACTGCGTACCAATTCACA
*Mse*I-CGA	GATGAGTCCTGAGTAACGA
*Mse*I-CCA	GATGAGTCCTGAGTAACCA

[a]*EcoRI* and *Mse*I selective primers were used in all possible combinations.

After selective amplification, 5µl of each selective amplification product was added to 1µl Rox standard and 24µl formamide. The samples were then denatured at 94°C for 10 minutes with quick cooling on ice. Fragments were visualised on an ABI310 automatic capillary (PE Biosystems) sequencer.

AFLP scoring and data analysis

The AFLP data was scored into a binary matrix as discrete variables ("1" for presence and "0" for absence)(Appendix 10.2). All reproducible fragments, above a thresh hold fluorescence intensity of 35, were scored using a peak height of 180. The AFLP genotypic data was used to calculate pairwise genetic distances according to the formula of Nei and Li (1979). Estimates of similarity between genotypes were based on the probability that an amplified fragment from one genotype would also be present in another. Associations among the 20 varieties were determined from cluster analysis based on the genetic distance estimates. The UPGMA clustering method was used for hierarchical clustering, and the necessary computations were performed using the NCSS program (Hintze, 1998).

Results and discussion

Results of the AFLP markers scored as present (1) or absent (0) for the 20 wheat entries are given in Appendix 9.3. The genetic distances between all possible pairs of the wheat entries, Detail of the cluster section, and a dendrogram representing the genetic distances among the wheat entries are given in Table 6.1, Table 6.2, and Fig 6.1, respectively.

Table 6.1 Pairwise distance matrix of the greenhouse experiment based on the F statistic of Nei and Li (1979).

No	Cultivar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	HAR 1685	0																			
2	HAR 1709	0.35	0																		
3	Pavon 76	0.46	0.46	0																	
4	Foka	0.56	0.58	0.56	0																
5	Dashen	0.57	0.57	0.59	0.56	0															
6	HAR 710	0.55	0.58	0.56	0.48	0.50	0														
7	ET-13-A2	0.49	0.52	0.47	0.46	0.46	0.44	0													
8	DZ-2023	0.50	0.56	0.51	0.53	0.54	0.51	0.41	0												
9	DZ-393-4	0.56	0.59	0.57	0.52	0.47	0.51	0.46	0.54	0											
10	DZ-1052	0.62	0.62	0.63	0.56	0.49	0.58	0.51	0.56	0.53	0										
11	K-6290-bulk	0.53	0.60	0.58	0.56	0.52	0.56	0.48	0.48	0.49	0.59	0									
12	DZ-04-118	0.52	0.59	0.56	0.54	0.50	0.52	0.47	0.47	0.49	0.56	0.42	0								
13	HAR 1522	0.52	0.54	0.54	0.59	0.47	0.46	0.46	0.54	0.45	0.58	0.47	0.48	0							
14	K-6195-4A	0.48	0.50	0.54	0.51	0.46	0.51	0.47	0.51	0.51	0.59	0.50	0.49	0.49	0						
15	DZ-1050	0.55	0.58	0.56	0.51	0.51	0.55	0.51	0.56	0.52	0.63	0.53	0.49	0.43	0.51	0					
16	Kilinto	0.51	0.56	0.55	0.51	0.47	0.52	0.49	0.51	0.48	0.61	0.51	0.45	0.46	0.46	0.38	0				
17	Cocorit 71	0.55	0.61	0.60	0.48	0.51	0.52	0.47	0.53	0.48	0.50	0.56	0.54	0.45	0.52	0.51	0.49	0			
18	HAR 1407	0.52	0.54	0.48	0.49	0.47	0.52	0.46	0.50	0.49	0.57	0.51	0.51	0.41	0.43	0.43	0.43	0.46	0		
19	Boohai	0.49	0.53	0.51	0.48	0.47	0.51	0.46	0.50	0.51	0.54	0.53	0.48	0.46	0.43	0.45	0.41	0.45	0.34	0	
20	HAR 604	0.51	0.54	0.51	0.56	0.51	0.56	0.51	0.54	0.55	0.62	0.56	0.55	0.52	0.48	0.54	0.48	0.51	0.43	0.38	0

Table 6.2 Detail of the cluster section provided by UPGMA analysis of the AFLP markers.

Row	Cluster	C1
18	1	HAR 1407
19	1	HAR-1522
1	2	HAR 1685
2	2	HAR 1709
15	3	DZ-1050
16	3	Kilinto
3		Pavon 76
4		Foka
5		Dashen
6		HAR 710
7		ET-13-A2
8		DZ-2023
9		DZ-393-4
10		DZ-1052
11		K-6290-bulk
12		DZ-04-118
13		Boohai
14		K-62954-A
17		Cocorit 71
20		HAR 604

