THE GLIADIN COMPOSITION OF SOUTH AFRICAN WHEAT CULTIVARS

by

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Chapter 1

Introduction

The need for varietal identification is probably far greater for wheat (*Triticum aestivum* L.em.Thell.) than for any other cereal grain because it is grown so widely and because of the differences among varieties in their quality and other agronomic properties. The need for identification or verification of varietal identity arises throughout the sequence of events from breeding, through variety release, pure-seed propagation and sowing, at harvest, and into marketing and processing of the harvested grain. With the introduction of Plant Breeders' Rights, even more exacting requirements for genotype identification and distinctness testing in seed certification are required.

The storage proteins in the wheat endosperm constitute over 80% of the total protein in the grain, which can be classified as either gliadins or glutenins according to their solubilities in different solvents (Payne, Holt, Lawrence & Law, 1981; Lásztity, 1986).

Three protein fractions of wheat: gliadins, glutenins and grain-albumin are suitable for varietal identification. Of these proteins, the gliadins are the best and most often used because they are readily extracted and fractionated and the genetic control of their synthesis is well understood (Wrigley & Shepherd, 1973). This is being done with the help of electrophoretic techniques that distinguish between the gliadin banding patterns of different cultivars.

An important aim of genotype identification is the elimination of irrelevant factors, such as the effects on phenotype of growing conditions. Factors such as these can complicate the task of visual identification by grain morphology, but are

generally not affecting the results of electrophoretic techniques (Lookhart & Finney, 1984).

The gliadin electropherogram is unaffected by the growth conditions of the grain, its protein content, sprouting or fumigation of the grain, or heat treatment up to and beyond that required to destroy baking quality, making it ideal for cultivar identification (Lookhart & Finney, 1984). Stability is therefore one of the main features of seed protein profiles (Ladizinsky & Hymowitz, 1979). Only in cases where severe sulphur deficiencies occur during growth, significant changes occur in the relative intensities of gliadin banding patterns (Wrigley, Du Cros, Archer, Downie & Roxbourgh, 1980).

The discrimination power of this approach is, however, remarkable and is certainly far greater than for any comparable morphological character or marker. Furthermore, the embryo at the end of the kernel can be excised and germinated to produce a new plant while the remaining endosperm can be used for analysis (Joppa, Khalil & Williams, 1983). Electrophoretic identification can provide assurance that seed is true to label and can also indicate the nature of off-type plants during propagation (Cooke & Draper, 1986).

A comparison between the electropherograms of wheat cultivars showed specific differences in the gliadin banding patterns, which provided the possibility of a clear classification system (Harsch, Günter, Kling, Rozynek & Hesemann, 1992). Because of this, a great deal of attention has been paid to the development of laboratory-based methods for cultivar characterisation, which have the following potential advantages: laboratory-based analysis are likely to be much quicker and require less personnel than traditional techniques used and an appropriate biochemical parameter is able to eliminate environmental effects completely in some cases. The most widely applied techniques used for laboratory cultivar characterisation have been seed protein analysis by various forms of electrophoresis.

The use of protein markers provides the basis for a rational, straightforward and practical system for the registration of genetic resources. Registration of cultivars and biotypes in the form of protein formulas lends itself to computer storage and retrieval of information. This can be important, especially in programs of germplasm conservation for wheat and other cereals where such information must be stored for use in the future (Konarev, Gavrilyuk, Gubareva & Peneva, 1979).

In South Africa high molecular weight glutenin subunits (HMW-GS) have to a large extent been used for cultivar identification in the past. This is an unreliable method, as many cultivars have identical banding patterns. Low molecular weight glutenin subunits (LMW-GS) have also been studied (Maartens, 1997), but this classification system is quite complicated and needs expert interpretation. Therefore the aim of this study was to determine the gliadin composition of South-African wheat cultivars by SDS-PAGE. The gliadin subunit composition has never previously been studied in South Africa. This study served to isolate and characterise these subunits for the possible use of cultivar identification and to use this information to determine genetic relationships between cultivars.

Chapter 2

Literature review

2.1 Protein structure

In order to understand wheat protein structure it is necessary to study the endosperm (morphology), polypeptide subunits, amino acids, as well as total nitrogen content. Criteria being met on these proteins include cellular function and location, processing value, chemical characteristics and amino acid content. Genetic aspects have been examined at most of these levels. It is important to build up an integrated picture of the genetics of protein composition at all of these levels, because of interactions occurring between these levels. The study of the genetics of these components complements the direct study of this character which itself is inherited in a complex manner. Genetic studies of all of these fractions as dissociated polypeptides must obviously be related to studies of the native proteins for the results to have proper significance for cultivar evaluation (Shepherd, 1988).

The application of genetics to the study of protein composition and wheat quality has concentrated on the storage proteins as they are probably equivalent to the proteins of gluten, the viscoelastic mass that makes wheat flour uniquely suitable for bread making. In spite of its complexity, this aspect of grain quality has been the most actively investigated (Wrigley, 1982).

Proteins are a complex group of natural polymers of which each protein is unique and performs a specific function in the plant from which it is derived. All proteins consist of more or less 20 different L-alpha amino acids that differ from one another in the side chain attached to the tetrahedral alpha-carbon, along with a

hydrogen atom and the amino and carboxyl groups. The numbers of each amino acid incorporated into the polymer chain give origin to the uniqueness of each protein (Poehlman, 1986).

The primary structure of the protein is the number of each species of amino acid and the sequence of its incorporation through formation of peptide bonds. This unique sequence of amino acid residues determines the three-dimensional structure of the protein through defining the possibilities for interaction of any residue with other residues in the chain. Much of the complexity of gluten proteins are due to the extensive duplication and diversification of structural genes needed for their synthesis. Gliadins of homologous amino acid sequence are coded for by genes on chromosomes of genomes A, B and D of hexaploid wheats. Together with the duplication of genomes that has led to polyploidy in wheat, duplication and mutation of ancestral genes on individual chromosomes has taken place, leading to groups ("blocks") of tightly linked genes (Wrigley & Bietz, 1988).

Interactions such as hydrogen, ionic and apolar bonds attribute to the secondary structure of a protein. Alpha-helix or beta-structures are formed when these interactions cause a part or all of a peptide chain to fold into highly ordered helical structures.

The tertiary structure of the protein occurs when portions of the polypeptide chain which are not involved in ordered, secondary arrangements, fold back on one another to interact with other amino acid residues in a nonregular manner to form random, but stable structures. The tertiary structure might be stabilised by intramolecular disulfide cross links formed between the side chains of cysteine residues in the polypeptide chain. These cross links sometimes join parts of the same polypeptide chain, or link two or several polypeptide chains intermolecularly to form a complex molecule.

Quaternary structures are ordered complexes of proteins which form through the association of protein molecules. The forming of protein structure at any level is dependent on interactions by means of secondary forces. These interactions can occur between the peptide backbone and the side chains, the side chains with one another, with water, ions and other molecules. This is possible because the side chains range from polar to nonpolar groups (Kasarda, Nimmo & Kohler, 1971).

2.2 Wheat protein composition

A protein is a primary product of a structural gene and therefore it serves as a marker for that particular gene. Genes are coupled into genetic systems and because of this, proteins may also serve as markers for such systems, including chromosomes and the genome as a whole. Because of this, the totality of protein markers gives considerable insight into genome or genotype structure and could be used to resolve genetic and breeding problems (Konarev, Gavrilyuk, Gubareva & Peneva, 1979). The protein band pattern of the electropherogram shows only genotypic variations, while environmental factors can be excluded to a large extent (Harsch *et al*, 1997).

The starchy endosperm of mature wheat grains contains several types of proteins apart from the storage proteins (gliadins and glutenins). It also contains proteins and enzymes that have survived from the metabolically active endosperm of the developing grain, and structural proteins, such as those found in the membranes (Payne, Holt, Jarvis & Jackson, 1985).

The storage proteins in the wheat endosperm constitute over 80% of the total protein in the grain. These proteins can be classified into different groups according to their solubility in different solvents (Payne, Holt, Lawrence & Law, 1981).

Osborne made the first systematic study of proteins and classified them into groups on the basis of their extraction and solubility (Osborne, 1907). These groups were called albumins (soluble in water), globulin (soluble in salt solutions), gliadins (soluble in aqueous ethanol), glutenins (soluble, or rather dispersable in dilute acid or alkali), as well as insoluble residues (Eliasson & Larsson, 1993).

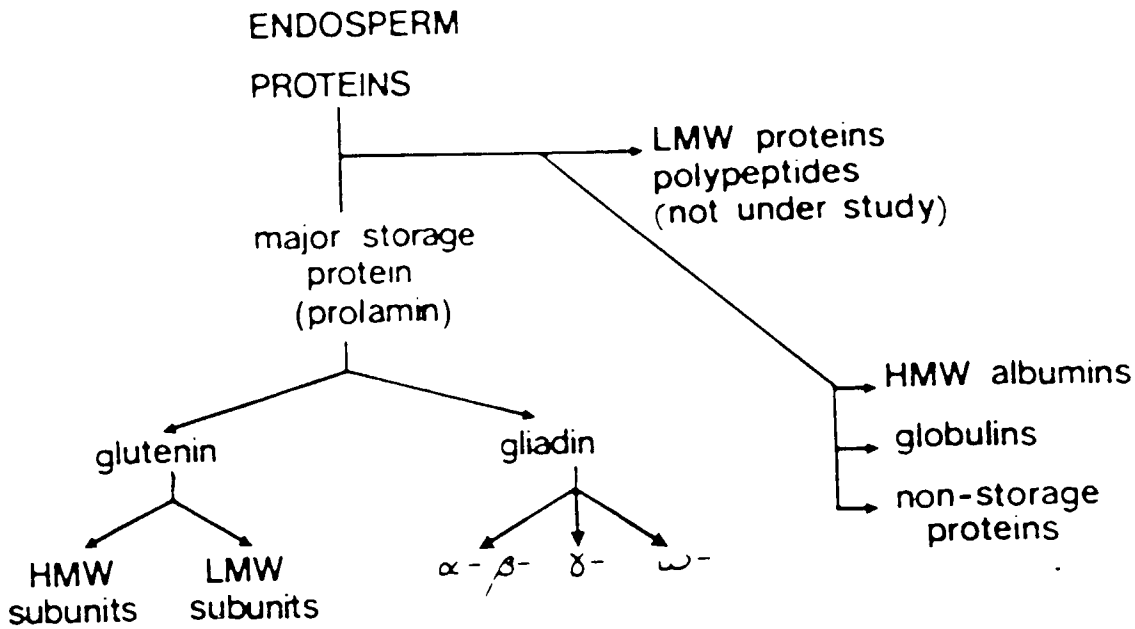


Figure 2.2.1 Classification of the major endosperm proteins of wheat (Payne *et al*, 1985).

The suitability of wheat for bread-making purposes is determined largely by the properties of the major storage proteins, which, when mixed with water form a cohesive mass, the gluten (Shewry, Tatham, Forde, Kreis & Miflin, 1986). This viscoelastic mass is composed mainly of storage proteins which can be classified into two groups depending on whether they are present as monomers (gliadins) or as disulphide-stabilised aggregates (glutenins).

Conceptually, gluten proteins are those proteins that impart the unique visco-elastic properties to dough made from wheat flour. In practice, gluten is the mass remaining when dough is thoroughly washed under running water. Gluten proteins are the proteins in this mass. The term gluten generally refers to the relevant proteins from wheat grain, and not to that of other crop species (Wrigley, Bushuk & Gupta, 1996).

In practice, the distinction between glutenins and gliadins means that gliadins have molecular sizes smaller than those of glutenin proteins. The dividing line between the two groups of proteins being an "apparent molecular weight" of more or less 100 kDa. This means that a practical separation of gliadins from glutenins can be achieved by any method that separates proteins according to size (Wrigley *et al*, 1996).

Gliadins and glutenins, the two traditional storage protein groups, belong to the prolamin family of storage proteins and are synthesised on the endoplasmatic reticulum in the developing endosperm, whereafter they are deposited in protein bodies (Shewry & Miflin, 1985). The name "prolamin" is due to the high content of pro*line* and gluta*mine* found in these proteins (Payne *et al*, 1985).

Prolamines, which comprise 80% of the total grain proteins are subdivided into glutenins and gliadins (Payne *et al*, 1981).

The difference in solubility between the gliadin and glutenin fractions of gluten is distinctive and these fractions have been found convenient for study, as well as serving as a base from which to fractionate the component proteins of gluten.

2.3 The gliadins

Traditionally gliadins were defined as the wheat proteins soluble in aqueous ethanol in the classic Osborne extraction procedure (Osborne, 1907). Gliadins constitute about 30-35% of the total protein content when they are extracted in

70% ethanol according to this procedure. In addition to this, Shewry *et al* (1986), proposed that gliadins can be defined as monomeric proteins. For this reason it is assumed that all the disulfide bonds present are intramolecular and that the gliadin conformations are stabilised by hydrogen bonding and hydrophobic interactions.

The gliadin group of proteins is very complex. Due to the complexity of the gliadins, the isolation of totally homogeneous components is very difficult. Usually preparations containing some components very similar in amino acid composition and structure are obtained showing only one major band with gel electrophoresis. To solve this problem, two dimensional gel electrophoresis is being used.

Gliadins can be divided into four groups, the α-, β-, γ- and ω-gliadins when fractionated by gel electrophoresis (Mosleth & Uhlen, 1990). In this group, the α-gliadins are the fastest moving, while the β-gliadins are the slowest moving gliadins (Eliasson & Larsson, 1993). The ω-gliadins are found in the lowest amount and constitute about 8-13% of the total protein, followed by the other gliadins which constitute about 34-38% of the total protein content (Eliasson & Larsson, 1993).

Gliadins are composed of proteins of relatively low molecular weight in comparison with the HMW proteins of the glutenin fraction (Hamauzu, Arakawa & Yonezawa, 1972). The molecular weight of most gliadins is in the range of 30-40 kDa, but the ω-gliadins have a molecular weight of about 60-70 kDa (Bietz, 1979). The fractions also differ slightly in amino acid composition. All gliadin components have extremely high proline, glutamic acid and glutamine contents. The high proline content affects the secondary structure of gliadin polypeptides because the formation of alpha helices is hindered by the presence of proline side chains. Almost all of the glutamic acid content of the gliadins is present as glutamine. The aspargine and aspartic acid content of all gliadins are relatively

low. Gliadins also tend to have larger amounts of cystine, isoleucine, phenylalanine and amide nitrogen than glutenin (Ewart, 1967).

Gliadins are poor in basic amino acids like lysine, glycine, tryptophan, arginine and histidine, but especially lysine. The low levels of these amino acids, along with the low levels of free carboxyl groups place the gliadins among some of the least charged proteins. Therefore the specific amino acid sequence of gliadins contain many neutral amino acids, especially glutamine and proline. As a result of this gliadins are less mobile than other grain proteins (Patey & Waldron, 1976).

The molecular structure of the gliadin components is characterised by a globular conformation. Because of the high level of proline in all gliadin components, the proportion of the alpha helical parts are relatively low. Intramolecular disulfide bonds occur in every case.

Gliadins belong to the less valuable protein fractions in wheat due to the low lysine content. Gliadin and glutenin differ in their physical properties, especially their viscoelasticity. Gliadin is cohesive, but with low elasticity, whereas glutenin is both cohesive and elastic. Furthermore, gliadins are composed of proteins of relatively low molecular weight (Crow & Rothfus, 1968).

The surface hydrophobicities of α-, β- and γ-gliadins depend on both aromatic and aliphatic amino acid side chains, whereas those of the ω-gliadins depend mainly on aromatic side chains (Popineau & Pineau, 1987).

The α-gliadins are the most abundant class of wheat endosperm-specific prolamines (Anderson, Green & Litts, 1990). The ω-gliadins constitute the S-poor prolamins of wheat, while the γ-gliadins are the S-rich prolamins (Tatham, Shewry & Field, 1990).

The chromosomes of homeologous group 1 control the synthesis of ω-gliadins and slow-moving γ-gliadin components, while chromosomes of group 6 mainly control α-, β-, and the fast-moving γ-gliadin components. As a consequence of the localisation of genes for gliadin proteins, six groups of gliadin protein components correspond to the 1A, 1B, 1D, 6A, 6B and 6D chromosomes (Nieto-Taladriz & Carrillo, 1996). Each locus on these chromosomes controls the synthesis of a group (block) of jointly inherited components in the spectrum. Multiple allelism might occur at each of these loci (Metakovsky, 1991).

The variation in gliadin patterns provides a means to identify biotypes and cultivars and to discover heterogeneity within cultivars. The gliadin spectrum is also useful for studying the intracultivar heterogeneity of cross-polinated crops, such as rye. Some gliadin components are, however, monomorphic in that they appear in all representatives of species, subspecies, genomes or cultivars. These monomorphic components then serve as markers for the corresponding chromosome of the species or genome (Konarev *et al*, 1979).

Gliadins are also of physiological interest, since it is toxic to individuals with celiac disease. α-Gliadin is particularly toxic, but β-gliadin and the other gluten fractions also elicit immunological responses associated with celiac disease (Bietz , Huebner, Sanderson & Wall, 1977).

2.4 High molecular weight glutenin subunits

Glutenins are the proteins that remain after the albumins, globulins and gliadins have been extracted according to the classification of Osborne (1907). Glutenins are dispersible in dilute acid or alkali, in denaturants such as urea and in surfactants (Bietz, 1985). The solubility of glutenins are very low due to their high molecular weight, but also due to the fact that some of the individual subunits are only sparingly soluble in aqueous alcohols (Shewry, Field & Tatham,

1987). Therefore glutenins belong to the polymeric prolamins (Eliasson & Larsson, 1993).

After reduction, glutenin subunits can be sub-divided by electrophoretic fractionation into two groups on the basis of their apparent molecular mass: The HMW glutenin subunits (80-120kDa) and the LMW glutenin subunits (30-50 kDa).

*Glu-A*1, *Glu-B*1 and *Glu-D*1 are the genes controlling the synthesis of high molecular weight glutenin subunits and can be found on the short arms of Group 1 chromosomes (McIntosh, Hart & Gale, 1994). These loci are located close to the centromeres (Shewry *et al*, 1986).

The HMW glutenins differ from the gliadins in their high content of glycine and low content of proline (Shewry *et al*, 1986). Glutenin consists of polymers with molecular weights extending into millions which is the product of polymerisation of polypeptides through intermolecular disulfide linkages (Hamauzu *et al*, 1972).

In addition to Osborne's nomenclature, Shewry *et al* (1986), suggested that the glutenins belong to the polymeric prolamines. Transmission electron microscopy has shown that the glutenins are aggregates built up of spherical particles (Graveland & Henderson, 1987). If these aggregates are reduced, the subunits can be studied. The HMW subunits have a molecular weight in the range of 90 000-150 000.

HMW glutenin conformation is similar to that of the ω-gliadins, which are characterised by a large proporion of β-turns in the central domain. The existance of these β-turns has been suggested as the reason for the elasticity of glutenins (Tatham, Miflin & Shewry, 1985).

The genes coding for HMW glutenins are located on the long arms of chromosomes 1A, 1B and 1D. Since the HMW glutenin subunits in any variety are controlled by genes on only these three chromosomes, the banding pattern

can be envisaged as being the sum of three subpatterns, where the bands in any subpattern are controlled by genes on the same chromosome. It has been possible to clearly identify the different forms of subpattern controlled by each chromosome because each variety posesses only three to five major HMW glutenin bands (Payne *et al*, 1981).

The HMW subunits constitute only about 1% of the dry matter content of the endosperm with a total number of different HMW subunits around 20. A single variety usually contains three to five different subunits that are linked together with disulfide bonds to the huge polymeric glutenin molecules (Eliasson & Larsson, 1993).

Glutenins influence the baking performance of a wheat cultivar in the following ways: through the gliadin:glutenin ratio, through the molecular weight distribution of glutenins and through the presence of certain HMW glutenin subunits (Schepers, Keizer & Kolster, 1993). It has long been recognised that the glutenins control the mixing requirements, but they may also influence loaf volume (Eliasson & Larsson, 1993). It is therefore easy to see that HMW glutenins play an important role in quality breeding.

2.5 Low molecular weight glutenin subunits

LMW glutenin subunits are controlled by genes found on the short arms of the chromosomes of homoeologous group 1 and are closely linked to genes controlling gliadins found on the same chromosomes (Rodriguez-Quijano & Carillo, 1996). The loci coding for LMW glutenins, *Glu-A*3, *Glu-B*3 and *Glu-D*3 are found on chromosomes 1A, 1B and 1D (McIntosh *et al,* 1994).

The LMW subunits are subdivided into B, C and D subunits when analised by two-dimensional electrophoresis. The B subunits are the major group and consist of basic proteins, whereas the D subunits are minor, have slightly larger molecular weights and are the most acidic protein group in the endosperm. The

minor C subunits are a diffuse group of widely different isoelectric points (Payne *et al*, 1985).

Two other groups of LMW glutenin subunits have been described recently: The minor D subunits which are acidic and controlled by *Gli-B3* and *Gli-D3*, as well as the minor C subunits which have lower apparent molecular weights and appear to be controlled by genes on either Group 1 or Group 6 chromosomes (Pogna, Redaelli, Vacano, Biancardi, Peruffo, Curioni, Metakovsky & Pagliaricci, 1995).

The basic LMW subunits of glutenin are encoded by genes of the *Gli*-1 loci, while the acidic subunits are encoded by loci *Glu-B2* and *Glu-D2*, which are located between the *Gli*-1 loci and the centromeres on chromosomes 1B and 1D (Shewry *et al*, 1986).

LMW glutenin subunits, as components of glutenin, play an important role in determining the technological quality of dough. The function of LMW glutenin subunits is related to their structure and probably also to their quantity. The amount and allelic type of LMW glutenin subunits present in durum wheats were found to be tightly linked to good pasta-making quality (Autran, Laignelet & Morel, 1987).

The LMW subunits have molecular weights in the range of 30 000-51 000 (Graveland & Henderson, 1987).

2.6 Gene localisation

Localisation of genes coding for gliadins has been made possible by the availability of many wheat aneuploid lines in which specific chromosomes or segments are known to be deleted, duplicated or substituted by corresponding ones from other genotypes. Studies of gene linkages have relied largely on segregation studies of progeny from specific crosses and indicated independent inheritance of proteins with genes located on different arms of chromosomes.

