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THE INHERITANCE AND INFLUENCE OF LOW MOLECULAR WEIGHT GLUTENIN SUBUNITS ON THE BREADMAKING QUALITY OF SOUTH AFRICAN WHEAT CULTIVARS

by

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Contents

Chapter		Page
1	**The expression and inheritance of LMW-GS in parents and F_2 progeny.**	
1.1	Introduction.	1
1.2	Literature review.	3
1.2.1	Wheat proteins	3
1.2.2	Monomeric proteins.	6
1.2.2.1	Gliadin	6
1.2.3	Polymeric proteins.	8
1.2.3.1	The high molecular weight glutenin subunits (HMW-GS)	8
1.2.3.2	The low molecular weight glutenin subunits (LMW-GS)	10
1.2.3.2.1	Gene location of low molecular weight glutenins.	12
1.2.3.2.2	The inheritance of low molecular weight glutenins	14
1.3	Materials and methods	18
1.3.1	Materials	18
1.3.2	Methods	19
1.3.2.1	Extraction of glutenins	19
1.3.2.2	Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE)	20
1.3.2.3	Staining of the gel	21
1.3.2.4	Gel analysis	21
1.4	Nomenclature	22
1.5	Results and discussion	23
1.6	Conclusion	47
2	**Quality analysis of $F_{2:3}$ derived lines of eight wheat crosses differing in LMW-GS.**	
2.1	Introduction	49
2.2	Protein quantity	50
2.3	Protein quality	50
2.4	Quality tests	55
2.4.1	Hectoliter mass (test weight)	55
2.4.2	Flour extraction (flour yield)	55
2.4.3	Falling number (endosperm starch content determination)	55
2.4.4	The mixograph	56
2.4.5	The alveograph	57

2.4.6 The farinograph 57
2.4.7 Flour colour 58
2.4.8 The baking tests 58

2.5 Materials and methods 59
2.5.1 Materials 59
2.5.2 Methods 59

2.6 Results and discussion 62

2.7 Conclusion 84

2 Conclusion **86**

3 Summary **88**

References **93**

Appendix A
Appendix B
Appendix C

CHAPTER 1

The expression and inheritance of LMW-GS in parents and F_2 progeny.

1.1 INTRODUCTION

There is more wheat grown worldwide than any other single crop. It has provided a range of staple foods for man through the centuries and is also used as a food for meat-producing animals. Bread and macaroni (durum) wheats are the most important commercial wheats. For bread wheats one of the basic breeding objectives is, improved milling and baking quality (Wright, 1983).

During the last two decades, many advances have been made in our understanding of the chemical and molecular basis of functional quality in durum and bread wheats (Shepherd, 1988).

The rheological properties of gluten (i.e. its combination of viscous, elastic and cohesive properties) are generally considered to be responsible for conferring breadmaking quality on wheat flour. Both the gliadin and glutenin fractions contribute to the viscoelastic nature of gluten. However, differences in the glutenin fraction appear to account for most of the differences in gluten quality between wheat varieties, rather than the gliadin proteins (Schofield, 1986). This discovery has prompted an intensive effort to understand the chemical basis for gluten's functionality and its variation from one wheat variety to another, which is largely genetically controlled.

Glutenin proteins are separated into two groups, the high molecular weight glutenin subunits (HMW-GS) encoded by *Glu-1* loci and the low molecular weight glutenin subunits (LMW-GS) encoded by *Glu-3* loci. It has been found that the HMW-GS composition variation is partly responsible for differences in breadmaking quality among bread wheat cultivars (Peña *et al*, 1995). Previous work has demonstrated that the Glu-1 score (which is a quality score from adding

a score for each HMW-GS) accounts for 50 to 70% of the variation in breadmaking quality for wheats from many countries. The results for Australian wheats, however, gave contrasting results, showing that the Glu-1 score accounted for only 19% of the variation in breadmaking resistance (Rmax) (Campbell *et al,* 1987). The differences in baking quality of genotypes primarily stemmed from the LMW-GS (Gupta and Shepherd, 1988).

The LMW-GS are thus an important, but relatively unknown, class of wheat proteins representing about 70% of glutenins and 20 to 30% of the total protein in wheat seed (Melas *et al*, 1994). The importance of the LMW-GS on dough quality has been shown only recently (Gupta and Shepherd, 1988). The delay in obtaining this information has been due mainly to the lack of suitable procedures of separating the LMW-GS from gliadins, which have similar extractabilities and electrophoretic mobilities. This was overcome with the addition of a much simplified one dimensional separation (SDS-PAGE) of glutenin subunits, suitable for rapid screening of a large number of samples (Singh *et al*, 1991).

Glutenin subunit inheritance in F_1 and F_2 generations is co-dominant, thus subunits are expressed whatever their gene dosage in the endosperm. The specific alleles that are associated with breadmaking quality, permit screening of early generation progeny for desirable characteristics (Jackson *et al*, 1983; Bietz, 1987).

The aim of this study was:

(i) To determine the inheritance of LMW-GS in 50 F_2 lines of eight differentTugela crosses. Inheritance of the LMW-GS was determined comparing SDS-PAGE derived band patterns (grouped according to the Gupta and Shepherd (1990b) classification system) in highly homozygous parents and their F_2 progeny population.

(ii) A further aim was to determine the effects of the different LMW-GS on breadmaking quality using $F_{2:3}$ derived lines to determine the usability of LMW-GS as markers for baking quality.

1.2 LITERATURE REVIEW

1.2.1 WHEAT PROTEINS

Wheat cultivars display considerable differences in breadmaking potential. This is largely due to variation in the composition of endosperm proteins (Morel, 1994). The endosperm is the largest tissue in the grain, which contains the majority of the protein (Shewry and Miflin, 1985).

The starchy endosperm of mature wheat grains contains several types of protein. These include the storage proteins, gliadin and glutenin, proteins and enzymes that have survived from the metabolically active endosperm of the developing grain and structural proteins, such as those in membranes (Payne *et al*, 1985). There are five classes of proteins, namely albumins (soluble in water), globulins (soluble in salt solutions), gliadins (soluble in aqueous ethanol), glutenins (soluble, or rather dispersible, in dilute acid or alkali) and an insoluble residue (Eliasson and Larson, 1993). In figure 1.1 the major endosperm proteins of wheat are classified.

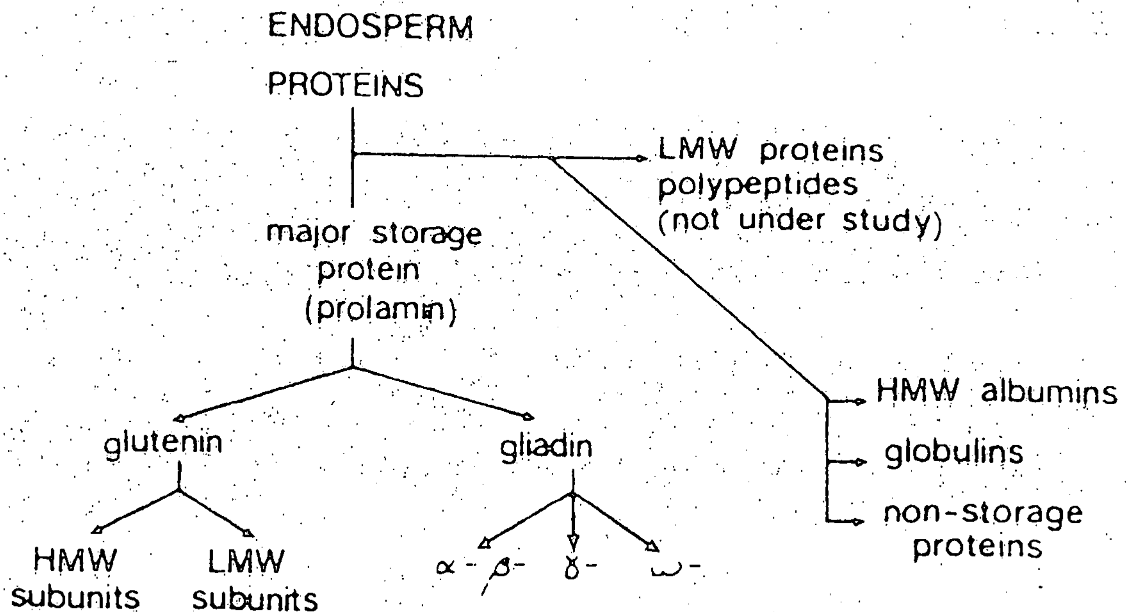

Figure 1.1 Classification of the major endosperm proteins of wheat (Payne *et al*, 1985).

Osborne (1907) divided gluten proteins into two main classes based on their solubility in 70% ethanol. The alcohol-soluble class is gliadin and the residue is considered to be glutenin, a part of which can be solublized in 0.1M of acetic acid. However, it has long been realized that distinction between solubility classes is not sharp and that there is an overlap of components. Consequently, there has been a tendency to define these classes on the basis of molecular size. Proteins larger than 100 kDa were considered to be mainly glutenin, those between 100 and 25 kDa, mainly gliadin and proteins smaller than 25 kDa, were classed as albumin and globulin. This definition minimized the overlap of components, but did not completely eliminate it (Bietz and Wall, 1972).

Glutenin proteins are separated into two groups, the high molecular weight glutenins (HMW-GS) encoded by *Glu-1* loci and the low molecular weight glutenins (LMW-GS) encoded by *Glu-3* loci (Peña *et al,* 1995). The HMW-GS have a molecular weight in the range 90 kDa to 150 kDa, whereas the LMW-GS have molecular weights in the range 30 kDa to 51 kDa.

Genes for LMW-GS on the short arms of homoeologous group 1 chromosomes have been found to be closely linked to the group 1 gliadin genes (Singh and Shepherd, 1988). It is conceivable that some polypeptides are common to both classical gliadin and glutenin, as considered by Bietz and Rothfus (1970), since alpha (α-), betta (β)-, gamma (γ)-gliadins and LMW-GS have similar electrophoretic mobilities and are both soluble in aqueous ethanol. In such a scheme, any one polypeptide in the developing endosperm could either become aggregated, *via* disulphide bonds, with other species of LMW and HMW glutenin subunits to form glutenin, or form intramolecular disulphide bonds and become part of the classical gliadin fraction. The results of Jackson *et al* (1983) showed that this is not the case since all the 14 major LMW-GS have different positions to the α-, β-, γ- and ω-gliadins on the composite two-dimensional map. Their conclusion was that they are different and distinctive proteins.

Dimers of some LMW glutenin subunits (30-50kDa) have molecular weights similar to those of ω-gliadins (69-78 kDa) and minor triplet band protein (globulin) form oligomers of 150 – 160 kDa and longer size (Singh and Shepherd, 1985).

Recently all gluten proteins, apart from minor globulin components, have been classified as prolamins (Shewry *et al*, 1986). Prolamins comprise 80% of the total grain proteins (Payne *et al*, 1981). Gliadins and glutenins in wheat flour are the major components of gluten, which determines the quality of the flour when used for various technological processes, including breadmaking. Glutenins are aggregating (more accurately polymeric) proteins, which are linked by disulfide bonds. Gliadins are non-aggregating or monomeric proteins and are associate by hydrogen bonding and hydrophobic interactions. This classification does not, however, reflect the chemical and genetic relationship of the component polypeptides.

Shewry *et al* (1986) therefore reported that the classification of storage proteins should be based on their biological and functional properties rather than their solubility characteristics. This classified storage into HMW-GS, the sulphur-poor prolamins (ω-gliadins) and sulphur-rich prolamins LMW-GS, ∝-, β- and γ-gliadins (Payne *et al*, 1985).

Secondary structure is ∝-helix, a structure where the backbone is arranged in a helic coil, and the β-sheet, where an extended polypeptide chain forms complementary hydrogen bonds with another parallel chain (Tatham *et al*, 1985).

Gluten from poor-quality flour has higher solubility than gluten from good-quality flour. The differences in protein solubilisation between different quality flours suggest that the proteins in poor-quality flour might have smaller molecular weights or possess less of a tendency to interact with themselves. The surface of the starch granule is presumed to be more hydrophilic. This means that the poor-quality gluten, which has a greater tendency to interact with starch, is more hydrophilic than is the good-quality gluten. The good-quality gluten is therefore more difficult to solubilise (He and Hoseney, 1990).

1.2.2 MONOMERIC PROTEINS

1.2.2.1 Gliadins

Gliadins are defined as the wheat proteins soluble in aqueous ethanol in the classic Osborne extraction procedure and are thus true prolamins. Gliadins are non-aggregating, monomeric proteins and are associated with hydrogen bonding and hydrophobic interactions. Where present, the disulfide bonds are intra-chain (Schofield, 1986). Four main groups of gliadins are usually distinguished in electrophoresis. These are α, β, γ and ω in decreasing order of mobility and therefore increasing molecular size (Eliasson and Larsson, 1993). The ω-gliadins have the highest molecular weight group and are the sulfur-poor prolamins as classified by Shewry *et al* (1986). They are clearly separated from the other gliadins in SDS electrophoresis, whereas there is often some overlap of the ∝-, β- and γ-gliadins (MacRitchie, 1992).

The ω-gliadins are found in the lowest amount and constitute about eight to 13% of the total protein, followed by the other gliadins which constitute about 34 to 38% of the total protein content. The molecular weight of gliadins are between 30 and 40 kDa and the ω-gliadin with a molecular weight of about 60 to 70 kDa (Eliasson and Larsson, 1993), similar to those of LMW-GS (30 to 50 kDa).

Genes coding for the gliadin proteins are located on the short arms of the group one and six chromosomes. The group one chromosomes control all the ω-gliadins, most of the γ-gliadins and a few of the ω-gliadins. Genes on the group six chromosomes code for all the ∝–gliadins, most of the β-gliadins and some of the γ-gliadins. The genes coding for gliadin proteins occur as a single complex locus on each of the short arms of group one and six chromosomes, rather than at two or more loci (MacRitchie, 1992). These loci which contain tightly linked genes, are designated *Gli-1 and Gli-2,* respectively. Most of the ∝– and β-gliadins are encoded at *Gli-2,* while most γ– and ω-gliadins are encoded at the *Gli-1* loci. (Lafiandra *et al*, 1993).

In addition to the tight linkage between gliadins, there is also tight linkage between gliadin genes and genes coding for LMW-GS on chromosome one. This tight linkage between LMW-GS, γ-gliadins and ω-gliadins are also encoded by genes at equivalent loci and homoeologous chromosomes 1A and 1D in bread wheats. Breadmaking quality could be associated with the presence of certain LMW-GS, whose genetic linkage to the particular gliadin components may so far have gone unrecognized (Schofield, 1986).

The gliadin composition is characteristic of the wheat variety. It has been found that groups of gliadins are characterized by high stability, such that they remain unchanged throughout repeated generations (MacRitchie, 1992). The gliadin patterns are not affected by growth conditions, by total protein content, or by sprouting. Gliadins can thus be used as a means of identification of wheat varieties. Tatham *et al* (1990) found that more than 30 components could be separated by two-dimensional electrophorectic techniques.

Gliadin is inherited, as a rule, in the form of definite groups or blocks of components. Practically no recombination between components of allelic variants of blocks, have been observed (Payne *et al*, 1984a). These blocks are inherited co-dominantly, in accordance with a gene dosage in triploid endosperm (Metakovsky *et al*, 1984). A small amount of recombination occurred within a block between the ω- and γ-gliadins and is thought to be coded by chromosome 1B (Payne *et al*, 1984b).

Gliadin mainly confers extensibility to dough. It was found that a cultivar with strong dough properties and good baking performance was associated with a high glutenin to gliadin ratio (Peltonenand Virtanen, 1994). It seems more likely that it is the glutenin subunits that affect gluten quality rather than the gliadin proteins.

1.2.3 POLYMERIC PROTEINS

1.2.3.1 The high molecular weight glutenin subunits (HMW-GS)

The average molecular weight of the LMW-GS (average 40 kDa) is about half the average of the HMW-GS (average 85 kDa). Due to the formation of large polymers and dough strength, the HMW-GS have a greater influence on breadmaking quality than the LMW-GS. It can be calculated that the effect of one HMW subunit molecule is four times that of one LMW subunit molecule (Gupta *et al*, 1995).

HMW-GS are controlled by genes at loci, *Glu-A1, Glu-B1* and *Glu-D1* located on the long arms of chromosome 1A, 1B and 1D, respectively. HMW-GS composition variation is partly responsible for differences in breadmaking quality (Peña *et al*, 1995).

The conformation of the HMW-GS is similar to that of the ω-gliadins. The conformation is characterized by a large proportion of β-turns in the central regions and α-helix structure at the terminal regions (Tatham *et al*, 1985). The β-turn conformation appears to be a feature common to proteins possessing elasticity (Schofield, 1986; Tatham *et al*, 1985). The other reason is the number and distribution of cross-links formed between cysteine residues, which are predominantly located in the N-terminal and C-terminal domains (Buonocore *et al,* 1996).

The HMW-GS constitute only one percent of the dry matter content of the endosperm with a total number of different HMW-GS around 20. A single variety usually contains three to five different subunits. This is consistent with the location of the disulphide bonds in the terminal domains of each subunit, as is evident from the cysteine residues located there (Eliasson and Larsson, 1993). The disulphide bonds are very important. Cleavage of disulphide bonds causes a decrease in viscosity, because the polymers break into smaller pieces (Bietz and Lookhart, 1996).

Each HMW-GS has been assigned a number, and the presence of certain combinations of these subunits is related to different quality aspects (Payne *et al*, 1981). Among allelic HMW-GS controlled by chromosome 1A (*Glu-A1* locus), band 1 and 2* have an equal positive quality effect over the null allele, suggesting a quantitative effect that was related to better breadmaking quality. Similarly, among several alleles at the *Glu-B1* locus on chromosome 1B, those producing double bands or intensely staining bands (e.g., subunits 7+8, 13+16 and 17+18) are associated with superior breadmaking quality compared to those with single faint bands (e.g. subunits 7, 20 and 6+8). An exception to the quantitative basis of allele superiority is the *Glu-D1* locus on chromosome 1D. On this chromosome HMW subunits 5+10 produce better quality than 2+12, but there is no drastic differences in their staining intensities (Singh *et al*, 1990).

It is significant that subunits 5+10 and subunits 2+12 are coded by genes on the D-genome. This is the genome that distinguishes bread wheats from durums. This probably explains why HMW-GS has not been found to be associated in any way with dough properties in durum wheats. The differential effects of the HMW subunits of glutenin appear to be strongest for those coded by chromosome 1D, followed closely by chromosome 1A with the 1B chromosome the least effective (Payne *et al*, 1981).

Randall *et al* (1993) found that bands 13+16 and 17+18 were much more prevalent in South African wheats than researchers have published for American (Khan *et al,* 1989), British (Payne *et al*, 1987a), and Canadian (Lukow *et al,* 1989) wheats.

Depending on the Glu-1 bands present in a genotype, a total Glu-1 score can be calculated. Previous work has demonstrated that the Glu-1 score (HMW-GS) accounts for a substantial proportion (50 to 70%) of the variation in breadmaking quality for wheats from many countries. The studies of Australian wheats, however, gave contrasting results, showing that the Glu-1 score accounted for as little as 11% variation (Campbell *et al*, 1987). It was also seen in Southern African wheats, that the HMW-GS explains less than 20% variability in breadmaking quality (Randall, personal communication).

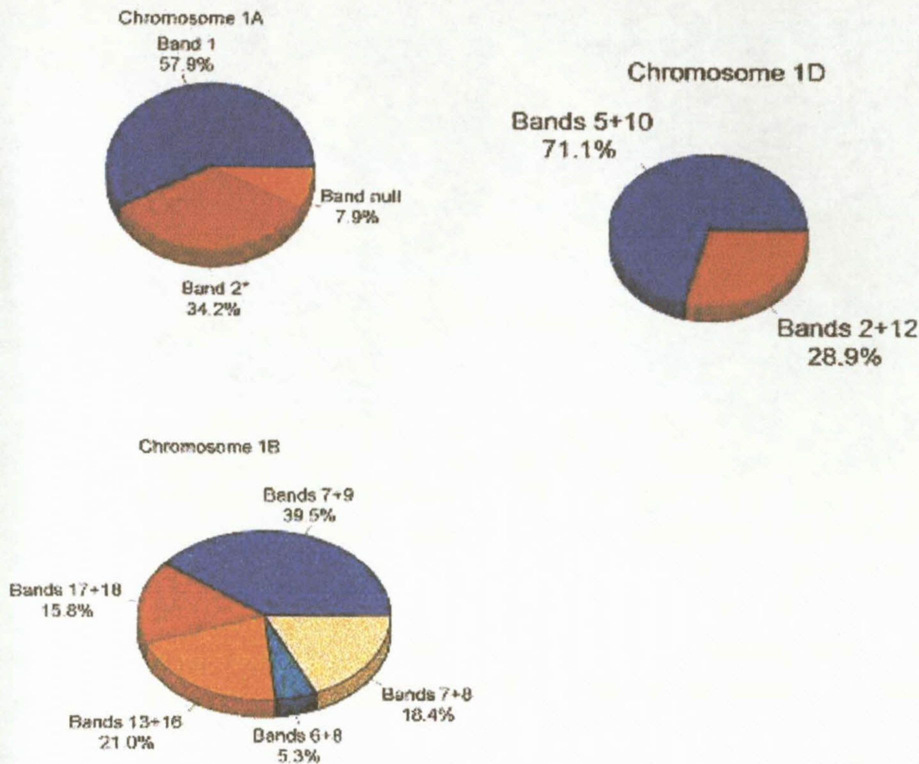

Figure 1.2. Frequency of alleles for high molecular weight glutenin subunits in South African wheats (Randall *et al,* 1992).