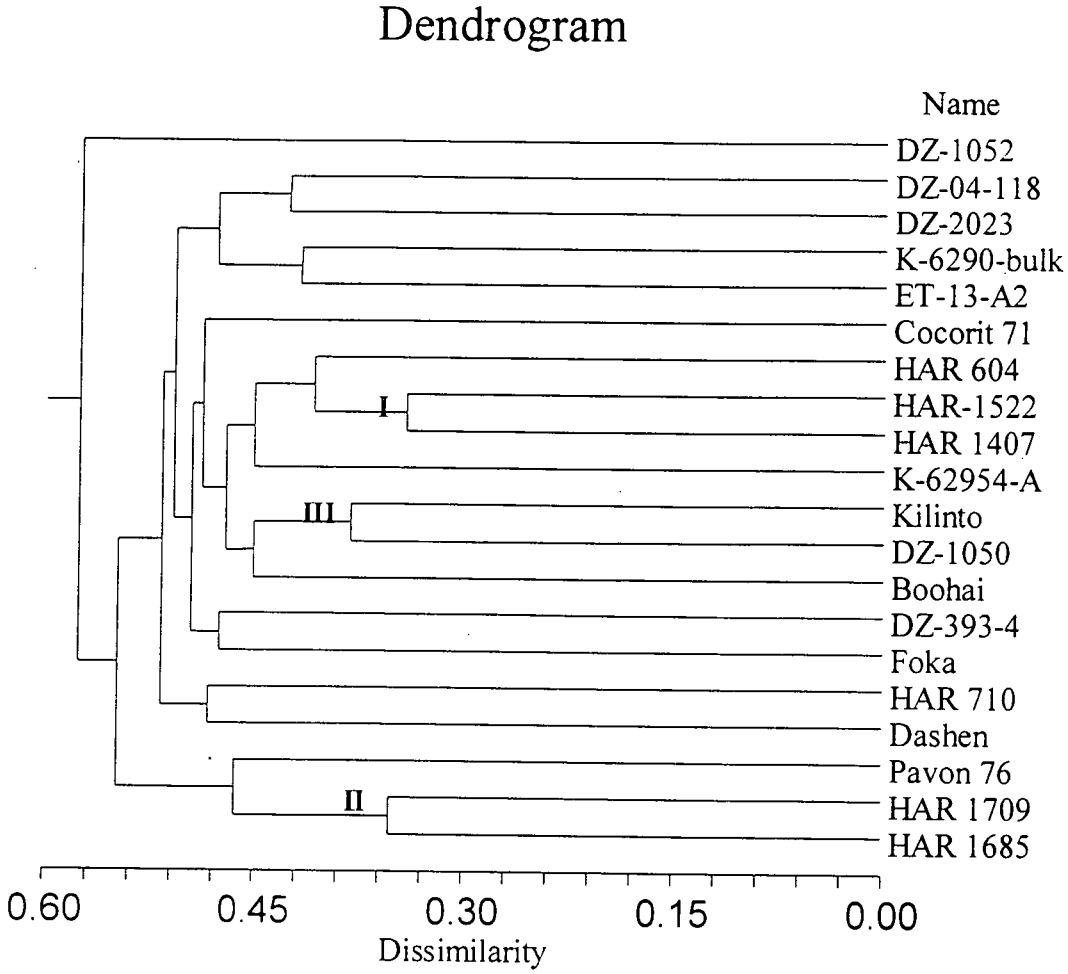

Fig 6.1 Dendrogram generated by UPGMA analysis of the AFLP markers.

Genetic distance analysis

The genetic distances among the bread wheat cultivars (Table 6.2) based on the F statistic of Nei and Li (1979) ranged from 0.35 (35%) between HAR 1685 and HAR 1709 to 0.60 (60%) between HAR 1709 and K-6295-bulk, with an average genetic distance of 0.50 (50%). Among the durum wheat cultivars, the average genetic distance was also found to be 0.50 (50%), ranging from 0.30 (30%) between DZ-1050 and Kilinto to 0.63 (63%) between DZ-1050 and DZ-1052.

Dendrogram

A dendrogram constructed using the AFLP markers was given in Fig. 6.1. In this dendrogram, the range of dissimilarity of all the wheat entries was from 0.00 to 0.60. Results from the cluster section (Table 6.2) indicated three clusters, each of which consisted of two cultivars. The rest of the cultivars stood individually as a separate cluster. All the cultivars were clustered according to their genetic relationships except DZ-1052 (Fig. 6.1). Although DZ-1052 is closely related to DZ-1050 (Table 3.2), it was clustered far apart from DZ-1050. This can be ascribed to insufficient amplification of the DNA to yield proper AFLP data, contamination of the DNA, or incorporation of off type.

The first cluster consisted of two bread wheat cultivars, HAR 1407 and HAR 1522. The second cluster had also two bread wheat cultivars, HAR 1709 and HAR 1685. In the third cluster, two durum wheat cultivars, Kilinto and DZ-1050, were included. The rest of the wheat entries stood individually as different clusters, and this indicates that they are significantly dissimilar from one another and also from the three clusters mentioned above.

An attempt was also made to identify and determine the genetic divergence of 10 Ethiopian tef cultivars on the basis of morphological and AFLP markers. Although preselective amplification of tef samples proved successful, the selective amplification did not yield any fragments that could be visualized reproducibly. Repeated digestion ligation reactions, preselective amplification reactions and attempts to vary the amount of preselective template in selective reactions, made little difference.

Conclusion

The discrimination of wheat cultivars with two primer combinations demonstrated that the AFLP technology is a powerful tool for wheat cultivar identification. There was a large genetic diversity among the test materials. The two primer pairs amplified 780 AFLP markers, out of which 144 (18.4%) were polymorphic across all the genotypes tested. Among the polymorphic fragments, 42 (29.1%) were unique to individual accessions. These large numbers of individual fragments are one explanation why these accessions are distinct from others in the dendrogram (Fig. 6.1).

It is clear that the AFLP analysis determines direct measures of genetic similarity between individuals. Thus, compared to morphological or protein markers, AFLP provides a more detailed coverage throughout the genome, which in turn provides a more reliable estimation of the genetic distances among genotypes. AFLP markers are, therefore, a powerful tool for measuring the genetic diversity and determining relationships within and among species.

Chapter 7

General conclusion

In the research reported here, the identity and genetic divergence of 20 Ethiopian wheat varieties were surveyed based on morphological, protein, and DNA markers. Each of these three classes of genetic markers uniquely identified all the wheat entries tested. However, there were significant differences in the results of the cluster analysis based on morphological, protein, and DNA data.

It is clear that morphological traits are limited in number and have interaction with the environment. Thus, they cannot adequately represent genetic relationships among different cultivars, and they are also inconsistent across environments. In this study, for instance, it was found that the average genetic distance among the durum wheat entries was 1.01 for the greenhouse and 1.11 for the field experiment. Similarly, the average genetic distance among the bread wheat entries varied from 1.24 to 1.16 for the greenhouse and field experiment, respectively. The clustering patterns of the wheat entries were also not consistent for the greenhouse (Fig 3.1) and field (Fig 3.2) experiment. Thus, morphological traits cannot efficiently detect the genetic distance values among different cultivars.

As far as biochemical characterization is concerned, the gliadins and HMW-GS provided a more objective and stable way of identification among the wheat varieties. In the dendrogram shown in Fig. 5.1, the wheat genotypes were clustered in a fashion that is in a general agreement with their genetic relationships. However, the limitation with the use of protein markers is that they represent only part of the gene, which code for the synthesis of a specific protein. In hexaploid wheat, for instance, the gliadins are coded by six genes, 1A, 1B, 1D, 6A, 6B, and 6D, on the short arms of the group 1 and group 6 chromosomes; whereas the HMW-GS are coded by three genes at the Glu-A1, Glu-B1, and Glu-D1 loci on the long arms of the chromosomes 1A, 1B, and 1D, respectively (Payne *et al*, 1982). This indicates that protein markers are insufficient to represent the

whole genome. However, for genetic distance analysis, the use of more than one kind of protein marker can provide more information about the genome, since different proteins are coded by different genes. In this study, protein markers were not used to characterise the tef cultivars, because it was already reported that the low number of protein markers detected could not allow the separation of individual tef accession into distinct classes (Bekele *et al*, 1995).

In the case of AFLP analysis, more variations were detected among the cultivars. This can be seen from the fact that cultivars which fell into the same cluster in the morphological (Fig 3.1 and 3.2) and biochemical (Fig 5.1) analysis were fell apart individually as a different cluster in the AFLP (Fig 6.1) data. This suggests that AFLP markers are able to detect a large number of polymorphisms in a more efficient way in comparison to morphological and biochemical markers, due to the higher number of loci assayed. The clustering data can be used by the breeder to plan his breeding programme. It gives him the data to make informed choices for the crossing block so that parents are included which are genetically diverse. In summary, results from this study suggest that AFLP technology offers a reliable and effective means of cultivar identification and genetic distance analysis. Thus, AFLP can be a good supplement to morphological and protein markers.