When gluten proteins, with genes occurring on the same chromosome arm, are inherited as a group, it indicates that these groups of genes are clustered together at the same locus within about 1cM of each other. These complex loci are found in hexaploid wheat on the long and short arms of group 1 chromosomes and on the short arm of the group 6 chromosomes (Wrigley & Bietz, 1988).

Further information about the genetic control of protein synthesis in the wheat grain was revealed by studying the segregation of storage proteins between F1 and F2 progeny. These studies revealed that the endosperm is triploid due to the combination of one nucleus from the pollen cell with two nuclei from the centre cell of the ovary. The segregation of gliadin bands in the total number of F2 grains showed a close approximation to the results expected for a one-locus situation for both ω- and α-regions. Examination of the joint segregation results showed that the α-gliadins were inherited independently of those in the omega region (Wrigley, 1982; McKinnon & Henry, 1995). Gliadins are inherited codominantly and protein synthesis proceeds in the heterozygous endosperm according to the maternal and paternal gene dosage ratio (Sozinov & Poperelya, 1980). What is significant, is that certain gliadins are inherited as a group or block, without any changes in composition. This may indicate that one gene product is transformed into a few distinguishable proteins by genetically determined post-translational processing or even by non-specific artefactual modification during extraction and fractionation. It seems more likely that the gliadins inherited as a block are products of a cluster of structural genes (isoloci) that have arisen from a single ancestral gene by mutation and duplication (Wrigley, 1982).

Wheat originated from three separate genomes, all of which had a common precursor. The diploid species *Triticum boeoticum* (AA) hybridised with a BB species (of which exact identity is not certain yet) to form a tetraploid (AABB) wheat, similar to durum. This later crossed with the DD diploid species *Aegilops*

squarrosa to produce *Triticum aestivum* (AABBDD), common hexaploid wheat (Bietz *et al*, 1977).

Therefore bread wheat is hexaploid with three genomes A, B and D, each containing seven pairs of chromosomes (Reddy & Appels, 1990). For an overall picture of the gliadin genes, two-dimensional analysis are needed (Wrigley, 1982). This has shown that six chromosomes (numbers 1 and 6 from each genome) of two homeologous groups of chromosomes are present in hexaploid wheat. Examination of ditelocentric lines showed that the appropriate genes were located on the short arms of the chromosomes (Brown & Flavell, 1981; Khelifi, Branlard & Bourgoin-Greneche, 1992). These genes are found at the *Gli-A*1, *Gli-B*1, *Gli-D*1 loci situated on the short arms of chromosomes 1A, 1B and 1D respectively, as well as on the short arms of chromosomes 6A, 6B and 6D at the *Gli-A*2, *Gli-B*2 and *Gli-D*2 loci (Rodriguez-Quijano & Carillo, 1996). Recently more genes have been described which account for some of the minor gliadins which segregate separately from the main group of genes at the *Gli*-1 loci, including *Gli-A*3, *Gli-B*3 and *Gli-B*5 (Jackson, Morel, Sontag-Strohm, Branlard, Metakovsky & Redaelli, 1996).

Due to duplication and mutation of ancestral genes on individual chromosomes, groups or blocks of tightly linked genes has formed. Genes for some ω-gliadins are found on chromosome 1B. The chromosomes of homeologous group 1 control ω-gliadins and slow-moving γ-gliadin components, whereas chromosomes of group 6 mainly control α-, β-, and fast-moving γ-gliadin components. As a consequence of this localisation of genes for gliadin proteins, six groups of gliadin protein components correspond to the 1A, 1B, 1D, 6A, 6B and 6D chromosomes. Each locus on these chromosomes controls the synthesis of a group (block) of jointly inherited components in the spectrum. Multiple allelism might occur at each of these loci (Metakovsky, 1991).

The synthesis of the γ- and ω-gliadin components are controlled by genes on the short arms of group 1 chromosomes, namely the *Gli*-1 genes, whereas the

synthesis of α- and β- gliadins are mostly controlled by genes on the short arms of homoeologous group 6 chromosomes, the *Gli*-2 genes (Jackson, Holt & Payne, 1983). There is now evidence that there is at least one other homoeolocus on chromosomes 1A and 1B, which controls ω gliadins. These loci are located approximately midway between the centromere and the *Gli*-1 locus (Metakovsky, Akhmedov & Sozinov, 1986). Recent work of Metakovsky *et al* (1986) indicates that there might be two other loci on chromosome 1A, controlling ω-gliadins which shows recombination with the *Gli-A*1 locus.

The *Gli*-1 loci contain a complex of genes coding for three protein groups, the ω- and γ-gliadins and LMW subunits of glutenin. The *Gli*-2 loci consists of a complex of genes coding for α- and β-gliadins. It is not yet clear how many genes are present in each locus and the degree to which recombination may occur between the genes within a locus (Payne, Jackson, Holt & Law, 1984a).

The most complex loci are those located on the long arm of chromosomes 1A, 1B and 1D, the *Gli*-1 loci, which encodes for γ-, as well as ω-gliadins, as well as LMW glutenin subunits. Recombination within a locus is rare, in particular between genes encoding the gliadins. Therefore the encoding gliadins are inherited as tightly linked groups or blocks (Doekes, 1973; Mosleth & Uhlen, 1990) in a Mendelian way (Metakovsky, Novoselskaya, Kopus, Sobko & Sozinov, 1984). Different approaches, like the use of substitution lines can be used to identify the subunits encoded by each allele. It is then possible to recognise the components of the alleles when the standard and substituted cultivars are compared (Jackson *et al*, 1996).

Because the components of many blocks of gliadin occur within rather narrow ranges of electrophoretic mobility, it seems likely that their structure is similar. If the components of a gliadin block are the products of a series of point mutations in a gene, they would be expected to differ principally in charge and isoelectric point and to be similar in size (Metakovsky, Akhmedov & Sozinov, 1986).

Table 2.6.1 Chromosomal control of gliadin components (cv. Chinese Spring)

TABLE III
Chromosomal Control of Gliadin Components (cv. Chinese Spring)

By Shepherd (1968)			By Mitrofanova (1976)	
Components		Chromosome and Its Arm*	Components	Chromosome and Its Arm*
I	2	1D(S)	ω 9	1D(S)
	3	1D(S)	8	1D(S)
	4		7	1D(S)+?
K	1	1A(S)	5	1B(S)
	2	0	4	1B(S)+?
	3	1B(S)		
	4	1B(S)	3	1B(S)
	6		γ 5	1A(S)
L	1	1A+1D	3	1A+1D
	2	1A+B	2	1B+1D+6B(S)
	3a	0	β 5	6B(S)+?
	3b	1B	4	6B(S)+?
	4	6B+?	3	6B(S)
	6	0	2	0
	7	1A+6A		
M	2	1B(S)	α 7	1B(S)
	3	0	6	6D(α)+?
	5	6D(α)		
	6	6A(α)	4	6A
	7	6A(α)	2	6A

*0 = Control by three pairs of homoeologous chromosomes is assumed. ? = Control by other chromosomes is assumed.

According to Konarev *et al* (1979), α-gliadins 2 and 4 are found on chromosome 6A α-gliadin 6 is probably found on chromosome 6D, α-gliadin 7, γ-gliadin 2, ω-gliadin bands 3, 4 and 5 are found on chromosome 1B(S). β-Gliadins 3, 4 and 5, as well as γ-gliadin 2 are found on chromosome 6B(S). γ-Gliadins 2 and 3 and ω-gliadins 7, 8 and 9 are found on chromosome 1D(S). γ-Gliadins 3 and 5 are found on chromosome 1A and 1A(S) respectively.

2.7 Cultivar identification with gliadins

Because wheat is the world's most important crop, the need for varietal identification is probably far greater than for any other cereal grain. The breeder therefore needs to recognise specific features of a variety that either make it attractive and desired by consumers or unsuited to their needs (Cooke, 1984).

Traditional breeding relied on the breeder's ability to observe differences that may have economic value in plants of the same species. This criteria is however unreliable and alternative selection methods and criteria are needed (Maunder, 1992).

Prolamine allelic blocks are phenotypical markers of polygenic loci at molecular level, so that this genetic classification enables us to obtain information on the characteristics of genotypes. Thus they serve as markers of gene clusters or non recombinant parts of chromosomes that play an important role in the forming of some characters like technological qualities of grain, low temperature tolerance, spike colour and disease resistance (Sozinov & Poperelya, 1980).

Gliadin components have been found to be inherited as linked groups (blocks), co-dominantly and in accordance with a gene dosage in triploid endosperm. Mutations in individual genes of gliadin-coding loci and processes changing the number of expressing genes and the sizes of their structural part have apparently occurred in the course of evolution, causing the development of blocks. In the case of gliadins the blocks are controlled by chromosomes 1A, 1B, 1D, 6A, 6B and 6D (Sozinov & Poperelya, 1980). Components differing in their electrophoretic mobility and molecular weight are included in blocks.

The actual segregation by a genotype in electrophoretic patterns corresponds to the theoretically expected segregation ratio by genotype of endosperm, in this case I:I:I:I. Due to this it could be assumed that the groups of components are controlled by allelic loci which are apparently polygenic. Practically no

recombination occurs between components of allelic variants of blocks. This suggests the existence of clusters of closely linked genes which have been mapped in the distal region of the short arms of corresponding chromosomes (Payne *et al*, 1984a). Therefore it can be assumed that the block is actually a group of components in the electrophoretic profile which are inherited in a linked, Mendelian and non-recombinant way in the process of crossing over. Since a block of components is taken as a classification unit, it is not so important whether or not each component of the electrophoretic pattern is represented by one polypeptide, or by several proteins similar in electric charge. The block is actually a phenotypical marker of a polygenic locus at the level of protein molecules and therefore enables the breeder to obtain the information on the characteristics of a genotype and can be used to solve many problems of plant breeding. Gliadin blocks serve as markers of gene clusters or non recombinant parts of chromosomes that play an important role in the formation of some characters like technological qualities of grain, low temperature tolerance, spike colour and disease resistance (Sozinov & Poperelya, 1980).

The allelic blocks may differ for the number of components present. It is therefore easy to explain the nature of varietal differences in gliadin electrophoretic patterns. Some allelic variants of blocks differ only in the presence of a few additional components or in the electrophoretic mobility of components with similar molecular weights, while other variants may contain no similar components. Several blocks might share some constituents and it could be assumed that they originate through intralocus recombination, while other blocks may originate from one another through single mutation events (Metakovsky, 1991).

Some members of the gliadin-coding genes are being located at a distance from the clusters and are able to recombine with the clusters. These genes are known as "selfish" genes and several of them may occur on one chromosome at once. The detection of such "selfish" genes, removed from the main gene cluster will

make it possible to use them as additional independent markers (Metakovsky *et al*, 1986).

Recently, specific gliadin proteins have also been reported to be associated with dough strength in bread wheats. The quality-protein associations may be due to close linkage between the genes for protein synthesis and quality-conferring genes, or to the direct contributions of these proteins to dough properties, or both. It is however, highly unlikely that a few grain proteins would confer quality attributes in a simple manner, since many non-protein factors also contribute to quality. The knowledge of such protein-quality associations is, however, likely to revolutionise quality-type segregation in breeding and at harvest, since they would indicate genotypes of high quality without the need to consider environmental effects (Wrigley, 1982; Pushman & Bingham, 1976).

In some cases, gliadin blocks can be used as reliable genetic markers of other economically important genes. It was found that block *Gld* 1A1 has a negative effect on flour quality, while block *Gld* 1B1 is of a better technological and baking quality. Block *Gld* 1B3 is a reliable marker of gene(s) responsible for stem rust resistance in wheat. All of the lines studied which contained the block *Gld* 1B3 turned out to be resistant to stem rust.

Allelic gliadins apparently influence certain aspects of bread-making quality such as dough strength and sedimentation volume. It is generally agreed that glutenin, rather than gliadin is associated with dough strength and large sedimentation volumes (reflecting good quality). The gliadins coded by genes on the group 1 chromosomes are tightly linked to LMW glutenin subunit genes and therefore there is a distinct possibility that the quality associations with gliadins might be due to linked genes and that the causal proteins are actually LMW glutenin subunits (Payne *et al*, 1984b).

The ω-gliadins 11, 13.5 and 16 influence baking quality favourably and correspond to the first three bands of group *Gld* 1D4, which is known to have a

positive effect on quality. Opposite to this, groups *Gld* 1D2 and *Gld* 1D3 have been described as having an unfavourable effect on baking quality and includes the 14.5 and 17.5 ω-gliadins. Group *Gld* 1B1 and *Gld* 1B2, which are thought to have a favourable influence on quality contain the low mobility bands ω30 and ω32, which are positively correlated with quality. Band ω35.5, which is negatively correlated with quality seems to correspond with the more concentrated band of *Gld* 1B6, which has an unfavourable effect on quality. It is important to keep in mind that results of different authors might differ due to differences in mobility resulting from the use of different electrophoretic methods or different nomenclature systems (Branlard & Dardevet, 1985).

Variation at the *Glu*-1 loci (coding for LMW-GS, ω- and γ-gliadins) can have large effects on the balance of elasticity and extensibility in doughs, causing major differences in bread-making quality. In contrast to this, allelic variation at *Gli-A2* (genes coding for α- and β-gliadins on chromosome 6A) has a negligible effect on dough rheology (Payne, Seekings, Kaur, Krattiger & Rogers, 1990).

Gliadins have a small effect on bread-making quality and mainly confer viscosity to the dough (Reddy & Appels, 1990). γ- And ω-gliadins are encoded at the *Gli*-1 loci on the short arms of the chromosomes of group 1. LMW glutenins are encoded at the *Glu*-3 loci, which are very closely linked to the *Gli*-1 loci. It has been suggested that the LMW-GS, associated with the γ- and ω-gliadins, were primarily responsible for the effects of gliadins on baking quality (Johansson, 1996).

Gliadins can be used as markers for LMW-GS because of the linkage existing between *Gli*-1 and *Glu*-3. This is because cultivars carrying the same allele at one *Gli*-1 locus are expected to show identical LMW-GS patterns at the corresponding *Glu*-3 locus (Singh & Shepherd, 1988).

The attribution of a series of allelic variants of blocks to a particular gliadin-encoding locus is determined by comparing the spectra of a cultivar and its nullitetrasomic lines. The attribution of an allele to an allelic series is determined through analysis of the segregation of gliadin components in hybrid crosses and F2-seeds of crosses (Metakovsky, 1991).

Gene blocks are groups of coupled genes that are transferred from the parents to the offspring as a unit. Components of each allelic series (block) occupy a definite position in the gliadin spectrum. This facilitates the identification of alleles in the spectrum of a cultivar. However, the use of catalogues of gliadin alleles represented only as schemes of blocks may in practice cause many problems due to genetic variation in the gliadin spectrum of the same genotype. The use of gliadin block schemes are therefore not recommended for practical day-to-day use (Metakovsky, 1991).

The major aim in durum breeding is the production of cultivars with superior gluten properties. The cooking quality of durum is based largely on gluten quality, which is associated with the quality and quantity of gliadins and glutenins (Kosmolak, Dexter, Matsuo, Leisle & Marchylo, 1980).

Electrophoresis of gliadins have been recommended for quality assessment of durum wheats at breeding stage (Autran & Feillet, 1987). These studies have revealed a close relationship between gluten strength and gliadin protein band 45 and also between gluten weakness and gliadin band 42. Cultivars containing band 45 have better viscoelastic properties than those containing band 42, while cultivars which contained both bands had superior viscoelastic properties. Cultivars lacking both bands have less than optimum viscoelastic properties (Kosmolak *et al*, 1980; Yupsanis & Moustakas, 1988; Du Cros, Joppa & Wrigley, 1983).

Wild relatives of common wheat represent an accesible source of many valuable genes for the extending of genetic variability of cultivars. The brown spike gene

*Rg*2, is claimed to have tight linkage with the *Gli-D*1 locus (Payne *et al*, 1981). It is further well-known that the *Rg*2 gene is tightly linked with two genes for rust resistance, *Lr*21 and *Sr*33 (Jones, Dvorak & Qualset, 1990; Czarnecki & Lukow, 1992). The *Gli-D*1 allele was found to be tightly linked tc a gene for glume colour in durum wheat and can therefore be used as a genetic marker during selection (Pshenichnikova & Maystrenko, 1995).

Freezing tolerance in wheat is controlled by an additive-dominance genetic system and is a complex trait which is influenced by at least 10 of the 21 pairs of wheat chromosomes (Sutka, 1994). Wheat lines containing chromosomes 6A amongst other chromosomes accumulated more apoplastic protein than other lines, while plants with chromosome substitutions 1A, 1D and 6D amongst other chromosomes exhibit much higher antifreeze activity (Chun, Yu & Griffith, 1998).

The identification of quality-promoting factors would enable the cereal chemist, plant breeder, as well as the end user to plan more effectively and reduce costs in selecting the most desirable wheat cultivars in a variety development program (Khan, Figueroa & Chakraborty, 1990). A comparison between the electrophoregrams of wheat cultivars showed specific differences in the gliadin band patterns which provided the possibility of a clear classification system (Harsch *et al*, 1997).

Many practical applications have stemmed from the use of electrophoresis to characterise and compare genotypes. The fact that these are based on some understanding of the genetic control and heritability of the proteins enhances their reliability.

Gliadin banding pattern identification with the help of SDS-PAGE thus serves as the basis for the improvement of a genome composition of cultivated as well as wild cereal species and could therefore be of great help in selecting and breeding new cultivars and overcoming the shortcomings of traditional visual selection.

2.8 Extraction and separation of gliadins

The application of zone electrophoresis to separate the monomeric proteins of wheat seeds in starch gels at acid pH started the modern era of wheat protein genetics (Elton & Ewart, 1960). For the fist time α-, β-, γ- and ω-peaks in moving boundary electrophoresis could be visualised as a series of discrete bands in a gel and through this opened the way for qualitative genetic studies on these proteins.

Long after its first use, polyacrylamide gel electrophoresis continues to play a major role in the experimental analysis of proteins. Although two-dimensional gel separations of proteins have the highest resolving power, one-dimensional gel protein separations is still the most widespread form of the technique used. This is because of its ability to offer sufficient resolution for most situations and combine the ease of use and the ability to process many samples for comparative purposes (Hames, 1990). Furthermore the basic components for the polymerisation reaction are commercially available at reasonable cost and in addition, polyacrylamide gels have the advantage of being chemically inert, stable over a wide range of pH, temperature, and ionic strength and is transparent. For these, as well as other reasons, polyacrylamide gels have become the medium of choice for electrophoresis of most proteins.

When denatured in the presence of sodium dodecyl sulphate (SDS), the most polypeptides binds SDS in a constant weight ratio, such that they have essentially identical charge densities and migrate in polyacrylamide gels according to polypeptide size. Doubt of whether the polypeptides move according to size or charge can therefore be eliminated when SDS-PAGE is used as a tool in protein, like gliadin separation (Hames, 1990).

Any charged ion or group will migrate when placed in an electric field. Since proteins carry a net charge at any pH other than their isoelectric point, they too will migrate and the rate of their migration will depend upon the charge density of

the proteins concerned. The higher the ratio of charge to mass, the faster the molecule will migrate. Therefore the application of an electric field to a protein mixture in solution will result in different proteins migrating at different rates towards one of the electrodes (Wrigley, 1992).

Gliadins can be classified into α-, β-, γ- and ω-gliadins according to the order of their decreasing electrophoretic mobility in acidic buffers (Bietz *et al*, 1977).

The specificity of electrophoretic patterns for species may be due to differences in electrophoretic mobilities resulting from differences in structural state (oligomers) or other polypeptide modifications that do not necessarily change the antigenic character of the proteins (Konarev *et al*, 1979).

In order to understand intraspecific differences in terms of proteins, it is necessary to understand the genetic variability of these proteins as manifested in a variety of molecular forms. Molecular forms of proteins resulting from different loci usually are representative of species and these multiple forms may be used as markers.

Woychik, Boundy & Dimler (1961) were some of the first to adapt gel electrophoresis in aluminium lactate buffer (pH 3.1) to native gliadin proteins. They grouped the gliadin patterns from Ponca wheat variety into four major fractions with subfractions designated as: α_1, α_2, β_1, β_2, β_3, β_4, γ and ω. During the same time, the cultivar specificity of gliadins was demonstrated by Cluskey, Taylor, Charley & Senti (1961).

Eventually a number of laboratories proposed schemes for nomenclature that could be used with gel electrophoresis to designate a gliadin "formula" for any cultivar, better known as the "nomenclature" of a cultivar, which made the comparison of different cultivars with one another possible (Konarev, 1973).

2.9 Gliadin nomenclature

Nomenclature is the language we use to communicate in describing research, in this case referring to individual gliadin proteins. It combines concepts (what is the protein we refer to and how may it differ in function from others?) and methods (how do we single it out in practice?). The system of naming must be uniform and agreed to prevent confusion in the literature, as well as poor interaction between research groups (Wrigley *et al*, 1996).

Furthermore, it is very important to decide on a common classification system in order for different research groups to be able to understand the results of other research groups (Jackson *et al*, 1996).

In order to understand intraspecies differences in terms of the proteins it is necessary to understand the genetic variability of these proteins as manifested in a variety of molecular forms. Differences in protein components that have the same function may result from gene variability associated with multiple loci. Proteins coded for by genes at the same locus may therefore have different forms (multiple alleles). Molecular forms of proteins resulting from different loci are usually representative of species and these multiple forms may serve as markers of species, genomes, genetic groups of organisms, and separate chromosomes. Variability that results from multiplicity of allelic components tends to be more specific for cultivars, biotypes and lines and this molecular forms may therefore be used for cultivar identification. Polymorphism of protein components may result from gene mutations or from quaternary structures composed of associated subunits, or secondary modification of proteins by amidation, deamidation, acetylation, phosphorolation, etc., of amino acid side chains.

The results of identification of blocks of components in wheat cultivars and data from the monosomic analysis enabled breeders to use a genetic principle for gliadin classification, the basis of which is a block of components. Sozinov and

Poperelya (1980), used the symbol Gld (an abbreviation of gliadin), followed by a figure and a letter indicating chromosome number and the genome responsible for the synthesis of the block of components, and the figure following the letter, a number of the block of components controlled by this chromosome and registered in the catalogue of blocks.