Recent studies have indicated that a more effective predictive model of dough properties should include the composition of both the low and high molecular weight subunits of glutenin (Gupta *et al*, 1991).

1.2.3.2 The low molecular weight glutenin subunits (LMW-GS)

LMW-GS are an important, but relatively little known class of wheat proteins representing about 70% of glutenins and 20 to 30% of total proteins and are the second most abundant class of storage protein after gliadin. They are polypeptides with a molecular mass of less than 60 kDa. LMW-GS and HMW-GS are large polymers linked by disulphide bands or by non-covalent association (Melas *et al,* 1994). The importance of LMW-GS in determining dough quality has been shown only recently (Gupta and Shepherd, 1988).

The *Glu-3* LMW-GS accounted for a higher proportion of variation in breadmaking quality (42%) than did the Glu-1 score in a set of 48 Australian wheats. Several of the Australian wheat cultivars, which have very high Glu-1 scores, were found to carry the *Glu-3* alleles in low proportions. These cultivars have low to average maximum dough resistance (Rmax) and extensibility (Ext). These data thus emphasize the previously little recognized importance of LMW-GS as significant components to the assessment of the breadmaking potential of wheat flour (Gupta *et al*, 1991).

Screening for LMW-GS (B and C subunits) has been restricted, because they do not fractionate adequately in SDS-PAGE and because they have mobilities similar to those of some gliadins (Melas *et al*, 1994). The first determination of the chromosomal location of genes encoding LMW-GS was made using two-dimensional electrophoretic techniques. However, these techniques are complicated and slow, allowing only one or two samples to be analyzed on each gel, and therefore they are unsuitable for screening a large number of samples. At present a more simplified procedure for one dimensional separation of glutenin subunits, suitable for rapid screening of a large number of samples, is available. It gives a more improved resolution for both HMW and LMW subunits of glutenin, and has been tested on a large number of bread and durum wheat cultivars and wild tetraploid wheats (Singh *et al*, 1991).

Recent years have brought advances in our understanding of the genetics and relationship to dough quality of the LMW-GS in bread and durum wheats. Most of these subunits are controlled by *Glu-3 loci* on the short arms of group 1 chromosomes in bread wheat, i.e. *Glu-A3, Glu-B3* and *Glu-D3 loci*. They are closely linked to genes controlling gliadins found on the same chromosomes (Gupta *et al*, 1991).

The LMW subunits are subdivided into B, C and D subunits when analyzed by two-dimensional electrophoresis. The B subunits are the major group and consist of basis proteins, whereas the D subunits are minor, have slightly larger molecular weights than the B subunits 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).

The simplified procedure for one-dimensional separation of glutenin subunits, provides a much simpler alternative, to those used in the past, for screening both HMW-GS and LMW-GS on a single gel. However, the positive identification of individual alleles of LMW subunits controlled by the *Glu-3* homeoloci on chromosome 1A, 1B and 1D, remains difficult. Their identification are facilitated by analyzing the same sample for gliadin patterns also, especially for ω-gliadins, which are coded by very tightly linked *Gli-1* genes. This approach is particularly useful for identifying the *Glu-B3* alleles and some of the *Glu-D3* alleles. The *Glu-A3* alleles are easily identified without any help from gliadin patterns, although its null alleles (*Glu-A3e*) are linked with a prominent ω-gliadin band (Singh *et al*, 1991).

Two other groups of LMW-GS 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 *et al*, 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-D3*, which are located between the *Gli-1 loci* and the centromeres on chromosomes 1B and 1D (Shewry *et al*, 1986).

1.2.3.2.1 Gene location of low molecular weight glutenin

The genes controlling the synthesis of the major, basic structure of LMW-GS are located on the short arms of chromosomes 1A, 1B and 1D, like the genes for the ω-gliadins and the majority of the γ-gliadins. The only genes of LMW-GS not located on group 1 chromosomes, are the minor components, which migrate to similar positions on IEF x SDS-PAGE gels as the group 6 α- and β-gliadins (Jackson *et al*, 1983).

The ω- and γ-gliadins are encoded by the loci, *Gli-A1, Gli-B1* and *Gli-D1*, located on the short arms of chromosome 1A, 1B and 1D, respectively. The α- and β-gliadins are encoded by the loci, *Gli-A2, Gli-B2* and *Gli-D2*, located on the short arms of chromosome 6A, 6B and 6D, respectively. Each of the *Gli-1* loci is furthermore, closely linked to a locus coding for LMW glutenin subunits (*Glu-3*). There are several alleles at each of the *Glu-3* (located on chromosome 1), *Gli-1* (located on chromosome 1) and *Gli-2* (located on chromosome 6) loci and the potential number of permutations is enormous (Rogers *et al*, 1989).

Tabel 1.1 Chromosomal location of the storage-protein genes of the wheat endosperm (Blackman and Payne, 1987).

Gene locus	Approx. no. of alleles	Chromo-some	Arm	Position	Storage proteins coded for
Glu-A1	6	1A	Long	Close to centromere	HMW-GS
Glu-B1	11	1B	Long	Close to centromere	
Glu-D1	6	1D	Long	Close to centromere	
Gli-A1	7	1A	Short	Towards the end	ω-gliadin, γ-gliadin and LMW-GS
Gli-B1	7	1B	Short	Towards the end*	
Gli-D1	3	1D	Short	Towards the end	
Gli-A2	3	1A	Short	Towards the end	α-gliadin and β-gliadin
Gli-B2	4	1B	Short	Towards the end*	
Gli-D2	3	1D	Short	Towards the end	

*The genes on these chromosomes are located on the short-arm satellites (the terminal part of the chromosome arm beyond the nucleolar organising region). The three homoeologous sets of loci are called collectively *Glu-1, Gli-1* and *Gli-2.*

All the loci encoding storage protein polypeptides are composed of tightly linked genes, which rarely recombine, thus they are called complex loci (Galili and Feldman, 1983; Maghaub and Odenbach, 1988). The genes are co-dominant

and products of each gene are present in the grain endosperm (Day *et al*, 1986; Payne, 1987; MacRitchie *et al*, 1990).

The *Gli-1 loci* and the *Glu-3 loci* are tightly linked on the short arms of group 1 chromosomes. It was found that the *Glu-B3* locus was 1.8 to 2.0 cM from the *Gli-B1* locus. So far, no recombination has been found between *Gli-1* and *Glu-3* loci on chromosome 1A and 1D. *Gli-3* encoded gliadins can therefore be considered as reliable markers of LMW glutenin subunits (Radaelli *et al*, 1995).

1.2.3.2.2 The inheritance of low molecular weight glutenins

All the storage protein genes that occur on the short arms of each of the group one chromosomes occur together at a single locus (Payne *et al*, 1984b). Gliadin polypeptides are strictly inherited in blocks (Metakovsky *et al*, 1984; Payne *et al*, 1984a). In segregating progenies of many crosses no recombination is detected between ω- and γ-gliadins coded by chromosome 1B, or between ω-gliadins on chromosome 1D (Payne *et al*, 1984a).

As the genes of the LMW-GS, the ω-gliadins, the γ-gliadins and the group 1 β-gliadins are adjacent to each other, they may have arisen from a common ancestral gene. It will only be possible to assess this properly when the primary structures of the different proteins are known, either from amino acid sequencing or by sequencing the DNA of the storage protein genes cloned in plasmids. The ω-gliadins and γ-gliadins can at best be only distantly related. Much less still is known about the biochemistry of LMW-GS. As discussed by Jackson *et al* (1983) they appear to resemble the γ-gliadins more than the ω-gliadins on the basis of amino acid composition and electrophoretic mobility.

Since a number of LMW-GS and gliadin bands are controlled by a cluster of very tightly linked genes, for practical screening purposes, any protein band of a specific gene cluster (or "block") should give an indication of the LMW subunit alleles present. The inherent danger with this approach is that a low level of recombination can occur within gliadin/LMW subunit blocks. Consequently, the gliadin bands can only be used as an indicator, rather than confirmatory evidence

of the presence of specific LMW subunit alleles, especially when dealing with cultivars/lines of unknown origin, where rare recombinants may have been fixed. This approach is very convenient for analyzing segregating progenies from crosses between cultivars of known LMW subunits/gliadin patterns, because the level of recombination is extremely low (Singh *et al,* 1990).

In wheat the *Gli-1* locus may be envisaged as consisting of a set of three major families of genes coding for the three major protein groups, each family having arisen by gene duplication and mutation (Payne *et al,* 1984a). Rare recombination between genes controlling gliadins, LMW-GS and hybridization of the cDNA's to different mRNA and DNA fragments of wheat, suggests that these genes, although derived from a common gene, have undergone duplication and are now distinct (Gupta and Shepherd, 1988). The presence of B and C subunits in all wheats (Gupta and Shepherd, 1990b), their different iso-electric points (Jackson *et al,* 1983), their ability to recombine with each other (Singh and Shepherd, 1988) and the differences in their size suggest that they might be encoded by two gene subfamilies (Gupta and Shepherd, 1990b).

Glutenin subunit inheritance in F_1 and F_2 generations is co-dominant, so subunits are expressed whatever their gene dosage in the endosperm (Bietz, 1987). It should be noted that because of the overlapping of some components belonging to different blocks in any cross, an analysis of F_2 grains from a series of crosses of a given cultivar with others is required, in order to determine the composition of all blocks in this variety by one-dimensional electrophoresis (Metakovsky *et al,* 1985). The inevitable future discovery of a recombination within the main cluster and estimation of genetic distance between genes composing it, will compel us to regard this cluster also as a group of individual "loci". Considerable differences in the number of genes composing clusters in different varieties (Metakovsky *et al,* 1984) will markedly complicate the nomenclature used to describe this family of genes.

The most complex loci are those located on the long arm of chromosome 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 genes encoding the gliadins are inherited as tightly linked groups or blocks in a Mendelian way (Metakovsky *et al,* 1984). Different approaches, such as the use of substitution lines, can be used to identify the subunits encoded by each allele. It is then possible to recognize the components of the alleles when the standard and substituted cultivars are compared (Jackson *et al*, 1996).

Allelic variation in LMW glutenin subunits have been studied for more than 200 cultivars by Gupta and Shepherd (1990b), using two-step one-dimensional SDS-PAGE. A total of 40 different B and C subunits were detected, the number in a given cultivar ranging from seven to 16. The subunits could be divided into 20 band patterns, which could be classified into three groups, namely Glu-A3 on chromosome 1A, Glu-B3 on chromosome 1B and Glu-D3 on Chromosome 1D, based on their mutual exclusiveness and available inheritance data (Gupta and Shepherd, 1988), with six, nine and five patterns. Examination of the banding combinations of some cultivars revealed that some LMW bands, or band combinations, were not present together in the same cultivar these combinations occurred as alternatives to each other.

By analyzing substitution lines, it was determined that the different patterns in the groups were controlled by genes on chromosome 1A, 1B and 1D, respectively. Many allele designations and the band patterns for three standard cultivars on the extreme left are shown in Figure 1.3. Six combinations were assigned to group 1. Pattern "a", *"b"* and *"d"* correspond to Chinese Spring, Gabo and Orca bands, respectively and are controlled by genes on chromosome 1AS (Gupta and Shepherd, 1988).

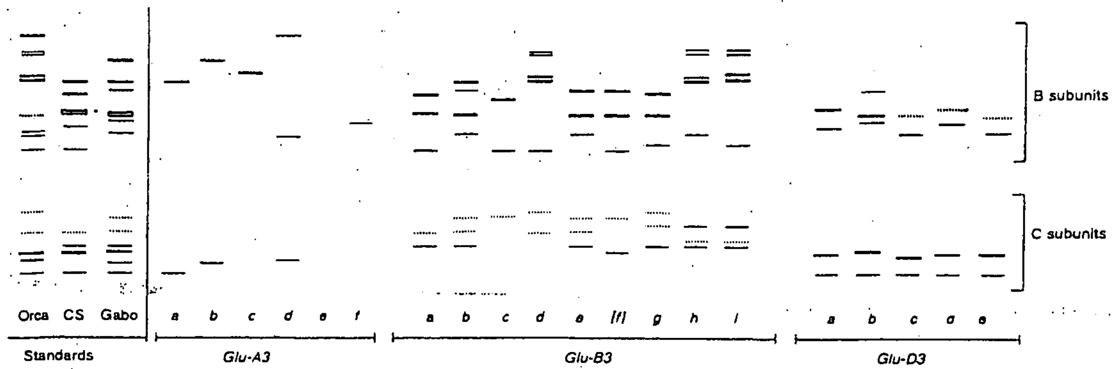

Figure 1.3. Identification by two step SDS-PAGE of the B and C subunits of the LMW-GS isolated from 222 bread wheat cultivars sourched from 32 countries. The grouping is based on the mutual exclusiveness of these bands or band combinations among the cultivars. This diagram also incorporates the information obtained on these subunits from analysis of substitution lines and the test-cross pogeny. Combinations *"a"* and *"b"* in each group are from Chinese Spring and Gabo, respectively. The [*f*] = direct evidence for the chromosomal location of pattern *"f"* has not been obtained. Faintly stained bands are shown by broken lines. * Denotes that this thick band represents two bands of the same mobility, one controlled by 1BS and the other by 1DS in Chinese Spring and Gabo. These two bands have been included in group 2 and group 3, combinations of these as well as other cultivars having the denoted thick band (Gupta and Shepherd, 1990b).

The least number of subunits was controlled by chromosome 1A and about 40% of the cultivars examined, contained no band controlled by those chromosome. These cultivars that did not have any bands, are termed null phenotypes (pattern "*e*"). The faster moving band in pattern "*a*" usually overlaps with a band controlled by 1DS in Chinese Spring and it's therefore not completely absent in these substitution lines (Gupta and Shepherd, 1990b).

The greatest polymorphism is shown by chromosome 1B. Nine different combinations have been assigned to group 2 and each pattern had two or more B subunits bands (Figure 1.3). Combinations "*a*" and "*b*" corresponding to the bands in Chinese Spring and Gabo, respectively, were controlled by genes on chromosome 1BS. It is clear from segregation data, that the majority of the B

subunits are inherited as a group and, moreover, the genes controlling them are closely linked to those controlling gliadins (Gupta and Shepherd, 1988).

The five different combinations allocated to group 3 are associated with chromosome 1D. Combinations "*a*" and "*b*", corresponding to the bands in Chinese Spring and Gabo, respectively, were controlled by genes on the short arms of chromosome 1D (Gupta and Shepherd, 1988).

Some difficulties were encountered when the LMW subunit combinations of these cultivars were analyzed. The *Glu-B3* bands (group 2, Figure 1.3) represented a wide range of mobilities, and some of them overlapped with *Glu-A3* and *Glu-D3* bands (groups 1 and 3, Figure 1.3).

The number of band combinations found in the LMW-GS of hexaploid wheat, are much lower than the expected number of such combinations on the basis of random association, indicating that the genes coding for these bands are closely linked. Such a close linkage, has been demonstrated by Singh and Shepherd (1988) by detecting a low level of recombination between LMW-GS on chromosome 1B. The exact genetic and molecular nature of variation of LMW-GS is not yet known, however, rare recombination and point mutation can generate new LMW subunit combinations. Moreover, the analysis of nucleotide sequences of a LMW glutenin gene has revealed the presence of repetitive sequence. Differences in their size and number also exist between the genes. Unequal crossing-over between these repeats might produce new subunits and will thus contribute towards the multiplicity and variation of LMW glutenin subunits (Gupta and Shepherd, 1990b).

1.3 Materials and methods

1.3.1 Materials

Nine hard red wheat cultivars were chosen for their similarity in HMW-GS. The reason for this was to compare the LMW-GS without the influence of the HMW-GS. Tugela, a South African wheat cultivar, which was removed from the market

due to over-stability, was crossed with eight other commercial cultivars. Tugela DN is isogenic to Tugela, expect that a *Diuraphic noxia* resistance gene was crossed into it. Tugela fg is a selection from Tugela, which has a shorter growing time than Tugela. Tugela, however, has excellent yield and disease resistance and if the quality can be improved, it can be re-introduced for commercial production.

Tugela was crossed with the eight other cultivars (Table 1.2). The F1 progeny was then self-pollinated. From each of the eight F2 progeny, 50 kernels were randomly selected and halved. The halved kernels without the embryos were used to identify the LMW-GS patterns, for the inheritance studies. The other half was labeled and stored for later use.

Table 1.2. Summary of the HMW-GS of the wheat cultivars used.

Cultivar	A genome	B genome	D genome	Growth type
Tugela	2*	7+8	5+10	Winter
Tugela DN	2*	7+8	5+10	Winter
Tugela fg	2*	7+8	5+10	Winter
Flamink	1	7+9	5+10	Intermediary
Gariep	1	7+9	5+10	Spring
Inia	1	7+9	5+10	Spring
Palmiet	2*	13+16	5+10	Spring
Letaba	2*	7+9	5+10	Winter
Gamtoos DN	1	7+9	5+10	Spring

Two control cultivars, Chinese Spring and Gabo were included. Chinese Spring originated from China and Gabo from Australia. Their LMW-GS patterns are known and were used to compare the LMW composition of the South African cultivars tested in this study.

1.3.2 Methods

1.3.2.1 Extraction of glutenins

An adapted method of Singh *et al* (1991) was used. The advantage of this technique, is that the HMW and LMW glutenin subunits can be read from the

same gel. All the protein extractions were done in a waterbath at 60°C. Half a kernel was crushed to a fine powder. The gliadins were removed with a 70% ethanol extraction procedure, as they overlapped with the LMW-GS. After the removal of the gliadins, the glutenins were washed with 1-propanol, twice for half an hour each time. During the washing procedure, the eppendorf tubes were vortexed for 30 s every 10 min. The eppendorf tubes were then centrifuged for 2 min at 10 000 rpm's. The 1-propanol and all of the remaining supernatant were removed by suction and 75 μl extraction buffer [80 mM tris-HCl (pH 8.0)] containing 1.25% dithiothreitol was added. The tubes were again vortexed to loosen the seed material. After an hour, 75 μl extraction buffer containing 16.8 μl/ml vinylpyridine was added. The tubes were vortexed and left for an hour in the waterbath. After the extraction of the glutenins, the seeds were centrifuged for 2 minutes at 10 000rpm. Of the supernatant, 110 μl was transferred to a new eppendorf tube containing a 100 μl sample buffer [80 mM tris-HCl (pH 8.0), 40 g glycerol, 2 g SDS, 0.02 g bromophenol blue]. After 15 minutes in the waterbath, the samples were ready for loading (20 μl or more as required).

1.3.2.2 Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE)

A 10% uniform separating gel was used. It consisted of 14 ml separating buffer (45.412 g Tris in 460 ml H_2O, titrate to pH8.88, add 1.0 g SDS), 9.4 ml separating acrylamide (30% Ac/1% crosslinked), 75 g acrylamide, 0.75 g bisacrylamide, 181 ml H_2O and 4.7 ml water. TEMED (N,N,N',N' – Tetra methylethylenediamine) 55 μl and 65 μl 10% amonium persulphate (APS) were used as catalysts.

The gel was run at 66 mA at a constant temperature of 15 °C for approximately 3 hours. The cathode stock buffer consisted of 30.28 g Tris, 144 g glysine and 10 g SDS made up to 1 l with dH_2O. The anode buffer consisted of 30.28 g Tris and 800 ml dH_2O, titrated to pH 8.4. The cathode and anode buffers were diluted 10x before use.

1.3.2.3 Staining of the gel

The staining method of Wrigley (1992) was used. The gel was immersed in a fixing solution (400 ml methanol, 100 ml glacial acid and 500 ml dH_2O) for one hour. It was then stained overnight in a staining solution (30 g trichloricacetic acid made up to 200 ml with water and 0.1 g Coomassie Blue made up to 10 ml in methanol). The stained gel was rinsed in distilled water for a few hours before being examined and photographed.

1.3.2.4 Gel analysis

The "Molecular Analyst Fingerprinting" software of Biorad was used to analyze the gels. The gels were scanned with the Gel Doc 1000 using an UV-gel camera and VGA graphics in 256 colours as recommended. The analysis procedure consisted of three steps, namely:

a) The conversion of the gel.

b) The normalisation of the tracks.

c) The analysis of the tracks.

Using the conversion program a rectangle was drawn around the LMW-GS on the gel. The identification process was easier as only the LMW-GS were screened and not the HMW-GS, albumins and globulins.

The normalisation setting was as follow: the resolution was set at 200 points and a smoothing factor of three was chosen (this implied that one point on either side of a data point would be averaged with the data point). To remove the background the rolling disk method was chosen. The principle of the rolling disk mechanism 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. This method gives very stable and reliable background subtraction. The intensity of the background subtraction was set at 10 (typical settings 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 the standard reference. Normalization of a gel is achieved by aligning the bands of all reference patterns on the gel to the corresponding ones of the standard (Chinese Spring). Non-reference tracks are interpolated gradually according to both surrounding references. At least three references were loaded on each gel for the best normalization results.