Chapter 8

Summary

In this study, morphological, seed storage proteins, and AFLP markers were used to identify and determine the genetic divergence among 20 Ethiopian wheat varieties. In the same way, an attempt was also made to characterize 10 Ethiopian tef cultivars using morphological and AFLP markers. Seed storage proteins were not used to characterize the tef cultivars, because it was already reported that the low number of protein markers detected could not distinguish individual tef cultivars.

The morphological traits have uniquely identified all the wheat and tef accessions, although it is found that morphological distances are not accurate estimate of genetic distances owing to their limited number and environmental interaction. Thus, results obtained from morphological data have to be confirmed by biochemical or DNA markers, depending on the need of identification.

From the protein markers, the gliadins uniquely distinguished all the individual wheat accessions. Thus, gliadins can be effectively used to discriminate wheat cultivars. However, some of the wheat cultivars had the same HMW-GS banding patterns, and therefore, HMW-GS should not be used for cultivar identification. The HMW-GS can rather be used to determine quality characteristics. In general, compared to morphological markers, protein markers are more reliable as the effect of environmental influence is absent or minimal. The limitation with the protein markers is that only part of the genome that codes for a specific protein can be detected.

With the AFLP markers, more variations were detected among the wheat entries, due to the potential of the AFLP markers to survey the entire genome and also due to the larger number of loci assayed. Thus, AFLP markers are more reliable and hence they can be used to supplement and refine morphological- and protein-based classifications.

Opsomming

In hierdie studie is morfologiese, saad storings proteïene en AFLP merkers gebruik om identifikasie en vasstelling van genetiese variasie tussen 20 Etiopiese koring variëteite te doen. Op dieselfde wyse is 'n poging aangewend om 10 tef cultivars te identifiseer met morfologiese en AFLP merkers. Saad storings proteïene is nie gebruik om die tef cultivars te identifiseer nie omdat daar alreeds gevind is dat die lae getal merkers nie geskik is om tef cultivars te onderskei nie.

Die morfologiese eienskappe het al die tef en koring cultivars effektief onderskei, alhoewel daar gevind is dat morfologiese afstande nie 'n akkurate bepaling van genetiese afstande gee nie a.g.v. 'n beperkte getal en omgewings interaksie. Daarom sal resultate van morfologiese data bevestig moet word met biochemiese of DNA merkers, afhangend van die identifikasie behoeftes.

In terme van die proteïen merkers kon die gliadiene al die koring cultivars effektief onderskei. Daarom kan gliadiene effektief gebruik word vir cultivar identifikasie. 'n Aantal van die koring cultivars het dieselfde HMW-GS bandpatrone gehad, en die HMW-GS kan daarom nie effektief vir cultivar identifikasie gebruik word nie. Die HMW-GS kan beter gebruik word vir kwaliteits bepaling. In die algemeen, in vergelyking met morfologiese merkers, is proteïen merkers meer betroubaar omdat die invloed van die omgewing minimaal of afwesig is. Die beperking van proteïen merkers is dat net 'n gedeelte van die genoom wat kodeer vir die spesifieke proteïen bestudeer kan word.

Met die AFLP merkers is meer variasie opgetel tussen die koring cultivars, a.g.v. die potensiaal van die AFLP tegniek om die hele genoom vir merkers te toets en a.g.v. die groot aantal loci wat ingesluit word. Daarom is AFLP merkers meer betroubaar en kan dit gebruik word om aanvullend te dien tot morfologiese en proteïen gebasseerde klassifikasie.

Chapter 9

List of references

Ainsworth, C.C. and P.J. Sharp, 1989. The potential role of DNA probes in plant variety identification. *Plant Varieties and Seeds* 2:27-34.

Arus, P., S.D. Tanksley, T.J. Orton, and R.A. Jones, 1982. Electrophoretic variation as a tool for determining seed purity and for breeding hybrid varieties of *Brassica oleracea*. *Euphytica* 31:417-428.

Assefa, K. S. Ketema, H. Tefera, H.T. Nguyen, A. Blum, M. Ayele, G. Bai, B. Simane and T. Kefyalew, 1999. Diversity among germplasm lines of the Ethiopian cereal tef [*Eragrostis tef* (Zucc.) Trotter]. *Euphytica* 106:87-97.

Ayele, M., J. Doležel, M. Van Duren, H. Brunner and F.J. Zapata-Arias, 1996. Flow cytometeric analysis of nuclear genome of the Ethiopian cereal tef [*Eragrostis tef* (Zucc.) Trotter]. *Genetica* 98:211-251.

Bai, G., M. Ayele, H. Tefera and H.T. Nguyen, 1999. Amplified fragment length polymorphism analysis of tef [*Eragrostis tef* (Zucc.) Trotter]. *Crop Science* 39:819-824.

Bailey, D.C., 1983. Isozymic variation and plant breeders' rights. In: Isozymes in plant Genetics and Breeding. Eds. S.D. Tanksley and T. J. Orton. Elsevir, Amsterdam. PP 425-440.

Ballester, J. and M. Carmen de Vicente, 1998. Dtermination of F1 hybrid seed purity in pepper using PCR-based markers. *Euphytica* 103:223-226.

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

Bechere, E., G. Belay, D. Mitiku and A. Merker, 1996. Phenotypic diversity of tetraploid wheat landraces from northern and north-central regions of Ethiopia. *Hereditas* 124:165-172.

Bechere, E., T. Tesemma and D. Mitiku, 1994. Improved varieties of durum wheat in Ethiopia. Release of 1966-1994. Research report series, No. 2. Debrezeit Agricultural Research Center, P.O. Box 32, Debrezeit, Ethiopia.

Becker, J., P. Vos, M. Kuiper, F. Salamini and M. Heun, 1995. Combined mapping of AFLP and RFLP markers in barley. *Molecular and General Genetics* 249:65-73.

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

Bekele, E., 1995. Essential and nonessential amino acids in a free state and in the major protein fractions of tef seeds. SINET. *Ethiopian Journal of Science* 18(1):79-99.

Bekele, E., R.J. Fido, A.S. Tatham and P.R. Shewry, 1995. Heterogenity and polymorphism of seed proteins in tef (*Eragrostis tef*). *Hereditas* 122:67-72.

Bellamy, A., F. Vedel and H. Bannerot, 1996. Varietal identification in *Cichorium intybus* L. and determination of genetic purity of F1 hybrid seed samples, based on RAPD markers. *Plant Breeding* 115:128-132.