It is quite possible that groups of proteins which are represented by multiple alleles can also be found in other plant species. These groups of proteins controlling variability in the manifestation of quantitative characters are called "hot spots" and are of great interest for plant breeding. They could be used in the search for necessary polymorphic proteins, developments of their genetic classification, studying of the relationship between their polymorphism and variation in characters.

Three basically different systems of nomenclature for gliadins have already been described:

- The designation of gliadin zones by a Greek letter as α, β, γ and ω. This nomenclature was used by Woychik *et al* (1961). To make this system more precise, bands within zones were subsequently identified by numbers, which identify bands within the zones, originally identified by Greek letters (Konarev *et al*, 1979). To provide additional flexibility to the standard formula, additional conventions are used to indicate deviations from the standard such as higher intensity (band number underlined), lower intensity (band number overlined), slightly greater mobility (subscript 1 to band number), slightly lesser mobility (subscript 2 to band number). The standard spectrum can also be used to write formulas for cultivars and biotypes of *Hordeum, Secale, Avena, Agropyron, Aegilops* and *Triticale.*

- The allelic block system was introcduced by Sozinov and Poperelya (1971). According to this system, each allelic block that may contain several bands is identified by the chromosome and the block number. This system is however

very complex and requires the use of aneuploid lines to develop the allelic block formula for each variety.

- The third system is based on the relative mobility of each band. In the original system of Autran and Bourdet (1973), a prominent reference band present in most wheat varieties was used. In attempting to apply this system in other countries, it was found that the reference band was not found in all cultivars and furthermore that it was not clearly visible and distinguishable with some of the methods being used. The nomenclature then proposed is similar to that of Autran and Bourdet (1973), but instead of using a γ-gliadin band as reference, it was proposed to use one of the major bands, which is readily identifiable in different systems of electrophoresis as a reference. The new band used is readily identifiable in electropherograms, and assigned a mobility of 0.5. Other gliadin bands are then identified by a mobility relative to this reference (Bushuk & Zillman, 1978).

The proposed nomenclature for the gliadin bands in the electropherogram have the advantage of the use of standard reference which would serve to improve inter-laboratory agreement of results, because adjustments to electrophoretic procedures could be made until identical electropherograms are obtained.

The high degree of polymorphism and specificity of gliadin proteins suggests that they must contain a rich supply of genetic and phylogenetic information that has yet to be discovered.

The essence of genetic nomenclature is that the electrophoretic gliadin spectrum of a given variety of specimen may be presented as a set of blocks of components determined by gliadin-coding loci.

Woychik *et al* (1961), distinguished between α-, β-, ω- and ω-gliadins, with α-gliadins closest to the cathode. These designations have been expanded by Konarev *et al* (1979), by adding sequential digits.

Another approach is that of Harsch *et al* (1992), which suggests the following: The demarcations between the groups are formed by marked bands, which, due to their position within the gel make it possible to compare their own classification with those of others. The bands within one group are numbered according to their migration speed within the gel. The band with the highest mobility therefore is always given the number "1". When a change in the mobility of single proteins occur, an increased migration speed is marked with a plus(+) and a decreased migration speed with a minus(-) after the band number. Sometimes one component splits and two or three bands instead of one are revealed. These subdivisions are then given Roman numerals, e.g. I, II, III (Harsch *et al*, 1997).

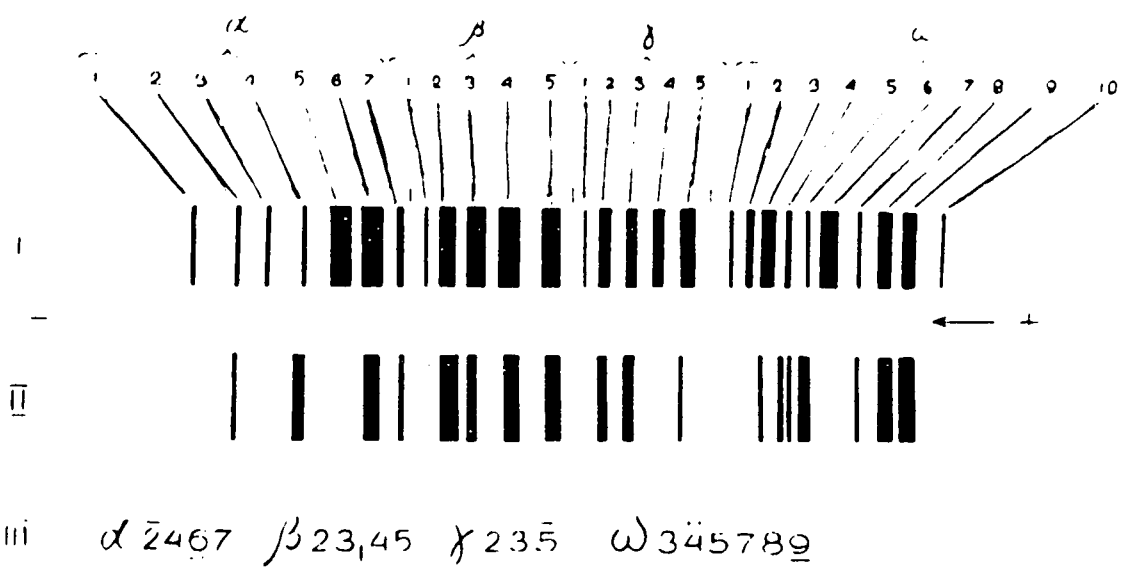


Figure 2.9.1 Standard spectrum of (I) gliadin: (II) gliadin spectrum of Chinese Spring; and (III) gliadin formula of this cultivar (Konarev *et al*, 1979).

Differences between accessions of the same taxon in intensity and thickness of various bands occur mainly because the formation of many of the bands in the

seed protein profile are under control of quantitative gene systems. Caution should be taken in interpreting intensity and thickness of bands as quantitative gene systems, because this kind of variation may be due to differential extraction or solubility of seed protein from different accessions. Differences in the thickness and darkness of bands might also be due to the lack of separation on the gels of several proteins having similar migration rates (Ladizinsky & Hymowitz, 1979).

Some authors (Metakovsky, 1991), prefer the use of catalogues represented as schemes of blocks. This system, however, is very difficult to use in practice and involves the making of crosses and analysis of hybrid seeds; the analysis of gliadin biotypes; the analysis of related cultivars or/and the identification of new blocks. These practices are however labour and time consuming and therefore unsuitable for routine experiments (Wrigley – personal communication).

Chapter 3

Materials and methods

3.1 Materials

Thirty-four commercial South African wheat cultivars were screened for gliadin subunit composition. A summary of the cultivars used, their classification and origin are given in Table 3.1.

Table 3.1 Summary of the cultivars screened for gliadin subunit composition.

Cultivar	Classification	Origin
Adam Tas	HRS	RSA
Belinda	HRW	USA
Betta	HRI	Argentinia
Betta DN	HRW	RSA
Chinese Spring	HRW	China
Flamink	HRI	RSA
Gamka	HRS	RSA
Gamtoos	HRS	RSA
Gamtoos DN	HRS	RSA
Gariep	HRS	RSA
Hartz	HRS	RSA
Hugenoot	HRW	RSA
Inia	HRS	RSA
Karee	HRI	RSA
Kariega	HRS	RSA
Letaba	HRW	RSA
Limpopo	HRS	RSA
Marico	HRS	RSA
Molen	HRW	RSA
Molopo	HRW	RSA
Nantes	HRS	RSA
Oom Chart	HRW	RSA
Palmiet	HRS	RSA
PAN 3211	HRI	RSA
PAN 3232	HRW	RSA
PAN 3235	HRW	RSA
PAN 3342	HRW	RSA
PAN 3349	HRW	RSA
Schoepers	HRW	RSA
SST 66	SRS	RSA
SST 86	HWS	RSA
Tugela	HRW	RSA
Tugela DN	HRW	RSA
T4	HRS	RSA
Wilge	HRI	RSA

HRW = Hard, red winter wheat.
HRI = Hard, red intermediary wheat.
HRS = Hard, red spring wheat.
HWS = Hard white spring wheat.
SWS = Soft, white spring wheat.

Chinese Spring, originating from China was used as control cultivar. The gliadin composition of Chinese Spring is well known and studied. The gliadin bands of Chinese Spring was used to compare the gliadin banding patterns of the South African wheat cultivars tested.

3.2 Methods

Electrophoresis involves four basic steps: 1) gel preparation (choosing the type and concentration of polymeric network to separate the proteins is important); 2) sample preparation (extraction of proteins to obtain a solution containing either all of the proteins or only a particular class of proteins); 3) sample separation (fractionation is provided by different rates of movement of the proteins in a gel matrix caused by differences in charge and size) and 4) gel staining and interpretation (protein stains are used to colour the protein bands, yielding a banding pattern or "fingerprint" for comparison with those of authentic samples separated under the same conditions) (Lookhart & Wrigley, 1995).

The following procedure is based on a combination that is being considered for adoption into the Rules of the International Seed Testing Association (Wrigley, 1992) and that of Singh, Shepherd & Cornish (1991).

3.2.1 Protein extraction

Stock solution for extraction:

70% Ethanol:

70 ml ethanol

30 ml water

Extraction procedure:

- Crush wheat kernel into a fine powder and add to tube.
- Add 120µl 70% ethanol to each tube and place in 60°C waterbath for 1 hour. Vortex at 20 and 40 minutes.
- Centrifuge tubes 2 min at 10 000 r.p.m. Transfer 75µl supernatant to tube containing 80µl sample buffer. Mix well and centrifuge as before. Samples are now ready for loading.

3.2.2 Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE)

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

Gel preparation

A discontinuous gel system is used, which requires the formation of two gel layers: the main (resolving) gel, in which band separation takes place, and the short upper (stacking) gel, on which samples are applied and in which the protein zones are concentrated to give very thin starting zones.

Separating gel:

Stock solutions for separating gel:

Separating buffer pH 8.88:

- Dissolve 45.412 g tris in 460 ml water.
- Titrate to pH 8.88 then add 1.0 g SDS.
- Makes total 500 ml.
- Store at 4°C.

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

- Dissolve 75g acrylamide and 0.75 g bisacrylamide in 181 ml water.
- Makes total 250 ml.
- Store in dark at 4°C.

Separating gel (For 2 gels)

Separating buffer	38 ml
Separating acrylamide	28.1 ml
Water	14 ml

TEMED	165 µl
Ammonium persulphate (APS)(10%)	190 µl

- Mix the above mentioned amounts of chemicals and add APS just before casting.
- The gel is then casted and left to set.

Stacking gel:

Stock solutions for stacking gel:

Stacking buffer (2x) pH 6.8:

- Dissolve 6.06 g tris in 190 ml water.
- Titrate to pH 6.8 then add 0.4 g SDS.
- Makes total 200 ml.
- Store at 4°C.

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

- Dissolve 87.5 g acrylamide and 1.32 g bisacrylamide in 181 ml water.
- Makes total 250 ml.
- Store in dark at 4°C.

Stacking buffer	10 ml
Stacking acrylamide	2.6 ml
Water	7.4 ml
TEMED	40 µl
APS (10%)	100 µl

- Mix the above mentioned amounts of chemicals and add APS just before the casting of the gel.
- Cast the stacking gel on top of the separating gel and insert sample-loading positions.

Electrode buffers:

Anode buffer (pH8.3):

30.3 g tris

140 g glycine

10 g SDS

Dissolve in 883 ml water. Makes 1 l.

Cathode buffer:

30.28 g tris

144 g glycine

10 g SDS

Fill up with water till 1 l.

It is used diluted with water: 1 part of buffer: 10 parts of water.

Running of the gel

Sample buffer:

80 mM tris-HCl (pH8.0)

40 g glycerol

2 g SDS

0.02 g bromophenol blue

Protein samples of 20 µl, as well as 20 µl of sample buffer are then loaded in the sample positions. The gel is run at 80 mA. Voltage turned to maximum should read between 120-140 Volts. The running time is approximately three to four hours. The temperature must be kept stable at 15°C. The current should be switched off once the dye (sample buffer) reaches the bottom of the gel (Singh *et al*, 1991).

Staining of the gel

Fixing solution:

400 ml methanol

100 ml glacial acetic acid

500 ml water

Staining solution:

30 g trichloroacetic acid made up to 200 ml with water.

0.1 g Coomassie Blue made up to 10 ml in methanol.

- The gel is removed from the glass plates and immersed for about 1 hour in the fixing solution and then overnight in the staining solution.
- The efficiency of shaking is very important in order to get uniform results and should therefore be optimised so that the fluid circulates efficiently without breaking the gels during both steps.
- The stained gel is rinsed in distilled water for a few hours before examination and photography (Wrigley, 1992).

3.2.3 Gel analysis

The gels were analysed with the help of "Molecular Analyst Fingerprinting" software of Biorad. Gels were scanned with the help of Gel Doc 1000 using a UV-gel camera and VGA graphics in 256 colors as recommended. The analysing procedure consisted of three steps: 1) the conversion of the gel, 2) the normalisation of the tracks and 3) the analysis of the tracks.

A rectangle was drawn around the gliadin subunits on the gel by using the conversion program. This made the identification process easier since the gliadin subunits were screened in isolation of the HMW and LMW glutenin subunits, as well as albumin and globulin residue.

The normalisation settings were as follow: the resolution was set at 200 points and a smoothing factor of three was chosen (implying that one point at either side of a data point would be averaged with the data point). The rolling disk method was chosen to subtract the background. The principle of this method is that a disk is rolled on the inside across the curve. Every area of the curve below the imaginary trace left behind the disk will be subtracted as background. Very stable and reliable background subtraction is guaranteed by using this method.

The intensity of the background subtraction was set at ten (typical setting for SDS-PAGE protein patterns are between eight and 12). The clearest Chinese Spring pattern was used as the standard reference pattern and all the other Chinese Spring reference patterns were aligned to this standard reference. Normalisation of a gel is achieved by aligning the bands of all reference patterns on the gel to the corresponding ones of the standard. Non-reference tracks are interpolated gradually according to both surrounding references.

The gels were analysed using the main programme of the "Molecular Analyst Fingerprinting" software after normalisation. A densitometric curve of every replication of every cultivar was drawn and from this the migration distances were determined. Only bands with an intensity of more than 15 percent were accepted. Peak positions with a repeatability of 50 percent and higher in the replications were accepted as representative of a specific cultivar. Repeatability was calculated as the percentage of occurrence of a band in the replications.

An average of the band positions (thus the migration distance of the bands on the gel) of the seven replications of each cultivar was calculated. These values were used to compare the cultivars with one another.

3.2.4 Nomenclature

A detailed discussion of the nomenclature used is given in chapter 2.9. The nomenclature suggested by Konarev *et al* (1979), was used for the purpose of this study. Modifications made to this nomenclature was the exclusion of the densities of the gliadin bands, since this parameter often differs for wheat of the same cultivar from year to year, location to location, protein amount per kernel, kernel size, extractability of kernel proteins and different staining methods.

3.2.5 Calculation of genetic distance

The index of genetic similarity (F) is suitable to calculate the pairwise distance matrixes from SDS-PAGE data. The formula is:

$$F = 2n_{xy} / (n_x + n_y)$$

Where F is the ratio of shared bands between individuals x and y, $2n_{xy}$ is the number of shared bands and n_x and n_y are the numbers of bands observed in cultivars x and y respectively (Nei & Li, 1979).

$$D = -\ln (F)$$

The F-ratio resembles the coefficient of similarity between two cultivars and is used to determine the genetic distance D for a pairwise combination.

The data obtained by these procedures was then analysed using "Phylip", a program that uses distance and parsimony analysis methods. Bootstrapping of 100 replicates was performed to test the statistical significance of trees resulting from the analyses.

Chapter 4

Results and discussion

4.1 Results

Typical movement of the different gliadin bands are shown on the following photograph.

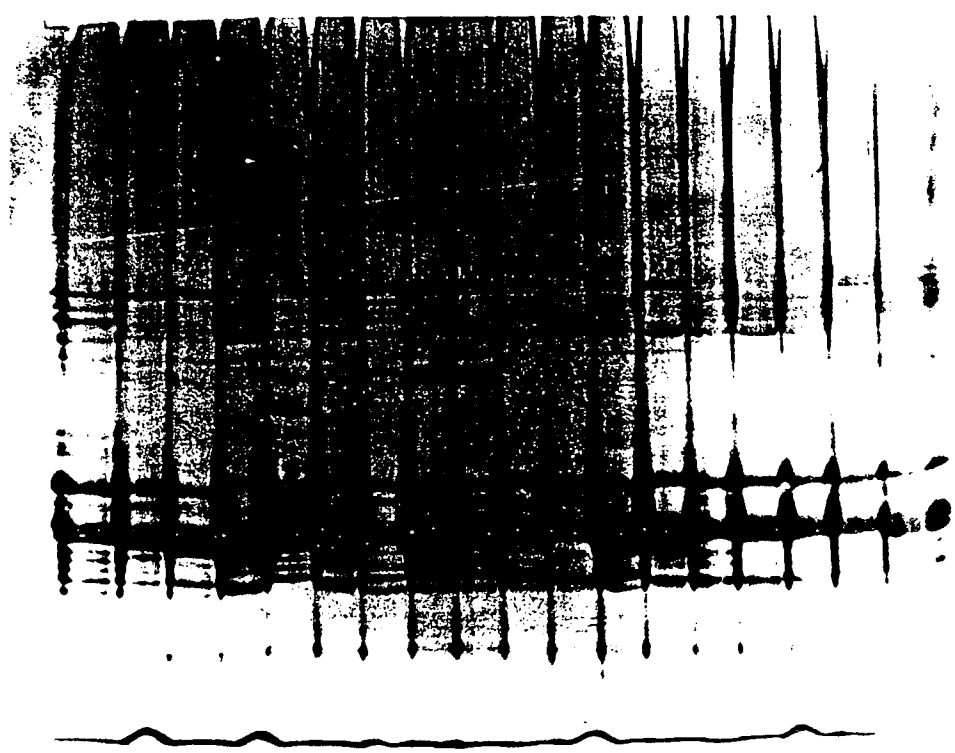


Figure 4.1 Photograph of typical electrophoretic banding patterns for cultivars Chinese Spring, Molen, Molopo, Nantes, Oom Charl, Palmiet, PAN 3211, PAN 3232, PAN 3235, PAN 3324, PAN 3349, Scheepers, SST 66, SST 86, Tugela, Tugela DN, T4 and Wilge obtained by the procedure described previously.

The data of each cultivar are summarised in the following tables. In the first seven columns, the migration distances of the peaks on the gel are stated. Seven replications are shown. In the next column, an averaged value that was accepted as the specific peak position is stated, while the last column shows the repeatability of the replications.

Adam Tas

Table 4.1 Migration distances of the gliadin subunits of Adam Tas.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
8			6		10	6	6	57.14
	26	28		26	28	30	27.6	71.43
	36	39	40			38	38.25	57.14
50	48	46	48				48	57.14
	63	63	62		60	61	61.8	71.43
		104	103	103	105	105	105	71.43
202		198	200			201	200.33	57.14
		262	265	266	263		264.5	57.14
275	278	278	274		278		276.6	71.43
285	289		289	286		285	286.8	71.43
321	323	319	321		323		321.4	71.43
380	378	382		382			380.5	57.14

Belinda

Table 4.2 Migration distances of the gliadin subunits of Belinda.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
24	24		28	24	24	24	24.67	85.71
98			101	100	102	98	99.8	71.43
112	115		114	111	111	114	112.83	85.71
	123	124		122	123		123	57.14
206	203		204			203	204	57.14
	191	192	194	194	194	193	193	85.71
256	257			257	258		257	57.14
283	285			286	287		285.25	57.14
293	295	291	291	295	294	292	293	100
315	315	317	319	317	317	317	317	100
352	353	353			352	353	352.6	71.14
	369	367	370	366		369	368.2	71.14
380	383	383	379	379	380	379	380.43	100

Betta

Table 4.3 Migration distances of the gliadin subunits of Betta.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
31	32	34	30	31		33	31.83	85.71
50	50	51		54		51	51.2	71.43
		85	86	82	83	83	83.8	71.43
116			112		112	113	113.25	57.14
242	242	241	242		245	243	242.5	85.71
267	269	269		269			268.5	57.14
	282	283	280	279	279	280	280.5	85.71
289	292	293	293	291	291		291.5	85.71
304				306	306	305	305.25	57.14
321	319	318			318	318	318.8	71.43
373		375			375	374	374.25	57.43

Betta DN

Table 4.4 Migration distances of the gliadin subunits of Betta DN.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
26	27			26	24		25.75	57.14
66	68	67				67	67	57.14
	123	126		126		122	124.25	57.14
154	156			156	157		155.75	57.14
245	245	243	242	244		245	244	85.71
258	259		262	260			259.75	57.14
269	271	272	270				270.5	57.14
		277	280	280	281	281	279.8	71.43
		297	297		295	295	296	57.14
306			307	303	305	307	305.6	71.43
324	326	323			326		324.75	57.14
358	359		358	357			358	57.14
384	385	388		386		385	385.6	71.43

Chinese Spring

Table 4.5 Migration distances of the gliadin subunits of Chinese Spring (reference).