After normalization the gels were analyzed using the main program of the "Molecular Analyst Fingerprinting" software. A densitometric curve of every F_2 replication from every cross with Tugela and each parent, was drawn and from this the migration distances were determined. The program gave the migration distance of each peak and the intensity of the band. Peak positions with a repeatability of more than 67% in the replications were accepted as representative of a specific parent cultivar. Repeatability was calculated as the percentage of the occurrence of a band across the replications. An average of the band position (the migration distance of the bands on the gel) of the 10 replications of each of the nine parental cultivalrs were calculated. These values were used to compare the cultivars with each other.

1.3.3 Nomenclature

A detailed discussion of the nomenclature used, is given in Chapter 1 (section 1.2.3.2.2.). This nomenclature was used to score the banding patterns in order to determine how the protein subunits were inherited by the F_2 segregating progeny from the parents.

1.4 Results and discussion

The migration distances of each of the eight F2 population and their parents were summarized in tables in Appendix A. The LMW subunit combinations (Gupta and Shepherd, 1990b) for each parent and F2 replication are given in Appendix B. All the parents of the eight crosses were confirmed to be pure breeding for the HMW-GS and the LMW-GS. No segregation of any subunit therefore occurred in the parents.

A summary of the results for chromosome 1A, 1B and 1D.

MacRitchie (1992) found that the least number of subunits were controlled by chromosome 1A. About 40% of the cultivars he examined contained no bands controlled by this chromosome. This was, however, not the case in this study. All the cultivars examined, contained bands that were controlled by this chromosome. Only four percent of the F2 progeny from the Tugela x Tugela fg cross contained no bands on the A genome. LMW subunit combination *e*, (group 1), is an indication of no bands controlled by chromosome 1A. In this study, polymorphism was also found for chromosome 1A.

It was also found that more than one banding combination could appear together in one cultivar, for example Tugela had combinations *a, c* and *f*. This cannot be due to segregation, as all the cultivars were selfed and were found to be pure breeding, for both the HMW-GS and the LMW-GS. It was noted that more than one band combination was expressed in this material.

This was also found by Maartens (1997, 1999) also found that banding combinations *c* and *f* (group1) appeared together in 13.3 % of the South African cultivars. These combinations were therefore not alternatives (i.e. or allelic) to each other.

From the results of the A genome it is obvious that the nomenclature of Gupta and Shepherd (1990b) could not be fully applied to South African wheats. It is also possible that there are combinations that are not yet included in the

nomenclature of Gupta and Shepherd (1990b). MacRitchie (1992) and Gupta and Shepherd (1990b) found that the greatest polymorphism is shown by chromosome 1B. This was also the case in this study. Polymorphism was also found for subunits coded by chromosome 1D. The polymorphism of this chromosome could, however, not be confirmed through literature. The results of individual crosses are discussed in a later section.

❖ ***Tugela x Palmiet***

Table 1.3. Separation by SDS-PAGE of LMW subunit combinations in two parent wheat cultivars and their F2 progeny.

PARENTS	A genome	B genome	D genome
Tugela	a/c/f	b	d/e
Palmiet	a/b/f	c	d/e
F2 progeny	a (1) b (1) c (1) f (1) a/b (1) a/c (1) a/d (3) a/f (8) b/c (1) b/d (1) b/f (4) c/d (2) c/f (4) d/f (3) a/b/d (1) a/b/f (1) a/c/d (2) a/c/f (1) a/d/f (2) b/c/f (2) b/d/f (1) a/b/c/f (2) b/c/d/f (1)	a (1) c (39) a/b (1) a/c (1) b/c (1) unknown (7)	a (4) a* (1) c (3) e (19) a/e (1) b/e (1) c/e (3) d/e (2) d*/e* (7) unknown (6)

* a band is missing from the combination.

Chromosome 1A, group 1 (A genome)

Tugela had the combination *a/c/f* and Palmiet *a/b/f*. The simultaneous expression ot these banding patterns contrasts with the findings of Gupta and Shepherd (1990b), who found that only one banding pattern for each chromosome was expressed in a pure breeding cultivar. Four F2 progeny had either a or b or c or f band combinations. They therefore lost the accompanying combinations, which were present in the parents. An *a/b, a/c, b/c* and *b/d* combination occurred in one of the progeny each. It is interesting to note that the *d* pattern was present in neither parent, it must therefore be a product of recombination. An *a/d* combination was found in three of the F2 lines, an *a/f* combination in eight of the lines, a *b/f* combination in four, *c/d* in two, *c/f* in four and *d/f* in three of the progenies. A combination of *a/b/d, a/b/f, a/c/f, b/d/f* was found in one F2 line each. An *a/c/d, a/d/f* and *b/c/f* combination was found in two F2 lines each. A four band combination of *a/b/c/f* was found in two cases and *b/c/d/f* in one case.

It was conspicuous that the number of banding combinations decreased in the F2 progeny relative to the parentslines. Of the lines 71% had only two or less banding combinations and 22% had the same number of combinations as the parents, while 6% had more than three combinations (hybrids). The *a/f* combination occurred most frequently, which was expected, as they occurred in both parents. Combination *b/f* and *c/f* also occurred frequently, which was also expected, given the parent combinations. An *a/d* and *d/f* combination also occurred quite frequently, which indicates that the new *d* combination was expressed in a number of cases, and that it must be due to interaction between the parent types. The full hybrid *a/b/c/f* was expressed in two progenies, while another hybrid *b/c/d/f* was expressed in one individual. In the latter one the expected combination was replaced with a new combination *d*. It also became evident that an expected 1:2:1 ratio in the case of co-dominant inheritance with half the progeny expressed as hybrids, was not true in this recorded case. In fact, only 6% of the progeny were hybrids, in the sense that they were full combinations of the parents. It would seem as though none of the "normal" inheritance patterns were followed, but rather that there is some kind of suppression of banding patterns in the F2 lines. It seems as though less protein

was expressed in the F2 and it is probable that a large number of the progeny will keep these banding patterns to homozygosity. This in turn may influence the quality of the progeny, as a decreased protein expression may lead to decreased breadmaking quality. This will be looked at in chapter 2.

Chromosome 1B, group 1 (B genome)

Tugela had the combination *b* and Palmiet *c*. In the F2 progeny 39 lines had combination *c*. That is 78% of the progeny, which could be an indication of the dominance (3:1 ratio) of combination *c* over combination *b*. However, combination *b* occurred only twice in combinations *a/b* and *b/c*. Combination *a* occurred only once alone and once in *a/b* and *a/c*. As it was present in neither parent, it must therefore be a product of recombination.

The *c* band was dominant to *b* with a 3:1 inheritance ratio (χ_2 = 10.52, $p<0.05$). However, where *c* was dominant, the recessive combination, which should have been *b* was replaced by a new combination *a*. A hybrid *b/c* was expressed once in the progeny.

Seven of the progeny did not correspond to any of the combinations in group 2 (nomenclature of Gupta and Shepherd, 1990b). In Maartens (1999) it was found that in some South African cultivars the nomenclature of Gupta and Shepherd (1990b) could not be used to identify certain combinations. Some cultivars had no matching combinations in group 2 and some had no matching combinations in group 3. Polymorphism is common in wheat and new banding combinations are quite possible. Gupta and Shepherd (1990b) found that for LMW glutenins, chromosome 1B showed the greatest polymorphism. Rare recombination and point mutations can generate new LMW glutenin combinations. This could be the reason why new combinations were found, but it is also possible that there are combinations that are not yet included in the nomenclature of Gupta and Shepherd (1990b).

Chromosome 1D, group 3 (D genome)

Both Tugela and Palmiet had the combination *d/e*. In the F2 progeny 19 lines had combination *e*, that is 38%. Four lines had *a*, one line had *a** and three lines had only *c*. Both pattern *a* and *c* were not present in either parent. The only difference between combination *d* and *a* was that the second slowest moving band of *a*, on the SDS-PAGE was a bit lower on the gel than the second slowest moving band of combination *d*. There was also one line with combination *a* where the second fastest band was missing.

Combination *c* was also present in neither parent; it must therefore be a product of recombination. An *a/e* and *b/e* combination was found in one of the F2 lines each. As combination *b* was not found in either parent, it must therefore also be a product of recombination.

The expected parental combination *d/e* occurred only twice in the F2 progeny, but a combination *d*/e** occurred seven times. Both *d** and *e** were missing a band from the standard combination.

Six of the progeny did not correspond to any of the combinations in group 3 of the classification of Gupta and Shepherd (1990b). This again highlighted the possibility that there are other combinations not yet included in the nomenclature of Gupta and Shepherd (1990b).

It was also observed that the number of banding combinations decreased in the F2 lines. Only 28% had the same number of combinations as the parents and 54% had one combination.

It was expected that combination *d/e* should have occurred the most frequently, but this was not the case. None of the expected inheritance patterns were found. It appears that suppression of banding patterns and specific LMW bands occured. It is also possible that most of the progeny will retain these banding patterns to homozygosity.

❖ *Tugela x Gariep*

Table 1.4 Separation by SDS-PAGE of LMW subunit combinations in two parent wheat cultivars and their F2 progeny.

PARENTS	A genome	B genome	D genome
Tugela	a/c/f	b	d/e
Gariep	f	a/c	a
F2 progeny	a (5)	a (10)	a (18)
	c (2)	b (1)	b (4)
	f (1)	c (21)	d (3)
	a/d (1)	e (3)	e (12)
	a/f (7)	e* (1)	a/d (2)
	c/f (6)	a/b (1)	c/e (5)
	b/f (9)	a/c (6)	d*/e* (1)
	a/b/f (9)	e/f (1)	a*/d*/e* (3)
	a/c/d (1)	b*/c* (2)	unknown (1)
	a/b/d (1)	unknown (6)	
	b/c/f (4)		
	a/b/c/d/f (1)		

*a band is missing from the combination.

Cromosome 1A, group 1 (A genome)

Tugela had the combination *a/c/f* and Gariep *f*. In the F2 progeny *a, c* and *f* each also occurred separately, as was recorded in the previous cross. These individuals therefore lost the accompanying combinations that were present in Tugela. Combination *a* occurred in five, *c* in two and *f* in one of the progeny. Combination *a/d* occurred in one progeny. It was interesting to note that the first

two bands of this combination occurred in Tugela and the last one in Gariep. As suggested previously, *d* may be a recombination product. Combination *a/f* occurred in seven of the F2 lines, a *c/f* combination in six and a *b/f* combination in nine of the progeny. It's noted that combination *b* was found in neither parent. An *a/c/d* and *a/b/d* combination was found in one F2 line each. Combination *a/b/f* was found in nine F2 lines. A *b/c/f* combination was found in four F2 lines. It was also interesting to note that all the combinations detected for the A genome of this cross were found in one plant. Combination *a/b/c/d/f* was found in one F2 line. Of the F2 lines 62% had two or less banding combinations, 30% had three banding combinations and 2% had five combinations.

It was interesting to note that although the LMW-GS were inherited as groups of LMW subunits, more than one combination could be found in one line. It was also noted that recombination did occur and that it was not exactly a rare event. Interestingly combinations *a/b/f* and *b/f* occurred most frequently, as the combination *a/c/f* or *a/f* or *c/f* was expected more frequently. The new combination *b* was expressed in a number of cases.

Chromosome 1B, group 2 (B genome)

Tugela had the combination *b* and Gariep *a/c*. In the F2 progeny combination *a* occurred in 10 F2 lines. Combination *b was* found once and combination *b* without its last band, *b**, was also found only once. Combination *c* was found in 21 of the F2 lines.

A new combination *e* was found in three F2 lines. Interesting to note, is that combination *e's* first four bands were also found in Tugela and the last one in Gariep. Combination *e* may be the product of recombination. Combination *e**, that is, without the second slowest moving band, was found once. An *a/b* was found once and *a/c* was found in six F2 lines. Combination *b*/c** was found twice in the progeny. Both combination *b** and *c** were missing a band.

Six of the progeny did not correspond to any combination in group 2 classification of Gupta and Shepherd (1990b). It was again evident that some combinations are yet to be included in the nomenclature of Gupta and Shepherd (1990b).

It was again seen that the single banding combinations occurred most frequently. Thus, the number of banding combinations decreased in the F2 lines. Of the lines 74% had only one banding combination and 18% had two banding combinations. For the expected 1:2:1 ratio in the case of co-dominance, combination *b* must be present in 25%, but was found in only two percent and combination *a/c* was found in only 12% of F2 progeny. The hybrid *a/b* and *b*/c** was found in 2 and 4% of the F2 progeny, respectively. Thus, the expected 1:2:1 ratio for co-dominant inheritance was not found.

Chromosome 1D, group 3 (D genome)

Tugela had the combination *d/e* and Gariep *a*. In the F2 progeny 36% had combination *a* and only 2% had combination *d*/e**. Both *d** and *e** had one missing band.

Combination *b* was found in 4% of the F2 progeny. This combination is present in neither parent. Interestingly the first two bands in *b* were also found in Tugela, the next two in Gariep and the last band in both parents. Combination *b* must again be the product of recombination. A *d* combination occurred in 6% and combination *e* in 24% of the progeny. A combination *a/d* occurred in 4% and *a*/d*/e** in 6% of the progeny. One band is missing out of each of the combinations. Finally, combination *c/e* was present in 10% of the progeny. Combination *c* was found in neither parent, but the first two bands in *c* were found in Tugela, the next one in Gariep and the last one in both parents. Thus, this combination must be the product of recombination.

Only one line did not correspond to any of the combinations in group 3. Again it may be concluded that some combinations have not been included in the nomenclature of Gupta and Shepherd (1990b).

❖ ***Tugela x Flamink***

Table 1.5 Separation by SDS-PAGE of LMW subunit combinations in two parent wheat cultivars and their F2 progeny.

PARENTS	A genome	B genome	D genome
Tugela	a/c/f	b	d/e
Flamink	f	c	e
F2 progeny	a (2)	a (5)	a* (1)
	f (3)	b (9)	d (2)
	a/c (3)	c (16)	e (18)
	a/d (4)	e (5)	a/e (8)
	a/f (6)	f(1)	a*/e* (2)
	b/f (1)	a/c (2)	d*/e* (5)
	c/f (2)	b/c (1)	d/e (8)
	a/b/d (1)	b/e (2)	unknown 6
	a/b/f (1)	a/b/c (2)	
	a/c/d (2)	unknown 11	
	a/c/f (4)		
	a/d/f (7)		
	b/c/f (1)		
	b/d/f (1)		
	c/d/f (1)		
	a/c/d/f (10)		

* a band is missing from the combination.

Chromosome 1A, group 1 (A genome)

Tugela had the combination *a/c/f* and Flamink *f*. In the F2 progeny *a* was found in two lines and *f* in three. Combination *a* lost it's accompanying combinations, which were present in the parents. An *a/c* combination was found in three of the F2 lines, *a/d* in four, *a/f* in six, *b/f* in one and *c/f* in two of the progeny. The *b* pattern was present in neither parents. This pattern has two bands, the first band was also found in both parents and the last band could be found in Tugela, but the corresponding band in Tugela was a bit higher on the gel. This combination must therefore be a product of recombination. The same could be said of combination *d* that was present in neither parent. This pattern has three bands, the first was found in Flamink, the next one on both parents and the last one in Tugela.

Combination *a/b/d, a/b/f, b/c/f, b/d/f* and *c/d/f* were found in one F2 line each. Combination *a/c/d* was found in two F2 lines, *a/c/f* in four and *a/d/f* in seven. A four band combination *a/c/d/f* was found in 10 lines.

Tugela's combination *a/c/f* was found in 8% and Flamink's combination *f* in 6% of the progeny, where it would have been 25% each, if a 1:2:1 segregation pattern, had been followed. The hybrids, *a/f, c/f* and *a/c/f* were found twice, six and four times respectively in the progeny. Thus, the hybrid was present in 24% of the progeny and not in 50% as expected.

Unexpectedly the new combination *d* was expressed in 26 lines, that is 52%, it could be due to interaction between parent types. A full hybrid combination, with *d* included, *a/c/d/f* was expressed in 10 lines, which is quite unusual. The other new combination *b* was found in only five lines.

Chromosome 1B, group 2 (B genome)

Tugela had the combination *b* and Flamink *c*. In the F2 progeny nine lines had combination *b* and 16 lines combination *c*. There were also three new

combinations, which were found in neither parent. These combinations, *a, e* and *f* were expressed in five, five and one F2 progeny respectively.

Combination *a* has five bands, collectively expressed in both parents. The first of the five bands was found in both parents, the next in Tugela, the next in Flamink, the next in both parents and the last band again in Tugela. The same principal is found in combinations *e* and *f.* Thus, again these new combinations may be the product of recombination.

The *b/c* hybrid was expressed only once in the F2 progeny. The new combinations were also expressed as hybrids, *a/b/c, a/c* and *b/e* in two lines each.

Eleven of the progeny did not correspond to any of the combinations in group 2. This may again indicate that some combinations in South African wheat are not yet included in the nomenclature of Gupta and Shepherd (1990b).

Chromosome 1D, group 3 (D genome)

Tugela had the combination *d/e* and Flamink *e*. In the progeny, *d* was expressed twice, *e* 18 times, the hybrid *d/e* eight times and *d*/e** five times. Both *d** and *e** were missing their first bands.

A new combination *a*, found in neither parent, was also expressed once in the progeny with its hybrid *a/e* eight times and *a*/e** twice in the progeny. In *a** the third band and in *e** the first band were missing.

Again a small difference between *d* and *a* was noted (as in the Tugela x Palmiet cross). The second band of *a* migrated slightly more on the SDS-PAGE gel than did the second slowest moving band of *d.* Six of the progenies did not correspond to any of the combinations in group 3.

❖ ***Tugela x Gamtoos DN***

Table 1.6 Separation by SDS-PAGE of LMW subunit combinations in two parent wheat cultivars and their F2 progeny.

PARENTS	A genome	B genome	D genome
Tugela	a/c/f	b	d/e
Gamtoos DN	a/b	a	a
F2 progeny	a (2)	a (17)	a (9)
	b (1)		
	c (1)	b (9)	a*(1)
	a/c (1)		
	a/d (6)	c (10)	e (4)
	a/f (3)		
	b/f (5)	a/b (1)	a/b(1)
	c/d (2)		
	c/f (6)	a/c (3)	a/e (9)
	d/f (2)		
	a/b/d (1)	a/b/c (8)	d/e (13)
	a/b/f (4)		
	a/c/d (1)	unknown (2)	a/d/e (1)
	a/c/f (1)		
	a/d/f (6)		a*/d*/e*(10)
	b/d/f (1)		
	c/d/f (1)		unknown (3)
	a/b/d/f (3)		
	a/c/d/f (3)		

*a band is missing from the combination.

Chromosome 1A, group 1 (A genome)

Tugela had the combination *a/c/f* and Gamtoos DN *a/b*. In the F2 progeny *a/c/f* was expressed once and *a/b* was absent. The hybrids, *a/b/f, a/c, a/f, b/f* and *c/f* were expressed four, one, three, five and six times in the progeny, respectively. Therefore once again, no expected genetic inheritance patterns were expressed.

Combination *a* was found in two lines and *b* and *c* each in one line; they, therefore have lost the accompanying combinations which were present in the parents.

As in the first three crosses, the new combination *d* was again present. It was expressed as a hybrid once in *a/b/d, a/c/d, b/d/f* and *c/d/f* and three times in *a/b/d/f* and *a/c/d/f*, six times in *a/d, a/d/f* and twice in *c/d* and *d/f*, that is in 52% of the progeny. Combination *d* was also expressed in 52% of the Tugela x Flamink F2 progeny. Combination *d* may again be the product of recombination, as its first of three bands found in Gamtoos DN, although, a bit lower on the SDS-PAGE gel and the next two bands were found in both parents.

As none of the expected genetic inheritance patterns were followed, it is apparent that extra-genetic factors determined the inheritance of the band combinations or patterns. It is also evident that recombination did occur and that a large number of the progeny will keep these banding patterns to homozygosity.

Chromosome 1B, group 2 (B genome)

Tugela had the combination *b* and Gamtoos DN *a.* In the F2 progeny 17 lines had combination *a* and nine *b*. The hybrid *a/b* occurred only once in the progeny.

A new combination *c* found in neither parent,appear ten times in the progeny. Combination *c* has three bands, the first two bands are found in Gamtoos DN and the last one in Tugela. It may therefore be a product of recombination. It was also expressed in hybrid form *a/b/c* eight times and *a/c* three times in the progeny.

Two of the progeny did not correspond to any of the combinations in group 2. It again showed that there might be some combinations that are not yet included in the nomenclature of Gupta and Shepherd (1990b).

Recombination did occur in the progeny. Metakovsky *et al* (1984) stated that, "The future discovery of a recombination within the main cluster would compel us to regard this cluster also as a group of individual "loci"".

Chromosome 1D, group 3 (D genome)

Tugela had combination *d/e* and Gamtoos DN *a*. In the F2 progeny nine lines had combination *a* and one *a** (missing band 2) and 13 lines had combination *d/e*. The hybrid *a/d/e* occurred once, *a*/d*/e** 10 times and *a/e* nine times in the F2 progeny. Combination *d** and *e** are missing their slowest moving band. This was the first case where a 1:2:1 ratio was actually expressed as evident from the Chi-square test $p < 0.05$ ($\chi_2 = 1.8$).