Bernatzky, R. and S.D. Tanksley, 1989. Restriction fragments as molecular markers for germplasm evaluation and utilisation. In: The Use of Plant Genetic Resources. Eds. A.D. Brown, D.R. Marshall, O.H. Frankel and J.T. Williams. PP 353-362.

Bietz, J.A., 1985. High performance liquid chromatography: How proteins look in cereals. *Cereal Chemistry* 62:201-212.

Bietz, J.A., and D.G. Simpson. 1992. Electrophoresis and chromatography of wheat proteins: Available methods and procedures for statistical evaluation of the data. *Journal of Chromatography* 624:53-80.

Bietz, J.A., T. Burnouf, L.A. Cobb and J.S. Wall, 1984. Wheat varietal identification and genetic analysis by reversed-phase-high performance liquid chromatography. *Cereal Chemistry* 61(2):128-135.

Blears, M.J., S.A. De Grandis, H. Lee and J.T. Trevors, 1998. Amplified fragment length polymorphism (AFLP): a review of the procedure and its applications. *Journal of Industrial Microbiology and Biotechnology* 21:99-114.

Bohn, M., H.F. Utz and E. Meichinger, 1999. Genotypic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs, SSRs and their use for predicting progeny variance. *Crop Science* 39:228-237.

Botstein, D., R.L. White, M. Skolnick and R.W. Davis, 1980. Construction of genetic map in man using restriction length polymorphisms. *American Journal Human Genetics* 32:314-331.

Bretting, P.K. and M.P. Widrlechner, 1995. Genetic markers and horticultural germplasm management. *HortScience* 30(7):1349-1356.

Brown, A.H.D., 1978. Isozymes, plant population structure and genetic conservation. *Theoretical and Applied Genetics* 52:145-157.

Central Statistical Authority, 1996. Agricultural sample survey 1995/96 (1988 E.C.) on area and production of major crops, private peasant holdings, *Meher* season, Vol.I. statistical Bulletin 152, Addis Ababa, Ethiopia.

Chauhan, K.P.S., M.C. Gopinath and C.R. Babu, 1985. Electrophoretic variants of proteins and enzymes in relation to seed quality. *Seed Science and Technology* 13:620-641.

Clements, R.L., 1987. A study of gliadins of soft wheat from the Eastern United States using a modified polyacrylamide gel electrophoresis procedure. *Cereal Chemistry* 64:442-448.

Cooke, R.J., 1983. The characterization of *Pisum sativum* L. (field pea) cultivars by SDS-PAGE. *Journal of the National Institute of Agricultural Botany* 16:213-220.

Cooke, R.J., 1984. The characterization and identification of crop cultivars by electrophoresis. *Electrophoresis* 5:59-72.

Cooke, R.J., 1985. Characterization of cultivars of Allium cepa L. (onions) by ultrathin-layer isoelectric focusing of seed esterases. *Electrophoresis* 6:572-573.

Cooke, R.J., 1988. Electrophoresis in plant testing and breeding. *Advances in Electrophoresis* 2:171-261.

Cooke, R.J., 1989. The use of electrophoresis for the distinctness testing of varieties of autogamous species. *Plant Varieties and Seeds* 2:3-13.

Cooke, R.J., 1995a. Variety identification: Modern techniques and applications. In: Seed Quality: Basic Mechanisms and Agricultural Implications. Ed. A.S. Basra. Food Products Press, An Imprint of the Haworth Press, Inc. New York. PP 279-318.

Cooke, R.J., 1995b. Introduction: The reasons for variety identification. In: Identification of Food-Grain Varieties. Ed. C.W. Wrigley. American Association of Cereal Chemists, Inc. St. Paul, Minnesota. PP 1-17.

Costanza, S.H., J.M.J. Dewet and J.R. Harlan, 1980. Literature review and numerical taxonomy of *Eragrostis tef* (tef). Economic Botany 33(4):413-424.

Damania, A.B., E. Porceddu and M.T. Jackson, 1983. A rapid method for the evaluation of variation in germplasm collections of cereals using polyacrylamide gel electrophoresis. *Euphytica* 32:877-883.

Davis, M.G. and I. Simpkins, 1975. Electrophoretic techniques. In: Principles and Techniques of Practical Biochemistry. Eds. B.L. Williams and K. Wilson. Edward Arnold Press, London. PP 99-123.

De Villiers, O.T. and M. Bosman, 1993. Wheat cultivar identification by electrophoretic analysis of gliadin proteins. *South African Journal of Plant and Soil* 10(3):99-104.

Demissie, A. and BjØrnstad, 1996. Phenotypic diversity of Ethiopian barleys in relation to geographical regions, altitude range, and agro-ecological zones: As an aid to germplasm collection and conservation strategy. *Hereditas* 124:17-29.

Ebba, T., 1969. Tef (*Eragrostis tef*). The cultivation, usage and some of its known disease and insect pests. Part I. Experimental Station Bulletin No. Addis Ababa University, College of Agriculture, Dire Dawa, Ethiopia.

Ebba, T., 1975. Tef (*Eragrostis tef*) cultivars: Morphology and classification. Part II, Agricultural Experiment Station Bulletin, 66, Addis Ababa University, College of Agriculture, Dire Dawa, Ethiopia.

Edwards, K., C. Johnstone and C. Thompson, 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Research* 19:1349.

Ehdaie, B. and J.G. Waines, 1989. Genetic variation, heritability and path-analysis in landraces of bread wheat from southwestern Iran. *Euphytica* 44:183-190.

Eliasson, A.C. and K. Larsson, 1993. Cereals in bread making. A molecular colloidal approach. Marcel Dekker, Inc. New York.

Fullington, J.G., E.W. Cole and D.D. Kasarda, 1980. Quantitative sodium dodecylsulphate polyacrylamide gel electrophoresis of total protein from different wheat varieties. *Journal of the Science of Food and Agriculture* 31:43-53.

Gao, L. and W. Bushuk, 1993. Polymeric glutenin of wheat lines with varying number of high molecular weight glutenin subunits. *Cereal Chemistry* 70(4):475-480.

Gardiner, S.E. and M.B. Forde, 1988. Identification of cultivars and species of pasture legumes by sodium dodecylsulphate polyacrylamide gel electrophoresis of seed proteins. *Plant Varieties and Seeds* 1:13-26.

Gelfand, D.H., 1989. *Taq* DNA polymerase. In: *PCR Technology Principles and Applications for DNA Amplification*. Ed. H.A. Erlich. Stockton Press, New York. PP. 17-22.

Gore, P.J. and K.H. Sutton, 1995. Wheat identification in Australia and New Zealand. In: Identification of Food-Grain Varieties. Ed. C.W. Wrigley. American Association of Cereal Chemists, Inc. St. Paul, Minnesota. PP 209-219.