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
30	30	25		26			30.25	57.14
41	40	43	44				42	57.14
59	58			58	55		57.5	57.14
	67	65	62		66	67	65.4	71.43
96		94	93	95	93	92	93.83	85.71
	111	113		109		109	110.5	57.14
141	137			140		138	139	57.14
168		170	172			168	169.5	57.14
193	190	190	189	189	190		190.17	85.71
202	201		200			200	200.75	57.14
242		243	240	239			241	57.14
	279		279	279	281	283	280.2	71.43
	291	291	289	289		292	290.4	71.43
299	298		298	300	297	301	299	85.71
307		309	307		307	305	307	71.43
326			327	329	329	328	327.8	71.43
		342	338	340		340	340	57.14
	386	386	385		384		385.25	57.14

Flamink

Table 4.6 Migration distances of the gliadin subunits of Flamink.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
22			24	26	25		24.25	57.14
	105			109	109	108	107.75	57.14
155		159	159	158			157.75	57.14
186		182	182	182			183	57.14
	208	204	206	206			206	57.14
	228	230	228		230	232	229.6	71.43
269	270	271			270	272	270.4	71.43
	281	281	283	281	281	285	282	85.71
315	314	314	314	311	313		313.5	85.71
	322	323	321	321		321	321.6	71.43
339	343				340	341	340.75	57.14
377		373	373	374		375	374.4	71.43
385	388	388				385	386.5	57.14

Gamka

Table 4.7 Migration distances of the gliadin subunits of Gamka.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
43	42	43				40	42	57.14
62		63		62	66		63.25	57.14
86	87	86			87	87	86.6	71.43
124	125	124	127	126	124	128	125.43	100
	179	179	178	181	180		179.4	71.43
	273	275	274	273	277		274.4	71.43
293	295	292			290	291	292.2	71.43
305			308	304		307	305.8	57.14
362			362	361	364		362.25	57.14
384	380	382	380	383		384	382.6	85.71
390	393	393	393	390		390	391.5	85.71

Gamtoos

Table 4.8 Migration distances of the gliadin subunits of Gamtoos.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
48		49		47	47	48	47.8	71.43
75	75	76	73			76	75	71.43
84	85	83	83	85	84		84	85.71
128	129		128			126	127.75	57.14
147	145	149	147				147.75	57.14
162	162	160	161				161.25	57.14
		183	185	182	182		183	57.14
192	190		189			190	190.25	57.14
206	206			206	206	210	206.8	71.43
249	249		250	250	248	252	249.67	85.71
263				263	263	265	263.5	57.14
272	272	274	274				273	57.14
301	299	301	302		301	303	301.17	85.71
320	320	324	322	321	319	320	320.86	100
380	380		380	379	380		379.8	71.43
386	387	385		384		386	385.8	71.43

Gamtoos DN

Table 4.9 Migration distances of the gliadin subunits of Gamtoos DN.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
54	55	55	56		54	58	55.33	85.71
88		85	85	85	83		85.2	71.43
129	129	129	130				129.25	57.14
147	147	146	144			144	145.6	71.43
	158	158	155	156	157		156.8	71.43
163	165	162				161	162.75	57.14
207	208			206	207		207	57.14
249	252	249	250	249	249		249.67	71.43
274	274	273	276	277		275	274.83	71.43
294	294		297			292	294.25	57.14
301	301	301			301		301	57.14
		312	312	309	310		310.75	57.14
323	325	323	323		320	321	322.5	85.71
350	351		352	351			351	57.14
	363	362	361	360			361.5	57.14
390		390	390	386	386		388.4	71.43

Gariep

Table 4.10 Migration distances of the gliadin subunits of Gariep.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
14		14		16	16	18	15.6	71.43
73		75	74		72		73.5	57.14
126	127	123	126			124	125.2	71.43
158	162			158		160	159.5	57.14
		243		242	242	244	242.75	57.14
256	255		255	258		256	256	71.43
	266	268	265		267	265	266.2	71.43
285	284			286	285	286	284.4	71.43
295	293	295	292	294		295	294	85.71
318	316	317			316		316.75	57.14
343	342		345		344		343.5	57.14
356			357	353	357		355.75	57.14
369	368	369			366		368	57.14
386		384	385	382	384	386	384.5	85.71

Harts

Table 4.11 Migration distances of the gliadin subunigs of Harts.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
	28	24	28		24		26	57.14
74		75		77		77	75.75	57.14
88	88	86	85			89	87.2	71.43
139	137		139	138	138		138.2	71.43
154	154	156	158	158		156	156	85.71
172	172			170	171	172	171.4	71.43
208	207		206	209	208		207.6	71.43
243		244	243	242	243	245	243.33	85.71
260	260	259	262		261		260.4	71.43
270	269	271		268		269	269.4	71.43
287	287			289	286		287.25	57.14
299	299			300	299	299	299.2	71.43
309	313	309	313		313	309	311	85.71
379	380		381		381	378	379.8	71.43

Hugenoot

Table 4.12 Migration distances of the gliadin subunits of Hugenoot.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
31		34	30		34	32	32.2	71.43
47	47	43	43	43			44.6	71.43
73	72	73			72	73	72.6	71.43
		110	107		108	107	104.4	57.14
165	164			164	164	166	164.6	71.43
248	245		249	246			247	57.14
269	265			265		267	266.5	57.14
291	290		289	289	292	292	290.5	85.71
317		317			317	317	317	57.14
348	349	349	349	348	346	347	348.14	100
376	374	376	375				375.25	57.14
	385		388	387	388		387	57.14
399	399	399	396		396	394	398.5	85.71

Inia

Table 4.13 Migration distances of the gliadin subunits of Inia.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
28		26			30	27	27.75	57.14
64			68		67	67	66.5	57.14
91	91	91	91	91	89	87	90.14	100
99	97	99				96	97.75	57.14
194	192		196	192			193.5	57.14
245	244	243	244	245	245		244.33	85.71
289	288	288	289				288.5	57.14
299		301	301	297	298	300	299.33	85.71
329	326	326		327	326	325	326.33	85.71
347	345		346	344	344		345.4	71.43
375	375	375	374	378		376	375.33	85.71
386	388	388			384	383	385.8	71.43

Karee

Table 4.14 Migration distances of the gliadin subunits of Karee.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
	38			40	40	38	39	57.14
		69	69	71	69		69.25	57.14
83	84	85	83	83			83.8	71.43
96	95	94				95	95	57.14
115	114				116	115	115	57.14
190	188			192	190		190	57.14
242	241	242	243				242	57.14
268				268	269	269	268.5	57.14
284	286	285	287	287			285.8	71.43
	292	294	295		291	292	292.33	71.43
314	314	315	314	313		317	314.5	85.71
348	349	350	351		349	347	349	85.71
384	383	381	380	380		384	382	85.71

Kariega

Table 4.15 Migration distances of the gliadin subunits of Kariega.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
63			66	66		66	65.25	57.14
74	71	73			71	73	72.4	71.43
	82	80	83	82		80	81.4	71.43
	128	129	129	125		127	127.6	71.43
191	188	187	191	188	187		188.67	85.71
241	239		242	238			240	57.14
249		249			251	248	249.25	57.14
264	262	265	265	265	263		264	85.71
286	285		285	284			285	57.14
291		292	294	290		294	292.2	71.43
316	315	316		314	318	314	315.5	85.71
336	336		338	335	335	334	335.67	85.71
347	346		349	347	347	346	347	85.71
383	380	382		384	384	380	381.86	87.5
		399	396	398	396	396	397	71.43

Letaba

Table 4.16 Migration distances of the gliadin subunits of Letaba.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
56			54		56	58	56	57.14
81	82	80		83			81.5	57.14
91		88			89	91	89.75	57.14
132	134	130	131				131.75	57.14
148	148				149	145	147.5	57.14
183	182	185		184	182		183.2	71.43
258	258			257		259	258	57.14
		276	277		278	277	277	57.14
287	286			287		284	286	57.14
294	294	294	290	293		294	293.8	85.71
306	308	306		308	304		306.4	71.43
335	335				335	335	335	57.14
378			374	374		374	375	57.14
	382	381	380	381		379	380.6	71.43

Molopo

Table 4.20 Migration distances of the gliadin subunits of Molopo.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
41	41	42		40	44		41.6	71.43
57	60	60	57		59		58.6	71.43
75	75	76		75	77		75.6	71.43
118	119	118	117		120	116	117.83	85.14
	125	130		125		127	126.75	57.14
		158	154	156		156	156	57.14
216	218		214		215		215.75	57.14
237	235	238	239	236	235		236.85	85.14
261	259			258	261	259	259.6	71.43
303	301	305			302	303	302.8	71.43
		327	328	328	324	325	325.3	71.43
353	356			353	354	356	354.4	71.43
375	377	376	376	374			377.6	71.43

Nantes

Table 4.21 Migration distances of the gliadin subunits of Nantes.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
48	50	47	48			49	48.4	71.43
71	71	71	73	73		72	71.83	85.71
91	92	92	92	90	91		91	85.71
	174			176	174	176	175	57.14
218	220	220			218	222	219.6	71.43
238		237	237	239	235	234	236.57	85.71
260		258	258		262		259.5	57.14
	273	276	277		274	273	274.6	71.43
285	286	285	286	289		287	286.66	85.71
		300	299	300	299	301	299.8	71.43
334	337	337	337	333			335.6	71.43
	380	380		378		377	378.75	57.14

Oom Charl

Table 4.22 Migration distances of the gliadin subunits of Oom Charl.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
	49		48	50	51		49	57.14
62	62	63				63	62.5	57.14
75		75		76	75		75.25	57.14
	79		79	79	81	78	79.2	71.43
87	87	90	91			89	88.8	71.43
	128	129	129	127	129	125	127.83	85.71
		166	165	169		166	166.25	71.43
263	266			265	263	264	264.2	71.43
274	277	277	276	277	277		276.33	85.71
			290	290	293	293	291.5	57.14
304	307	307	306			307	307	71.43
		326	326	327	328		326.75	57.14
361	362		364	362	361		362	71.43
377	376	374				375	375.5	57.13

Palmiet

Table 4.23 Migration distances of the gliadin subunits of Palmiet.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
54	54		57	57	55	55	55.33	85.71
76	77			79	79	80	78.2	71.43
95	94	91			95	95	94	71.43
		204	200	203	200		201.75	57.14
224	225			222	220		222.25	57.14
268	267			265	266	267	266.6	71.43
274	274	274	272		276	276	274.33	85.71
286	287	286	285	284			285.8	71.43
315	315		316		313		314.75	57.14
335	332	333	331	333	331		332.5	85.71
		363	360		360	362	361.25	57.14
380	380	384	384	380	384		382	85.71

PAN 3211

Table 4.24 Migration distances of the gliadin subunits of PAN 3211.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
56	58		57		59		57.5	57.14
66	68	67		65	68		66.6	71.43
75	73	76		73	76	73	74.33	85.71
83	84		85	81		85	85.6	71.43
127	126	126	125	125	125	124	125.43	100
174	175	177		175	175		175.6	71.43
187	187		184	184	184		185.8	71.43
222			222	221	224		221.8	71.43
		254	257	258		252	255.25	57.14
269	271	271		267	272	271	270.17	85.71
277	281	280	281			279	279.6	71.43
309	310			308	310	307	308.8	71.43
320	321	323	320		321		321.4	71.43
	346	349	345			349	346.75	57.14
364		367	368			366	366.25	57.14
382	383	380	380	381	384		381.67	85.71

PAN 3232

Table 4.25 Migration distances of the gliadin subunits of PAN 3232.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver..	Repeat
51	52	54	53		52	51	52	85.71
85	86		84	85	83	83	85.2	85.71
130	131	131	129		129	128	129.67	85.71
158		159	160	158			158.75	57.14
233	233	238	238			236	237.6	71.43
242	246			245	244		244.25	57.14
274	274	274	276	279	278		275.83	85.71
285	285	286	288	287	287	286	286.5	100
310	311	311	307		307		309	71.43
321	323	323	324	321			322.4	71.43
	362	360	360	358	358	360	359.67	85.71
380	381			378	378		379.25	57.14
		393	397	396	394	394	394.8	71.43

PAN 3235

Table 4.26 Migration distances of the gliadin subunits of PAN 3235.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
16			14	16	17	16	15.8	71.43
51	50		54	51	53	54	52.17	85.71
83	83	87		84		87	84.8	71.43
106		108	109		107		107.5	57.14
	158	157			158	155	157	57.14
	191		188	192		192	191	57.14
	223		222	224		225	223.5	57.14
240		240	241	239		240	240	71.43
	256	251	255			253	253.75	57.14
279	279	276	278		276	278	277.67	85.71
310	311			309	310	309	309.8	71.43
319		321			319	321	320	57.14
370	367			369	367		368.25	57.14
389	386		388			386	387.25	57.14

PAN 3342

Table 4.27 Migration distances of the gliadin subunits of PAN 3342.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
	36	35	35	34	33		34.6	71.43
47		45	47	48	44	47	46.33	85.71
56			57	57	58		57	57.14
75		74		71	75		73.75	57.14
	85			86	86	85	86.75	57.14
		111	111		108	108	109.5	57.14
	122	125	121	121		123	122.4	71.43
147		144			146	147	146	57.13
208	206	204				204	205.5	57.14
237		236	235	234		238	236	71.43
254		255			256	257	255.25	57.13
	274	279	275	275	278	274	276	85.71
		299	297	297	297		297.5	57.14
305	307	307	307	307	308	307	306.85	100
	324	325	325	327			325.25	57.14
	376	375	372	374	376	375	374.67	85.71

PAN 3349

Table 4.28 Migration distances of the gliadin subunits of PAN 3349.

Repl.1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
34	35	31			33	34	33.4	71.43
49	51	51		48	49		49.6	71.43
64			64	64		60	63	57.14
		76	76		79	74	76.25	57.14
80	83		83	82		83	82	71.43
92		91	91			93	91.75	57.14
165	164			167		166	165.75	57.14
		194	196		198	194	195.5	57.14
215	215	216		214	218		215.6	71.43
		243	244	242	241		242.5	57.14
254	257	253	256			255	255	71.43
276	278		278	275		277	277.2	71.43
		287	289	289	289		288.5	57.14
299	303	303	299		302	303	301.8	71.43
	357			356	356	353	355.75	57.14
	378	380	378	378	379	380	380.83	85.71
			386	384	384	388	385.5	57.14

Scheepers

Table 4.29 Migration distances of the gliadin subunits of Scheepers.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
56	53	54	54				54.25	57.14
		66	66	66	67	62	65.4	71.43
	77	78			77	78	77.5	57.14
85	85	86	86	87	84		85.83	85.71
	105	105	105		106	105	105.2	71.43
127			131	130	128	127	128.6	71.43
	188	190		188		190	189	57.14
251	247	247	248		251		248.8	71.43
275	279	279	279			275	277.4	71.43
	292	291	292		291		291.5	57.14
325	327	327			329		327	57.14
	358	354		357		356	356.75	57.14
371			374		376	372	373.25	57.14
		384	384	385	386	384	384.6	71.43

SST 66

Table 4.30 Migration distances of the gliadin subunits of SST 66.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
50			48	51	50	51	50	71.43
	65	65			64	63	64.25	57.14
	78	78	75		77	78	78.2	71.43
86			87	83		85	85.25	57.14
	107	107	107			105	106.5	57.14
	127		126	125		124	125.75	57.14
		196	199	198		196	197.25	57.14
239	242	243	239		239		240.2	71.43
280	279	277	280	276	276	277	277.85	100
290	289	289	291		290	289	289.67	85.71
302	302	303	303	306			303.2	71.43
332	331			333		333	332.25	57.14
	362	365		363	364	363	363.4	71.43
376	378	376	378	374		376	376	85.71
		385	384	387	383	383	384.6	71.43

SST 86

Table 4.31 Migration distances of the gliadin subunits of SST 86.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
12		16	12	12	14	16	13.67	85.71
22	23	24	25		21	24	23.28	85.71
42	43	43		46			43.5	57.14
53			53	56	54		54	57.14
67	69	69	68	69	69	67	68.25	100
95	94	95				97	95.25	57.14
105	107	104		107	105	107	105.83	85.71
113		117	117		113	115	115	71.43
164	167		166		165		165.5	57.14
180	184	184	184				183	57.14
198	199	195			196	196	196.8	71.43
214	215		214	216	218		215.4	71.43
259	261	262		261			260.75	57.14
279	281	279	279	277		277	278.67	85.71
287		287	287	287	285		286.6	71.43
	317	316	315		315	315	315.6	71.43
	334	335	337		333	337	335.2	71.43
374		374		374		377	374.75	57.14

Tugela

Table 4.32 Migration distances of the gliadin subunits of Tugela.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
22		22	22	19	21		22	57.14
51	50			50		53	51	57.14
69				69	71	69	69.95	57.14
85	87	87			86	83	85.6	71.43
	106	107		107		105	106.25	57.14
	167		164	164	166		165.25	57.14
214		212	214	216	215	212	213.83	85.71
	248	248			247	249	248	57.14
256		256	256		253	257	256	71.43
	283	283	285	285		283	283.8	71.43
	293	295			297	295	295	57.14
305	306	307	307				306.25	57.14
	335	335	332	334			334	57.14
362			364		361	362	362.5	57.14

Tugela DN

Table 4.33 Migration distances of the gliadin subunits of Tugela DN.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
52	54			50	53	51	52	71.43
67	67	67		66	65	65	66.17	85.71
83	79	80	79	80	80	83	80.88	100
	95	95	96	97	95	97	95.83	85.71
112	113	111			111	115	114.4	71.43
212		208	212	210		212	210.8	71.43
251	249	249			249	249	245.4	71.43
262				267	267	264	265	57.14
	274		275	276		276	275.25	57.14
296	295	296		294	297	294	294.83	85.71
	336	338	334	337		337	336.4	71.43
355		357		357	354	353	355.2	71.43
365		365	362		366		364.5	57.14
392		396	394	393		395	394	71.43

T4

Table 4.34 Migration distances of the gliadin subunits of T4.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
30			30	26	29	28	28.6	71.43
42	42	39		38	42		40.6	71.43
69	69	67	71			69	69	71.43
87	83	87	83		83	83	84.67	85.71
97			99	95	95	97	96.6	71.43
	107	107			111	107	108	57.14
	184	182	180			185	182.75	57.14
226	225			222	222		223.75	57.14
251	254			251		254	252.5	57.14
		268		271	271	270	270	57.14
278	282	282		279	281		280.4	71.43
287	288	290	289	288		290	288.67	85.71
305	309	308	307			308	307.8	71.43
332		330		330	335		331.75	57.14
	347	345	344	346			345.5	57.14
353		352		356	356	352	353.8	71.43
397	393		392	397		394	394.8	71.43

Wilge

Table 4.35 Migration distances of the gliadin subunits of Wilge.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7	Aver.	Repeat
40	43		44	40	42		41.8	71.43
53	54	51		53			52.75	57.14
61	61	58	62			61	60.6	71.43
70	70		72	70			70.5	57.14
80		78	80	83	83	78	80.33	85.71
	105	107		105		105	105.5	57.14
118	117				115	115	116.25	57.14
	144	146		146		148	146	57.14
217	216		216	215			216	57.14
267	262	262		265		265	264.2	71.43
281	283		280	281	281		281.2	71.43
		295	292	293	295		293.75	57.14
302	301				303	305	302.75	57.14
311	312	315	316	313	311		313	85.71
	325	328			325	325	325.5	57.14
360			364	360		364	362	57.14
374	373	375			375		374.25	57.14
386			387	384		384	385.25	57.14

Table 4.36 Summary of the cultivars tested and their gliadin subunit combinations (Bands within five units of deviation were considered to be the same).

Cultivar	α-gliadins	β-gliadins	γ-gliadins	ω-gliadins
Adam Tas	1; 2; 3; 5; 6	4	3	1; 2; 3; 7; 9
Belinda	2	4	2; 3	3; 4; 8; 9
Betta	3; 5	3; 4	5	1; 3; 4; 5; 6; 9
Betta DN	2; 7	4	5	1; 2; 4; 5; 7; 8; 10
Chinese Spring	2; 4; 6; 7	2; 3; 4; 5	2; 3; 5	3; 4; 5; 7; 8; 9
Flamink	2	4	2; 4	3; 6; 7; 8; 9; 10
Gamka	4; 7	3; 4	1	2; 4; 5; 8; 9; 10
Gamtoos	5	1; 3; 5	1; 2; 4; 5	1; 2; 5; 7; 9; 10
Gamtoos DN	5	3; 5	1; 4; 5	2; 4; 5; 6; 7; 8; 9; 10
Ganep	1	1; 4	1; 5	1; 3; 4; 6; 8; 9
Harts	2	1; 3; 5	1; 4; 5	1; 2; 4; 6; 9
Hugenoot	2; 4	1; 4	1; 5	1; 4; 6; 8; 9; 10
Inia	2; 7	3; 4	2; 5	3; 7; 8; 9
Karee	3	3; 4	2; 5	1; 3; 4; 6; 8; 9
Kariega	7	1; 3; 5	2; 5	1; 3; 4; 6; 8; 9; 10
Letaba	6	3; 5	2	2; 3; 4; 5; 8; 9
Limpopo	2	3; 4; 5	2	3; 5; 9; 10
Marico	1; 5; 7	1; 3	1; 2; 3; 4	3; 5; 9
Molen	1; 5	1; 3; 4	1	2; 3; 5; 7; 8; 9; 10
Molopo	4; 6	1; 4; 5	4; 5	5; 7; 8; 9
Nantes	5	1; 4	1; 4; 5	2; 3; 4; 8; 9
Oom Charl	5; 7	2; 3; 5	1	1; 2; 4; 5; 7; 9
Palmiet	5	2; 4	3; 4	1; 2; 3; 6; 8; 9
PAN 3211	6; 7	1; 3; 5	1; 2; 4	1; 2; 5; 7; 8; 9
PAN 3232	5	3; 5	5; 5	1; 3; 5; 7; 8; 9; 10
PAN 3235	1; 5	3; 4	2; 4; 5	2; 5; 6; 9; 10
PAN 3342	3; 5; 6	1; 3; 4	4; 5	2; 4; 5; 7; 9
PAN 3349	3; 5; 7	2; 3; 4	1; 3; 4; 5	2; 3; 5; 8; 9; 10
Scheepers	5; 7	2; 3; 4; 5	2; 5	2; 4; 7; 8; 9
SST 66	5; 7	2; 3; 4; 5	3; 5	2; 3; 5; 8; 9
SST 86	1; 2; 4; 5; 7	4	1; 2; 3; 4	1; 2; 3; 6; 8; 9
Tugela	2; 5	1; 3; 4	1; 4; 5	3; 4; 5; 8; 9
Tugela DN	5; 7	3; 4	4; 5	1; 2; 4; 8; 9; 10
T4	2; 4	3; 4	2; 4	1; 2; 3; 5; 7; 8; 10
Wilge	4; 5; 6	1; 4	4	1; 3; 4; 5; 6; 7; 9

Table 4.37 The percentage of occurance of the different gliadin banding patterns in Southern African wheat cultivars.