Combination *e* was also found four times and an *a/b* combination once in the F2 progeny. Combination *b* was a new combination found in neither parent. It has five bands. The two slowest moving bands were found in Tugela, the next band in Gamtoos DN, the next in Tugela and the fastest moving band in both parents. It appears, therefore to be a product of recombination.

The bands if three progenies did not correspond to any of the known combinations in group 3. The possibility of some combinations not included in the classification of Gupta and Shepherd (1990b) are again raised.

Some combinations (*a**, *d** and *e**) were again missing one band within their banding patterns. Again, it appears that band suppression or non-expression has occurred within these combinations in the F_2 progeny.

❖ *Tugela x Tugela DN*

Table 1.7 Separation by SDS-PAGE of LMW subunit combinations in two parent wheat cultivars and their F2 progeny.

PARENTS	A genome	B genome	D genome
Tugela	a/c/f	b	d/e
Tugela DN	f	a/c	a/e
F2 progeny	a (10)	a (5)	a (5)
	c (1)	b (2)	a* (1)
	f (1)	c (28)	e (9)
	a/c (5)	a/b (1)	a/e (1)
	a/d (1)	a/c (13)	c/e (1)
	a/f (27)	unknown (2)	a*/d* (2)
	b/f (1)		d*/e* (22)
	c/f (2)		a*/d*/e* (5)
	a/b/f (1)		unknown (6)
	a/c/f (1)		

* a band is missing from the combination.

Chromosome 1A, group 1 (A genome)

Even though these two cultivars are near-isogenic (only differing for the DN-gene), they had different LMW-GS banding patterns. Tugela had combination *a/c/f* and Tugela DN *f*. In the F2 progeny, combinations *a/c/f* and *f each* occurred only once. The hybrids *a/f* and *c/f* occurred in 27 lines and two lines, respectively.

Combination *a* occurred in 10 lines and *c* in one line They therefore lost the accompanying combinations that were present in Tugela. Combination *a/c* was found in five lines and had lost the *f* combination.

The *b* and *d* patterns were present in neither parent and may therefore be the product of recombination. Combination *b* has two bands, the first was also found in both parents and second band could also be found in Tugela, although, the corresponding band on the SDS-PAGE gel was a bit higher on the gel. Combinations *a/b/f* and *b/f* occurred once each in the progeny.

Combination *d* has three bands, the first could be found in Tugela, but the corresponding band in Tugela was a bit lower on the SDS-PAGE gel. The next band was found in both parents and the last one in Tugela. Combination *a/d* occurred in one line. There was again some kind of suppression of banding patterns in the progeny. Recombination also occurred.

Chromosome 1B, group 2 (B genome)

Tugela had the combination *b* and Tugela DN *a/c*. In the F2 progeny *b* occurred in two lines and *a/c* in 13 lines. The hybrid *a/b* occurred once. The combinations *a* and *c* also occurred separately in five and 28 lines, respectively.

Two of the progeny did not correspond to any of the combinations in group 2. It again showed that there might be some combinations that are not yet included in the nomenclature of Gupta and Shepherd (1990b).

Chromosome 1D, group 3 (D genome)

Tugela had the combination *d/e* and Tugela DN *a/e*. In the F2 progeny *d*/e** occurred in 22 lines (*d** is missing band one and *e** band two). Combination *a/e* was found only once. The hybrid *a*/d*/e** was found in five lines and *a*/d** in two lines (*a** is missing band 2, *d** band two and *e** band one).

Combination *a* was found in five lines, *a** in one line and *e* in nine lines. These combinations lost their accompanying banding combinations. Six of the progeny did not correspond to any of the combinations in group 3.

A new combination *c,* found in neither parent, was present in one line as *c/e*. This must be the product of recombination. Combination *c* has four bands, band one was found in Tugela, the next in both parents, the next could be found in Tugela, but the corresponding band on the SDS-PAGE gel was a bit higher, the last band was found in both parents.

❖ ***Tugela x Tugela fg***

Table 1.8 Separation by SDS-PAGE of LMW subunit combinations in two parent wheat cultivars and their F2 progeny.

PARENTS	A genome	B genome	D genome
Tugela	a/c/f	b	d/e
Tugela fg	a	a/c	a
F2 progeny:	a (7)	a (6)	a (2)
	b (1)	a*(1)	a*(4)
	c (3)	b (3)	c*(3)
	e (2)	c (19)	d*(1)
	f (11)	c*(4)	e (8)
	a/b (1)	a/c (8)	e*(9)
	a/f (5)	a*/c*(3)	a/e (3)
	b/f (8)	a*/b*/c*(1)	d/e (6)
	c/f (8)	6 unknown	a*/d*/e*(5)
	a/b/f (3)		12 unknown

*a band is missing from the combination.

Chromosome 1A, group 1 (A genome)

Tugela fast growing, is a selection from Tugela, which has a shorter growing season. However, the LMW-GS were totally different for the two cultivars, although, their HMW-GS were the same. Tugela had combination *a/c/f* and Tugela fg *a.* In the F2 progeny combination *a* was found in seven lines and *a/c/f* in none. The hybrid *a/f* was found in five lines.

A combination *c* was found in three lines, *f* in 11 lines and *c/f* in eight lines. Some kind of suppression of banding patterns did again occur in the F2 progeny.

A new combination *b,* which was found in neither parent, was present in the F2 progeny. Combination *b* and *a/b* occurred eight times and *a/b/f* in three lines. Combination *b* may be a product of recombination, because it has two bands, the first was found in Tugela and the next one could also be found in Tugela, but the corresponding band was a bit higher on the SDS-PAGE gel.

Two of the progeny did not have any banding combination on the A genome (combination *e*).

Chromosome 1B, group 2 (B genome)

Tugela had combination *b* and Tugela fg *a/c.* In the F2 progeny *b* was found in three lines, *a/c* in eight and *a*/c** (*a** is missing band five and *c** band three) in three lines.

Combinations *a* and *a** occurred in six and one line, respectively, and *c* and *c** in 19 and four lines, respectively. Again, it appears that band suppression or non-expression has occurred within these combinations in the F2 progeny.

Six of the progeny did not correspond to any of the banding patterns in the nomenclature of Gupta and Shepherd (1990b).

Chromosome 1D, group 3 (D genome)

Tugela had combination *d/e* and Tugela fg *a*. In the F2 progeny *d/e* was expressed in six lines and *a* in two and *a** in four lines (*a** is missing band four). The hybrid *a*/d*/e** was found in five lines and *a/e* in three lines. Combination *d** was missing band one and e* band four.

Combination *d** occurred in one, *e* in eight and *e** in nine lines. Therefore, there was a reduction in the number of banding patterns, which were expressed.

A new combination *c**, found in neither parent, was found in three F2 lines. Combination *c* may be the product of recombination. It has four bands, the first was found in Tugela, the next in both parents and the next could also be found in Tugela, but the corresponding band was a bit higher on the SDS-PAGE gel. The last band was found in both parents, but was missing from *c**.

❖ ***Tugela x Letaba***

Table 1.9. Separation by SDS-PAGE of LMW subunit combinations in two parent wheat cultivars and their F2 progeny.

PARENTS	A genome	B genome	D genome
Tugela	a/c/f	b	d/e
Letaba	a/f	c	a/d
F2 progeny	a (7)	a (1)	a (7)
	b (1)	b (4)	a* (1)
	d (1)	b* (1)	c (12)
	e (2)	c (26)	c* (1)
	f (3)	c*(2)	d (5)
	a/c (7)	g (2)	e (3)
	a/b (2)	h (1)	c/e (3)
	a/d (3)	a/c (2)	a*/d*/e* (1)
	a/f (7)	b/c (1)	c*/d*/e* (7)
	b/f (2)	b*/c* (1)	unknown (9)
	c/d (2)	unknown (8)	
	c/f (4)		
	d/f (1)		
	a/c/f (4)		
	a/c/d (3)		
	a/b/d (1)		
	b/c/f (1)		

* a band is missing from the combination.

Chromosome 1A, group 1 (A genome)

Tugela had the combination *a/c/f* and Letaba *a/f*. In the F2 progeny four lines had *a/c/f* and seven *a/f*. The hybrids *a/c* occurred in seven lines and *c/f* in four. Again the expected 50% hybrids were not found.

Combination *a* and *f* occurred separately seven and three times, respectively. They had lost the accompanying combinations that were present in the parents.

Two lines had no bands (combination *e*) controlled by this chromosome. The new combination *d* was again present in the F2 progeny as in the first five crosses. It occurred once alone and as the hybrids *a/d, c/d, d/f, a/c/d* and *a/b/d* it occurred in two, two, one, three and one F2 progeny. This combination is also a product of recombination as the first band was found in Letaba, the next two in both parents.

Combination *b* is also new as it was found in neither parent. It was present in just one line and as hybrids *a/b, b/f* and *b/c/f* it occurred in two, one and one lines each. Again this combination probably is the product of recombination.

No genetical inheritance patterns were followed. Recombination of band patterns was again evident as was the suppression of certain bands and banding patterns in the F2 progeny, due to polimorphism.

Chromosome 1B, group 2 (B genome)

Tugela had the combination *b* and Letaba *c*. In the F2 progeny four lines had *b* and one *b** and 26 lines had c and two *c** (*b** and *c** are both missing band two). The hybrid *b/c* occurred once and *b*/c** also once in the F2 progeny. From the Chi-square test for the 3:1 ratio, (χ_2 = 7.44; $p<0.01$), it was evident that a 3:1 ratio was true in this case.

New combinations found in neither parent were also present. Combination *a* was found once and in hybrid *a/c* twice in the F2 progeny. It has five bands, the first

found in Tugela, the next in both parents, the next two in Tugela and the last one in Letaba. This may again be the product of recombination.

Combination *g* and *h* were found in neither parent and twice and once in the progeny respectively. These combinations were probably the products of recombination.

Eight of the progeny did not correspond to any of the combinations in group 2.

Chromosome 1D, group 3 (D genome)

Tugela had combination *d/e* and Letaba *a/d*. In the F2 progeny combination *c* occurred 12 times, *c** once and *d/e* not once in the F2 progeny. The expected 25% were only true in one case. The hybrid *c*/d*/e** occurred seven times (*c** and *d** are missing its second band and *e** its last band) and *c/e* only three times. Again the expected 50% hybrids were not found.

Combinations *d* and *e* occurred separately, five and three times respectively in the progeny. Again suppression of certain LMW subunits and banding patterns did occurred in the progeny.

A new combination present in neither parent did also occurred. Combination *a* was present in seven lines and *a** in one line. As a hybrid *a*/d*/e** occurred only once in the F2 progeny. It has four bands; the first is present in both parents, the next in Letaba, the next in Tugela and the last band again in both parents. Thus, this may again be the product of recombination. Nine of the progeny did not correspond to any of the combinations in group 3,according to the classification of Gupta and Shepherd (1990b).

❖ ***Tugela x Inia***

❖ Table 1.10 Separation by SDS-PAGE of LMW subunit combinations in two parent wheat cultivars and their F2 progeny.

PARENTS	A genome	B genome	D genome
Tugela	a/c/f	b	d/e
Inia	a/f	a	a
F2 progeny:	a (3)	a (11)	a (4)
	b (1)		
	c (1)	b (15)	d (1)
	d (2)		
	a/c (1)	c (7)	e (14)
	a/d (3)		
	a/f (5)	e (1)	e* (3)
	b/f (5)		
	c/f (3)	a/b (6)	a/b (1)
	d/f (2)		
	a/c/f (2)	a/c (5)	a/e (14)
	a/b/d (2)		
	a/b/f (1)	a/e (1)	d/e (8)
	a/d/f (9)		
	b/c/f (1)	a/b/c (1)	d*/e* (2)
	b/d/f (3)		
	c/d/f (1)	unknown 3	unknown 3
	a/b/d/f (1)		
	a/c/d/f (3)		

* a band is missing in the combination.

Chromosome 1A, group 1 (A genome)

Tugela had the combination *a/c/f* and Inia *a/f*. In the F2 progeny *a/c/f* was expressed twice and *a/f* five times. The hybrids *a/c* and *c/f* occurred once and three times, respectively.

Combination *a* and *c* occurred separately in the progeny from their accompanying combinations present in the parents. Some kind of suppression of banding combinations may have occurred.

It is again found, as in the first four crosses, that the new combination *d*, which was found in neither parent, is present. Combination *d* occurred twice alone and as hybrids *a/d, d/f, a/b/d, b/d/f, c/d/f, a/b/d/f* and *a/c/d/f* it occurred three, two, two, nine, three, one, one and three times in the F2 progeny. That is 50% of the progeny. In the Flamink and Gamtoos DN crosses combination *d* was also present in 52% of the progeny. Combination *d* may again be the product of recombination, as its first band could be found in Inia and the next two bands in Tugela.

Combination *b* was found once alone and in hybrid form *b/f, a/b/d, a/b/d/f, a/b/f, b/c/f, b/d/f* occurred five, two, one, one and three times respectively in the F2 progeny. That is 28% of the progeny. This must again be the product of recombination. It has two bands, the first found band was in Inia and the next in Tugela. Again no genetic inheritance patterns were followed.

Chromosome 1B, group 2 (B genome)

Tugela had the combination *b* and Inia *a*. In the F2 progeny 11 lines had combination *a* and 15 lines combination *b*. A new combination *c* found in neither parent was present in seven lines. In the hybrid form, *a/b/c* and *a/c* were found five times in the F2 progeny. This combination has three bands. The first band could be found in Tugela, but the corresponding band in Tugela was a bit higher on the SDS-PAGE gel. The next band could also be found in Tugela, but the corresponding band was a bit lower on the SDS-PAGE gel. The fastest moving band was found in Inia.

Another new combination *e*, found in neither parent, was present in one of the F2 progenies. The hybrid form, *a/e* was present only once. It has six bands and may again be the product of recombination.

Three of the progeny did not correspond to any of the combinations in group 2. It showed that some combinations might not yet be included in the nomenclature of Gupta and Shepherd (1990b).

New combinations may have replaced some hybrid forms and these combinations may be the products of recombination, thus influencing the inheritance patterns of the blocks.

Chromosome 1D, group 3 (D genome)

Tugela had combination *d/e* and Inia *a*. In the F2 progeny combination *a* occurred in four, *d/e* in eight and *d*/e** (*d** and *e** were both missing band one) in two lines. The hybrid *a/e* occurred in 14 lines and *a/d/e* in none of the progeny.

Combination *d* and *e* or *e** occurred separately in the progeny. Combination *d* occurred once, *e* 14 times and *e** (*e** was missing its fastest moving band) three times in the F2 progeny.

A combination *a/b* occurred once. Combination *b* was found in neither parent. This may again be a product of recombination as the first two bands were found in Tugela, the next band in Inia, the next in Tugela, but the corresponding band on the SDS-PAGE gel was a bit lower on the gel. The last band was again found in both parents. There was a large reduction of LMW-GS in the progeny.

Again three of the progeny did not correspond to any known combination in group 3.

1.5 Conclusion

From this study it was evident that the inheritance of LMW-GS in South African material was much more complex than expected. Firstly, the pure line cultivars had combinations of patterns witin groups (genomes), whereas according to Gupta and Shepherd (1990b) classification system only a single band pattern witin a froup should occure. There is therefore no possibility that the cultivars were still segregating, or that hybrids were expressed.

A further complicating factor was that, in a number of cases, bands from each parent were recombined in the progeny to form new banding patterns. In some

cases the new combinations matched the banding patterns described by Gupta and Shepherd (1990b), but in other cases new banding patterns were formed, which have not been described by them. This phenomenon also precludes the expression of clear genetic inheritance patterns, such as a 1:2:1 ratio, which may otherwise have been expressed (Bietz, 1987). It was also found that the parents often contained more than one banding pattern which were not inherited by the progeny in the same combinations.

This was also confirmed by Gupta and Shepherd (1990b) who stated that polymorphism is common in wheat and new banding combinations are quite possible. They also stated that rare recombinations and point mutations could generate new LMW-GS combinations. In this study the recombinations were quite frequent.

Most of the banding combinations matched those found by Gupta and Shepherd (1990b), but clearly the nomenclature requires extension to include combinations of band patterns that apparently occur uniquely in South African wheat lines.

For almost all the crosses, there was a reduction in the number of LMW-GS band patterns in the progeny relative to the parent. The reason for this are not entirely clear. The expected hybrids, where a full compliment of the banding patterns of the parents are present, realised only in a few progeny, which again emphasises the complexity of the pre- or post-translation genetics of the LMW-GS.

The multiple bands found in the arents and the apparent lack of strong linkage between the controlling genes makes the likehood of simple genetic inheritance patterns in the F_2 lines slim.

Chapter 2

Quality analysis of F2:3 derived lines of eight wheat crosses differing in LMW-GS.

2.1 Introduction

Wheat quality can be defined as the suitability of the cultivar for the intended procedure and product manufactured from the grain. Milling performance, dough rheology and the baking quality are the main criteria used to describe wheat quality. Quality testing thus provides important information that is used to facilitate selection during breeding. Furthermore, prediction of inherent end-use quality is important to the miller, baker and breeder as it affects income directly (Peterson *et al,* 1992; Graybosch *et al*, 1996)

Quality wheat is used principally by the breadmaking industry, which sets the standards that plant breeders strive to meet. Millers and bakers require a stable, assured supply of wheat of high specific weight (hectolitre mass) and good milling texture that is low in α-amylase.

The proteins of wheat flour are crucial in determining a flour's breadmaking quality, both quantity and qualitative characteristics of protein being important. Quality is mainly controlled genetically whereas quantity is largely influenced by enviromental or related factors (MacGuire and MacNeal, 1974; Fowler *et al*, 1975; Baenziger *et al*, 1985; Peterson *et al*, 1992). The ability of wheat flour to be baked into bread is due to the physico-chemical properties of its gluten protein fraction and differences in this same fraction. This discovery has prompted an intensive effort to understand the chemical basis for gluten's functionality and its variation from one wheat variety to another, which is largely controlled genetically (Schofield, 1986).

The aim of this chapter is to determine the breadmaking quality for the eight Tugela crosses, using the F2:3 derived lines. The usability of this very early

generation quality test will be determined and also as far as possible the influence of the LMW-GS on quality in this material.

2.2 Protein quantity

Protein quantity is important due to the nutritional value of the protein (Eliasson, 1990). Thus, wheats that do not have a high or acceptable protein quantity, are undesirable for consumers (MacGuireand MacNeal, 1974). The protein content in wheat can vary from 6% up to as much as 27%. Most commercial wheat cultivars in South Africa, however, contain between eight to 16% protein. A protein content higher than 11% is sufficient for the production of well leavened bread (Koekemoer, 1997).

Most of the differences in breadmaking quality between flours are explained by variation in the protein content. Protein content and breadmaking quality of flour are positively correlated, but selection for a higher protein content is hampered by a negative relationship with kernel yield (Schepers *et al*,1993).

Fowler and De la Roche (1975) found that most of the variation in loaf volume could be explained by variation in protein quantity. Flour protein content and SDS-sedimentation volumes were often correlated with quality parameters (Graybosch *et al*, 1996). A significant correlation was found between loaf texture and glutenin content. Loaf texture and flour protein content were, however, negatively correlated (Peterson *et al*, 1992; Graybosch *et al*, 1996).

2.3 Protein quality

Differences in breadmaking quality can also be attributed to differences in protein quality. Dough development is influenced by protein quality, which emphasizes the importance of protein quality in cultivar breeding (Fowler and De la Roche, 1975).

Protein quality is almost as important as protein quantity in baking (Campbell *et al*, 1987). It has long been accepted that the breadmaking quality of wheat flour

is primarily determined by its proteins. Therefore, considerable effort has been made to elucidate which protein constituents are responsible for the quality differences (Singh *et al,* 1990).

Although protein content is important, there is now ample evidence that the kinds of protein present are also very important (Day *et al,* 1986). One explanation was that flour quality varies with the glutenin-gliadin ratio. Attempts to correlate glutenin-gliadin ratio with flour quality of wheat cultivars produced conflicting results. Reconstitution studies demonstrated that rheological properties of the reconstituted flours are highly influenced by the relative amount of these fractions (Singh *et al,* 1990).

Gliadin mainly confers extensibility to dough. It was found that a cultivar with strong dough properties and good baking performance was associated with a high glutenin to gliadin ratio (Peltonen and Virtanen, 1994). To make leavened bread a strong flour is required which, after mixing with water to form a dough, has elasticity (conferred by glutenin) and extensibility (mainly conferred by gliadin) (Payne *et al,* 1985).

It seems more likely that it is the glutenin subunits that affect gluten quality rather than the gliadin proteins. The observation that for certain wheats some gliadin bands are tightly linked to certain LMW subunits of glutenin, suggests the possibility that the observed association between the presence of certain gliadin bands and indices of breadmaking quality may be spurious. Rather, breadmaking quality could perhaps also be associated with the presence of certain LMW subunits of glutenin whose genetic linkage to the particular gliadin components may so far have gone unrecognized (Schofield, 1986).