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

Gupta, R.B. and F. MacRitchie, 1991. A rapid one-step one-dimensional SDS-PAGE procedure for analysis of subunit composition of glutenin in wheat. *Journal of Cereal Science* 14:105-109.

Hamauz, Z.I., T. Arakawa and D. Yonezawa, 1972. Molecular weights of glutenin and gliadin polypeptides estimated by SDS polyacrylamide gel electrophoresis. *Agricultural and Biological Chemistry* 10:1829-1830.

Hartl, L., and S. Seefelder, 1998. Diversity of selected hop cultivars detected by fluorescent AFLPs. *Theoretical and Applied Genetics* 96:112-116.

Hashimuze, T., T. Sato and M. Hirai, 1993. Determination of genetic purity of hybrid seed in watermelon (*Citrullus lanatus*) and tomato (*Lycopersicum esculentum*) using random amplified polymorphic DNA (RAPD). *Japanese Journal of Breeding* 43:367-375.

Hayes, P.M., J. Cerono, H. Witsenhoer, M. Kuiper, M. Zabeau, K. Sato, A. Kleinhofs, D. Kudrna, A. Kilian, M. Saghai-Maroof, D. Hoffman and the North American Barley Genome Mapping Project, 1997. Characterizing and exploiting genetic diversity and quantitative traits in barley (*Hordeum vulgare*) using AFLP markers. *Journal of Quantitative Trait Loci*, Volume 3, Article 2. http://probe.nalusda.gov:8000/otherdocs/jqtl

He Zhong-hu, R.J. Peña and S. Rajaram, 1992. High molecular weight glutenin subunit composition of Chinese bread wheats. *Euphytica* 64:11-20.

Hill, M., H. Witsenboer, M. Zabeau, P. Vos, R. Kesseli and R. Michelmore, 1996. PCR-based fingerprinting using AFLP as a tool for studying genetic relationships in *Lactuca* spp. *Theoretical and Applied Genetics* 93:1202-1210.

Hill, P.J. and G.S.A.B. Stewart, 1992. The polymerase chain reaction in molecular and micro-biology. *Biotechnology and Genetic Engineering Reviews* 10:343-377.

Hintze, J.L., 1997. NCSS 2000 user's guide-III. Number Cruncher Statistical Systems. Kaysville, Utah.

Hongtrakul, V., G.M. Huestis, and S.J. Knapp, 1997. Amplified fragment length polimorphisms as a tool for DNA fingerprinting sunflower germplasm: genetic diversity among oilseed inbred lines. *Theoretical and Applied Genetics* 95:400-407.

Huaman, Z. and H. Stegemann. 1989. Use of electrophoretic analysis to verify morphologically identical clones in a potato collection. *Plant Varieties and Seeds* 2:155-161.

Huebner, F.R. and J.A. Bietz, 1988. Quantitative variation among gliadins of wheats grown in different environments. *Cereal Chemistry* 65:362-366.

Hussain, A., H. Ramirez, W. Bushuk and W. Roca, 1986. Field bean (*Phaseolus vulgaris* L.) cultivar identification by electrophoregrams of cotyledon storage proteins. *Euphytica* 35:729-732.

Jackson, E.A., L.M. Holt, and P.I. Payne, 1983. Characterization of high molecular weight gliadin and low-molecular-weight glutenin subunits of wheat endosperm by two-dimensional electrophoresis and the chromosomal localisation of their controlling genes. *Theoretical and Applied Genetics* 66:29-37.

Jaradat, A.A., 1991. Levels of phenotypic variation for developmental traits in landrace genotypes of durum wheat (*Triticum turgidum* ssp. *turgidum* L. conv. *durum* (Desf.) MK.) from Jordan. *Euphytica* 51:265-271.

Jarman, R.J., 1995. Visual identification by grain characteristics. In: *Identification of Food-Grain Varieties*. Ed. C.W. Wrigley. PP 35-54.

Jarvie, J.K., and M.E. Barkworth, 1992. Morphological variation and genome constitution in some perennial Triticeae. *Botanical Journal of the Linnean Society* 108:167-180.

Juliano, B.O., 1995. Identification of rice varieties.In: *Identification of Food-Grain Varieties*. Ed. C.W. Wrigley. American Association of Cereal Chemists, Inc. St.Paul, Minnesota. PP 265-274.

Ketema, S., 1997. Tef. *Eragrostis tef* (Zucc.) Trotter. Promoting the conservation and use of underutilised and neglected crops. 12. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resource Institute, Rome, Italy.

Kochert, G., 1994. RFLP technology. In: DNA-Based Markers in Plants. Eds. R.L. Phillips and I.K. Vasil. Advances in Cellular and Molecular Biology of Plants, Vol.1. Kluwer Academic Publishers, London. PP. 3-38.

Kumar, L.S., 1999. DNA markers in plant improvement: An overview. *Biotechnology Advances* 17:143-182.

Larkins, B.A., 1981. Seed storage proteins: Characterization and biosynthesis. In: The Biochemistry of Plants, (A comprehensive Treatise) Vol.6, Proteins and Nucleic Acids. Ed. A. Marcus. Academic Press. PP 449-489.

Law, R.J., P. Donini, R.M.D. Koebner, J.C. Reeves, and R.J. Cooke, 1998. DNA profiling and plant variety registration. III: The statistical assessment of distinctness in wheat using amplified fragment length polymorphisms. *Euphytica* 102:335-342.

Lawrence, G.J. and K.W. Shepherd, 1980. Variation in glutnin subunits of wheat. *Australian Journal of Biological Sciences* 33:221-223.

Lee, D., J.C. Reeves and R.J. Cooke, 1996. DNA profiling and plant variety registration: 1. The use of random amplified DNA polymorphisms to discriminate between varieties of oilseed rape. *Electrophoresis* 17:261-265.

Lew, E.J.L., D.D. Kuzmicky and D.D. Kasarda, 1992. Characterization of low molecular weight glutenin subunits by reversed phase high performance liquid chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophopresis, and N-terminal amino acid sequencing. *Cereal Chemistry* 69(5):508-515.

Lin, C.S. and Binns, 1984. The precision of cultivar trials within eastern cooperative tests. *Canadian Journal of Plant Science* 64:587-591.

Litt, M. and J.A. Luty, 1989. A hypervariable microsatelite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics* 44:397-401.

Livneh, O., Y. Nagler, Y. Tal, S.B. Harush, Y. Gafni, J.S. Beckmann and I. Sela, 1990. RFLP analysis of a hybrid cultivar of pepper (Capsicum annuum) and its use in distinguishing between parental lines and in hybrid identification. *Seed Science and Technology* 18:209-214.