	1	2	3	4	5	6	7	8	9	10
α	17%	31%	14%	20%	54%	20%	34%			
β	37%	17%	69%	74%	37%					
γ	40%	43%	23%	49%	63%					
ω	49%	57%	63%	54%	60%	34%	43%	66%	91%	40%

Table 4.38 Pairwise distance matrix on the F (coefficient of similarity) and D (genetic distance) statistics of Nei and Li (1979).

F Values

	Chin Spr	Adam Tas	Belinda	Beta	Beta DN	Flamink	Gamka	Gamtoos	Gamt DN	Ganep	Herts	Hugenoot	Inia	Karee	Kenega	Letaba	Limpopo	Manco	Molen	Molopo	Nantes	Oom Char	Palmiet	PAN 3211	PAN 3232	PAN 3235	PAN 3342	PAN 3349	Scheeper	SST 66	SST 86	Tugela	Tugela D	T4	Wilge
Chinese Spring		0.69	0.69	0.85	0.59	0.69	0.56	0.83	0.65	0.78	0.92	0.65	0.34	0.59	0.58	0.48	0.48	0.79	0.79	0.44	0.85	0.79	0.88	0.58	0.59	0.88	0.66	0.5	0.44	0.37	0.86	0.51	0.79	0.68	0.72
Adam Tas	0.5		0.79	0.79	1.06	0.79	1.3	1.18	1.14	0.88	1.1	1.1	0.79	1.06	1.47	0.79	0.92	0.92	0.59	1.01	0.79	1.14	0.65	1.18	1.06	0.83	0.58	0.66	0.92	0.77	0.47	0.88	0.92	0.92	0.81
Belinda	0.5	0.45		0.79	1.34	0.61	0.3	1.87	1.43	0.83	1.39	0.88	0.79	0.65	0.96	1.06	0.69	1.14	1.43	1.7	1.01	1.83	0.83	1.87	1.75	1.06	0.92	1.22	1.14	1.18	0.88	0.88	1.43	1.14	0.99
Beta	0.43	0.45	0.45		0.83	1.3	0.79	0.77	0.73	0.53	0.69	0.88	0.79	0.25	0.77	0.88	0.92	1.14	0.73	1.01	0.61	0.92	0.83	1.18	0.45	0.65	0.45	0.52	0.73	0.62	0.88	0.41	0.58	0.92	0.52
Beta DN	0.55	0.35	0.26	0.43		0.83	0.49	0.99	0.62	0.92	0.73	0.58	0.65	0.88	0.81	1.1	0.97	1.87	0.77	0.83	0.83	0.77	1.1	0.81	0.69	0.88	0.77	0.69	0.62	0.81	0.92	0.73	0.49	0.49	1.03
Flamink	0.5	0.45	0.54	0.27	0.43		1.01	0.96	0.73	0.88	1.1	0.89	0.45	0.65	0.77	1.11	0.51	1.43	0.73	0.79	0.79	1.83	0.65	0.96	0.83	0.65	1.14	0.99	0.92	1.18	0.59	0.89	0.92	0.45	0.81
Gamka	0.57	0.27	0.27	0.45	0.61	0.36		0.77	0.44	0.88	0.88	0.54	0.79	0.83	0.77	0.65	0.69	0.92	0.45	0.79	0.61	0.58	1.06	0.62	1.07	0.65	0.73	0.41	0.58	0.62	0.73	0.58	0.58	0.73	0.99
Gamtoos	0.44	0.31	0.15	0.46	0.37	0.38	0.46		0.28	1.03	0.44	0.85	0.96	0.99	0.63	0.81	0.69	0.59	0.48	0.62	0.62	0.48	0.99	0.31	0.41	0.41	0.48	0.54	0.59	0.76	0.86	0.56	0.59	0.59	0.79
Gamt DN	0.52	0.32	0.24	0.48	0.54	0.48	0.64	0.76		0.99	0.41	0.81	0.92	0.77	0.59	0.62	0.83	0.83	0.44	0.58	0.44	0.44	0.77	0.47	0.47	0.37	0.44	0.41	0.44	0.63	0.83	0.41	0.44	0.69	0.92
Genep	0.47	0.42	0.43	0.58	0.4	0.42	0.42	0.36	0.37		0.62	0.37	0.88	0.58	0.58	1.14	1.3	0.99	0.68	0.88	0.41	1.22	0.73	1.03	0.92	1.14	0.99	0.85	0.99	1.03	0.68	0.49	0.81	1.22	0.73
Herts	0.4	0.33	0.25	0.5	0.48	0.33	0.42	0.64	0.66	0.54		0.62	1.1	0.73	0.56	0.92	1.01	0.99	0.99	0.88	0.54	0.66	0.92	0.56	0.92	0.73	0.66	0.88	0.88	1.03	0.79	0.49	0.66	0.99	0.88
Hugenoot	0.52	0.33	0.42	0.42	0.56	0.5	0.58	0.43	0.44	0.89	0.54		0.88	0.58	0.56	1.43	1.01	1.5	0.81	0.89	0.54	1.22	0.92	1.03	0.92	0.92	0.88	0.85	0.99	1.25	0.66	0.48	0.66	0.81	0.73
Inia	0.71	0.45	0.45	0.45	0.52	0.63	0.45	0.38	0.4	0.42	0.33	0.42		0.49	0.62	0.83	0.51	0.92	0.73	0.79	0.79	1.14	1.06	0.77	0.65	0.83	0.92	0.68	0.45	0.62	0.73	0.54	0.73	0.58	0.99
Karee	0.55	0.35	0.52	0.78	0.41	0.52	0.43	0.37	0.46	0.56	0.48	0.58	0.61		0.41	0.69	0.74	1.18	0.96	1.06	0.65	1.18	0.69	0.99	0.89	0.69	0.77	0.69	0.82	0.81	0.78	0.58	0.77	0.96	1.03
Kenega	0.56	0.23	0.38	0.46	0.44	0.46	0.46	0.53	0.55	0.57	0.57	0.57	0.54	0.66		0.66	0.88	1.06	0.88	1.18	0.77	0.88	0.99	0.63	0.52	0.81	1.06	0.79	0.59	0.76	0.86	0.65	0.59	0.73	1.13
Letaba	0.62	0.35	0.35	0.42	0.33	0.35	0.52	0.44	0.54	0.32	0.4	0.24	0.43	0.5	0.52		0.74	0.96	0.77	0.83	0.83	0.77	1.09	0.52	0.69	0.88	0.77	0.85	0.62	0.66	1.09	0.73	0.96	0.77	1.03
Limpopo	0.62	0.4	0.5	0.4	0.38	0.6	0.5	0.5	0.43	0.27	0.36	0.36	0.6	0.48	0.42	0.48		0.83	0.65	0.92	1.2	1.34	1.25	0.88	0.58	0.56	1.06	0.73	1.06	0.88	0.99	0.45	1.06	0.5	1.14
Manco	0.45	0.4	0.32	0.32	0.15	0.24	0.4	0.55	0.43	0.37	0.37	0.22	0.4	0.31	0.34	0.38	0.43		0.56	1.14	0.73	0.85	0.96	0.59	1.03	0.62	0.85	0.51	1.03	0.49	0.58	0.52	1.03	1.03	0.92
Molen	0.45	0.56	0.24	0.48	0.46	0.48	0.64	0.82	0.64	0.52	0.37	0.44	0.48	0.38	0.41	0.46	0.52	0.57		0.73	0.45	0.69	0.85	0.59	0.49	0.49	0.56	0.41	0.69	0.59	0.69	0.41	0.69	0.56	0.76
Molopo	0.64	0.38	0.18	0.38	0.43	0.45	0.45	0.54	0.56	0.42	0.42	0.5	0.45	0.35	0.31	0.43	0.4	0.32	0.48		0.61	1.14	1.34	0.49	0.65	0.83	0.45	0.81	0.73	0.77	0.77	1.06	0.92	0.73	0.52
Nantes	0.43	0.45	0.36	0.54	0.43	0.45	0.54	0.54	0.64	0.67	0.58	0.58	0.45	0.52	0.46	0.43	0.3	0.48	0.64	0.54		0.92	0.5	0.77	0.83	0.65	0.5	0.41	0.58	0.62	0.59	0.18	0.45	0.92	0.66
Oom Char	0.45	0.32	0.16	0.4	0.46	0.16	0.56	0.82	0.84	0.3	0.52	0.3	0.32	0.31	0.41	0.46	0.26	0.43	0.5	0.32	0.4		0.96	0.48	0.62	0.96	0.76	0.56	0.73	0.59	0.98	0.81	0.89	1.03	0.96
Palmiet	0.41	0.52	0.43	0.43	0.33	0.52	0.34	0.37	0.48	0.48	0.4	0.4	0.35	0.57	0.37	0.33	0.29	0.38	0.43	0.26	0.61	0.38		0.99	0.88	0.69	0.96	0.44	0.77	0.52	0.41	0.73	0.62	0.77	0.69
PAN 3211	0.56	0.31	0.15	0.31	0.44	0.38	0.54	0.73	0.82	0.36	0.57	0.36	0.48	0.37	0.53	0.59	0.42	0.55	0.55	0.62	0.46	0.62	0.37		0.66	0.81	0.59	0.54	0.59	0.76	0.72	0.69	0.73	0.59	0.79
PAN 3232	0.55	0.35	0.17	0.63	0.5	0.43	0.35	0.86	0.82	0.4	0.4	0.4	0.52	0.5	0.59	0.5	0.57	0.38	0.62	0.52	0.43	0.54	0.42	0.52		0.69	0.77	0.69	0.82	0.52	1.09	0.58	0.62	0.82	0.85
PAN 3235	0.41	0.34	0.35	0.52	0.42	0.52	0.52	0.87	0.89	0.38	0.48	0.4	0.43	0.5	0.44	0.42	0.57	0.54	0.62	0.43	0.52	0.38	0.5	0.44	0.5		0.49	0.44	0.82	0.66	0.63	0.58	0.49	0.82	0.85
PAN 3342	0.52	0.56	0.4	0.64	0.48	0.32	0.48	0.82	0.64	0.37	0.52	0.41	0.4	0.46	0.34	0.46	0.35	0.43	0.57	0.64	0.61	0.47	0.38	0.55	0.46	0.82		0.51	0.56	0.73	0.98	0.4	0.59	0.85	0.51
PAN 3349	0.81	0.52	0.3	0.59	0.5	0.37	0.67	0.58	0.67	0.43	0.41	0.43	0.52	0.5	0.45	0.43	0.48	0.6	0.67	0.44	0.67	0.57	0.64	0.58	0.5	0.64	0.6		0.51	0.26	0.53	0.37	0.41	0.63	0.98
Scheeper	0.65	0.4	0.32	0.48	0.54	0.4	0.58	0.55	0.64	0.37	0.41	0.37	0.64	0.54	0.55	0.54	0.35	0.36	0.5	0.48	0.56	0.48	0.46	0.55	0.54	0.54	0.57	0.6		0.37	0.83	0.66	0.44	0.84	1.09
SST 66	0.69	0.46	0.31	0.54	0.44	0.31	0.54	0.47	0.53	0.38	0.36	0.29	0.54	0.44	0.47	0.52	0.42	0.61	0.55	0.46	0.54	0.55	0.59	0.47	0.59	0.52	0.48	0.77	0.69		0.72	0.56	0.59	0.88	1.13
SST 86	0.51	0.62	0.41	0.41	0.4	0.55	0.48	0.42	0.44	0.52	0.45	0.52	0.48	0.47	0.42	0.33	0.37	0.56	0.5	0.46	0.55	0.38	0.67	0.48	0.33	0.53	0.38	0.59	0.44	0.48		0.66	0.69	0.58	0.75
Tugela	0.6	0.42	0.42	0.66	0.48	0.5	0.56	0.57	0.67	0.62	0.62	0.62	0.58	0.56	0.43	0.48	0.64	0.59	0.67	0.34	0.83	0.44	0.48	0.5	0.56	0.56	0.67	0.69	0.52	0.57	0.52		0.52	0.66	0.59
Tugela D	0.45	0.4	0.24	0.56	0.61	0.4	0.56	0.55	0.64	0.44	0.52	0.52	0.48	0.48	0.55	0.38	0.35	0.36	0.5	0.4	0.64	0.5	0.54	0.48	0.54	0.62	0.57	0.87	0.84	0.55	0.5	0.59		0.69	0.92
T4	0.52	0.4	0.32	0.4	0.62	0.64	0.48	0.55	0.5	0.3	0.37	0.44	0.38	0.38	0.48	0.46	0.61	0.36	0.57	0.48	0.4	0.36	0.46	0.55	0.54	0.54	0.43	0.53	0.43	0.41	0.56	0.52	0.5		0.76
Wilge	0.48	0.44	0.37	0.59	0.35	0.44	0.37	0.45	0.4	0.48	0.41	0.48	0.37	0.36	0.32	0.38	0.32	0.4	0.48	0.59	0.52	0.4	0.5	0.45	0.43	0.43	0.6	0.38	0.33	0.32	0.47	0.55	0.4	0.46	

D Values

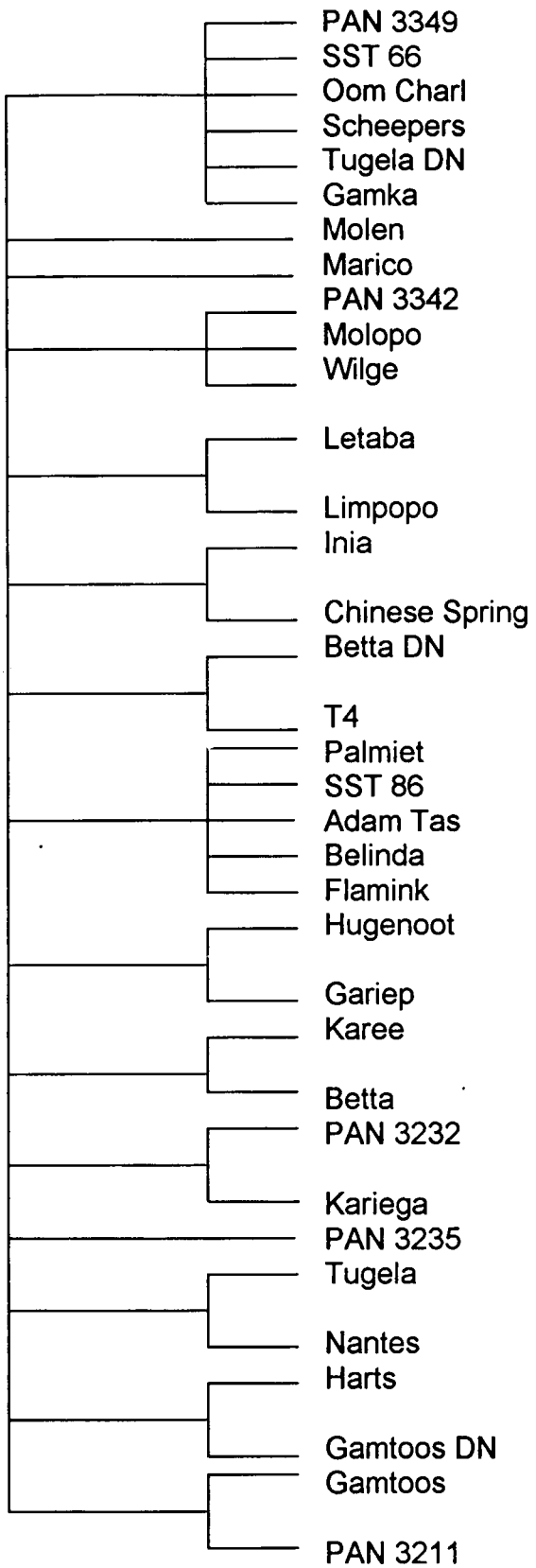


Figure 4.3 Consensus tree generated by the use of distance and parsimony analysis methods using the "Phylip" programme.

Refer to chapter 2.9 for an explanation of the nomenclature.

The α-gliadin combination of bands 3 and 5 were found in Adam Tas, Betta, PAN 3342 and PAN 3349 (Table 4.36). The α-gliadin 1 and 5 band combination were found in Adam Tas, Marico, Molen, PAN 3235 and in SST 86. However in Adam Tas bands 2 and 3 and in SST 86, bands 2 and 4 were found between these two bands. The α-gliadin combination of bands 2 and 7 were found in Betta DN, Inia and SST 86. In SST 86 bands 4 and 5 were found between bands 2 and 7. The banding combination of bands 5 and 7 were found in Marico, Oom Charl, PAN 3349, SST 86 and Tugela DN.

The β-gliadin combination of bands 3 and 4 were found in Betta, Gamka, Inia, Karee, Limpopo, Molen, PAN 3235, PAN 3342, PAN 3349, Scheepers, SST 66, Tugela and T4. The combination of bands 3 and 5 were found in the cultivars Gamtoos, Gamtoos DN, Harts, Kariega, Letaba, Limpopo, Oom Charl, PAN 3211, PAN 3232, Scheepers and SST 66. In Gamtoos, Harts, Kariega and PAN 3211, band 1 also formed part of the combination, while in the cultivars Limpopo, Scheepers and SST 66, band 4 was present between bands 3 and 5. The β-gliadin combination of bands 1 and 4 were present in Gariep, Hugenoot, Molen, Molopo, Nantes, PAN 3342, Tugela and Wilge. In Molen, PAN 3342 and Tugela band 3 was present between these two bands.

The combination of γ-gliadins 1, 4 and 5 were found in Gamtoos, Gamtoos DN, Harts, Nantes, PAN 3349 and Tugela. In PAN 3349, band 3 was found between band 1 and band 4. γ-Gliadin banding combinations of band 2 and band 5 were found in Gamtoos, Inia, Karee, Kariega, PAN 3235 and Scheepers. In Gamtoos as well as in PAN 3235 band 4 was found between bands 2 and 5.

In Adam Tas, Palmiet, SST 86 and T4, banding combinations of ω-gliadin bands 1, 2 and 3 were found. In Belinda, Gariep, Harts, Hugenoot, Karee and Kariega banding combinations of bands 4 and 6 were found.
In Betta, Gariep, Karee and Kariega banding combinations of ω-gliadins 1, 3 and 4 were found.

In Betta DN, Flamink, Gamka, Gamtoos, Gamtoos DN, Hugenoot, Kariega, Molen, Molopo, PAN 3211, PAN 3232, PAN 3349, Scheepers, Tugela DN and T4 banding combinations of ω-gliadin bands 7, 8, 9 and 10 were found. In Hugenoot, Gamka, Kariega, PAN 3349 and Tugela DN band 7 was absent, while in Betta DN and T4 band 9 was absent. Band 8 was absent in Gamtoos, while in Inia, Molopo, PAN 3211 and Scheepers band 10 was missing.

Banding combinations of ω-gliadin bands 2, 4 and 5 were found in Betta DN, Gamka, Gamtoos DN, Harts, Molen, Palmiet, PAN 3211, PAN 3235, PAN 3342, Scheepers, Tugela DN, T4 and Wilge. In Molen, PAN 3211, PAN 3235, band 4 was missing. In Harts, Scheepers, Tugela DN, band 5 was missing. In Betta DN two ω-gliadin band 2's were found.

Banding combinations of ω-gliadins 2, 3 and 4 were found in cultivars Letaba and Nantes. In T4, SST 66, PAN 3349 and Molen banding combinations of ω-gliadins 2, 3 and 5 were found. ω-Gliadin banding combinations of bands 1, 2 and 4 were found in cultivars Betta DN, Harts, Oom Charl and Tugela DN.

The α-gliadin banding patterns of cultivars Adam Tas, Betta, PAN 3342 and PAN 3349 were very similar to one another, all containing the α-gliadin combination of bands 3 and 5. The α-gliadin patterns of cultivars Betta DN and Inia were similar to one another in containing the α-gliadin banding pattern combination of bands 2 and 7. Cultivars Adam Tas, Marico, Molen, PAN 3235 and SST 66 all contained the banding combination of α-gliadin bands 1 and 5.

The β-gliadin banding patterns of Betta, Gamka, Karee, Limpopo, Molen, PAN 3342, PAN 3349, SST 66, Tugela, Tugela DN and T4 were similar in containing β-gliadin bands 3 and 4. Cultivars Gamtoos, Harts, Kariega, Letaba, Oom Charl and PAN 3211 all contained β-gliadin bands 3 and 5. Gariep, Hugenoot, Molopo, Nantes and Wilge were all similar in containing β-gliadin bands 1 and 4.

γ-Gliadin banding patterns for cultivars Gamtoos, Gamtoos DN, Harts, Oom Charl, PAN 3349, Tugela and Tugela DN were all similar in containing γ-gliadin bands 4 and 5 and most of them also band 1. Cultivars Inia, Kariega, Karee and Scheepers were all similar in containing γ-gliadin bands 2 and 5.

The cultivars Adam Tas, Palmiet, SST 86 and T4 were all similar in containing ω-gliadin banding combinations of bands 1, 2 and 3. ω-Gliadin bands 1, 3 and 4 were all found in cultivars Betta, Gariep, Karee and Kariega. Cultivars Betta DN, Flamink, Gamka, Gamtoos, Gamtoos DN, Hugenoot, Inia, Kariega, Molen, Molopo, PAN 3211, PAN 3232, PAN 3349, Scheepers, Tugela DN and T4 all contained at least three of the ω-bands 7, 8, 9 and 10. Betta DN, Gamka, Gamtoos DN, Oom Charl and PAN 3342 all contained ω-gliadin bands 2, 4 and 5. Letaba and Nantes were similar to one another in both containing ω-gliadin bands 2, 3 and 4. Cultivars Molen, PAN 3349, SST 66 and T4 all contained ω-gliadin bands 2, 3 and 5. Betta DN, Harts, Oom Charl and Tugela DN were similar to one another in all containing ω-gliadin bands 1, 2 and 4.

Genetic distance analysis

According to the results obtained from the calculation of the D(genetic distances)- and F(coefficient of similarity)-values for gliadins with the method of Nei and Li (1979) (Table 4.38) high coefficients of similarity, F>0.65, (65 percent) were found between Gamtoos and Gamtoos DN; Hugenoot and Gariep; Karee and Betta;Kariega and Karee; Nantes and Gariep; PAN 3211 and Gamtoos, PAN

3349 and Gamka; PAN 3349 and Molen; PAN 3349 and Nantes; Scheepers and Chinese Spring; SST 66 and Chinese Spring; SST 66 and PAN 3349; SST 66 and Scheepers; SST 86 and Chinese Spring; Tugela and PAN 3342; Tugela and PAN 3349; and Tugela DN and PAN 3349. This means in effect that these cultivars were more than 65 percent genetically similar.