The elasticity, or tendency of a dough to recoil after stretching, results from the presence of long fibrils of gluten molecules. The molecules consist of two groups of subunits, HMW-GS and LMW-GS, which are connected together by disulphide bonds (Day *et al,* 1986).

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The prominent role of glutenin subunits in determining dough strength lies almost entirely in their capacity to create a large variability in the size of the polymers (hence overall protein) by forming intermolecular disulphide linkage. There is a loss of cohesive elastic properties of the dough or polymeric protein (native glutenin) when the disulphide linkages are cleaved even partially. This clearly supports the positive roles of disulphide linked polymers, particularly of the polymers, in governing dough strength. Monomeric proteins, on the other hand, do not form intermolecular, disulphide linkage, thus do not have positive effects on strength properties of wheat flours (Singh *et al*, 1990).

The different HMW-GS have been ranked in order of their influence on breadmaking quality. However, similar relationships have not been found for Australian wheat, but, rather, stronger associations have been observed between glutenin subunit composition and grain hardness and dough strength, although these apparently are mainly pedigree associations (Schofield, 1986).

The importance of *Glu1*-loci in determining protein quality was assessed by Randall *et al* (1993) and Schepers *et al* (1993). Allelic bands 1 and 2* on chromosome 1A, are equally desirable in contrast with the null allele (Moonen *et al*, 1983; Lukow *et al*, 1989). Bands 7+8 as well as 7+9 (Lukow, 1991) and 13+16 (Lukow *et al*, 1989) are desirable combinations on chromosome 1B. Dong *et al* (1992) reported, however, that subunits 17+18 on chromosome 1B have the strongest positive association with loaf volume. Interaction of subunits 8 and 9 has a detrimental effect on the quality parameters and is associated with a reduced protein content (Khan *et al*, 1989).

The HMW-GS 5+10 are said to be present in varieties of good baking performance, whereas 2+12 are present in varieties of poor performance (Campbell *et al*, 1987; Cressey *et al*, 1987). The presence of subunits 5+10 is also associated with a high sedimentation volume in the SDS sedimentation test, whereas the presence of subunits 2+12 is associated with a low sedimentation volume (Payne *et al*, 1981). On the basis of these results, quality scores are assigned to each of the HMW-GS and by adding these scores up for the different HMW-GS present, a quality score called the Glu-1 score, is obtained for each

wheat variety. The highest score is obtained for the subunits 5+10. The Glu-1 score is positively correlated with baking performance in the case of bread and 47 to 60% of the variation in breadmaking qualities could be accounted for by the variation in HMW subunits. The quality score is negatively correlated with the baking performance in the case of biscuits (Payne *et al*, 1987a), which is in accordance with what is expected. The results for Australian wheats, however, gave contrasting results, showing that the Glu-1 score accounted for as little as 11% variation (Campbell *et al,* 1987). It has be noted in southern African wheats that the effect of HMW-GS on breadmaking quality is less than 20% (Randall, personal communication).

The correlation between subunits 5+10 and baking quality is lower in some countries. For Australian wheats a somewhat weaker relation was observed, perhaps related to the presence of subunit 17+18 (Campbell *et al*, 1987). The HMW glutenins seem not to influence loaf volume during fermentation or proofing (Singh *et al*, 1990). Lines with poor combinations of HMW-GS generally were poor in quality. The presence of favorable combinations of HMW-GS did not ,however, guarantee acceptable quality (Primard *et al,* 1991).

HMW-GS appears to also have additive effects on dough quality, which enhance their value for predicting dough properties. Identification of proteins that confer good breadmaking quality of flour at an early stage will thus accelerate the process of developing new and better varieties. Recent studies have indicated that a more effective predictive model of dough properties should include the composition of both the LMW-GS and HMW-GS (Gupta *et al,* 1991).

These difficulties in accessing the relevance of the *Glu-1* composition can be explained by the *Glu-3* (LMW glutenin) composition. The *Glu-3* composition accounted for a higher proportion of variation (42%), than did the Glu-1 score for a set of 48 Australian wheats. These data thus emphasize the previously little recognized importance of the LMW glutenin as significant components to the assessment of the breadmaking potential of wheat flour (Gupta *et al*, 1991). The effect of the LMW and HMW-GS on dough quality appear to be additive (Pogna *et al,*1990).

LMW-GS (*Glu-3*) affected the quantity and/or size distribution of the polymers due to differences in the amounts and/or probably the quality of the subunits produced. The LMW-GS allelic comparison at the *Glu-A3* and *Glu-B3* loci showed differences in %UPP (unextractable polymeric protein) values. Alleles at the *Glu-B3* locus also differed with respect to the absolute quantity of the total polymer, however, their allelic effects on dough strength can be attributed to either quantity or quality or the polymers. The alleles at the *Glu-D3* and *Glu-A1* loci showing similar effects on Rmax (maximum dough resistance) gave similar %UPP values. This suggests that the allelic differences in Rmax for all these loci could be accounted for by the variation in the relative size distribution of the polymeric protein (UPP%) alone (Gupta and Shepherd, 1988).

The alleles at the *Glu-B3* locus affected both quantity and the size of the polymers. These could be attributed to differences in the amounts of the B type (not the C type) of LMW-GS and the ratio's of the B and C subunit quantities (Figure 1.3), respectively (Gupta and Shepherd, 1988).

The effects of individual glutenin loci seem to be largely additive for dough strength, indicating that dough strength can be improved by selecting for the glutenin subunit alleles with superior positive effects. These loci interact with each other in the manifestation of dough strength (Gupta and MacRichie, 1994).

The Australian wheat alleles at the *Glu-3* loci provided better prediction for Rmax and Ext (extensibility), than did the *Glu-1* loci. However, the best prediction of these values was obtained when it was based on both LMW and HMW subunits together.

Relative ranking of the alleles, occurring at a frequency of five or more at each LMW glutenin locus, was almost identical between the Australian and World wheat sets: thus, they were summarized as follow with respect to their effects on Rmax: *Glu-A3*, $b > [d = e] > c$; *Glu-B3*, $b = i >> g = h >> c$ and *Glu-D3*, $b = a >> c$. Similarly, these alleles can be ranked with respect to their effect on Ext: *Glu-A3*, $d > b > c > e$ and *Glu-D3*, $e > c > b = a = d$ (Gupta *et al*, 1991).

The information available on the effects of LMW-GS on what is much more limited than for the HMW-GS, thus further analysis is needed of the relationship between the LMW glutenin alleles and dough properties so that LMW and HMW subunits can be considered together so as to better predict flour quality (Gupta *et al,* 1991).

2.4 Quality tests

2.4.1 Hectolitre mass (test weight)

Hectolitre mass (test weight) is an economically important parameter, because it is used to predict the potential flour yield (Finney *et al,* 1987; Nel *et al,* 1998). Hectoliter mass depends on the density of kernels and their packing efficiency. It is widely recognized as an important consideration for the grading of wheat grain (Ghaderi and Everson, 1975), but may be influenced strongly by the environment (Jalaluddin and Harrison, 1989). Due to its economical importance these authors also stated that grain hectolitere mass must be used as a selection criterion. De la Roche and Fowler (1975) found that hectoliter mass, kernel weight and mixograph values were positively correlated. Wheats with a higher hectolitre mass also tended to mill better (Gaines *et al,* 1996).

2.4.2 Flour extraction (flour yield)

During the milling process the endosperm is separated from the bran before its conversion to flour. The extraction percentage reflects the ease with which this is achieved. The main objective is to achieve maximum flour yield with minimum bran and germ contamination. The more effective the extraction, the more flour is produced (Rubenthaler and King, 1987).

2.4.3 Falling number (endosperm starch content determination)

The falling number measures the alpha-amylase concentration. It gives an indication of the starch to sugar conversion in the wheat grain (Lukow and Bushuk, 1984). Starch is the major storage compound in wheat and other cereal

grains. When flour, water and all the other ingredients required for breadmaking are being mixed, the storage proteins hydrate and yield a continuous filmlike matrix in which the starch granules are embedded (Hoseney,1985). These characteristics together with higher water absorption enhanced by damaged starch granules, such as when hard wheat is milled, causes unsprouted wheat flour to have a higher falling number.

Under rainy conditions prior to harvesting wheat grain may begin to germinate, a phenomenon known as preharvest sprouting (Derera *et al,* 1977). The α-amylase in sprouted wheat results in degration of starch into simple sugars. Consequently sprouted wheat will have a higher sugar content that is unacceptable to the baking industries.

2.4.4 The mixograph

The mixograph measures the mixing time, tolerance to mixing and the optimum water absorption (Gras *et al,* 1990; Spies, 1990). The mixing curve (mixogram) also indicates optimum development time (point of minimum mobility); tolerance to over-mixing, descending graph width, other dough characteristics (such as being weak or strong) and estimates baking absorption. The mixograph has been used to study dough rheology, blending, quality control and for the evaluation of hard, soft and durum wheats (Finney *et al,* 1987).These advantages make the mixograph a valuable selection criterion for wheat breeders to use when assessing breadmaking quality in early generation lines (Wikström and Bohlin, 1996).

During dough mixing, the resistance of the system to extension increases progressively until the point of minimum mobility is reached. This is referred to as the point where dough is optimally mixed (Finney *et al,* 1987). The mixing time of the mixograph (in minutes) indicates the rate at which the flour and water are blended together into a quasi-homogeneous mixture in order to develop a gluten matrix and to incorporate air (Spies, 1990). This method proved to be a valuable tool for the selection of wheat cultivars with superior quality (Van Lill and Purchase, 1995).

2.4.5 The alveograph

The alveograph is used mainly to evaluate the breadmaking quality of flours. The alveograph is designed to measure the resistance to bi-axial extension of a thin sheet of flour-water-salt dough and gluten fraction (Bettge *et al*, 1989). A stiff dough is prepared with a mixer containing a sigmoidal blade. This process is similar to the effect of sheeting, rounding and moulding in the baking process.

Disks are cut from the sheet of dough, allowed to relax for 20 minutes and then clamped above a valve mechanism (Walker and Hazelton, 1996). Air is blown at a constant rate from below the disk, creating a bubble, until it ruptures and is then recorded.

The length of the alveograph curve (L) is related to extensibility, the height (height x 1.1 = P) is associated with stiffness, shortness and tightness of the dough and W is a measure of resistance to extensibility, or the strength of the dough. The P value should be a measure of water absorption capacity of flour when the standard alveograph is used (Chen and D'Appolonia, 1985). It is important to distinguish between extensibility as measured for viscoelastic material and plasticity, which refers to drawing out of nonelastic material. Extensibility corresponds to elongation at break in a tensile stress test. As a dough is stretched, the protein network strands become thinner until failure occurs (MacRitchie, 1992). Randall *et al* (1993) considered the alveograph a suitable tool for predicting breadmaking quality.

2.4.6 The Farinograph

The farinograph is one of the most widely used physical dough instruments in the world. It measures and records the resistance of dough to mixing. It is used to evaluate water absorption of flours and to determine stability and other characteristics of dough during mixing (Finney *et al,* 1987)

Water absorption is among the indicators of baking quality (Finney *et al,* 1987; Van Lill *et al*, 1995). Water absorption gives an indication of the potential of the

protein molecules to absorb moisture. As it is with the mixograph, the farinograph evaluates dough stability. Dough stability estimates the ability of dough to resist mechanical mixing (Brunori *et al,* 1989).

2.4.7 Flour colour

A white flour colour is desired and both millers and bakers use the degree of colour as an important indication of flour quality. Colour change may occur due to genotype, environment, or their interaction and several factors are involved such as: the carotenoid pigment inherent in the wheat kernel; discoloration caused by microbial infestation, particles of bran, darker mill streams; the percent extraction of the flour; etc. (Patton and Dishaw, 1968; Shuey and Skarsaune, 1973). Different methods are used for colour determination and taking the Small Grain Institute quality lab as an example, colour grader series III is used.

Flour categories; Cake flour = -2.5 to 1.0

White bread = 1.5 to 4.5

Brown bread = 9 to 14

2.4.8 The baking tests

Loaf volume is a major selection criterion in identifying cultivars with superior quality (McGuire and McNeal, 1974). The loaf volume and protein content of a single sample of wheat flour establishes its relative gas-retention or protein quality (Finney *et al,* 1987).

Good gluten quality is needed to produce a well-shaped loaf of bread with high volume and a good, fine and resilient crumb structure. Many components are important determinants of the texture of a slice of bread, for example the stickiness, springiness and most importantly, the firmness of the crumb (Spies, 1990). Schepers *et al* (1993) reported a low correlation between loaf volume and grain yield.

2.5 Materials and methods

2.5.1 Materials

From the materials described in section 2.4.1, F 2:3 lines were developed as follow:

After the screening for LMW-GS, the remaining seed from the F2 progenies were randomly selected. The eight progenies consisting of F2 lines were planted in a randomized block design with three replications. The F2-.plants were allowed to self-pollinare to generate F2:3 derived seeds. Each entry was harvested separately as a bulk. Only entries with an excess of 500 g seed were used for quality analyses. The LMW-GS for the F2 progenies and their parents are given in Appendix B.

Two control cultivars; Tugela and Kariega were included in the baking tests. Tugela is the parent cultivar with over stable dough and Kariega is the national control cultivar for baking quality.

2.5.2 Methods

The F2:3 derived lines of eight different crosses were analysed to assess the influence of LMW-GS of the A-, the B- and D-genome on breadmaking quality.

The samples were evaluated with the following tests and most of them using the indicated procedures of the American Association of Cereal Chemistry (AACC).

- **Hectoliter mass**

The hectoliter mass was determined with a two level funnel, a quality litre bucket and a mass meter.

- **Flour Extraction**

This is the flour yield after milling. Flour yield was calculated from the mass of total products and was expressed as a percentage. Extraction was measured to a maximum of 76%, since this is a standard.

- **Flour protein (AACC 39-11)**

An infrared reflectance spectrophotometer (Infra Alyser 360) was used which has been calibrated using Kjedahl data.

- **Falling number determination (AACC 56-81B)**

Falling number is a method based on the unique ability of α-amylase to liquefy a starch gel. The strength of the enzyme is measured by falling number (FN) apparatus, defined as time in seconds (sec) required to stir and allow the stirrer to fall a measured distance through a hot aqueous flour or meal gel undergoing liquefaction.

- **Mixograph method (AACC 54-40A)**

It is applicable to wheat flour of hard, soft and durum wheats. The mixograph measures and records dough development behaviour and its resistance to mixing. The equipment allows the use of either a 10 g or 35 g bowl, depending on flour quantity. The 35 g bowl was used for this test.

- **Farinograph method (AACC 54-21A)**

It is used to evaluate the water absorption capacity of wheat flours and other dough characteristics during mixing. The Barbender farinograph, with a large (300 g flour) or a small (50 g flour) mixing bowl is normally used, further adjustments (as directed in AACC 54-12) needs to be done when changing from one bowl to another. For this test the 50 g flour bowl was used.

- **The Alveograph (AACC 54-30A)**

It is designed to measure the resistance to a bi-axial extension of a thin sheet of flour-water-salt dough (generally at a constant hydration level). A sheet of dough of definite thickness prepared under specific conditions is expanded by air pressure into a bubble until it is ruptured and the internal pressure in the bubble is graphically recorded. The method uses the Chopin alveograph to blow the bubble.

- **Bread-making method (AACC 10-09)**

It is intended primarily for laboratory assessment of bread wheat flour quality under vigorous fermentation conditions. Dough mixing was done using a mixer, Swanson pin-type or equivalent, having a capacity for 100 to 500 g dough. Loaf volume was used for one analysis and for the second analysis the loaf volume was adjusted to 12% protein by adding 40 cm^3 for each 1% protein under 12% and 40 cm^3 was deducted for every 1% protein above 12% protein.

- **Statistical analysis**

The statistical analysis was done with the Agrobase 2000 program (Agronmix, Canada). ANOVA's and correlation analysis were done. The program also calculates the heritability or genotype contribution of each parameter from the ANOVA.

2.6 Results and discussion

The quality data for the three replications for each of the genotypes are summarized in Appendix C.

Table 2.6. Linear correlation coefficient for all the quality characters measured on F2 :3 derived lines. (*-p<0.05) (**-p<0.01)

Quality parameter	Correlation coefficient (r)	Quality parameter
Hectolitre mass	0.4732*	SDS-sedimentation volume
	-0.4147*	Flour colour
	-0.3836*	Falling number
	-0.4140*	Mixograph dough development time
Flour protein	-0.5683**	SDS-sedimentation volume
	0.7284*	Gluten 12%
	0.5685**	Loaf volume
Falling number	0.4283*	Vitreous kernels
	-0.3836	Hectolitre mass
Mixograph dough development time	-0.5229**	Break flour yield
	0.5253**	Farinograph water-absorption
	0.5550**	Alveograph P/L-value
	0.4673*	Alveograph strength
	-0.4140*	Hectoliter mass
	-0.4861*	Loaf volume 12%
Farinograph waterabsorption	0.4248*	Vitreous kernels
	-0.6174**	Breakflour yield
	-0.4761*	Loaf volume 12%
	0.6778**	Alveograph P/L-value
	0.8697**	Alveograph strength
	0.4840**	Gluten 12%
	0.7021**	Flour colour
	0.5253**	Mixograph dough development time
Alveograph P/L-value	-0.6600**	Loaf volume 12%
	0.4199*	Vitreous kernels
	-0.6636**	Breakflour yield
	0.4755*	Flour colour
	0.5550**	Mixograph dough development time
	0.6778**	Farino-waterabsorption
	-0.4761*	Loaf volume
Alveograph dough strength	0.6323**	Flour colour

(Table 2.6 continue)		
	0.4673*	Mixograph dough development time
	0.8697**	Farinograph waterabsorption
	0.8462**	Alveograph P/L-value
	0.5077**	Vitreous kernels
	-0.7235**	Breakflour yield
	-0.6736**	Loaf volume
Loaf volume	0.5685**	Flour protein content
	-0.4794*	SDS-sedimentation volume
	-0.4761*	Alveograph P/L-value
	0.5289*	Loaf volume 12%
	0.6259**	Gluten 12%
Loaf volume 12%	0.4628*	Breakflour yield
	-0.4861*	Mixograph dough development time
	-0.4761*	Farinograph waterabsorption
	-0.6600**	Alveograph P/L-value
	0.6736**	Alveograph strength
	0.5289*	Loaf volume
Gluten 12%	0.7284**	Flour protein content
	-0.6454**	SDS-sedimentation volume
	0.5533**	Flour colour
	0.4840**	Farinograph waterabsorption
	0.6259**	Loaf volume
SDS-sedimentation volume	-0.5683**	Flour protein content
	-0.6454**	Gluten12%
	-0.4732*	Hectolitre mass
Breakflour yield	-0.5229**	Mixograph dough development time
	-0.6174**	Farinograph waterabsorption
	-0.6636**	Alveograph P/L-value
	-0.7235**	Alveograph strenth
	0.4328*	Loaf volume 12%
Vitreous kernels	0.4519*	Flour yield
	0.4283*	Falling number
	0.4248*	Farinograph waterabsorption
	0.4199*	Alveograph P/L-value
	0.5077**	Alveograph strength
Flour colour	0.4677*	Mixograph dough development time
	0.7021**	Farinograph waterabsorption
	0.4755*	Alveograph P/L-value
	0.6323**	Alveograph strength
	0.5533**	Gluten 12%
	-0.4147*	Hectolitre mass
	0.4519*	Vitreous kernels

▫ Hectolitre mass

The hectolitre mass predicts the expected flour yield during milling. In South Africa, a minimum hectolitre mass of 76 kg/ha is required for wheat to be graded suitable for breadmaking (Koekemoer, personal communication).

The analysis of variance results (Table 2.6.1) show significant variance ($p < 0.01$) for entries. This implies different genotype performance. The genotype contribution of the parameter was 88.2%, indicating that the environment contributed only a small part of the variability.

Table 2.6.1 Analysis of variance for hectoliter mass. * ($p < 0.05$) ** ($p < 0.01$)

Source	DF	SS	MS	F-value	Pr>F
Total	29	30.932			
Block	2	0.003	0.001	0.00	0.9955
Entry	9	25.034	2.782	8.49	0.0001**
Residual	18	5.895	0.328		

The averages of the crosses (Table 2.6.2) ranged from 78.19 kg/ha to 80.82 kg/ha for Tugela x Flamink and Tugela x Letaba, respectively. Only the Tugela x Letaba cross had a value (80.82 kg/ha) significantly higher than the standard Tugela (79.83 kg/ha). The other standard (Kariega) had a value of 80.58 kg/ha. Tugela x Letaba had a significant higher hectolitre mass than Tugela. Letaba and Tugela differed by a *c* banding pattern on *Glu-A3*, Tugela had a hectolitre mass that was significant higher than Tugela x Gariep, Tugela x Tugela fg, Tugela x Inia, Tugela x Palmiet and Tugela x Flamink. This suggest that the contribution towards hectolitre mass from the second parent in each case must have reduced the hectolitre mass. Especially Tugela fg, which is genetically the closest related to Tugela.

The hectolitre mass is positively correlated with the SDS-sedimentation value and negatively with flour colour, falling number and mixograph dough development time (Table 2.6).

Table 2.6.2 Ranking and averages of the genotypes for hectolitre mass.