Lookhart, G.L. and C.W. Wrigley, 1995. Variety identification by electrophoretic analysis. In: *Identification of Food-Grain Varieties*. Ed. C.W. Wrigley. American Association of Cereal Chemists, Inc. St. Paul, Minnesota. PP 55-71.

Lookhart, G.L. and J.A. Bietz, 1990. Practical wheat varietal identification in the United States. *Cereal Foods World* 35:404-407.

Lookhart, G.L. and K.F. Finney, 1984. Polyacrylamide gel electrophoresis of wheat gliadins: The effect of environment and germination. *Cereal Chemistry* 61:496-499.

Lookhart, G.L. and Y. Pomeranz, 1985. Gliadin high performance liquid chromatography and polyacrylamide gel electrophoresis patterns of wheats grown with fertilizer treatments in the United States and Australia on sulfur-deficient soils. *Cereal Chemistry* 62:227-229.

Lookhart, G.L., 1985. Identification of oat cultivars by combining polyacrylamide gel electrophoresis and reversed-phase high performance liquid chromatography. *Cereal Chemistry* 62(5):345-350.

Lookhart, G.L., 1991. Cereal proteins: Composition of their major fractions and methods for identification. In: Handbook of Cereal Science and Technology. Eds. K.J. Lorenz and K. Kulp. Marcel Dekker, New York. PP 441-468.

Lookhart, G.L., B.A. Marchylo, V.J. Mellish, K. Khan, D.B. Lowe and L. Seguin, 1995. Wheat identification in North America. In. Identification of Food-Grain Varieties. Ed. C.W. Wrigley. American Association of Cereal Chemists, Inc. St. Paul, Minnesota. PP 201-208.

Ma, Z-Q. and N.L.V. Lapitan, 1998. A comparison of amplified and restriction fragment length polymorphism in wheat. *Cereal Research Communications* 26(1):7-13.

Mackill, D.J., Z. Zhang, Redona and P.M. Colowit, 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. *Genome* 39:969-977.

Maheswaran, M., P.K. Subudhi, S. Nandi, J.C. Xu, A. Parco, D.C. Yang and N. Huang, 1997. Polimorphism, distribution, and segregation of AFLP markers in a doubled haploid rice populations. *Theoretical and Applied Genetics* 94:39-45.

Marchylo, B.A., K.A. Handel and V.J. Mellish, 1989. Fast horizontal sodium dodecyl sulfate gradient polyacrylamide gel electrophresis for rapid wheat cultivar identification and analysis of high molecular weight glutenin subunits. *Cereal chemistry* 66(3):186-192.

Marshall, D.R. and A.H.D. Brown, 1975. The charge state model of protein polymorphism in natural populations. *Journal of Molecular Evolution* 6:149-163.

Martiniello, P., 1992. Morphological aspects involved in varietal registration and the production of certified seed of typical Mediterranean leguminous forage crops. *Plant Varieties and Seeds* 5:71-81.

Maughan, P.J., M.A. Saghai-Maroof, G.R. Buss and G.M. Huestis, 1996. Amplified fragment length (AFLP) in soybean: species diversity, inheritance, and near-isogenic lines analysis. *Theoretical and Applied Genetics* 93:392-401.

McCouch, S.R., G. Kochert, Z.H. Yu, Z.Y. Wang, G.S. Khush, W.R. Coffman and S.D. Tanksley, 1988. Molecular mapping of rice chromosomes. *Theoretical and Applied Genetics* 76:815-829.

McDonald, M.B., Jr., 1991. Blotting of seed proteins from isoelectrically focused gels for cultivar identification. *Seed Science and Technology* 19:33-40.

McFerson, J.R., 1998. From *In Situ* to *Ex Situ* and back: The importance of characterizing germplasm collections. *HortScience* 33(7):1134-1135.

Miralles, D.J. and G.A. Slafer, 1991. A simple model for nondestructive estimates of leaf area in wheat. *Cereal Research Communications* 19(4):439-444.

Molina-Cano, J.L. and J.M.E. Elena Rossello, 1978. A further contribution to the classification of barley cultivars: Use of numerical taxonomy and biochemical methods. *Seed Science and Technology* 6:593-615.

Morell, M.K., R. Peakall, R. Apples, L.R. Preston and H.L. Lloyd, 1995. DNA profiling techniques for plant variety identification. *Australian Journal of Experimental Agriculture* 35:807-819.

Morgunov, A.I., W.J. Rogers, E.J. Sayers and E.V. Metakovsky, 1990. The high-molecular-weight glutenin subunit composition of Soviet wheat varieties. *Euphytica* 51:41-52.

Mosleth, E. and A.K. Uhlen, 1990a. Associations between the composition of gliadin and HMW glutenin subunits and the gluten quality in wheat (*T. aestivum* L.). In: *Gluten Proteins 1990*. Eds. W. Bushuk and R. Tkachuk. American Assciation of Cereal Chemits, Inc. St. Paul, Minnesota. PP 112-128.

Mosleth, E. and A.K. Uhlen, 1990b. Identification of quality-related gliadins and prediction of bread-making quality of wheat from the electrophoretic patterns of gliadins and high molecular weight subunits of glutenin. *Norwegian Journal of Agricultural Science* 4:27-45.

Nei, M. and W-H. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings. Natlional Academy of Sciences [United States of America]* 76:5269-5273.

Nielsen, G. and H.B. Johansen, 1986. Proposal for the identification of barley varieties based on the genotypes for 2 hordein and 39 isozyme loci of 47 reference varieties. *Euphytica* 35:717-728.

Pathak, N.N. and D.P. Nema, 1985. Genetic advance in landraces of wheat. *Indian Journal of Agricultural Science* 55:478-479.

Patterson, H.D. and S.T.C. Weatherup, 1984. Statistical criteria for distinctness between varieties of herbage crops. *Journal of Australian Science* 102:59-68.

Payne, P. I. and G.J. Lawrence, 1983. Catalogue of allels for the complex gene loci, Glu-A1, Glu-B1, and Glu-D1 which code for high-molecular weight subunits of glutenin in hexaploid wheat: *Cereal Research Communication* 11:29-35.

Payne, P.I., L.M. Holt and C.N. Law, 1981. Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. Part 1. Allelic variation in subunits amongst variety of wheat (*Triticum aestivum*). *Theoretical and Applied Genetics* 60:299-236.

Payne, P.I., L.M. Holt, A.J. Worland and C.N. Law, 1982. Structural and genetical studies on the high-molecular weight subunits of wheat glutenin. *Theoretical and Applied Genetics* 63:129-138.

Payne, P.I., M.A. Nightingale, A.F. Krattiger and L.M. Holt, 1987. The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *Journal of the Science of Food and Agriculture* 40:51-65.