The genetic similarities were especially high, F>0.7, (70 percent) for Gamtoos and Gamtoos DN; Karee and Betta; Gamtoos and PAN 3211; Tugela and Nantes; and PAN 3349 and SST 66. Corresponding values of small genetic distances was found when the genetic distances was calculated.

Low coeffisients of similarity, F<0.3, (30 percent) were found for the cultivars Gamka and Adam Tas; Gamtoos and Belinda; Gamtoos DN and Belinda; Harts and Belinda; Kariega and Adam Tas; Letaba and Hugenoot; Limpopo and Gariep; Marico and Betta DN; Marico and Flamink; Molen and Belinda; Molopo and Belinda; Nantes and Limpopo; Oom Charl and Belinda; Oom Charl and Flamink; Oom Charl and Gariep; Oom Charl and Hugenoot; Oom Charl and Limpopo; Palmiet and Limpopo; Palmiet and Molopo; PAN 3211 and Belinda; PAN 3232 and Belinda; Tugela DN and Belinda; and T4 and Gamtoos DN.

Especially low genetic similarities, F<0.2, (20 percent) was found for the cultivars Belinda and Gamtoos; Belinda and Molopo; Belinda and Oom Charl; Belinda and PAN 3211; Belinda and PAN 3232; and for Flamink and Oom Charl.

Dendograms

When the pairwise analysis of Nei and Li (1979) was shown as a dendogram, it became clear that a high degree of similarity exists between the cultivars (Figure 4.3). There is however still a high enough degree of difference between the different cultivars to enable the breeder to distinguish clearly between the different cultivars (Table 4.37). To confirm these results, a parsimony analysis was done, where bootstrapping was done to create a 100 different dendograms.

In the end one single representative dendogram was drawn, which confirmed the results as shown in Figure 4.3.

4.2 Discussion

The use of gliadin electrophoregrams for the accurate identification of wheat cultivars requires a systematic nomenclature of the electrophoregram bands. The easiest way to note intercultivar differences is to examine the presence or absence of specific bands by examining the patterns on the electrophoretic gels. Patterns for most cultivars are quite distinct (Bushuk & Zillman, 1978).

Various cultivars contained bands not included in the original nomenclature: Belinda, Gariep, Letaba, Molen, Molopo, Nantes, PAN 3211, PAN 3235, PAN 3342, PAN 3349, Tugela and T4 contained a band located between γ-gliadin 5 and ω-gliadin 1, Betta DN, Flamink, Gamtoos, Harts, Letaba, Molopo, PAN 3232, PAN 3235, PAN 3342 and Wilge contained a band located between β-gliadin band 5 and γ-gliadin band 1, while Karee and T4 contained a band located between α-gliadin band 7 and β-gliadin band 1 and Gamtoos DN contained two bands in the region between β-gliadin 5 and γ-gliadin 1.

Because of the inability of the nomenclature system used to accommodate the above mentioned gliadin bands, it is recommended to use a modification of the present system. It is suggested that in the modified system, the different gliadin zones should follow each other directly without open zones between the α-, β-, γ- and ω-gliadin zones respectively. By doing this all the bands will be included in the nomenclature.

Not much similarity was found between cultivars Betta and Betta DN. β-Gliadin band 4, γ-gliadin band 5 and ω-gliadin bands 1, 4 and 5 corresponded to one another. Gamtoos and Gamtoos DN were similar for α-gliadin bands 5, β-gliadin

bands 3 and 5, γ-gliadin bands, 4 and 5, as well as ω-gliadin bands 2, 5, 6, 9 and 10. A relatively high degree of similarity were therefore found between these two cultivars. α-Gliadin band 5, β-gliadin bands 3 and 4, γ-gliadin bands 4 and 5, as well as ω-gliadin bands 4, 8 and 9 were found in both Tugela and Tugela DN. These results was confirmed between the data obtained by the methods of Nei and Li (1979).

The data obtained by the methods of Nei and Li (1979) suggested that enough differences occurred between the different cultivars in order to be able to distinguish between them by using their gliadin banding patterns, but that the level of genetic variation between the different cultivars was very low. The occurance of high levels of inbreeding is typical of recombinant populations and it is therefore impossible to form more groupings in a population with such a high level of inbreeding. No cultivar was found amongst those tested that differed statistically significant from the rest.

In spite of the high degree of genetic similarity obtained by using the method of Nei and Li (1979), major differences in the gliadin banding patterns of all the wheat cultivars that was studied suggested that gliadins can be used to successfully distinguish between different wheat cultivars. The repeatability for each cultivar between the different replications was very high. Therefore it could be assumed that gliadin electrophoregrams are stable within one cultivar for different replications. As a result of this, gliadin banding patterns can be used as a classification system for wheat varieties.

Whatever the basic explanations of these relationships, research has led us to practical conclusions. The electropherograms of gliadin proteins allowed an identification of the wheat cultivars examined, since each patttern was different for each cultivar (Yupsanis & Moustakas, 1988; Harsch *et al*, 1992). The extensive heterogeneity of gliadin electrophoretic composition combined with the stability of the patterns in response to environmental factors provides adequate

distinction of cultivars through the electrophoregrams (Sapirstein & Bushuk, 1985).

Many practical applications have stemmed from the use of electrophoresis to characterise and compare genotypes. The fact that these are based on some understanding of the genetic control and heritability of the proteins enhances their reliability (Khan *et al*, 1990).

It is possible that there are some components which are correlated to quality data, because of a genetic linkage between the genes that code gliadin bands with the genes coding for gluten strength, whereas some other components impart quality because of particular functional properties (Wrigley, 1982).

As gliadins are used internationally for cultivar identification, it may also be used in determining genetic distances between cultivars. In the germplasm we used, it became clear that a high degree of intercrossing and inbreeding has occurred, and that for gliadins, there is very little genetic variation. It sounds the alarm for South African breeders that the genetic base is becoming dangerously small, and that the continuous use of reliable and trusted wheat cultivars in crossing blocks should be discontinued in favour of new germplasm from totally different sources.

There should be a coordinated effort to achieve this goal. Genetic distance analysis should also be extended to DNA fingerprinting, which could give a bigger picture.

Chapter 5

Conclusion

The use of gliadins in cultivar identification is a well-known practice used in many countries, such as France and Italy (Autran, 1995), the USA and Canada (Lookhart, Marchylo, Mellish, Khan, Lowe & Seguin, 1995), as well as Australia (Gore & Sutton, 1995). The use of gliadin electrophoregrams offer a clear classification system, as well as a system from which quality characteristics can be determined.

Gliadin banding patterns of the cultivars tested were not identical for any two cultivars. Large differences existed even between near-isogenic lines. This study therefore showed that it is possible to distinguish between near-isogenic lines with the help of gliadin banding patterns. Cultivar identification and purity determination with gliadin banding patterns is sufficient to use as an only method of identification. Gliadin banding patterns also show more genetic variation than HMW and LMW glutenin banding patterns.

According to the genetic distance and parsimony analyses used in this study, high levels of inbreeding are present in South African wheat cultivars. It is therefore neccessary to use new genetic material from other sources in order to broaden the genetic base of South African wheat cultivars.

In summary, the use of protein markers, such as gliadins, provides the basis for a rational, straightforward and practical system of registration of genetic cereal resources. The system described in this study provides a means for designating genome and genotypic formulas based on protein components. These formulas reflect the genus, specie, cultivar and line or biotype of a sample and are accordingly rich in genetic and phylogenetic information. The significance of this

information will increase in the future as more research is done on the topic. Registration of cultivars and biotypes in the form of protein formulas lends itself to computer storage and retrieval of information. This can be especially important in programs of germplasm conservation for wheat (Sapirstein & Bushuk, 1985).

Although only six genes are described with the use of gliadin electrophoresis, it is assumed that these six genes are representative of the whole genome and that they are therefore sufficient for the use of cultivar identification. In addition to this method, DNA-procedures can also be used to give an even clearer description of the different cultivars.

Chapter 6

Summary

- The aim of this study was to identify the gliadin subunit composition of South African wheat cultivars and to use these banding patterns to determine genetic relationships between cultivars.

- Due to its solubility in 70 percent ethanol, gliadin was extracted from the wheat kernels and by using a simplified one-dimensional gel electrophoresis procedure the different gliadin subunits separated according to their relative mobilities on the gel. The gliadins were then analysed with the help of the "Geldoc"-system to identify their banding patterns according to the nomenclature of Konarev *et al* (1979). Further analyses was made with the help of the "Phylip"-system.

- The results were as follows:
  - α-Gliadin combinations of bands 3 and 5, bands 1 and 5, bands 2 and 7 and bands 5 and 7 were found frequently. In some of the cultivars novel bands sometimes occurred between the existing band combinations.
  - β-Gliadin combinations of bands 3 and 4, bands 3 and 5, bands 1, 3 and 5 and bands 1 and 4 were found frequently. Novel bands also occurred between the existing band combinations.
  - γ-Gliadin combinations of bands 1, 4 and 5 and bands 2 and 5 were found frequently. Novel bands sometimes occurred between these bands.
  - ω-Gliadin combinations of bands 1, 2 and 3, bands 7, 8, 9 and 10, bands 2, 4 and 5, bands 2, 3 and 4, bands 2, 3 and 5 and bands 1, 2 and 4 were found frequently. In some cases other bands occurred between these combinations and in some cases one of the bands were missing.

- Bands not included in the nomenclature system used also occurred at the following positions: between the α7- and β1-positions, between the β5- and γ1-positions, as well as between the γ5- and ω1-positions.
- In the genetic distance analyses it was found that high degrees of similarity and low genetic distances exist between the cultivars tested.

- The conclusions of this study are:
  - It is possible to distinguish between near-isogenic lines with the help of gliadin banding patterns, as was seen in the cases of Betta, Betta DN, Gamtoos, Gamtoos DN, as well as Tugela and Tugela Dn.
  - Gliadin banding patterns can be used as a means of cultivar identification on its own. Sufficient genetic information can be obtained from the banding patterns for this purpose, but if more information on quality is needed it is best to use the gliadin classification system in combination with HMW and LMW glutenin classification systems.
  - A dangerously high level of intercrossing and inbreeding has occurred in the South African wheat cultivars when measured by gliadin patterns. No genetically unique cultivars are found and it is therefore suggested that wheat cultivars from other genetic systems should in the future be used as parents in breeding programmes in order to lower the high level of inbreeding.

Opsomming

- Die doel van die studie was om die gliadien samestelling van Suid-Afrikaanse koring kultivars te bepaal en om hierdie bandpatrone te gebruik om die genetiese verwantskappe tussen die kultivars te bepaal.

- As gevolg van die oplosbaarheid van gliadiene in 70 persent etanol was dit moontlik om die gliadiene uit koring te ekstraheer. Die gliadiene is daarna met behulp van 'n eenvoudige een-dimensionele gel elektroforese prosedure

geskei. Die verskillende gliadien subeenhede skei op grond van hul relatiewe mobiliteite op die gel en is daarna geanaliseer met behulp van die "Geldoc"-sisteem om hul bandpatrone te identifiseer met behulp van die nomenklatuur van Konarev *et al* (1979). Verdere analises is met behulp van die "Phylip"-program gedoen.

- Die resultate was as volg:
  - α-Gliadien kombinasies van bande 3 en 5, bande 1 en 5, bande 2 en 7 en bande 5 en 7 het gereeld voorgekom. In sommige van die kultivars het nuwe bande soms tussen die bestaande bandkombinasies voorgekom.
  - β-Gliadien kombinasies van bande 3 en 4, bande 3 en 5, bande 1, 3 en 5 en bande 1 en 4 het dikwels voorgekom. In sommige van die kultivars het nuwe bande soms tussen die bestaande bandkombinasies voorgekom.
  - γ-Gliadien kombinasies van bande 1, 4 en 5 en bande 2 en 5 het dikwels voorgekom. Nuwe bande het somtyds tussen die bandkombinasies voorgekom.
  - ω-Gliadien kombinasies van bande 1, 2 en 3, bande 7, 8, 9 en 10, bande 2, 4 en 5, bande 2, 3 en 4, bande 2, 3 en 5 en bande 1, 2 en 4 het algemeen voorgekom. In sommige gevalle het ander bande tussen die bande voorgekom en in geval van sommige van die kombinasies was een van die bande soms afwesig.
  - Bande wat nie met behulp van die oorspronklike nomenklatuursisteem benoem kon word nie het op die volgende posisies voorgekom: tussen die α7- en β1-posisies, tussen die β5- en γ1-posisies en tussen die γ5- en ω1-posisies.
  - By genetiese afstandsbepalings is gevind dat hoë mates van genetiese ooreenstemming en klein genetiese afstande tussen die verskillende kultivars wat getoets is voorkom.

- Die gevolgtrekkings van die studie was die volgende:

- Dit is moontlik om met behulp van gliadien bandpatrone tussen naby-isogeniese lyne te onderskei.
- Gliadien bandpatrone kan op hul eie vir kultivar identifikasie gebruik word. Genoegsame genetiese inligting kan verkry word vanaf die gliadien bandpatrone vir hierdie doel, maar as meer inligting op kwaliteitsaspekte verkry moet word is dit beter om die gliadien identifikasie sisteem te kombineer met die van HMW en LMW gluteniene.
- 'n Gevaarlike hoë vlak van inteling kom voor by die Suid-Afrikaanse koring kultivars as waarnemings op grond van gliadien bandpatrone gemaak word. Geen geneties unieke kultivars is gevind nie en dit word daarom voorgestel dat koring kultivars van ander genetiese oorsprong in die toekoms as ouers in teelprogramme gebruik moet word om sodoende die hoë vlakke van inteling te verhoed.

References

ANDERSON, O.D., GREEN, F.C. & LITTS, J.C. 1990. Structure of the alpha-gliadin gene family from the bread wheat cultivar Cheyenne. In: Gluten proteins 1990. Eds. W. Bushuk & R. Tkachuk. American Association of Cereal Chemists, Inc. St. Paul, Minnesota. pp 640-645.

AUTRAN, J.C. 1995. Wheat identification in Europe. In: Identification of food-grain varieties. Ed. C.W. Wrigley. American Association of Cereal Chemists, Inc. St. Paul, Minnesota. pp 175-200.

AUTRAN, J.C. & BOURDET, A. 1973. Nouvelles donnees permettantl'exploitation de l'heterogeneite electrophoretique des gliadines du grain de ble en vue d'une identification varietale. *C.R. Acad. Sci. Paris. Ser. D.* 277:2081-2084.

AUTRAN, J.C. & FEILLET, P. 1987. Genetic and technological basis of protein quality for durum wheat in pasta. In: Protein evaluation in cereals and legumes. Ed. V. Pattakau. Commission of the European Communities. pp 59-71.

AUTRAN, J.C., LAIGNELET, B. & MOREL, M.H. 1987. Characterisation and quantification of low molecular weight glutenins in durum wheat. *Biochemie* 69:699-711.

BIETZ, J.A. 1979. Recent advances in the isolation and characterisation of cereal proteins. *Cereal Foods World* 24:199-206.

BIETZ, J.A. 1985. High performance liquid chromatography: How proteins look in cereals. *Cereal Chem.* 63:201-212.

BIETZ, J.A., HUEBNER, F.R., SANDERSON, J.E. & WALL, J.S. 1977. Wheat gliadin homology revealed through N-terminal amino acid sequence analysis. *Cereal Chem.* 54(5):1070-1083.

BRANLARD, G. & DARDEVET, M. 1985. Diversity of grain proteins and bread wheat quality. 1. Correlation between gliadin bands and flour quality characteristics. *Journal of Cereal Science* 3:329-343.

BROWN, J.W.S. & FLAVELL, R.B. 1981. Fractionation of wheat gliadin and glutenin subunits by two dimensional electrophoresis and the role of group 6 and group 2 chromosomes in gliadin synthesis. *Theor. Appl. Genet.* 59:349-359.

BUSHUK, W. & ZILLMAN, R.R. 1978. Wheat cultivar identification by gliadin electropherograms. 1. Apparatus, method and nomenclature. *Can. J. Plant Sci.* 58:505-515.

CLUSKEY, J.E., TAYLOR, N.W., CHARLEY, H. & SENTI, F.R. 1961. Electrophoretic composition and intrinsic viscosity of glutens from different varieties of wheat. *Cereal Chem.* 38:325-331.

CHUN, J.U., YU, X.M. & GRIFFITH, M. 1998. Genetic studies of antifreeze proteins and their correlation with winter survival in wheat. *Euphytica* 102:219-226.

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

COOKE, R.J. & DRAPER, S.J. 1986. The identification of wild oats species by electrophoresis. *Seed Sci. Technol.* 14:157-167.

CROW, M.J.A. & ROTHFUS, J.A. 1968. Chromatography of proteins from wheat gluten on polyacrylamide gel. *Cereal Chem.* 45:412-221.

CZARNECKI, E.M. & LUKOW, O.M. 1992. Linkage of stem rust resistance gene Sr33 and the gliadin (Gli-D1) locus on chromosome 1DS in wheat. *Genome* 35:565-568.

DOEKES, G.J. 1973. Inheritance of gliadin composition in bread wheat, Triticum aestivum L. *Euphytica* 22:28-34.

DU CROS, D.L., JOPPA, L.R. & WRIGLEY, C.W. 1983. Two dimensional analysis of gliadin protein associated with quality in durum wheat: Chromosome location of genes for their synthesis. *Theor. Appl. Genet.* 66:297-302.

ELIASSON, A.C. & LARSSON, K. 1993. Cereals in Breadmaking. A molecular colloidal approach. Marcel Dekker, Inc. New York.

ELTON, G.A.H. & EWART, J.A.D. 1960. Starch gel electrophoresis of wheat proteins. *Nature* 187:600-601.

EWART, J.A.D. 1967. Amino acid analysis of glutenins and gliadins. *J. Sci. Food Agr.* 18:111-119.

GORE, P.J. & SUTTON, K.H. 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 209-220.

GRAVELAND, A. & HENDERSON, M.H. 1987. Structure and functionality of gluten proteins. In: Proceedings of the third International Workshop on gluten proteins. Eds. R. Lasztity & F. Bekes. World Scientific. Singapoer. pp 238-246.

HAMAUZU, Z.I., ARAKAWA, T. & YONEZAWA, D. 1972. Molecular weights of glutenin and gliadin polypeptides estimated by SDS polyacrylamide gel electrophoresis. *Agr. Biol. Chem.* 10:1829-1830.

HAMES, B.D. 1990. One-dimensional polyacrylamide gel electrophoresis. In: Gel Electrophoresis of Proteins. A Practical Approach. Second Edition. Eds. B.D. Hames & D. Rickwood. IRL Press, Oxford. pp 1-147.

HARSH, S., GüNTHER, T., KLING, C.I., ROZYNEK, B. & HESEMANN, C.U. 1992. Characterisation of spelt (Triticum spelta L.) forms by gel electrophoretic analyses of seed storage proteins. I. The gliadins. *Theor. Appl. Genet.* 94:52-60.

JACKSON, E.A., HOLT, L.M. & PAYNE, P.I. 1983. Characterisation 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. *Theor. Appl. Genet.* 66:29-37.

JACKSON, E.A., MOREL, M.H., SONTAG-STROHM, T. BRANLARD, G., METAKOVSKY, E.V. & REDAELLI, R. 1996. Proposal for combining the classification systems of alleles of *Gli*-1 and *Glu*-3 loci in bread wheat (*Triticum aestivum* L.) *Journal of Genetics and Breeding* 50:321-336.

JOHANSSON, E. 1996. Qualitative evaluation of D-zone omega-gliadins in wheat. *Plant Breeding* 15:57-62.

JONES, S.S., DVORAK, J. & QUALSET, C.O. 1990. Linkage relationships of *Gli-D*1, *Rg*2 and *Lr*21 on the short arm of chromosome 1D in wheat. *Genome* 33:937-940.

JOPPA, L.R., KHALIL, K & WILLIAMS, N.D. 1983. Chromosomal location of genes for gliadin polypeptides in durum wheat *Triticum turgidum* L. *Theor. Appl. Genet.* 64:289-293.

KASARDA, D.D., NIMMO, C.C. & KOHLER, G.O. 1971. Proteins and the amino acid composition of wheat fractions. In: Wheat Chemistry and Technology. Ed. Y. Pomeranz. American Association of Cereal Chemists, St. Paul, Minnesota. pp 227-299.

KHAN, K., FIGUEROA, J. & CHAKRABORTY, K. 1990. Relationship of gluten protein composition to breadmaking quality of HRS wheat grown in North Dakota. In: Gluten Proteins 1990. Eds. W. Bushuk & R. Thackuk. American Association of Cereal Chemists. St. Paul, Minnesota. pp 81-95.

KHELIFI, D., BRANLARD, G. & BOURGOIN-GRENECHE, M. 1992. Division of some D-zone omega gliadins of bread wheats as revealed by two-step A-PAGE/SDS-PAGE technique. *Journal of Genetics and Breeding* 46:351-358.

KONAREV, V.G. 1973. The principle of protein markers in genome analysis. *Tr. Prikl. Bot. Genet. Sel.* 49(3):46-53.

KONAREV, V.G., GAVRILYUK, I.P., GUBAREVA, N.K. & PENEVA, T.I. 1979. Seed proteins in genome analysis, cultivar identification and documentation of cereal genetic resources: A review. *Cereal Chem.* 56(4):272-278.

KOSMOLAK, F.G., DEXTER, J.E., MATSUO, R.R., LEISLE, D., MARCHYLO, B.A. 1980. A relationship between durum wheat quality and gliadin electropherograms. *Can. J. Plant Sci.* 60:427-432.

LADIZINSKY, G. & HYMOWITZ, T. 1979. Seed protein electrophoresis in taxonomic and evolutionary studies. *Theor. Appl. Genet.* 54:145-151.

LáSZTITY, R. The Chemistry of Cereal Proteins. CRC Press, Inc. Florida, 1986.