Rank	Genotypes	Averages (kg/ha)
1	Tugela x Letaba	80.82
2	Kariega	80.58
3	Tugela x Tugela DN	80.56
4	Tugela x Gamtoos DN	80.17
5	Tugela	79.83
6	Tugela x Gariep	78.96
7	Tugela x Tugela fg	78.79
8	Tugela x Inia	78.76
9	Tugela x Palmiet	78.64
10	Tugela x Flamink	78.19

Grand mean = 79.529 LSD for entry = 0.8103

- **Flour protein content**

Apart from influencing some of the physical characteristics, protein content plays a major role in the functionality of wheat flour. Very high and very low protein content is not desirable for breadmaking in South Africa. A ± 12% flour protein content is recommended for good bread quality.

Table 2.6.3 Analysis of variance for flour protein content.

Source	DF	SS	MS	F-value	Pr>F
Total	29	91.110			
Block	2	1.161	0.580	0.53	0.5993
Entry	9	70.123	7.791	7.07	0.0002**
Residual	18	19.826	1.101		

* ($p < 0.05$) ** ($p < 0.01$)

The analysis of variance results (Table2.6.3) show significant variance ($p<0.01$) for entries. The genotype contribution to total variation for this for this parameter was 85.9%.

The averages of the crosses (Table 2.6.4) ranged from 12.17% to 17.17% for Kariega and Tugela x Letaba, respectively. The crosses Tugela x Inia and Tugela x Tugela fg had averages under 14%. The standard, Tugela, had a value of 14.03%.

Table 2.6.4 Ranking and averages of the genotypes for flour protein content.

Rank	Genotypes	Averages (%)
1	Tugela x Letaba	17.17
2	Tugela x Gariep	16.50
3	Tugela x Flamink	16.33
4	Tugela x Gamtoos DN	15.13
5	Tugela x Tugela DN	14.60
6	Tugela x Palmiet	14.57
7	Tugela	14.03
8	Tugela x Inia	13.27
9	Tugela x Tugela fg	13.20
10	Kariega	12.17

Grand mean = 14.697 LSD for entry = 1.4859

The crosses Tugela x Palmiet, Tugela x Tugela DN, Tugela x Gamtoos DN, Tugela x Flamink, Tugela x Gariep and Tugela x Letaba, had values significantly higher than Kariega and the last three, had values also significantly higher than Tugela. There were no significant differences between the lines, which were genetically closely related, i.e. Tugela, Tugela Dn and Tugela fg. In this trial the contribution of the genotype towards variability was relatively high (85.9%). Once again the contribution by the parent's LMW-GS was so diverse, that no one banding pattern can be identified, which definitely influenced the protein content.

Flour protein content is negatively correlated with SDS-sedimentation volume and positively with gluten strength at 12% protein and loaf volume (Table 2.6).

- **Falling number**

The falling number (in seconds) gives an indication as to what extent starch has been converted to sugar in the wheat grain, as determined by the alpha-amylase concentration (Hayberg, 1960). Usually values less than 250 s indicates flour that will be unacceptable for bread-baking purposes (Van Lill and Smith, 1997).

The analysis of variance results (Table 2.6.5) showed no significant variance for block and entries. The heritability of this parameter was 6.5%. This implies that the influence of the environment was very significant.

Table 2.6.5 Analysis of variance results for falling number.

Source	DF	SS	MS	F-value	Pr>F
Total	29	4463.867			
Block	2	176.867	88.433	0.57	0.5755
Entry	9	1493.867	165.985	1.07	0.4287
Residual	18	2793.133	155.174		

* ($p < 0.05$) ** ($p < 0.01$)

The averages of the crosses (Table 2.6.6) ranged from 392.67s to 410s, for Tugela x Letaba and Tugela x Gariep, respectively. These values are much higher than the minimum requirement. Falling number was positively correlated with vitreous kernels and negatively with hectoliter mass (Table 2.6).

Table 2.6.6 Ranking and averages for the genotypes for falling number.

Rank	Genotype	Average (s)
1	Tugela x Gariep	410.00
2	Tugela x Gamtoos DN	410.00
3	Tugela x Flamink	410.00
4	Tugela x Palmiet	410.00
5	Tugela x Inia	410.00
6	Tugela x Tugela DN	410.00
7	Tugela x Tugela fg	409.00
8	Tugela	395.67
9	Kariega	395.33
10	Tugela x Letaba	392.67

Grand mean = 405.267   LSD for entry = 17.6372

- **Mixograph dough development time**

During dough mixing, the resistance of the system to extension increases progressively until the point of minimum mobility is reached. This is referred to as the dough development.

The dough development time is considered as the point where dough is optimally mixed (Finney *et al*, 1987). In South Africa the optimal time required to mix a dough is two to three minutes (with 2.5 min as optimum). A shorter mixing time will result in sub-optimal dough development, whereas a longer mixing time is not desirable due to spending more time and energy resulting in financial losses to the baker.

The analysis of variance results (Table 2.6.7) show significant variation ($p < 0.01$) for entries. This implies different genotype performances. The genotype contribution to the total variation for this parameter was 90.7%.

Table 2.6.7 Analysis of variance results for mixo-dough development time.

Source	DF	SS	MS	F-value	Pr>F
Total	29	20.670			
Block	2	0.393	0.196	1.11	0.3508
Entry	9	17.096	1.900	10.75	0.0000**
Residual	18	3.181	0.177		

* ($p < 0.05$) ** ($p < 0.01$)

Mixograph dough development time is largely genetically determined (Van Lill, 1992). This signifies its importance as a selection criterion in the assessment of breadmaking quality in early generation wheat lines.

The averages of the crosses (Table 2.6.8) ranged from 2.20 min to 4.80 min, for Tugela x Tugela DN and Tugela x Tugela fg, respectively. It is interesting to note that the average of Tugela is 3.63 min, which is lower than the mixing time of Tugela x Tugela fg. This was, however, not the case with Tugela x Tugela Dn, was 2.20 min.

Table 2.6.8 Ranking and averages of the genotypes for mixo-dough development time.

Rank	Genotypes	Averages (min)
1	Tugela x Tugela fg	4.80
2	Tugela	3.63
3	Tugela x Flamink	3.20
4	Tugela x Palmiet	2.83
5	Tugela x Inia	2.60
6	Tugela x Letaba	2.57
7	Tugela x Gariep	2.50
8	Tugela x Gamtoos DN	2.37
9	Kariega	2.33
10	Tugela x Tugela DN	2.20

Grand mean = 2.903 LSD for entry = 0.5952

Six of the crosses and one standard (Kariega) had mixing times within the acceptable range of 2 min to 3 min. There were two crosses and one standard (Tugela) above these values.

Tugela x Tugela fg had a significant longer mixing time than Tugela. As this parameter is determined genetically to a large extent. There was also a significant difference between Tugela and Tugela DN, which would probably have been caused by the *a/c* on the *Glu-A3*, the *b* on *Glu-B3* and the *d* on *Glu-D3* in Tugela, which was not present in Tugela DN. Tugela x Palmiet, Tugela x Inia ,Tugela X Letaba, Tugela x Gariep and Tugela x Gamtoos DN also all had significant lower mixing times than Tugela.

The mixo-dough development time was negatively correlated with breakflour yield and hectolitre mass. It was also positively correlated with farinograph waterabsorption, alveograph P/L-value and alveograph strength (Table 2.6).

Mixing time decreases as flour protein content increases to about 12%, therefore remaining approximately constant with increases in flour protein. According to Van Lill (1992) low flour protein content appears to increase mixograph mixing time requirement. This could not be seen in this study, because the protein content for all the crosses were between 13.2% to 17.17%.

- **Farinograph waterabsorption**

The wheat flour waterabsorption capacity shows the potential of protein molecules to absorb moisture. In South Africa the ideal absorption value should reach approximately 60% as the optimum, but it can go as high as 63%.

Table 2.6.9 Analysis of variance results for farinograph waterabsorption.

Source	DF	SS	MS	F-value	Pr>F
Total	29	383.055			
Block	2	2.448	1.224	1.79	0.1955
Entry	9	368.229	40.922	59.84	0.0000**
Residual	18	12.309	0.684		

* ($p < 0.05$) ** ($p < 0.01$)

The analysis of variance results (Table 2.6.9) show significant variation ($p < 0.01$) for entries. The genotype contribution of this parameter was 98.3%. This parameter is therefore less sensitive to environmental variations.

Table 2.6.10 Ranking and averages of the genotypes for farinograph waterabsorption.

Rank	Genotypes	Averages (%)
1	Tugela x Tugela fg	74.33
2	Tugela x Tugela DN	70.32
3	Tugela x Letaba	69.95
4	Tugela	69.47
5	Tugela x Flamink	68.62
6	Tugela x Gamtoos DN	67.83
7	Tugela x Gariep	67.80
8	Tugela x Inia	67.03
9	Tugela x Palmiet	66.78
10	Kariega	59.70

Grand mean = 68.183 LSD for entry = 1.1708

The averages of the crosses (Table 2.6.10) ranged from 59.7% to 74.33% for Kariega and Tugela x Tugela fg, respectively. Only the standard, Kariega, was in the optimal range, all the other crosses had significant higher absorption values than Kariega. The other standard, Tugela, had a value of 69.47%, which is much higher than the specified peak value of 63%. The other two crosses, Tugela x Tugela DN and Tugela x Tugela fg, both had values higher than 70%. Tugela x Tugela fg had a significantly higher waterabsorption than Tugela. Tugela x Inia and Tugela x Palmiet both, also had significant lower waterabsorption than Tugela.

The farinograph waterabsorption was negatively correlated with breakflour yield and loaf volume at 12% protein content. Finney and Shogren (1972) reported that a higher flour content resulted in a higher waterabsorption and also that bread flour yield contributes to the waterabsorption capacity. In this study this was, however, not the case, as a higher waterabsorption gave a lower loaf volume. The farinograph waterabsorption was also positively correlated to the vitreous kernels, flour colour, mixo-dough development, alveograph P/L-value, alveograph strength and gluten at 12% protein content (Table 2.6).

- **The alveograph P/L value**

This value serves as an index of both protein quantity and quality. The "P" indicates a dough's tenacity and elastic resistance, which determine its ability to retain gas and "L" indicates a dough's extensibility. The ratio P/L, indicates the wheat quality. For South Africa, the P/L values between 0.5 and 0.8 (0.8 is optimal) produce good quality bread (Koekemoer, personal communication).

Table 2.6.11 Analysis of variance results for alveograph P/L-value.

Source	DF	SS	MS	F-value	Pr>F
Total	29	1.634			
Block	2	0.005	0.003	0.54	0.5933
Entry	9	1.541	0.171	34.81	0.0000**
Residual	18	0.089	0.005		

* ($p < 0.05$) ** ($p < 0.01$)

The analysis of variance results (Table 2.6.11) show that entries was highly significant ($p < 0.01$). The genotype contribution of this parameter was 97.1%.

All the crosses and Tugela had higher averages than the check, Kariega (Table 2.6.12). The cross, Tugela x Tugela DN had a P/L value of 0.8, which is optimal. Four other crosses, Tugela x Flamink, Tugela x Inia, Tugela x Letaba and Tugela x Gamtoos DN, had values between 0.5 and 0.8. The averages of the crosses ranged between 0.32 to 1.10 for Kariega and Tugela, respectively.

Table 2.6.12 Ranking and averages of the genotypes for alveograph P/L-value.

Rank	Genotypes	Averages
1	Tugela	1.10
2	Tugela x Tugela fg	0.95
3	Tugela x Gariep	0.82
4	Tugela x Tugela DN	0.80
5	Tugela x Flamink	0.60
6	Tugela x Inia	0.56
7	Tugela x Letaba	0.54
8	Tugela x Gamtoos DN	0.53
9	Tugela x Palmiet	0.49
10	Kariega	0.32

Grand mean = 0.672 LSD for entry = 0.0993

Tugela had a significant higher alveograph P/L-value than Tugela x Tugela fg, Tugela x Gariep, Tugela x Tugela DN and Tugela x Palmiet. This indicates that the contribution of LMW-GS in Tugela fg, Gariep, Tugela DN, Flamink, Inia, Letaba, Gamtoos DN and Palmiet decreased the P/L-value.

There were also significant differences between the lines, which were genetically closely related i.e. between Tugela, Tugela DN and Tugela fg. The only difference between these cultivars on *Glu-A3* were that Tugela had combinations *a/c/f*, Tugela DN only *f* and Tugela fg had only *a*. On the *Glu-B3*, Tugela had combination *b* and Tugela DN and Tugela fg had combinations *a/c*. On the *Glu-D3*, Tugela had combination *d/e*, Tugela DN *a/e* and Tugela fg had combination *a*. Again the contribution of the parents' LMW-GS were very diverse and no specific banding pattern could be identified that definitely influenced the alveograph P/L-value.

The P/L-value is positively correlated with vitreous kernels and alveograph strength. It is also negatively correlated with breakflour yield, loaf volume and the loaf volume at 12% protein (Table 2.6)

- **Alveograph dough strength (W value)**

This indicates the deformation action of the dough, based on one gram of dough. It indicates the amount of labour required for the deformation of the dough and is related to the baking "strength" of the dough. A W-value of close to 250 is usually recommended, so that it, when divided by 6.54, will result in a strength of ± 35 joules, which is popular with the bakers (Koekemoer, personal communication).

Table 2.13. Analysis of variance results for alveograph dough strength.

Source	DF	SS	MS	F-value	Pr>F
Total	29	8586.201			
Block	2	9.249	4.624	0.58	0.5691
Entry	9	8433.840	937.093	117.86	0.0000**
Residual	18	143.112	7.951		

* ($p < 0.05$) ** ($p < 0.01$)

The analysis of variance results (Table 2.6.12) show significant variation ($p < 0.01$) for entries. The averages of the crosses (Table 2.6.13) ranged from 36.29 to 93.74 for Kariega and Tugela x Tugela fg, respectively. All the crosses and Tugela had values higher than Kariega, which was the only one near the optimal value. The heritability of this parameter was 99.2%.

Table 2.6.14 Ranking and averages of the genotypes for alveograph dough strength.

Rank	Genotypes	Averages (joules)
1	Tugela x Tugela fg	93.74
2	Tugela x Tugela DN	93.12
3	Tugela	90.27
4	Tugela x Flamink	77.06
5	Tugela x Gariep	75.54
6	Tugela x Inia	73.09
7	Tugela x Letaba	68.35
8	Tugela x Gamtoos DN	64.27
9	Tugela x Palmiet	58.51
10	Kariega	36.29

Grand mean = 73.023 LSD for entry = 3.9923

From the results (Table 2.6.14) it is evident that the strength of all the Tugela crosses was high, especially Tugela x Tugela Dn and Tugela x Tugela fg , which had higher values than Tugela. The only cross with potential is the Tugela x Palmiet cross with a value of 58.51.

The crosses Tugela x Flamink, Tugela x Gariep, Tugela x Inia, Tugela x Letaba, Tugela x Gamtoos Dn and Tugela x Palmiet had significant lower dough strength than Tugela. There were no significant differences between the lines, which were genetically closely related i.e. Tugela, Tug\ela DN and Tugela fg.

The alveograph strength value was negatively correlated with the breakflour yield and loaf volume (12% protein content). It was also positively correlated with vitreous kernels, flour colour, mixo-dough development time and alveograph P/L-value (Table 2.6). It was also positively correlated with farinograph waterabsorption, which is actually an indication of high loaf volume (Finney *et al*, 1987; Van Lill and Purchase, 1995). This was, however, not the case in this study.

▫ Loaf volume

Loaf volume gives an indication of the gas retention capacity of the dough during the fermentation process and indicates the volume of bread determined by rapeseed displacement (Shogren and Finney, 1984). For two of the crosses the amount of flour was insufficient to bake bread, because of bird damage in the plots. The analysis was therefore done on only seven entries.

Table 2.6.15. Analysis of variance results for loaf volume.

Source	DF	SS	MS	F-value	Pr>F
Total	23	137295.833			
Block	2	764.583	382.292	0.30	0.7476
Entry	7	118512.500	16930.357	13.15	0.0000**
Residual	14	18018.750	1287.054		

* ($p < 0.05$) ** ($p < 0.01$)

The analysis of variance results (Table 2.6.15) show significant variation for entries. The genotype accumulated for 92.4% of the total variation measured. The averages ranged from 796.67 cm^3 to 1000.00 cm^3 for Tugela and Tugela x Palmiet, respectively. Four of the crosses had significantly higher loaf volumes than Kariega (895.00 cm^3).

The Tugela x Palmiet, Tugela x Letaba, Tugela x Gamtoos DN, Tugela x Flamink and Tugela x Tugela DN crosses had significant higher loaf volumes than Tugela. The genetically closely related cultivars i.e. Tugela and Tugela Dn, had significantly different loaf volumes.

A linear correlation between protein content and loaf volume generally exists, which indicates protein content to be a measure of quality of wheat (Finney, 1945). A positive correlation was also found with a loaf volume at 12% protein content and gluten at 12% protein content. A negative correlation was found with the alveograph P/L-value and the SDS-sedimentation volume (Table 2.6). In contrast De Villiers and Laubscher (1995) found that SDS-sedimentation volume was positively correlated with both protein content and loaf volume. From these results it were obvious that it was not the case in this study.

Table 2.6.16 Ranking and averages for the genotypes for the loaf volume at a 12% protein content.

Rank	Genotype	Average (cm^3)
1	Tugela x Palmiet	1000.00
2	Tugela x Letaba	1000.00
3	Tugela x Gamtoos DN	991.67
4	Tugela x Flamink	956.67
5	Tugela x Tugela DN	923.33
6	Kariega	895.00
7	Tugela x Inia	843.33
8	Tugela	796.67

Grand mean =924.58 LSD for entry = 51.5927

- **Loaf volume at 12% protein content (LFV 12)**

Most of the wheat flour characteristics (physical and chemical) are optimally observed at 12% protein content. According to Finney *et al* (1987) and Van Lill and Purchase (1995), the main indicators of baking quality include loaf volume and waterabsorption. Loaf volume as determined by rapeseed displacement (Shogren and Finney, 1984), gives an indication of the gas retention capacity of the dough.

Table 2.6.17 Analysis of variance for the results for the loaf volume at a 12% protein content.

Source	DF	SS	MS	F-value	Pr>F
Total	23	110557.833			
Block	2	2205.583	1102.792	0.49	0.6238
Entry	7	76731.167	10961.595	4.85	0.0059**
Residual	14	31621.083	2258.649		

* ($p < 0.05$) ** ($p < 0.01$)

The analysis of variance results (Table 2.6.17) show significant variation ($p < 0.01$) for entries. The genotype contribution of this characteristic was 79.4%. The first six entries were significantly better than Tugela (Table 2.6.18).

Tugela x Palmiet, Tugela x Gamtoos DN, Tugela x Tugela DN, Tugela x Letaba and Tugela x Inia had significant higher loaf volume at 12% protein content. As

this parameter is largely genetically determined, the higher loaf volume may then be the result of the differences in LMW-GS. The genetically closely related cultivars i.e. Tugela and Tugela DN had significantly different loaf volumes at 12% protein.

Table 2.6.18 Ranking and averages for the genotypes for the loaf volume at a 12% protein content.

Rank	Genotype	Average (cm^3)
1	Tugela x Palmiet	897.33
2	Kariega	888.33
3	Tugela x Gamtoos DN	856.33
4	Tugela x Tugela DN	819.33
5	Tugela x Letaba	796.00
6	Tugela x Inia	792.67
7	Tugela x Flamink	783.33
8	Tugela	715.33

Grand mean = 818.583 LSD for entry = 68.3462

The loaf volume at 12% protein content was negatively correlated to mixograph dough development time, farinograph waterabsorption, alveograph P/L-value and -dough strength. Although high waterabsorption also indicates high loaf volume (Finney *et al*, 1987; Van Lill and Purchase, 1995), in this study the loaf volume at 12% protein content and farinograph waterabsorption were negatively correlated. Loaf volume is also positively correlated with loaf volume at 12% protein content. This was confirmed in the study of Mamuya (2000), who found that genotypes that had higher waterabsorption had low to very low loaf volumes. This signifies the importance of protein quality in addition to protein content and waterabsorption when considering loaf volume. This was not the case in this study, because the P/L-value was negatively correlated to loaf volume at 12% protein contents. Loaf volume at 12% protein was positively correlated to breakflour yield (Table 2.6).

Gluten at 12% protein content

Gluten is an insoluble wheat protein, which consist of gliadin and glutenins. The former are responsible for extensibility and viscosity of the dough, whereas the latter determines its elasticity (Colt, 1990; Shewry *et al*, 1995).

The analysis of variance results (Table 2.6.19) show significant variance ($p < 0.01$) for entries. The genotype contribution of this parameter was 96.8%.

Table 2.6.19 Analysis of variance results for gluten at 12% protein content.

Source	DF	SS	MS	F-value	Pr>F
Total	29	251.562			
Block	2	1.004	0.502	0.60	0.5606
Entry	9	235.435	26.159	31.14	0.0000**
Residual	18	15.123	0.840		

* ($p < 0.05$) ** ($p < 0.01$)

The averages of the crosses (Table 2.6.20) ranged from 33.29% to 43.6%, for Kariega and Tugela x Gariep, respectively. Tugela had significantly higher gluten (35.29%) than Kariega. All eight of the crosses had significantly higher gluten values than the two standards. The value for the Tugela x Tugela DN cross for the Tugela x Tugela fg cross and Tugela were 38.37, 39.21 and 35.29%, respectively.