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

Peterson, D.M., W.K. Heneen and B. Mattsson, 1995. Identification of oat varieties. In: *Identification of Food-Grain Varieties*. Ed. C.W. Wrigley. American Association of Cereal Chemists, Inc. St. Paul, Minnessota. PP 239-251.

Poiarkova, H. and A. Blum, 1983. Landraces of wheat from the northern region in Israel. *Euphytica* 32:257-271.

Powell, W., M. Morgante, C. Andre, M. Hanafey, J. Vogel, S. Tingey and A. Rafalsky, 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* 2:225-238.

Qi, X., P. Stan and P. Lindhout, 1998. Use of locus-specific AFLP markers to construct a high density molecular map in barley. *Theoretical and Applied Genetics* 96:376-384.

Rao, T.N., Y.S. Nerkar, and V.D. Patil, 1990. Identification of cultivars of cotton by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) of soluble seed proteins. *Plant Varieties and Seeds* 3:7-13.

Redaelli, R., M.H. Morel, J.C. Autran and N.E. Pogna, 1995. Genetic analysis of low *Mr* glutenin subunits fractionated by two-dimensional electrophoresis (A-PAGE X SDS-PAGE). *Journal of Cereal Science* 21:5-13.

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

Rolfs, A., I. Schuller, U. Finckh, and I. Weber-Rolfs, 1992. PCR: Clinical Diagnostics and Research. Springer-Verlag, New York.

Ruano, G., D.E. Brash and K.K. Kidd, 1991. The first few cycles. Amplifications: A forum for PCR users 4:5-7.

Russel, J.R., J.D. Fuller, M. Macaulay, B.G. Hatz, A. Jahoor, W. Powell, R. Waugh, 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs, and RAPDs. *Theoretical and Applied Genetics* 95:714-722.

Schondelmaier, J., G. Steinrucken and C. Jung, 1996. Integration of AFLP markers into linkage map of sugar beet (*Beta vulgaris* L.). *Plant Breeding* 115:231-237.

Schut, J.W., X. Qi and P. Stam, 1997. Association between relationship measures based on AFLP markers, pedigree data and morphological traits in barley. *Theoretical and Applied Genetics* 95:1161-1168.

Sharma, S.K., M.R. Knox and T.H.N. Ellis, 1996. AFLP analysis of the diversity and phylogeny of *Lens* and its comparison with RAPD analysis. *Theoretical and applied Genetics* 93:751-758.

Sharopova, N.R., V.A. Portyanko and A.A. Sozinov, 1997. Classification of European oat (*Arena sativa* L.) varieties using biochemical and morphological markers. *Plant Varieties and Seeds* 10:33-38.

Shewry, P.R., A.J. Faulks, H.M. Pratt and B.J. Miflin, 1978. The varietal identification of single seed of wheat by SDS-PAGE of gliadin. *Journal of the Science of Food and Agriculture* 29:847-849.

Shewry, P.R., A.S. Tatham, J. Frde, M. Kreis and B.J. Mifilin, 1986. The classification and nomenclature of wheat glutenin proteins: A reassessment. *Journal of Cereal Science* 4:97-106.

Singh, N.K. and K.W. Shepherd, 1988. Linkage mapping of genes controlling endosperm storage proteins in wheat. 1. Genes on the short arms of group 1 chromosomes. *Theoretical and Applied Genetics* 75:628-641.

Singh, N.K., K.W. Shepherd and G.B. Cornish, 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. *Journal of Cereal Science* 14:203-208.

Smith, J.S.C. and H.H. Weissinger, 1984. Rapid monitoring of purity in seed lots of hybrid maze: Modifications of current technologies. *Maize Genetics Cooperation. News Letter* 58:103-105.

Smith, J.S.C. and O.S. Smith, 1989. The description and assessment of distance between inbred lines of maize: The use of morphological traits as descriptors. *Maydica* 34:141-150.

Smith, J.S.C. and O.S. Smith, 1992. Fingerprinting crop varieties. *Advances in Agronomy* 47:85-140.

Smith, J.S.C. and R.D. Wych, 1986. The identification of female selfs in hybrid maize: A comparison using electrophoresis and morphology. *Seed Science and Technology* 14:1-8.

Smith, J.S.C., 1986. Biochemical fingerprints of cultivars using reversed-phase high performance liquid chromatography and isozyme electrophoresis: A review. *Seed Science and Technology* 14:753-768.

Sneath, P.H.A. and R.R. Sokal, 1973. Numerical Taxonomy. The principles and practice of numerical classification. W.H. Freeman and company, San Francisco.

Sontag-Strohm,. T., 1996. Electrophoretic fractionation of wheat glutenin subunits after removal of glisadins by reversed SDS-PAGE. *Journal of Cereal Science* 24:87-89.

Stegemann, H., A.A. Shah, E. Krögerrecklenfort and M.M. Hamza, 1992. Sweet potato (*Ipomoea batatas* L.): Genotype identification by electrophoretic methods and properties of their proteins. *Plant Varieties and Seeds* 5:83-91.

Tadesse, D., 1993. Study on genetic variation of landraces of tef [*Eragrostis tef* (Zucc.) Trotter] in Ethiopia. *Genetic Res. Crop Evol.* 40:101-104.

Tanksley, S.D. and R.A. Jones, 1981. Application of alcohol dehydrogenase allozymes in testing the genetic purity of F1 hybrids of tomato. *HortScience* 16(2):179-181.

Tatham, A.S., R.J. Fido, C.M. Moore, D.D. Kasarda, D.D. Kuzmicky, J.N. Keen and P.R. Shewry, 1996. Characterisation of the major prolamins of tef (*Eragrostis tef*) and finger millet (Eleusine coracana). *Journal of Cereal Science* 24:65-71.

Tefera, H., M. Ayele and K. Assefa, 1995. Improved varieties of tef (*Eragrostis tef*) in Ethiopia. Release of 1970-1995. Research Bulletin No. 1. Debre Zeit Agricultural Research Centre, Alemaya University of Agriculture, Debre Zeit, Ethiopia.

Tefera, H., S. Ketema and T. Tessema, 1990. Variability, heritability and genetic advance in tef [*Eragrostis tef* (Zucc.) Trotter] cultivars. *Tropical Agriculture* (Trinidad) 67(4):317-320.

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

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

Thomas, C.M., P. Vos, M. Zabeau, D.A. Jones, K.A. Norcott, B.P. Chadwick and J.D.G. Jones, 1995. Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato *Cf-9* gene for resistance to *Cladosporium fulvum*. *The Plant Journal* 8(5):785-794.

Tohme, J., D.O. Gonzalez, S. Beebe and M.C. Duque, 1996. AFLP analysis of gene pools of a wild bean core collection. *Crop Science* 36:1375-1384.