LOOKHART, G.L. & FINNEY, K.F. 1984. Polyacrylamide gel electrophoresis of wheat gliadins: the effect of environment and germination. *Cereal Chem.* 61:496-499.

LOOKHART, G.L., MARCHYLO, B.A., MELLISH, V.J., KHAN, K, LOWE, D.B. & SEGUIN, L. 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.

LOOKHART, G.L. & WRIGLEY, C.W. 1995. Variety identification by electrophoretic analysis. In: Identification of Food-Grain Varieties. Ed. C.W. Wrigley. pp 55-71.

MAARTENS, H. 1997. Low molecular weight glutenin subunit composition of South African wheat cultivars. M.Sc. Thesis, UOFS, Bloemfontein.

MAUNDER, A.B., 1992. Identification of useful germplasm for practical plant breeding programs. In: Plant breeding in the 1990s. Eds. H.T. Stalker & J.P. Murphy. C.A.B.International, United Kingdom, pp 147-168.

MCINTOSH, R.A., HART, G.E. & GALE, M.D. 1994. Catalogue of gene symbols for wheat. Supplement. *Annual Wheat Genetics Newsletter* 40:362-375.

MCKINNON, G.E. & HENRY, R.J. 1995. Control of gene expression for the genetic engineering of cereal quality. *Journal of Cereal Science* 22:203-210.

METAKOVSKY, E.V. 1991. Gliadin allele identification in common wheat. II. Catalogue of gliadin alleles in common wheat. *Journal of Genetics and Breeding* 45:324-344.

METAKOVSKY, E.V., AKHMEDOV, M.G. & SOZINOV, A.A. 1986. Genetic analysis of gliadin-encoding genes reveals gene clusters as well as single remote genes. *Theor. Appl. Genet.* 73:278-285.

METAKOVSKY, E.V., NOVOSELSKAYA, A.Y., KOPUS, M.M., SOBKO, T.A. & SOZINOV, A.A. 1984. Blocks of gliadin components in winter wheat

detected by one dimensional polyacrylamide gel electrophoresis. *Theor. Appl. Genet.* 67:559-568.

MOSLETH, E. & UHLEN, A.K. 1990. 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 & R. Tkachuk. American Association of Cereal Chemists. St. Paul, Minnesota. pp 112-128.

NEI, M. & LI, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA* 76:5269-5273.

NIETO-TALADRIZ, M.T. & CARRILLO, J.M. 1996. Complexity of *Gli-A3* locus in bread wheat. *Plant Breeding* 115:192-194.

OSBORNE, T.B. 1907. The proteins of the wheat kernel. Carnegie Institute. Washington D.C. Publication no. 84.

PATEY, A.L. & WALDRON, N.M. 1976. Gliadin proteins from "Maris Widgeon" wheat. *J. Sci. Food Agric.* 27: 838-842.

PAYNE, P.I., HOLT, L.M., JACKSON, E.A. & LAW, C.N., 1984a. Wheat storage proteins: their genetics and potential for manipulation by plant breeding. *Philos. Trans. Roy. Soc. Lond. B* 304:359-371.

PAYNE, P.I., HOLT, L.M., JARVIS, M.G. & JACKSON, E.A. 1985. Two-dimensional fractionation of the endosperm proteins of bread wheat (Triticum aestivum L.): Biochemical and genetic studies. *Cereal Chem.* 62:319-326.

PAYNE, P.I., HOLT, L.M., LAWRENCE, G.J. & LAW, C.N. 1981. The genetics of gliadin and glutenin, the major storage proteins of the wheat endosperm. *Qualitas Pl. Plant Food Hum. Nutr.* 31:229-241.

PAYNE, P.I., JACKSON, E.A., HOLT, L.M. & LAW, C.N. 1984b. Genetic linkage between endosperm storage protein genes on each of the short arms of chromosome 1A and 1B in wheat. *Theor. Appl. Genet.* 67:235-243.

PAYNE, P.I., SEEKINGS, J.A., KAUR, H. KRATTIGER, A.F. & ROGERS, W.J. 1990. Contribution of allelic variation of glutenin subunits and gliadins to breadmaking quality. In: Gluten Proteins 1990. Eds. W. Bushuk & R. Tkachuk. American Association of Cereal Chemists. St. Paul, Minnesota. pp 504-511.

POEHLMAN, J.M. 1986. Breeding Field Crops. Third Edition. AVI Publishing Company, Inc. Conneticut.

POGNA, N.E., REDAELLI, R. VACANO, P., BIANCARDI, A.M., PERUFFO, A.D.B., CURIONI, A., MEVAKOVSKY, E.V. & PAGLIARICCI, S. 1995. Production and genetic characterisation of near-isogenic lines in the bread-wheat cultivar Alpe. *Theor. Appl. Genet.* 90:650-658.

POPINEAU, Y. & PINEAU, F. 1987. Investigation of surface hydrophobicities of purified gliadins by hydrophobic interaction chromatography, reversed-phase high performance liquid chromatography and apolar ligand binding. *Journal of Cereal Science* 5:215-221.

PSHENICHNIKOVA, T.A. & MAYSTRENKO, O. 1995. Inheritance of genes coding for gliadin proteins and glume colour introgressed into *T. aestivum* from a synthetic wheat. *Plant Breeding* 114:501-504.

PUSHMAN, F.M. & BINGHAM, J. 1976. The effects of a granular nitrogen fertilizer and a foliar spray of urea on the yield and bread-making quality of 10 winter wheats. *J. Agric. Sci.* 87:281-292.

REDDY, P. & APPELS, R. 1990. Structure and expression of genes coding for the glutenin and gliadin proteins in wheat. In: Gluten proteins 1990. Eds. W. Bushuk & R. Tkachuk. American Association of Cereal Chemists. St. Paul, Minnesota. pp 520-526.

RODRIGUEZ-QUIJANO, M. & CARRILLO, J.M. 1996. Linkage maps of prolamin loci Gli-D4 and Gli-D5 in hexaploid wheat. *Plant Breeding* 115:189-191.

SAPIRSTEIN, H.D. & BUSHUK, W. 1985. Computer-aided analysis of gliadin electropherograms. II. Wheat cultivar identification and classification comparison. *Cereal Chem.* 62:377-392.

SCHEPERS, J., KEIZER, L.C.P. & KOLSTER, P. 1993. The relation between high molecular weight glutenin subunits, bread-making quality and agronomic properties of winter wheat. *Cereal Research Communications* 21:289-237.

SHEWRY, P.R., FIELD, J.M. & TATHAM, A.S. 1987. The structures of cereal seed storage proteins. In: Cereals in a European context. First European Conference on Food Science and Technology. Ed. I.D. Morton. New York. pp 421-437.

SHEWRY, P.R. & MIFLIN, B.J. 1985. Seed storage proteins of economically important cereals. *Adv. Cereal Sci. Technol.* 7:1-84.

SHEWRY, P.R., TATHAM, A.S., FORDE, J., KREIS, M. & MIFLIN, B.J. 1986. The classification and nomenclature of wheat gluten proteins: a reassessment. *Journal of Cereal Science* 4:97-106.

SHEPHERD, K.W. 1988. Genetics of wheat endosperm proteins – in retrospect and prospect. In: Proc. Of the seventh Internat. Wheat Gen. Symp. (Vol.2) Eds. T.E. Miller & R.M.D. Koebner. Institute of Plant Science Research: Cambridge. pp. 919-931.

SINGH, N.K. & SHEPHERD, K.W. 1988. Linkage mapping of genes controlling endosperm storage proteins in wheat. 1. Genes on the short arms of group 1 chromosomes. *Theor. Appl. Genet.* 75:628-641.

SINGH, N.K., SHEPHERD, K.W. & CORNISH, G.B. 1991. A simplified SDS-PAGE procedure for separationg LMW subunits of glutenin. *Journal of Cereal Science* 14:203-208.

SOZINOV, A.A. & POPERELYA, F.A. 1980. Genetic classification of prolamines and its use for plant breeding. *Ann. Technol. Agric.* 29(2), 229-245.

SUTKA, J. 1994. Genetic control of frost tolerance in wheat (Triticum aestivum L.). *Euphytica* 77:277-282.

TATHAM, A.S., MIFLIN, B.J. & SHEWRY, P.R. 1985. The beta-turn conformation in wheat gluten proteins: Relationship to gluten elasticity. *Cereal Chem.* 62:405-412.

TATHAM, A.S., SHEWRY, P.R. & FIELD, J.M. 1990. Conformational studies of the repetitive domains of the cereal prolamines. In: Gluten proteins 1990. Eds. W. Bushuk & R. Tkachuk. Americal Association of Cereal Chemists. St. Paul. Minnesota. pp 376-388.

WOYCHIK, J.H., BOUNDY, J.A. & DIMLER, R.J. 1961. Starch gel electrophoresis of wheat gluten proteins with concentrated urea. *Arch. Biochem. Biophys.* 94:477-482.

WRIGLEY, C.W. 1982. The use of genetics in understanding protein composition and grain quality in wheat. *Qual. Plant. Foods Hum. Nutr.* 31:205-227.

WRIGLEY, C.W. 1992. Identification of Cereal Varieties by Gel Electrophoresis of the Grain Proteins. In: Modern Methods of Plant Analysis New Series, Volume 14. Seed Analysis. Eds. H.F. Linskens & J.F. Jackson. Springer-Verlag, Berlin Heidelberg. pp 17-41.

WRIGLEY, C.W. & BIETZ, J.A. 1988. Proteins and Amino Acids. In: Wheat Chemistry and Technology, Volume I. Ed. Y. Pomeranz. American Association of Cereal Chemist, Inc. St. Paul, Minnesota. pp 159-275.

WRIGLEY, C.W., BUSHUK, W. & GUPTA, R. 1996. Nomenclature: Establishment of a common gluten language. In: Gluten '96. Proceedings of the 6th International Gluten Workshop. Ed. C.W. Wrigley. Cereal Chemistry Division. North Melbourne. pp 403-407.

WRIGLEY, C.W., DU CROS, D.L., ARCHER, J.J., DOWNIE, P.G. & ROXBOURGH, C.M. 1980. The sulphur content of wheat-endosperm proteins and its relevance to grain quality. *Aust. J. Plant Physiol.* 7:755.

WRIGLEY, C.W. & SHEPHERD, K.W. 1973. Electrofocusing of grain proteins from wheat genotypes. *Ann. N.Y. Acad. Sci.* 209:154-161.

YUPSANIS, T. & MOUSTAKAS, M. 1988. Relationship between quality, colour of glume and gliadin electropherogram in Durum wheat. *Plant Breeding* 101:30-35.

Adam Tas

Appendix Table 1 Migration distances of the gliadin subunits of Adam Tas.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
8	26	28	6	16	10	6
21	36	39	13	26	28	16
50	48	46	26	32	42	30
70	63	63	40	68	60	38
106	77	78	48	83	87	61
115	91	104	62	103	93	90
143	146	111	83	127	105	105
159	177	115	99	140	126	128
180	206	174	103	171	143	153
202	231	198	111	184	154	190
208	247	220	119	200	167	201
227	258	238	143	228	206	232
239	279	262	159	250	263	261
255	289	271	167	266	278	285
275	307	278	181	286	301	303
285	323	303	190	305	311	313
302	342	307	200	311	323	328
321	360	319	223	329	362	346
337	378	335	246	353	380	363
380	386	350	265	382	389	
392	396	353	274	388		
399		368	289			
		382	307			
			321			
			367			
			373			
			376			
			382			
			392			

Belinda

Appendix Table 2 Migration distances of the gliadin subunits of Belinda.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
10	16	42	28	18	24	12
24	24	46	40	24	87	24
33	28	59	56	86	102	85
47	43	67	70	100	111	98
63	63	92	80	111	123	114
75	75	105	101	122	133	141
98	83	124	107	151	153	153
103	115	181	114	194	194	193
112	123	187	145	238	238	203
156	134	192	194	257	258	226
171	156	197	204	276	276	245
206	191	220	221	286	287	267
230	203	240	241	295	294	274
256	214	267	268	317	317	292
283	230	275	276	366	352	307
293	244	291	291	379	380	317
315	257	307	308			339
329	285	317	319			353
342	295	353	359			369
352	315	367	370			379
380	331	377	379			389
385	343	383	387			
390	353		396			
	369					
	383					
	391					

Betta

Appendix Table 3 Migration distances of the gliadin subunits of Betta.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
23	26	2	30	31	19	33
31	32	34	57	54	83	51
44	37	51	86	82	96	83
50	50	67	112	129	113	113
63	70	79	139	222	156	125
78	78	85	184	256	180	157
98	123	101	242	269	245	181
116	230	123	280	279	260	243
136	242	204	293	291	279	262
147	269	231	313	306	291	280
152	282	241	325	357	306	291
172	292	250	356		318	305
175	319	269	388		346	318
242	329	283			352	349
257	363	293			375	360
267	389	318				374
289		328				
304		364				
321		375				
346		385				
358		392				
373		398				
394						

Betta DN

Appendix Table 4 Migration distances of the gliadin subunits of Betta DN.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
26	27	37	57	26	24	8
44	43	67	89	85	69	67
66	68	126	133	97	99	79
99	116	166	224	101	145	98
115	123	231	242	126	157	109
121	156	243	262	156	181	122
154	178	249	270	182	212	146
174	194	272	280	244	234	165
204	247	277	297	260	246	183
216	259	283	307	280	273	234
245	271	297	358	291	281	245
258	298	320	379	303	295	273
269	312	323		317	305	281
301	326	367		378	318	295
306	348	371		386	326	307
324	359	388			367	320
333	385	396				369
347	399					385
358						
384						
398						

Chinese Spring

Appendix Table 5 Migration distances of the gliadin subunits of Chinese Spring

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
30	20	5	2	26	13	67
41	30	25	49	44	55	79
59	40	52	62	58	66	92
87	58	65	75	75	77	108
96	67	75	93	95	93	168
128	85	94	113	107	109	178
141	107	111	157	116	174	200
168	137	117	162	140	190	216
193	160	159	174	148	221	247
202	190	170	189	180	257	283
242	194	190	200	188	281	292
299	201	196	208	192	297	302
307	230	226	240	212	307	310
326	270	245	279	239	329	328
347	279	266	289	279	361	340
358	291	291	299	289	384	364
382	298	309	307	300		392
399	321	316	327	317		
	330	343	338	329		
	366	369	349	340		
	386	386	366	362		
			385	376		
			390			

Flamink

Appendix Table 6 Migration distances of the gliadin subunits of Flamink.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
22	43	43	24	13	25	13
28	58	89	91	25	68	28
33	65	96	101	75	109	75
58	85	135	117	109	113	108
66	98	159	127	158	152	144
118	105	182	159	182	230	197
142	140	196	182	206	270	232
145	200	204	206	274	281	272
155	208	230	226	281	313	285
186	228	271	245	311	326	309
243	249	281	283	321	340	321
269	270	288	295	340	363	341
295	281	314	314	369		369
308	287	323	321	374		375
315	314	329	350			385
339	322	373	376			
349	343	388	373			
359	358		382			
377	388					
385						
399						

Gamka

Appendix Table 7 Migration distances of the gliadin subunits of Gamka.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
43	42	17	35	62	34	40
46	87	21	108	80	66	54
62	125	43	127	126	87	67
86	179	63	144	181	124	78
124	188	86	178	190	156	87
131	233	124	233	224	180	99
159	252	160	274	259	205	128
188	273	179	289	273	243	138
193	284	194	308	304	277	161
243	295	205	324	331	290	166
265	315	232	362	361	320	197
293	329	255	380	383	364	252
305	369	275	393	390		269
312	380	285				291
337	393	292				307
349		325				315
362		382				352
384		393				384
390						390

Gamtoos

Appendix Table 8 Migration distances of the gliadin subunits of Gamtoos.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
16	55	40	73	47	12	48
36	75	49	83	85	24	76
48	85	67	88	121	47	118
75	129	76	108	138	84	126
84	145	83	128	156	119	187
128	153	102	147	182	168	210
147	162	149	161	206	182	246
162	190	160	185	216	206	252
192	206	183	189	250	248	265
206	249	200	237	263	263	287
249	272	224	250	287	285	303
263	291	237	274	311	301	309
272	295	256	302	321	309	320
291	299	264	305	352	319	329
301	320	274	322	379	356	359
320	336	281	380	384	380	380
337	349	301	396			386
349	380	314				
380	387	324				
386	398	379				
390		385				
399						

Gamtoos DN

Appendix Table 9 Migration distances of the gliadin subunits of Gamtoos DN.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
54	55	42	22	43	30	48
75	81	55	35	67	47	58
88	105	85	56	85	54	80
129	129	129	64	97	83	95
147	147	146	82	119	121	113
163	158	158	85	156	157	144
190	165	162	130	182	182	161
207	184	235	140	206	207	170
225	192	249	144	249	249	190
249	208	273	155	262	263	198
274	252	301	188	277	286	213
294	274	312	200	283	301	226
301	294	323	220	309	310	254
323	301	346	233	319	320	275
337	325	362	250	351	380	292
350	338	372	257	382	386	297
378	352	390	276	386		321
390	363	399	297	392		331
	382		212			342
	399		323			361
			352			370
			361			381
			390			390
			398			

Gariep

Appendix Table 10 Migration distances of the gliadin subunits of Gariep.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
14	67	14	55	16	16	18
54	127	55	74	101	72	91
73	136	75	126	109	79	103
126	162	118	213	158	97	115
139	233	123	226	180	131	124
158	246	194	234	242	204	160
162	255	219	255	258	242	184
235	266	243	265	286	267	244
256	284	250	277	294	285	256
285	293	268	292	307	303	265
295	298	279	345	320	316	286
303	316	295	357	353	344	295
318	332	317	378	376	357	328
343	342	369	385	382	366	358
353	363	384			374	386
369	368				379	
386	376				384	
395						

Harts

Appendix Table 11 Migration distances of the gliadin subunits of Harts.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
67	28	24	28	48	24	10
74	88	49	85	77	49	26
88	137	60	105	93	56	77
139	154	75	122	115	91	89
154	172	86	139	138	103	102
167	207	156	158	158	138	115
172	235	192	184	170	171	156
208	260	229	206	199	208	172
234	269	244	219	209	226	183
243	287	259	243	242	243	214
260	299	271	262	268	261	226
270	313	279	283	289	286	245
287	327	309	292	300	299	269
299	337	386	313	339	313	277
309	372	399	323	374	329	291
319	380		381	377	340	299
326	399			382	361	309
336				394	381	370
371					386	378
379						386
390						390
399						

Hugenoot

Appendix Table 12 Migration distances of the gliadin subunits of Hugenoot.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
31	47	34	30	43	34	32
49	72	43	43	80	72	37
73	84	73	79	101	90	73
85	164	97	92	129	108	85
97	218	110	107	164	153	107
138	245	150	150	246	164	115
143	265	217	176	265	174	123
165	290	283	224	289	180	166
183	310	317	249	300	254	175
248	328	349	289	348	275	254
259	349	376	311	387	292	267
269	374	399	330	396	301	292
291	385		349		317	317
304	399		375		346	347
317			388		370	388
339			396		383	394
348					388	
376					396	
382						
399						

Inia

Appendix Table 13 Migration distances of the gliadin subunits of Inia.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
28	20	26	8	12	30	10
54	62	55	68	57	45	27
64	91	83	79	74	67	48
71	97	91	91	91	89	67
81	117	99	105	124	103	75
91	122	119	117	142	166	87
99	132	149	138	150	181	96
129	167	172	145	192	219	106
194	179	180	196	215	245	113
245	192	201	200	223	266	119
257	212	222	220	245	298	142
270	221	243	244	286	310	204
289	227	281	289	297	326	254
299	244	288	301	311	344	277
309	255	301	325	327	384	300
314	288	310	346	344	390	325
329	295	325	364	378		341
347	309	356	368	390		376
375	326	370	374	396		383
386	345	375	390			390
	375	388				396
	388					

Karee

Appendix Table 14 Migration distances of the gliadin subunits of Karee.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
50	31	69	69	14	18	38
77	38	85	83	24	38	51
83	48	94	101	40	46	74
96	63	101	115	54	67	95
115	84	113	137	61	75	107
121	89	121	149	71	95	115
135	95	148	194	83	115	166
168	114	196	230	101	156	184
190	129	216	243	111	169	215
195	188	242	260	123	180	252
210	241	257	280	144	202	269
242	258	279	287	154	210	292
255	286	285	314	162	225	297
268	292	315	329	178	236	317
284	314	331	351	192	253	339
314	324	350	376	248	269	347
340	337	381	380	268	292	384
348	349			287	299	394
384	370			313	317	
	378			332	327	
	383			349	347	
	392			364	368	
				380	384	

Kariega

Appendix Table 15 Migration distances of the gliadin subunits of Kariega.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
38	28	19	21	16	20	14
55	47	33	39	24	55	41
63	71	53	53	42	71	57
74	82	73	66	57	100	66
91	128	80	83	66	107	73
101	164	103	129	73	122	80
133	188	114	153	82	153	87
147	210	120	188	97	168	103
178	239	129	191	111	178	107
191	262	153	242	117	187	127
199	285	166	265	125	204	151
241	310	176	285	145	229	162
249	315	187	294	153	251	210
264	325	190	313	168	263	223
286	336	227	338	188	274	248
291	346	249	349	214	297	260
316	380	265	384	221	318	269
336	392	274	392	227	335	294
347		292	396	238	347	314
383		316		265	365	334
390		324		284	381	346
		342		290	384	361
		363		306	396	376
		382		314		380
		385		335		387
		399		347		397
				362		
				384		

Letaba

Appendix Table 16 Migration distances of the gliadin subunits of Letaba.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
56	49	5	54	26	56	58
81	64	20	71	44	67	91
91	82	45	75	83	89	104
132	93	80	86	107	107	111
148	134	88	131	119	145	145
171	148	130	159	153	149	176
183	169	163	198	184	166	188
258	182	185	244	194	182	252
267	192	246	277	200	210	259
287	207	270	290	240	231	277
294	258	276	315	257	278	284
306	267	294	374	287	304	294
314	286	306	380	293	312	315
324	294	316		308	335	335
335	308	381		319	358	353
364	335			374	388	358
378	357			381	397	363
389	365					374
399	382					379
	387					
	399					