Table 2.6.20 Ranking and averages of the genotypes for gluten at 12% protein content.

Rank	Genotypes	Averages (%)
1	Tugela x Gariep	43.60
2	Tugela x Letaba	41.61
3	Tugela x Flamink	39.81
4	Tugela x Tugela fg	39.21
5	Tugela x Palmiet	39.16
6	Tugela x Tugela DN	38.37
7	Tugela x Gamtoos DN	37.58
8	Tugela x Inia	37.15
9	Tugela	35.29
10	Kariega	33.29

Grand mean = 38.505 LSD for entry = 1.2978

Tugela x Gariep, Tugela x Letaba, Tugela x Flamink, Tugela x Tugela fg, Tugela x Palmiet, Tugela x Tugela DN, Tugela x Gamtoos DN and Tugela x Inia had significant higher gluten content at 12% protein. The differences could be the result of the differences in LMW-GS, because this parameter was also largely genetically determined. The genetically closely related cultivars i.e. Tugela, Tugela DN and Tugela fg, also had significant differences. The only differences in the LMW-GS on the *Glu-A3* were that Tugela had combination *a/c/f*, Tugela Dn had combination *f* and Tugela fg had combination *a*. On *Glu-B3* Tugela had combination *b* and both Tugela Dn and Tugela fg had combination *a/c*. On *Glu-D3* Tugela had combination *d/e*, Tugela Dn had combination *a/e* and Tugela fg combination *a*.

Gluten at 12% protein content was positively correlated with flour colour, farinograph waterabsorption, loaf volume and flour protein content. It was negatively correlated with SDS-sedimentation volume (Table 2.6).

- **Sodium dodecyl sulphate (SDS)-sedimentation volume**

The SDS-sedimentation is used for measuring relative gluten strength as it indicates differences in the quantities of the polymeric glutenins (gel protein). Sedimentation values can range from 20ml or less for low-protein wheat of inferior bread-baking strength to as high as 70ml or more for high-protein wheat of superior bread-baking strength (Koekemoer, personal communication).

The analysis of variance results (Table 2.6.21) show highly significant variation ($p < 0.01$) for entries. The genotype contribution of this parameter was 94%.

Table 2.6.21 Analysis of variance results for SDS-sedimentation volume.

Source	DF	SS	MS	F-value	Pr>F
Total	29	242.167			
Block	2	0.867	0.433	0.30	0.7428
Entry	9	215.500	23.944	16.71	0.0000**
Residual	18	25.800	1.433		

* ($p < 0.05$) ** ($p < 0.01$)

The averages of the crosses (Table 2.6.22) ranged from 82.33ml to 91.00ml, for Tugela x Flamink and Tugela x Tugela Dn, respectively. The sedimentation values for all genotypes were all above 70 ml.

Tugela x Inia, Tugela x Gamtoos DN, Tugela x Letaba, Tugela x Palmiet, Tugela x Gariep and Tugela x Flamink had significant lower SDS-sedimentation values than Tugela. There were no significant differences between the lines, which were genetically closely related i.e. Tugela, Tugela DN and Tugela fg.

Table 2.6.22 Ranking and averages of the genotypes for SDS-sedimentation volume.

Rank	Genotypes	Averages (ml)
1	Tugela x Tugela DN	91.00
2	Kariega	90.67
3	Tugela	90.00
4	Tugela x Tugela fg	89.67
5	Tugela x Inia	88.67
6	Tugela x Gamtoos DN	88.67
7	Tugela x Letaba	86.67
8	Tugela x Palmiet	85.67
9	Tugela x Gariep	85.00
10	Tugela x Flamink	82.33

Grand mean = 87.833 LSD for entry = 1.6951

Interestingly, SDS-sedimentation volume was negatively correlated with flour protein content and loaf volume (Table 2.6).

- **Breakflour yield**

Breakflour yield is an important characteristic in wheat flour as it increases the waterabsorption capacity of the flour.

Table 2.6.23 Analysis of variance results for breakflour yield.

Source	DF	SS	MS	F-value	Pr>F
Total	29	65.815			
Block	2	0.098	0.049	0.71	0.5066
Entry	9	64.468	7.163	103.26	0.0000**
Residual	18	1.249	0.069		

* ($p < 0.05$) ** ($p < 0.01$)

The analysis of variance results (Table 2.6.23) show significant variance ($p < 0.01$) for entries. The genotype contribution of this parameter was 99%.

The averages of the crosses (Table 2.6.24) ranged from 19.13% to 24.27%, for the standards, Tugela and Kariega, respectively. All the crosses had significantly higher values than Tugela.

Table 2.6.24 Ranking and averages of the genotypes for breakflour yield.

Rank	Genotypes	Averages (%)
1	Kariega	24.27
2	Tugela x Gariep	23.60
3	Tugela x Letaba	23.10
4	Tugela x Inia	21.80
5	Tugela x Gamtoos DN	21.77
6	Tugela x Palmiet	21.37
7	Tugela x Flamink	21.27
8	Tugela x Tugela DN	20.73
9	Tugela x Tugela fg	20.47
10	Tugela	19.13

Grand mean = 21.750 LSD for entry = 0.3729

Tugela x Tugela fg, Tugela x Tugela DN, Tugela x Flamink, Tugela x Palmiet, Tugela x Gamtoos DN, Tugela x Inia, Tugela x Letaba and Tugela x Gariep, had a significant higher breakflour yield than Tugela. As breadklour yield was largely genetically determined, the contribution of the LMW-GS of the different parents may have caused these significant differences. There were significant differences between the lines, which were genetically closely related.

Breadklour yield was negatively correlated with mixo-dough development time, farinograph waterabsorption, alveograph P/L-value and alveograph dough strength. It was also positively correlated with loaf volume at 12% protein content (Table 2.6).

- **Vitreous kernels**

Hard and soft wheats are among the major groups of wheat. It is rare to find a genotype or cultivar that is 100% hard or soft and usually there is variation.

Vitreous kernels therefore indicate the percentage of hardness of a certain cultivar (Koekemoer, personal communication).

The analysis of variance results (Table 2.6.25) show significant variance ($p < 0.05$) for entries. The genotype contribution of this parameter was 67.79%. Hardness is a consequence of starch-protein interaction; kernel's vitreousness may decrease in an environment with poor grain filling conditions and less protein levels (Mamuya, 2000).

Table 2.6.25 Analysis of variance results for vitreous kernels.

Source	DF	SS	MS	F-value	Pr>F
Total	29	629.867			
Block	2	32.267	16.133	1.24	0.3133
Entry	9	363.200	40.356	3.10	0.0197*
Residual	18	234.400	13.022		

* ($p < 0.05$) ** ($p < 0.01$)

Tugela x Tugela Dn had a significantly higher percentage vitreous kernels than Tugela. The averages of the crosses (Table 2.6.26) ranged from 82.67% to 93.33% for, Tugela x Letaba and Tugela x Tugela DN, respectively. The standards, Kariega and Tugela had values of 82.67 and 87.33%, respectively. Vitreous kernels were postively correlated with flour yield, falling number, farinograph waterabsorption, alveograph P/L-value and alveograph dough strength (Table 2.6).

Table 2.6.26 Ranking and averages of the genotypes for vitreous kernels.

Rank	Genotypes	Averages (%)
1	Tugela x Tugela DN	93.33
2	Tugela x Gariep	92.00
3	Tugela x Tugela fg	90.67
4	Tugela x Flamink	89.33
5	Tugela x Gamtoos DN	88.00
6	Tugela	87.33
7	Tugela x Palmiet	86.00
8	Tugela x Inia	85.33
9	Kariega	82.67
10	Tugela x Letaba	82.67

Grand mean = 87.733 LSD for entry = 5.1093

- **Flour colour**

The analysis of variance results (Table 2.6.27) show highly significant variation for entries. This implies different genotype performances. The genotype contribution of the parameter was 94.4%. The averages of the crosses (Table 2.6.28) ranged from –1.03 to 4.80 for Kariega and Tugela x Tugela fg, respectively.

Table 2.6.27 Analysis of variance results for flour colour.

Source	DF	SS	MS	F-value	Pr>F
Total	29	76.463			
Block	2	0.962	0.481	1.14	0.3420
Entry	9	67.903	7.545	17.87	0.0000**
Residual	18	7.598	0.422		

* ($p < 0.05$) ** ($p < 0.01$)

Tugela x Tugela fg, Tugela x Gariep and Tugela x Inia had a significantly darker flour colour than Tugela.

Flour colour was positively correlated with the mixo-dough development time, farinograph waterabsorption, alveograph P/L-value, alveograph dough strength and gluten at 12% protein content. It was also negatively correlated with hectolitre mass (Table 2.6).

Table 2.6.28 Ranking and averages of the genotypes for flour colour.

Rank	Genotypes	Averages (%)
1	Tugela x Tugela fg	4.80
2	Tugela x Gariep	3.70
3	Tugela x Inia	3.63
4	Tugela x Letaba	2.57
5	Tugela Tugela DN	2.20
6	Tugela x Palmiet	1.90
7	Tugela	1.73
8	Tugela x Gamtoos DN	1.63
9	Tugela x Flamink	1.57
10	Kariega	-1.03

Grand mean = 2.270 LSD for entry = 0.9199

- **Flour yield**

Higher flour yield (extraction) is the primary objective for millers and in South Africa a flour yield of at least 76% for white flour is used as the selection norm of new cultivars (Koekemoer, personal communication).

The analysis of variance results (Table 2.6.29) show significant variation ($p < 0.01$) for entries. The genotype contribution of this characteristic was 85.1%.

Table 2.6.29 Analysis of variance results for flour yield.

Source	DF	SS	MS	F-value	Pr>F
Total	29	33.980			
Block	2	0.548	0.292	0.68	0.5172
Entry	9	25.713	2.857	6.69	0.0003**
Residual	18	7.683	0.427		

* (p < 0.05) ** (p < 0.01)

The averages of the crosses (Table 2.6.30) ranged between 76.4% to 79.27% for Tugela x Tugela fg and Kariega, respectively. All the crosses had values above the acceptable extraction value. Kariega, Tugela x Letaba, Tugela x Inia and Tugela x Tugela DN had values significantly higher than Tugela.

Table 2.6.30 Ranking and averages of the genotypes for flour yield.

Rank	Genotypes	Averages (%)
1	Kariega	79.27
2	Tugela x Palmiet	78.53
3	Tugela x Letaba	78.50
4	Tugela x Inia	77.90
5	Tugela x Tugela DN	77.83
6	Tugela x Flamink	77.30
7	Tugela x Gamtoos DN	77.20
8	Tugela	76.57
9	Tugela x Gariep	76.50
10	Tugela x Tugela fg	76.40

Grand mean = 77.600 LSD for entry = 0.9250

The crosses Tugela x Palmiet, Tugela x Letaba, Tugela x Inia and Tugela x Tugela Dn had a significant higher flour yield than Tugela. Flour yield was

positively correlated with the flour colour (Table 2.6) in this specific set of material.

2.7 Conclusion

There were a lot of significant differences between Tugela and the Tugela crosses. For all the quality parameters, but the falling number, the contribution of the genotype to variability was very high. This suggests that the environment made a relatively smaller contribution towards variability for three parameters.

For hectolitre mass, the Tugela x Letaba cross, had a significantly higher value than Tugela.

For flour protein content, crosses with Letaba, Gariep and Flamink had significantly higher values than Tugela.

For the mixograph development time, crossing with Palmiet, Inia, Letaba, Gariep, Gamtoos DN and Tugela DN resulted in more optimal mixing times.

For the alveograph P/L ratio, the introduction of all the other parents into crosses, caused a reduction in the P/L ratio from the value of Tugela.

In terms of the alveograph strength (W value), the three Tugela lines (Tugela, Tugela DN and Tugela fg) all had a very strong dough. The introduction of Flamink, Gariep, Inia, Letaba, Gamtoos DN and Palmiet, all caused a significantly reduction in the strength, which caused a shift towards more ideal values.

In terms of loaf volume, crosses with Palmiet, Letaba, Gamtoos DN, Flamink and also Tugela DN, caused a significant increase in the loaf volume, especially in the case of Tugela DN.

The loaf volume at 12%, is basically a test for gluten quality in the flour. The crosses with Palmiet, Gamtoos DN, Tugela DN and Inia caused significantly higher values than that of Tugela.

For the gluten content at 12% protein and the breakflour yield, all the crosses had significantly higher values than Tugela.

In the case of SDS-sedimentation, the crosses with Inia, Gamtoos DN, Letaba, Palmiet, Gariep and Flamink, caused a significant reduction in SDS-sedimentation. In this study, contary to evidence in the literature, this trait was not positively correlated with loaf volume.

The flour colour of the crosses with Tugela fg, Gariep and Inia were significantly darker than that of Tugela. Drrf colour in wheat is quantitively inherited and not controlled by LMW alleles.

The flour yield of crosses with Letaba, Inia and Tugela Dn were significantly higher than that of Tugela and especially in the Tugela x Tugela Dn cross.

The contribution of the parents to the LMW-GS were so diverse that no single banding pattern could be identified which was associated with the variation in baking quality.

It was already possible to observe an improvement in the stability of the dough. The unexpected segregation patterns in the F_2 generations, together with multiple banding combination expression will however, make marker assisted selection on ground of LMW-GS difficult at this early stage.

Chapter 3

General conclusion

The inheritance of LMW-GS in South African wheat material was much more complex than expected. The South African material did not always follow the inheritance and banding patterns as described in the nomenclature of Gupta and Shepherd (1990b). New combinations, recombinations, polymorphisms and more than one banding combination were observed in cultivars and the crosses.

Because of this, it was very difficult to determine the usability of LMW-GS as markers for baking quality. It was also difficult to make clear correlations between certain LMW-GS patterns and certain quality parameters.

It was still obvious that differences in LMW-GS composition contributed to a large extent to the differences in the breadmaking quality, because the HMW-GS (Table 1.2) were almost the same for all the crosses. This was also seen in the genetically closely related cultivars; Tugela, Tugela fg and Tugela DN. They have the same HMW-GS composition (Table 1.2), but there are differences in their LMW-GS composition (Table 1.7 and 1.8), which lead to significant differences in the measured quality characteristics. More research and the development of isogenic lines will be needed to prove this assumption. It would therefore seem that in South Africa, where less than 20% of the variability in breadmaking quality is attributed to the HMW-GS (Randall, personal communication), this percentage will probably be much higher, when the LMW-GS are also taken into account.

Thirty-nine different LMW bands are known (Maartens, 1999) and six, nine and five banding patterns are found on the A-, B- and D genome, respectively (Gupta and Shepherd,1990b). About 20 different HMW-GS are known and a single variety contains only three to five different subunits (Eliasson and Larson, 1993). There is also only three, five and two different HMW-GS banding patterns found on the A-, B- and D genome, respectively. The LMW-GS patterns are thus more complex than those of the HMW-GS.

This may be one of the reasons why the information available on the effects of LMW-GS on wheat, is much more limited than that of the HMW-GS. It is obvious that further analysis is needed on the relationship between the LMW glutenin alleles and dough properties for the better prediction of flour quality.

It was found in this study that early generation quality tests ($F_{2:3}$ derived lines) could give an indication of the advances made in the improvement of breadmaking quality. It was already possible to make selections from these lines, because most of the quality parameters were largely genetically determined.

The LMW banding patterns found in this material deviated largely from that found by Gupta and Shepherd (1990b). They found that only one combination could appear at one locus, but this was not the case on the South African material. Although the parental cultivars were all confirmed to be pure breeding, it was found that up to three banding patterns were expressed at one locus. The unexpected segregation patterns in the F_2 progeny, together with multiple band combination expression, will make marker assisted selection on grounds of the LMW-GS almost impossible. Clearly then, before assertion can be made that differences in LMW-patterns account for differences in quality traits, a direct correlation relationship has to be established. To establish a statistical relationship between the LMW patterns and the quality traits, the different LMW-GS in the genotypes would have to be extracted, isolated and quantified in the genotypes and then correlatied with the quality trait data. The presence or absence of band data could also be tested for correlation with quality trait data. It is also suggested that NIL's for South African wheat cultivars be developed for LMW-GS, to test the influence of specific banding combinations on quality.

Although the quality could be determined, the LMW-GS data was still too complex to make direct correlations between specific LMW-GS and specific quality parameters. Until the nomenclature system of Gupta and Shepherd (1990b) is updated with the possibly unique South African patterns, the correlation between certain LMW-GS and quality parameters will be very difficult.

Chapter 4

Summary

1 The aim of this study was to study:

a) The inheritance of LMW-GS in 50 F_2 lines of eight different Tugela crosses.

b) The effect of the different LMW-GS on breadmaking quality using $F_{2:3}$ derived lines in order to determine the usability of the LMW-GS as markers for baking quality.

c) The usability of $F_{2:3}$ derived lines in early generation selection for the improvement of breadmaking quality.

2 a) The cultivar and the F_2 progeny of the eight crosses were screened in a gliadin-free background using a simplified one-dimensional procedure of Singh *et al* (1991). The HMW and LMW glutenins were run on a single gel.

b) The $F_{2:3}$ derived lines of eight different Tugela crosses were evaluated for breadmaking quality using the procedures of the American Association of Cereal Chemistry (AACC).

3 The results were as follows:

a) Only four percent of the F_2 progeny of the eight Tugela crosses, contained no bands on the A genome.

b) Banding patterns were expressed simultaneously in the F_2 progeny and in the pure breeding cultivars. This contrasted with the findings of Gupta and Shepherd (1990b), who found that only one banding pattern for each chromosome was expressed in pure breeding cultivars.

c) Due to interaction between the parent types, new banding patterns were formed by recombination.

d) Some cultivars had no matching combinations in the B- and D genome. Polymorphisms are common in wheat and new banding combinations are quite possible.

e) It would seem that none of the "normal" inheritance patterns (co-dominant) were followed, but rather that there is some kind of suppression of banding patterns in the F_2 lines on some of the genomes.

f) The quality results showed many differences between the crosses of Tugela and the parent, Tugela. It was already possible in the early generation ($F_{2:3}$) to observe an improvement in the reduction of the stability of the dough of Tugela.

g) The complexity of the LMW-GS patterns made it difficult to correlate between certain LMW-GS and certain quality parameters.

4 The conclusions from this study:

a) The inheritance of LMW-GS in South African wheat material is much more complex than expected.

b) The South African material did not always follow the inheritance and banding patterns as described in the nomenclature of Gupta and Shepherd (1990b).

c) The differences in LMW-GS composition should explain most of the differences in breadmaking quality in the Tugela crosses, because there was little or no differences in the HMW-GS composition.

d) Early generation quality tests ($F_{2:3}$) could give an indication of the advances made in the improvement of breadmaking quality, although it is very difficult and much more research is needed..

e) The unexpected segregation patterns in the F_2 progeny, together with multiple combination expression will make marker-assisted-selection on ground of LMW-GS difficult at this early stage.

f) The contribution by the parents to the LMW-GS were so diverse, that no single banding pattern could be identified, which definitely influenced the baking quality.

g) Until the nomenclature system of Gupta and Shepherd (1990b) is updated with these possible unique South African LMW-GS patterns, the correlation of LMW-GS data with quality results will be very difficult.

Opsomming

1 Die doel van die studie was om:

a) Die oorerflikheid van die LMW gluteniene in 50 F_2 nageslagte van agt verskillende Tugela kruisings te bepaal.

b) Die effek van verskillende LMW gluteniene op broodbakkwaliteit te bepaal, deur $F_{2:3}$ ontwikkelde lyne te gebruik. So word dan bepaal wat die bruikbaarheid van die LMW gluteniene is as merkers vir broodkwaliteit.

c) Die bruikbaarheid van $F_{2:3}$ ontwikkelde lyne in vroeë generasie seleksies vir verbeterde broodbakkwaliteit te bepaal.

2 a) Verskillende cultivars is geëvalueer in 'n agtergrond wat vry was van gliadiene. 'n Vereenvoudigde een-dimensionele metode van Singh *et al* (1991) is gebruik. Die HMW en LMW gluteniene is op 'n enkele gel geëvalueer.

b) Die $F_{2:3}$ ontwikkelde lyne van agt verskillende Tugela kruising is geëvalueer vir broodbakkwaliteit deur gebruik te maak van die metodes van die "American Association of Cereal Chemists" (AACC).