Van Eck, H.J., J.R. van der Voort, J. Draaistra, P. van Zandvoort, E. van Enckevort, B. Segers, J. Peleman, E. Jacobsen, J. Helder and J. Bakker, 1995. The inheritance and chromosomal localization of AFLP markers in a non-inbred potato offspring. *Molecular Breeding* 1:397-410.

Van Hintum, Th.J.L. and A. Elings, 1991. Assessment of glutenin and phenotypic diversity of Syrian durum wheat landraces in relation to their geographical origin. *Euphytica* 55:209-215.

VanToai, T.T., J. Peng and S.K.S. Martin, 1997. Using AFLP markers to determine the genomic contribution of parents to populations. *Crop Science* 37:1370-1373.

Vavilov, N.I., 1951. The Origin, Variation, Immunity and Breeding of cultivated plants. Translated from the Russian by K. Starrchester. Ronald Press, New York. PP 37-38.

Vos, P., R. Hogers, M. Bleeker, M. Reijan, T. van de Lee, M. Hornes, A. Frijters, J.Pot, J. Peleman, M. Kuiper and M. Zabeau, 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23:4407-4414.

Wang, Z.Y. and S.D. Tanksley, 1989. Restriction fragment length polymorphism in *Oryza sativa* L. *Genome* 32:1113-1118.

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

Wills, A.B., S.K. Fyfe and E.M. Wiseman, 1979. Testing F1 hybrids of *Brassica oleracea* for sibs by seed isozyme analysis. *Annals of Applied Biology* 91:263-270.

Wrigley, C.W. and I.L. Batey, 1995. Efficient strategies for variety identification. In: Identification of Food-Grain Varieties. Ed. C.W. Wrigley. PP 19-33.

Wrigley, C.W., 1992. Identification of cereal varieties by gel electrophoresis of the grain proteins. In: Modern Methods of Plant Analysis (New Series), Vol. 14, "Seed Analysis". Eds. H.F. Linskens and J.F. Jackson. Springer-Verglag, Berlin Heidelberg. PP 17-41.

Appendices

Data matrix of the gliadin, HMW-GS, and AFLP markers scored for cluster analysis of the wheat varieties.

Table 10.1 Input data matrix of the gliadin and HMW-GS data used for cluster analysis.

Chinese spring
0100100100111010001010001010001000100000101001100100000

HAR 1685
0101001100110110010101010101010000000100010101001000000

HAR 1709
1001001000101010001101010101000000000000100101001010100000

Pavon 76
0100110000111110000011001000000000000100001010000100011

Foka
0000100001000100000100101011010100001000000000000001100

Dashen
1010101000000011010001010010100100000000100010101100000

HAR 710
0100001000011110010001010101000000000110000101000010001 1

ET-13-A2
1000100000110111000001111010010010010000001010011001000 0

DZ-2023
0000101101100010010010100101000101000000000010100000000

DZ-393-4
0000010010110010000110000001000010000010000000000001100

DZ-1052
0000010000010000001010100101000000000000000000000001100

K-6290-bulk
0010100100010100010101000101000000000000010101101000000

DZ-04-118
0000011000001100001001100010100000000000000001100000000

HAR 1522
0010001100001010000110000011000000000000001010000100011
K-6295-4A
0100101100010010001010001010000000000000101001100100000

DZ-1050
0000010000010000001100000110001000000000000000000001100

Kilinto
0001001101010010010100101010001000000000000010100000000

Cocorit 71
0000101001010110000110001010101001000010000001100000000

HAR 1407
0100100000000010000100010010000000000000011101011000000

Boohai
0000001010101001000100001100100001000000000000000001100

HAR 604
0100100100000001000001000010100010000000010101011000000

Table 10.2 Input data matrix of the AFLP data used for cluster analysis.

HAR 1685
0000000000101001001001001000100000001000010101000000000000000001000
0011000000101010001001001100101000101110001010100010010100101000000
000000010000

HAR 1709
0000000010001001010001101000100000001011010011000000000000000001000
0010000000001000101001001100101100101110001010010010000100101000000
000000010000

Pavon 76
0001000001110101001010100100101101010001010101000100000000000001010
0000000000001000101001000100101100101110001000010010010000101000000
000001010000

Foka
0100101001101000001101100100000000000001100011000000000000000000000
0000100100010010000100000010101000011010100000001000000000000000000
000100010000

Dashen
1000001001101110100010000110100000001001100100000000010000000000001
0000010010000001001001000000101000000000000010000000000000000001000
000000000000

HAR 710
0100001001100110100110001001000100001001100101000000000000000010000
0001100101000010101000000000100000100010000000000100000000000000010
000000010001

ET-13-A2
1000001001101001001000100100100000010001100101000000000000000001010
0000000001000010001000000000001000100010000000000100000000000000010
000000010000

DZ-2023
0100000101101000001000000100100000010001001000011000000000000010000
1000000001110010010000001000101000100100101001001000110000000000000
000000010000

DZ-393-4
0101001001001001001000010100000000000001101000010000000001000001000
0001000101010010001001000000000000000010000000000000000000000000001
000001110000

DZ-1052
0010101001101001001001100110110110010001100011000001000000000010000
0001000100000000000100101000000010010000000000000000000000010001001
101000100010

K-6290-bulk
0100001001101101001001000100101001010001100101000000000000000000000
0000000000100110010001001000101010100010100100100110100000001000010
000000010000

DZ-04-118
0100000101101000001000000100100000010001100101000000101000000010000
0001000101000010011001001100101000010100100000011010100000000000000
010010010000

HAR 1522
0000000001100101000001100110100010001001100101000000000000010010000
0000100000100010011001000100001000001101000000010000000000000000000
000001010000

K-62954-A
0100001100101001010011000100100000001001100101101000000000000000000
0000100000111010101001000100001000110101000000001000000001000000000
000000010000

DZ-1050
0100010001100000000000000100000000000000000010000000000011001000000
0100110000000000011001000100001000001101001000000011000000000000000
000010010000

Kilinto
0001010001001000000000000100000000000000100101000000000000000000000
0000100100100010011001000100101000001101000000001011000100000000000
000100010000

Cocorit 71
0100010001101101000001110110100010000100100100000000000000000000000
0001010100100010001010000010000001000000000000000000000000000000000
001000001000

HAR 1407
0000010001100101000001000100101000010001100100000000000000000100000
0000100000110000101000000100001000001101000000010000000000000000000
000001010000

Boohai
0100010001100100000000000100000000100010001000000000000000000000000
0001000001000010101000000100001000100100000000010000000000000000000
000000000100

HAR 604
0000000001101101001001100100100011001000100101010010000000010000
0000100000110010101011000110101100101101000000010001000010010100000
000001010001