Limpopo

Appendix Table 17 Migration distances of the gliadin subunits of Limpopo.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
31	25	26	22	45	24	24
48	48	67	60	73	57	35
80	65	93	80	82	79	73
132	79	100	91	121	91	81
161	92	135	105	222	107	90
189	131	154	132	268	117	103
199	188	188	147	283	130	115
236	194	196	196	307	160	158
284	202	224	262	371	252	187
307	238	265	282	353	265	190
323	258	283	304	376	285	200
331	291	298	312	388	306	210
359	307	330	333		329	248
377	332	356	357		356	283
388	276	376	368		374	306
392	386	385	382		379	327
	399		388		384	353
					388	375
						382
						388

Marico

Appendix Table 18 Migration distances of the gliadin subunits of Marico.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
18	40	8	16	18	19	16
48	49	17	32	63	30	36
73	65	24	50	76	39	40
81	87	49	76	89	62	50
89	188	53	91	104	71	73
127	198	62	103	138	79	91
158	224	69	136	190	85	99
164	229	88	187	226	90	109
186	272	94	200	288	111	13
236	293	106	224	305	121	121
249	301	119	267	326	135	127
278	310	129	287	341	150	140
287	322	135	305	362	169	165
301	334	157	350	369	192	178
306	358	166	364		200	206
332	368	184	371		210	216
368	383	199	380		216	228
372		208	384		240	249
390		221	388		275	261
399		242	394		278	285
		257			295	288
		260			309	319
		267			321	331
		281			349	339
		310			359	369
		328			368	380
		352			384	386
		367				

Molen

Appendix Table 19 Migration distances of the gliadin subunits of Molen.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
7	36	10	26	16	16	18
18	46	18	42	35	29	30
26	75	26	48	46	47	48
47	86	48	79	85	73	55
63	128	74	91	111	95	74
74	183	91	107	132	109	87
84	196	126	121	163	139	94
106	237	160	160	236	161	106
156	253	169	186	254	207	137
166	279	192	208	277	223	151
200	287	207	230	288	246	163
220	301	216	249	301	254	187
242	328	237	273	326	289	198
257	356	253	277	337	323	213
275	368	275	299	369	354	223
298	376	286	310	390	362	257
307	386	301	327	395	354	274
325	390	310	334		390	297
344		328	339			307
369		336	358			319
386		355	372			343
393		368	380			357
		394				362
						393

Molopo

Appendix Table 20 Migration distances of the gliadin subunits of Molopo.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
16	41	31	50	16	44	10
41	54	42	57	40	59	71
57	60	60	87	53	77	85
75	75	76	117	60	120	105
118	83	118	154	75	141	116
182	100	130	174	107	215	127
193	119	158	208	125	226	135
216	125	178	214	141	235	156
228	165	225	239	156	252	196
232	197	238	279	168	261	205
237	218	252	289	174	291	259
261	235	276	328	188	302	285
269	240	288	341	210	324	303
303	259	305	369	236	354	307
309	271	327	376	244	380	313
353	282	340	385	258		325
358	301	376	394	275		345
375	334			309		356
	356			328		
	372			338		
	377			353		
				362		
				368		
				374		
				380		
				386		
				394		

Nantes

Appendix Table 21 Migration distances of the gliadin subunits of Nantes.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
48	50	7	48	8	10	49
71	71	47	73	57	53	72
91	82	71	92	73	91	176
170	92	85	151	90	174	222
218	105	92	186	131	218	228
229	174	113	237	143	235	234
238	220	163	258	156	262	273
263	232	190	277	141	274	287
271	241	220	286	176	299	301
285	273	237	299	186	326	314
308	286	240	337	239	356	342
334	309	258	368	281		377
355	337	276		289		
372	358	285		300		
	362	300		317		
	374	337		333		
	380	363		378		
		380		392		

Oom Charl

Appendix Table 22 Migration distances of the gliadin subunits of Oom Charl.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
10	49	44	48	50	51	10
41	62	63	79	76	75	63
62	79	75	91	79	81	78
75	87	90	129	119	129	89
87	128	113	134	127	154	98
153	178	129	165	169	173	107
209	237	165	192	210	229	125
236	266	192	276	252	256	166
263	277	210	290	265	263	177
274	307	277	306	277	277	188
304	338	288	326	290	293	194
339	362	307	364	315	328	264
361	376	326	380	327	338	293
377		374		362	347	307
				368	361	313
				394		342
						351
						375

Palmiet

Appendix Table 23 Migration distances of the gliadin subunits of Palmiet.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
44	30	48	49	47	55	14
54	54	82	57	57	79	55
76	77	91	83	85	95	80
87	94	116	89	79	114	95
95	225	147	151	132	182	120
224	267	180	190	168	200	219
268	274	186	200	177	220	237
274	287	204	235	196	256	257
288	315	216	272	203	266	267
315	332	236	285	222	276	276
335	366	255	296	240	313	302
342	380	262	316	265	331	328
380		274	331	284	360	362
		286	360	290	384	
		299	376	321		
		333	384	333		
		363	391	369		
		378		380		
		384				
		398				

PAN 3211

Appendix Table 24 Migration distances of the gliadin subunits of PAN 3211.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
1	58	26	13	8	68	18
55	68	36	40	65	76	28
65	73	47	46	73	87	63
75	84	58	57	81	105	73
83	97	67	85	125	111	85
127	126	76	101	175	125	99
174	175	91	112	184	175	107
187	187	126	125	221	184	113
222	229	177	158	235	224	124
240	242	193	184	258	261	152
269	271	215	206	267	272	163
277	281	235	222	297	289	194
309	310	254	243	308	310	203
320	321	271	257	333	321	237
364	346	280	281	381	330	252
382	383	297	291		379	271
		323	320		384	279
		335	331			307
		349	345			349
		376	368			366
		380	380			378
			394			

PAN 3232

Appendix Table 25 Migration distances of the gliadin subunits of PAN 3232.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl 7
51	52	43	42	58	18	51
65	66	54	53	85	37	83
85	86	78	84	97	52	128
130	111	131	129	123	66	223
158	131	159	160	143	83	236
175	176	238	176	158	101	360
204	233	274	208	166	115	283
233	246	286	238	182	129	289
242	274	302	276	191	140	360
274	285	311	288	200	149	394
285	297	323	307	217	164	
295	311	360	324	226	194	
310	323	393	334	245	202	
321	362		360	279	244	
350	381		397	287	259	
380				321	278	
				330	287	
				341	307	
				358	328	
				373	358	
				378	369	
				396	378	
					394	

PAN 3235

Appendix Table 26 Migration distances of the gliadin subunits of PAN 3235.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
16	50	40	14	17	17	16
51	83	59	24	53	26	36
83	129	74	30	82	38	42
106	158	87	41	91	53	54
174	191	108	54	107	77	87
205	223	128	65	158	95	99
231	247	157	78	224	107	112
240	256	216	91	237	121	155
269	279	240	99	258	158	184
279	295	251	109	269	164	192
294	311	276	119	276	210	210
310	346	287	124	298	271	225
319	367	305	129	310	276	240
337	386	321	139	319	284	253
370		360	167	367	292	278
389		396	179		310	309
			188		319	321
			199		357	335
			208		367	375
			222			386
			241			
			255			
			278			
			288			
			328			
			340			
			357			
			388			
			396			

PAN 3342

Appendix Table 27 Migration distances of the gliadin subunits of PAN 3342.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
47	36	12	23	15	33	18
56	85	22	35	34	75	27
62	122	35	47	46	86	47
75	155	45	57	57	95	85
98	174	74	80	71	108	99
117	189	91	95	86	146	108
147	206	111	101	121	210	115
173	248	125	111	164	256	123
189	274	137	121	179	278	147
208	307	144	133	199	297	152
237	324	158	141	215	308	162
248	347	168	158	234	354	192
254	376	188	166	251	376	204
269	390	204	192	261		238
292		224	214	275		257
305		236	235	283		274
357		255	275	297		281
371		279	297	307		307
		286	307	321		313
		299	314	327		364
		307	325	351		375
		313	337	364		
		325	358	374		
		338	372	394		
		365	387			
		375				
		392				

PAN 3349

Appendix Table 28 Migration distances of the gliadin subunits of PAN 3349.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
34	38	14	64	12	6	24
49	51	31	76	21	10	34
64	83	51	83	38	18	60
80	164	76	91	48	33	74
92	215	91	103	82	49	83
125	230	107	143	93	56	93
165	257	133	176	119	66	109
215	278	172	196	139	79	148
225	303	194	244	170	109	166
254	323	216	256	198	131	194
276	357	243	265	218	143	201
299	368	253	278	226	167	231
347	378	287	287	241	198	255
351		303	299	289	218	277
368		333	329	302	241	294
		365	356	319	250	303
		380	378	332	275	353
		396	386	343	289	380
			396	356	332	388
				379	356	
				384	378	
				394	384	
					393	

Scheepers

Appendix Table 29 Migration distances of the gliadin subunits of Scheepers.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl 6	Repl. 7
22	53	22	19	49	66	13
56	77	54	54	60	77	62
85	85	66	66	66	84	78
127	105	78	72	87	106	96
179	188	86	86	130	128	105
220	211	105	95	166	200	127
251	247	190	105	188	209	190
275	279	212	131	222	251	275
299	292	247	158	254	281	310
325	327	279	199	266	291	321
371	337	291	248	298	319	358
	358	315	266	357	329	372
	395	327	279	385	376	384
		354	292		386	
		384	321			
			333			
			374			
			385			

SST 66

Appendix Table 30 Migration distances of the gliadin subunits of SST 66.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
50	65	10	48	51	41	10
71	78	19	87	83	50	51
85	95	48	95	125	64	63
148	107	65	107	198	77	78
182	127	78	126	264	93	85
220	160	107	160	276	178	105
230	196	146	199	306	239	124
239	242	196	239	333	270	196
259	260	243	260	363	276	202
280	279	277	280	374	290	236
290	289	289	291	387	364	275
302	302	303	303		383	289
332	331	344	331			313
376	362	365	362			333
	366	385	366			363
	378		378			376
	392		384			383
			392			392

SST 86

Appendix Table 31 Migration distances of the gliadin subunits of SST 86.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
12	23	16	12	12	14	16
22	43	24	25	46	21	24
27	69	43	53	56	31	38
42	94	60	68	69	36	67
53	107	69	101	87	47	73
60	184	95	117	107	54	97
67	199	104	130	216	59	107
74	215	117	166	234	69	115
83	229	143	184	255	85	123
95	248	159	195	261	105	137
105	261	184	202	277	113	154
113	281	195	214	287	127	171
120	292	240	238	301	147	196
137	310	262	253	347	165	223
146	317	279	268	374	177	237
164	334	287	279	386	196	252
174	381	316	287		203	269
180		335	315		218	277
198		374	337		234	291
214		383	365		269	315
254		390	382		285	337
259			392		301	345
279					309	355
287					315	363
299					333	377
303					346	
341					364	
374					386	
396					394	

Tugela

Appendix Table 32 Migration distances of the gliadin subunits of Tugela.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
22	50	22	8	19	21	21
51	61	79	22	50	71	53
69	79	87	34	69	86	69
85	87	95	60	75	166	83
214	95	107	77	95	215	105
256	106	111	150	107	253	144
266	144	197	164	123	260	212
305	167	212	186	131	297	249
349	248	248	214	164	322	257
362	283	258	238	216	340	270
393	293	283	256	238	361	281
	306	295	274	265		295
	335	307	285	277		319
	357	319	307	285		362
	392	335	332	317		382
		388	364	334		386
			384	378		
			392	384		
				391		

Tugela DN

Appendix Table 33 Migration distances of the gliadin subunits of Tugela DN.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
52	54	48	31	31	22	10
67	67	67	79	53	37	30
83	79	80	96	65	53	51
103	95	87	134	80	65	65
112	113	95	161	97	80	70
139	162	111	194	139	89	83
164	249	133	212	172	95	97
212	270	149	239	210	111	107
251	282	192	256	223	138	115
262	295	208	275	246	158	131
296	307	232	294	254	192	141
321	336	249	319	267	223	152
355	362	296	334	276	249	176
365		306	362	294	267	196
392		321	389	315	290	212
		338	394	337	297	228
		357		343	315	249
		365		357	345	254
		396		393	354	264
					366	276
					386	294
						301
						337
						348
						353
						370
						385
						395

T4

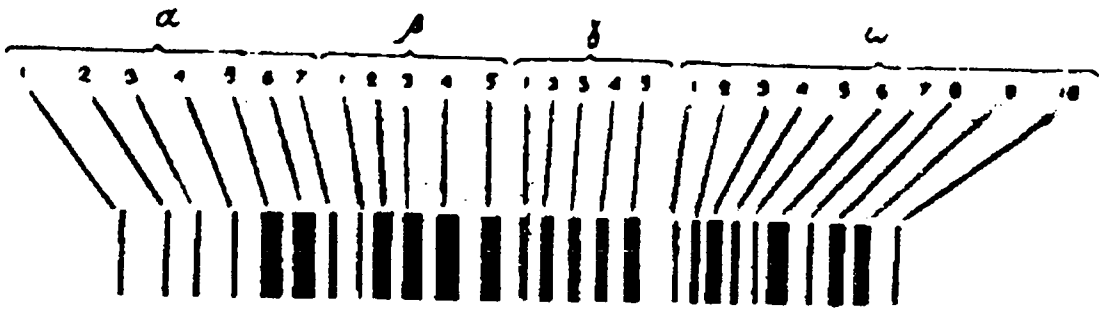
Appendix Table 34 Migration distances of the gliadin subunits of T4.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
30	42	39	30	26	29	13
42	69	67	51	38	42	21
69	83	76	71	53	83	28
87	107	87	83	71	95	69
97	153	107	99	81	111	83
178	175	144	126	95	164	97
213	184	169	148	127	174	107
226	215	182	165	135	200	119
232	225	247	180	153	211	130
251	232	268	200	168	222	144
258	254	282	245	180	271	185
278	260	290	282	193	281	198
287	282	303	289	222	312	254
296	288	308	307	251	335	270
305	299	317	330	271	356	290
332	309	330	344	279		299
353	325	345	362	288		308
391	347	352	379	307		352
397	371		392	330		358
	385			344		367
	393			356		375
				384		383
				390		390
				397		394

Wilge

Appendix Table 35 Migration distances of the gliadin subunits of Wilge.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Repl. 7
28	43	36	14	40	10	12
40	54	51	44	53	42	18
53	61	58	62	70	83	30
61	70	78	72	83	91	61
70	105	87	80	105	101	78
80	117	94	128	146	115	91
97	144	107	160	199	135	105
118	216	127	177	215	153	115
217	234	146	189	250	177	133
233	262	185	216	265	198	148
267	269	201	240	281	204	187
281	283	226	280	293	239	194
302	301	247	292	313	256	229
311	312	262	316	360	275	246
348	325	295	331	384	281	265
360	373	315	364	390	295	271
374	380	328	387		303	288
386	391	375			311	305
					325	325
					333	353
					342	364
					356	384
					375	392
					384	



Adam Tas

Belinda

Betta

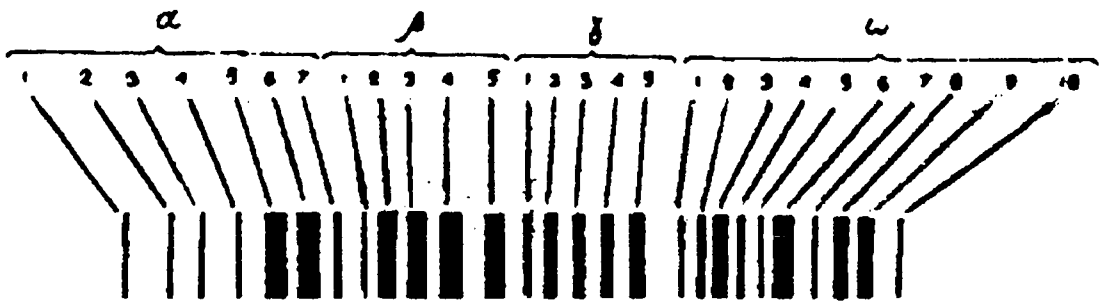
Betta DN

Chinese Spring

Flamink

Gamka

Gamtoos



Gamtoos DN

Gariep

Harts

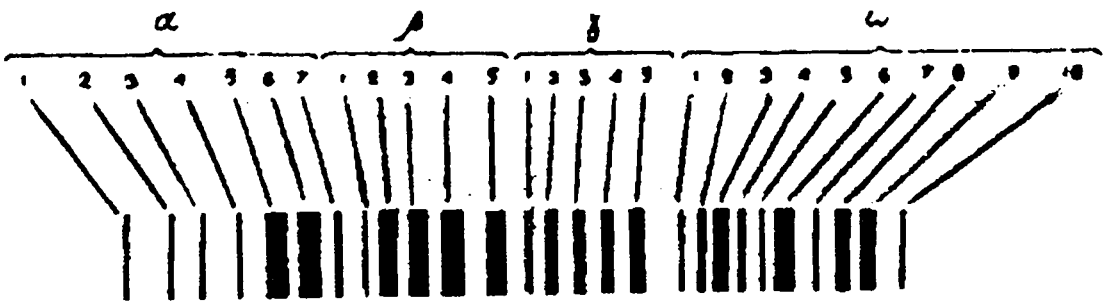
Hugenoot

Inia

Karee

Kariega

Letaba



Limpopo

Marico

Molen

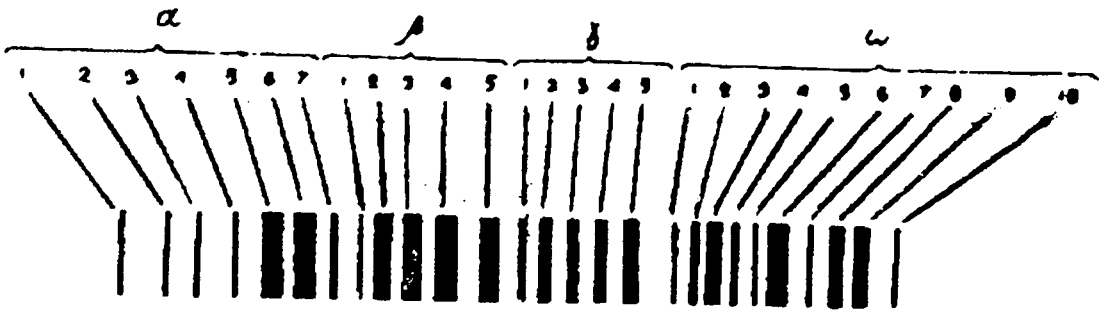
Molopo

Nantes

Oom Charl

Palmiet

PAN 3211



PAN 3232

PAN 3235

PAN 3342

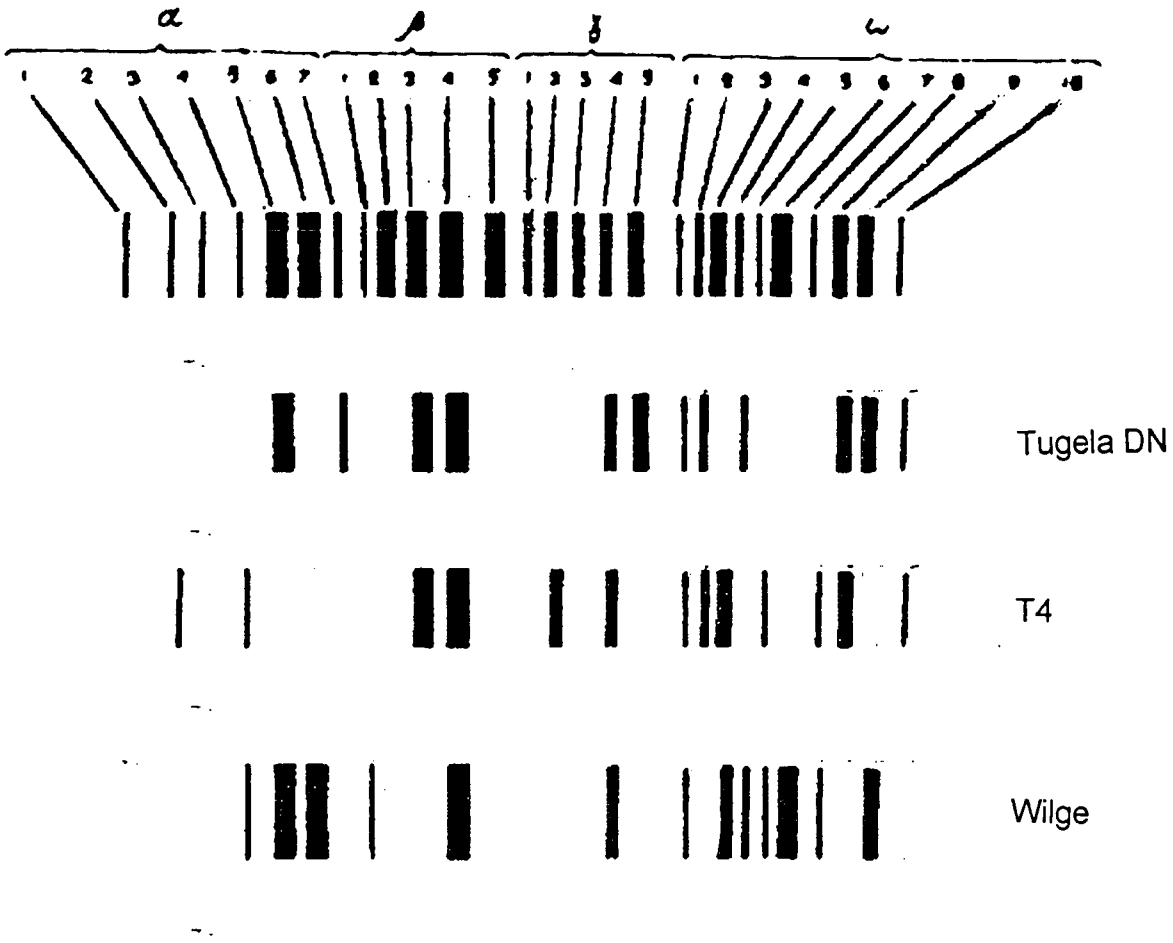
PAN 3349

Scheepers

SST 66

SST 86

Tugela



Appendix Figure 1 Gliadin banding combinations of all the cultivars tested.