3 Die resultate was as volg:

a) Slegs vier persent uit die F_2 nageslagte van die agt Tugela kruisings, het geen bande op die A genoom bevat nie.

b) Meer as een bandpatroon is terselfdetyd uitgedruk in die F_2 nageslag en in suiwertelende cultivars. Dit was kontrasterend met die bevindinge van Gupta en Shepherd (1990b). Hulle het bevind dat slegs een bandpatroon op elke chromosoom in 'n suiwertelende cultivar uitgedruk word.

c) As gevolg van interaksies tussen ouertipes word nuwe bandpatrone gevorm deur middel van rekombinasie.

d) Sekere kultivars het geen ooreenstemmende bandpatrone op die B- en D-genome getoon nie. Polimorfismes is algemeen in koring en nuwe bandkombinasies is moontlik.

e) Dit wil voorkom of daar geen van die normale oorerflikheidspatrone (kodominansie) gevolg is nie, maar dat daar eerder 'n tipe van onderdrukking van bandpatrone in die F_2 lyne was.

f) Die kwaliteit resultate het baie verskille tussen die Tugela kruisings en Tugela getoon. Dit was alreeds moontlik om in die vroeë generasies 'n verbetering in die stabiliteit (minder oorstabiel) in die deeg waar te neem.

g) Die kompleksiteit van die LMW-GS het dit bemoeilik om sekere LMW glutenien patrone met sekere kwaliteits parameters te korreleer.

4 Die volgende afleidings kan gemaak word:

a) Die oorerflikheid van LMW gluteniene in Suid-Afrikaanse koringmateriaal is baie meer kompleks as wat verwag is.

b) Die Suid-Afrikaanse materiaal het nie altyd die oorerflikheidspatrone en bandpatrone gevolg soos voorgeskryf deur die nomenklatuur van Gupta en Shepherd (1990b) nie.

c) Die verskille in LMW glutenien samestelling moet die verskille in broodbakkwaliteit verduidelik, want daar was min tot geen verskille in die samestelling van die HMW gluteniene nie.

d) Vroeë generasie kwaliteitstoetse ($F_{2:3}$) kon alreeds 'n aanduiding gee van die vordering wat gemaak is in die verbetering van die broodbakkwaliteit.

e) Die onverwagse segregasie patrone tesame met die veelvudige uitdrukking van kombinasies in die F_2 generasies sal merkerondersteunde seleksie op grond van LMW gluteniene moeilik maak in die vroeë stadium.

f) Die bydrae van ouers tot die LMW gluteniene was so divers, dat nie een bandpatroon geïdentifiseer kon word, wat spesifieke bakkwaliteits eienskappe beïnvloed nie.

g) Totdat die nomenklatuur sisteem van Gupta en Shepherd (1990b) opgegradeer is met die moontlike uniek Suid-Afrikaanse LMW glutenien patrone, sal die korrelasie van LMW glutenien data met kwaliteits resultate baie moeilik wees.

REFERENCES

American Association of Cereal Chemists (AACC) approved methods, Volume I and II.

Baenziger, P.S., Clements, R.K., McIntosh, M.S., Yamazaski, W.T., Starling, T.M., Sammons, D.J., and Johnson, J.W., 1985. Effect of cultivar, environment and their interaction and stability analysis on milling and baking quality of soft red winter wheat. *Crop Sci.* 25: 5-8.

Bettge, A., Rubenthaler, G.L. and Pomeranz, Y., 1989. Alveograph algorithms to predict functional properties of wheat in bread and cookie baking. *Cer. Chem.* 66: 81-86.

Bietz, J.A., 1987. Genetic and biochemical studies of nonenzymatic endosperm proteins. In: Wheat and wheat improvement (2nd Ed.). Ed. E.G. Heyne. Am. Soc. Of Agronomy, Madison, Wisconsin pp. 215 – 241.

Bietz, J.A. and Lookhart, G.L., 1996. Properties and non-food potential of gluten. *Cereal Foods World* 41(5): 376-382.

Bietz, J.A. and Rothus, J.A., 1970. Comparison of peptides from wheat gliadin and glutenin. *Cer. Chem.* 47:381-392.

Bietz, J.A. and Wall, J .S., 1972. Wheat gluten subunits: molecular weight determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis. *Cer. Chem.* 49 : 416 – 430.

Blackman, J.A. and Payne, P.I., 1987. Grain quality. In: Wheat breeding. Its Scientific Basis. Ed. F.G.H. Lupton, Chapman and Hall Ltd., University Press, Cambridge, Great Britain pp. 455 – 485.

Brunori, A., Galterio, G., Zannettino, C. and Pogna, N.E., 1989. Bread-making quality indices in *Triticum aestivum* progenies. Implications in breeding for better wheat. *Plant Breeding* 102:222-231.

Buonocore, F., Hickman, D.R., Caporale, C., Porceddu, E., Lafriandra, D., Tatham, A.S. and Shewry, P.R., 1996. Characterisation of a novel high Mr glutenin subunit encoded by chromosome 1D of bread wheat. *J. Cereal Science* 23:55-60.

Campbell, W.P., Wrigley, C.W., Cressey, P.J. and Slack, C.R., 1987. Statistical correlation between quality attributes and grain-protein composition for 71 hexaploid wheats used as breeding parents. *Cer. Chem.* 64 (5) :293 – 298.

Chen, J. and D' Appolonia, B.L., 1985. Alveograph studies on hard red spring wheat flour. *Cereal Foods World* 12: 862-867.

Colt, V., 1990. The genes encoding wheat storage proteins: towards a molecular understanding of breadmaking quality and its genetic manipulation. In: Genetic engineering: Principles and methods, vol.12. Ed. J.K. Seetlow. Plenum Press, New York, pp. 225-304.

Cressey, P.J., Campbell, W.P., Wrigley, C.W. and Griffin, W.B., 1987. Statistical correlation between quality attributes and grain-protein composition for 60 advanced lines of crossbred wheat. *Cer. Chem.* 64 (4):299-301.

Day, P.R., Bingham, J., Payne, P.I., and Thompson, R.D., 1986. The way ahead: Wheat breeding for quality improvement. In: Chemistry and physics of baking. Eds. J.M.V. Blanshard, P.J. Frazier and T. Gallard. The Royal Society of Chemistry, Londen pp. 251 – 261.

De La Roche, I.A. and Fowler, D.B., 1975. Wheat quality evaluation. I. Accuracy and precision of prediction tests. *Can. J. Plant Sci.* 55:241-249.

De Villiers and Laubscher, E.W., 1995. Use of the SDSS test to predict the protein content and bread volume of wheat cultivars. *S. Afr. J. Plant Soil* 12(4):140 – 142.

Derera, N.F., Bhatt, G.M. and McMaster, G.J., 1977. On the problem of pre-harvest sprouting of wheat. *Euphytica* 26: 299-308.

Dong, H., Sears, R.G., Cox, T.S., Hoseney, R.C., Lookhan, G.L. and Shogren, D., 1992. Relationships between protein composition and mixograph and loaf characteristics of wheat. *Cer. Chem.* 69: 132-136.

Eliasson, A., 1990. Rheology properties of cereal proteins. In: Dough rheology and baked products texture. Eds. H. Faridi and J.M. Faubion. Reinhold, New York. pp. 67-110.

Eliasson, A. and Larsson, K., 1993. Cereals in breadmaking: A molecular colloidal approach. Marcel Dekker, Inc. New York.

Fowler, D.B. and De La Rocha, I.A., 1975. Wheat quality evaluation. 2. Relationships among predictions tests. *Can. J. Plant. Sci.* 55: 251-262.

Finney, K.F., 1945. Methods of estimating and the effect of variety and protein level on the baking absorption of flour. *Cer. Chem.* 22: 149 – 158.

Finney, K.F. and Shogren, M.D., 1972. A ten-gram mixograph for determining and predicting functional properties of wheat flours. *Baker's Dig.* 46: 32-35.

Finney, K.F., Yamazaki, W.T., Youngs, V.L. and Rubenthaler, G.L., 1987. Quality of hard, soft and durum wheats. In: Wheat and Wheat Improvement (2nd Ed.). Ed. E.G. Heyne. Am. Soc. Of Agronomy, Madison, Wisconsin pp. 677-748.

Gaines, C.S., Finney, P.L. and Rubenthaler, G., 1996. Milling and baking qualities of some wheat developed in eastern and northwestern regions of the United States and grown at both locations. *Cer. Chem.* 73: 521-525.

Galili, G. and Feldman, M., 1983. Genetic control of endosperm proteins in wheat.I. The use of high resolution one dimension gel electrophoresis for the allocation of genes coding for endosperm protein subunits in common wheat cultivar Chinese Spring. *TAG* 64: 97 – 101.

Ghaderi, A., and Everson, E.H., 1975. Genotype-environment studies of test weight and its components in soft winter wheat. *Crop Sci.* 11: 617-620.

Gras, P.W., Hibberd, G.E. and Walker, C.E., 1990. Electronic sensing and interpretation of dough properties using 35 g mixograph. *Cereal Foods World* 35: 568-571.

Graybosch, R.A., Peterson, C.J., Shelton, D.R. and Baezinger, P.S., 1996. Genotypic and environmental modification of wheat flour protein composition in relation to end-use quality. *Crop Sci.* 11: 617-620.

Gupta, R.B., Bekes, F., and Wrigley, C.W., 1991. Prediction of physical dough properties from glutenin subunit composition in bread wheat: Correlation studies. *Cer. Chem.* 68(4) : 328 – 333.

Gupta, R.B. and MacRitchie, F., 1994. Allelic variation at glutenin subunits and gliadin loci, *Glu-1, Glu-3* and *Gli-1* of common wheats.II. Biochemical basis of the allelic effects on dough properties. *J. Cereal Science* 19: 19-29.

Gupta, R.B., Popineau, Y., Lefebvre, J., Cornec, M., Lawrence, G.J. and MacRitchie, F., 1995. Biochemical basis of flour properties in bread wheats. II. Changes in polymeric protein formation and dough/gluten properties associated with the loss of low M_r high M_r glutenin subunits. *J. Cereal Science.* 21: 103 – 116.

Gupta, R.B. and Shepherd, K.W, 1988. Low molecular weight glutenin subunits in wheat: their variation, inheritance and association with breadmaking quality. In Proc. of the Int. Wheat Genet. Symp. (vol2), 7th. Cambridge pp. 943 – 949.

Gupta, R.B. and Shepherd, K.W., 1990a. Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutenin. 1. Variation and genetic control of the subunits in hexaploid wheats. *TAG* 80 : 65 – 74.

Gupta, R.B. and Shepherd, K.W., 1990b. Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutenin. 2. Genetic control of the subunits in species related to wheat. *TAG* 80 : 183 – 187.

Hayberg, S., 1960. A rapid method for determining alpha-amylase activity. *Cer. Chem.* 37: 218-222.

He, H. and Hoseney, R.C., 1990. Gluten, a theory of how it controls breadmaking quality. In: Gluten proteins. Eds. W. Bushuk and W. Thackuk, R.Am. Ass. Cer. Chem. New York pp. 1 – 10.

Hoseney, R.C., 1985. The mixing phenomenon. *Cereal Foods World* 30: 453-457.

Jackson, E.A., Morel, M.H., Sontag-Ströhm, T., Branlard, G., Metakovsky, E.V. and Redaelli, R., 1996. Proposal for combining the classification system for alleles of *Gli-1* and *Glu-3 loci* in bread wheat (*Triticium aestivum* L.). *Journal of Genetics and Breeding* 50: 321–336.

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

Jalaluddin, M.D. and Harrison, S.A., 1989. Heritability, genetic correlation and genotype x environmental interaction of soft red wheat yield and test weight. *Cereal Res. Comm.* 17: 43-49.

Khan, K., Tamming, G. and Lukow, O., 1989. The effect of wheat flour proteins on mixing and baking - correlations with protein fractions and high molecular weight glutenin subunit composition by gel electrophoresis. *Cer. Chem.* 66:391-396.

Koekemoer, F.P., 1997. Important bread wheat quality characteristics affecting the producer and processing industry. Wheat Farmer's day 1997, ARC-Small Grain Institute, Bethlehem.

Lafiadra, D., Ciaffi, M. and Benedetelli, S., 1993. Seed storage proteins of wild wheat progenitors. In: Biodiversity and wheat improvement. Ed. A.B. Damania. Wiley-Sayce Publication, Chichester. pp. 329–340.

Lukow, O.M., 1991. Screening of bread wheats for milling and baking quality – A Canadian perspective. *Cereal Foods World* 36: 497-501.

Lukow, O.M. and Bushuk, W., 1984. Influence of germination on wheat quality. 1. Functional (breadmaking) and biochemical properties. *Cer. Chem.* 61: 336-339.

Lukow, O.M., Payne, P.I. and Tkachuk, R., 1989. The HMW glutenin subunit composition of Canadian wheat cultivars and their association with bread-making quality. *J. Sci. Food Agric.* 46:451-460.

Maartens, H., 1997. Low molecular weight glutenin subunit composition of South African wheat cultivars. M.Sc. Thesis, University of the Orange Free State, Bloemfontein, South Africa.

Maartens, H., 1999, The inheritance and genetic expression of low molecular weight glutenin subunits in South African wheat cultivars. Ph.D. Thesis, University of the Orange Free State, Bloemfontein, South Africa.

MacRitchie, F., 1992. Physicochemical properties of wheat proteins in relation to functionality. *Adv. in Food and Nutrition Research* 36 : 1 – 87.

MacRitchie, F., Du Cros, D.L. and Wrigley, C.W., 1990. Flour Polypeptides related to wheat quality. In: Advances in Cereal Science and Technology, Vol. 10. Ed. Y. Pomeranz. American Association of Cereal Chemists, Inc., St. Paul, MV. pp. 79–145.

Maghaub, E.S. and Odenbach, W., 1988. Genetic analysis of wheat endosperm storage proteins using reciprocal sets of inbred backcross. In: Proc. of the 7th Internat. Wheat Gen. Symp. Eds. T.E. Miller and R.M.D. Koebner. Institute of Plant Science Research, Cambridge. pp. 572 – 576.

Mamuya, I.N., 2000. Genotype x environment interaction for quality parameters of irrigated spring wheat. M.Sc. Thesis, University of the Orange Free State, Bloemfontein, South Africa.

Matuz, J., 1998. Inheritance of SDS-sedimentation volume of flours in crosses of winter wheats (*Triticum aestivum* L.) *Cereal Research Comm.* 26(2): 203-210.

McGuire, C.F. and McNeal, F.H., 1974. Quality response of 10 hard red spring wheat cultivars to 25 environments. *Crop Sci.* 14: 175-180.

Melas, V., Morel, M. Austran, J. and Feillet, P., 1994. Simple and rapid method for purifying low molecular weight subunits of glutenin from wheat. *Cer. Chem.* 77 (3): 234 – 237.

Metakovsky, E.V., Novoselskaya, A.Y.U. and Sozinov, A.A., 1984a. Genetic analysis of gliadin components in winter wheat using two-dimensional polyacrylamide gel electrophoresis. *TAG* 69: 31 – 37.

Metakovsky, E.V., Uzikova, G.A. and Sozinov, A.A., 1985. The genetics of gliadin components in winter bread wheat "Bezostaya". *Genetika* 21: 472–478.

Moonen, J.H.E., Scheepstra, A., and Graveland, A., 1983. Use of the SDS-sedimentation tests and SDS-polyacrylamide gel electrophoresis for screening breeder's samples of wheat for bread-making quality. *Euphytica* 31: 677-690.

Morel, M.H., 1994. Acid-polyacrylamid gel electrophoresis of wheat glutenins: A new tool for the separation of high and low molecular weight subunits. *Cer. Chem.* 71 (3): 238–242.

Nel, M.M, Agenbag, G.A. and Purchase, J.L., 1998. Sources of variation for yield, protein content and hectolitre mass of spring wheat (*Triticum aestivum* L.) cultivars of the Western and Southern Cape. *S. Afr.J.. Plant Soil* 15(2): 72-79.

Osborne, T.B., 1907. The proteins of the wheat kernel. Carnegie Inst., Washington D.C. no. 84.

Patton, J. and Dishaw, M., 1968. Flour colour evaluation with the green agtron. *Cereal Science Today* 13(4): 163-188.

Payne, P.I., 1987. Genetics of wheat storage proteins and the effects of allelic variation on bread-making quality. *Ann. Rev. Plant Phys.* 38: 141-153.

Payne, P.I., Corfield, K.G., Holt, L.M., and Blackman, J.A., 1981. Correlation between the inheritance of certain high-molecular weight subunits of glutenin and breadmaking quality in progenies of six crosses of bread wheat. *J. Sci. Food Agric.* 32: 51.

Payne, P.I., Holt, L.M., Jackson, E.A. and Law, C.N., 1984a. Wheat storage proteins: Their genetics and their potential for manipulation by plant breeding. Philos. Trans. R. Soc. London, Ser. B 304: 359–371.

Payne, P.I., Holt, L.M., Jarvis, M.G. and Jackson, E.A., 1985. Two-dimensional fractionation of the endosperm proteins of bread wheat (*Triticum aestivum*): Biochemical and Genetic Studies. *Cer. Chem.* 62 (5): 319–326.

Payne, P.I., Jackson, E.A., Holt, L.M. and Law, C.N., 1984b. Genetic linkage between endosperm storage protein genes on each of the short arms of chromosomes 1A and 1B in wheat. *TAG* 67: 235-243.

Payne, P.I., Nightingale, M.A., Krattiger, A.F.V., and Holt, L.M., 1987a. The relationships between HMW glutenin subunit composition and the breadmaking quality of British-grown wheat varieties. *J. Sci. Food Agric.* 40: 51-65.

Payne, P.I., Seeking, J.A., Worland, A.J., Jarvis, M.G. and Holt, L.M., 1987b. Allelic variation of glutenin subunits and gliadin and its effect on breadmaking quality in wheat: Analysis of F5 progeny from Chinese Spring x Chinese Spring (Hope 1A). *J. Cereal Science* 6: 103-118.

Peltonen, J. and Virtanen, A., 1994. Effect of nitrogen fertilisers differing in proteins in wheat. *Cer. Chem.* 71(1): 1-5.

Peña, R.J., Zarco-Henandez, J. and Mujeeba-Kazi, A., 1995. Glutenin subunit compositions and breadmaking quality characteristics of synthetic hexaploid wheats derived from *Triticum turgidum, Triticum tauschi* (cross.) Schmal crosses. *J. Cereal Science* 21(19):15–23.

Peterson, C.J., Graybosch, R.A., Baenzinger, P.S. and Grombacher, A.W., 1992. Genotype and environment effects on quality characteristics of hard winter wheat. *Crop Sci.* 32: 98-103.

Pogna, N.E., Radealli, R., Vacano, P., Biancardi, A.M., Peruffo, A.D.B., Curioni, A., Metakovsky, E.V. and Pagliaricci, S., 1995. Production and genetic characterization of near-isogenic lines in the bread-wheat cultivar Alpe. *TAG* 9: 650–658.

Pogna, N.E., Austran, J.C., Mellini, F., Lafiandra, D. and Feillet, P., 1990. Chromosome 1B-encoded gliadins and glutenin subunits in durum wheat: Genetics and relationship to gluten strength. *J. Cereal Science* 11: 15-34.

Primard, S., Graybosch, R., Peterson, C.J. and Lee, J., 1991. Relationship between gluten protein composition and quality characteristics in four populations of high-protein, hard red winter wheat. *Cer. Chem.* 66(3): 305-312.

Radealli, R., Morel, M.N., Austran, J.C. and Pogna, N.E., 1995. Genetic analysis of low M_r glutenin subunits fractionated by two-dimensional electrophoresis (A-PAGE x SDS-PAGE). *J. Cereal Science* 21: 5-13.

Randall, P.G., Manley, M., McGill, A.E.J. and Taylor, J.R.N., 1993. Relationship between the high M_r subunits of glutenins of South African wheats and end-use quality. *J. Cereal Science* 18: 251-258.

Randall, P.G., Manley M., Meiring, L. and Mcgill, A.E.J., 1992. The high molecular weight glutenin subunits of South African wheats. *J. Cereal Science* 16: 211-218.

Rogers, W.J., Payne, P.I. and Harinder, K., 1989. The HMW glutenin subunits and gliadin compositions of German-grown varieties and their relationship with breadmaking quality. *Plant Breeding* 103: 89–100.

Rubenthaler, G.L. and King, G.E., 1987. Computer characterization of mixograms and their relationships to baking performance. In: The alveograph handbook. Eds. H.A. Faridi and V.F. Rasper. Am. Assoc. Cereal Chem. St. Paul. MN. pp. 131-165.

Schepers, J., Keizer, L.C.P. and Kolster, P., 1993. The relation between high molecular weight glutenin subunits, bread-making quality and agronomic properties of winter wheat. *Cereal Res. Comm.* 21: 289-296.

Schofield, J.D., 1986. Flour proteins: Structure and functionality in baked products. In: Chemistry and Physics of baking. Eds. Blanshard, J.M.V., Fracier, P.J. and Galliard, T. The Royal society of Chemistry, Great Britain. pp. 14 – 29.

Shepherd, K.W., 1988. Genetics of wheat endosperm proteins in retrospect and prospect. In: Proc. of the 7th Internat. Wheat Gen. Symp. (vol. 2). Eds. T.E. Miller and R.M.D. Koebner. Institute of Plant Science Research: Cambridge. pp. 919-931.

Shewry, P.R. and Miflin, B.J., 1985. Seed storage proteins of economically important cereals. In: Advances in Cereal Science and Technology, Vol. 7. Ed. Y. Pomeranz. American Association of Cereal Chemists, Inc., St. Paul, MN. pp. 1 – 83.

Shewry, P.R., Tatham, A.S., Barro, F., Barcelo, P. and Lazzeri, P., 1995. Biotechnology of bread making: Unravelling and manipulating the multi-protein gluten complex. *Bio/Technology* 13: 1185-1190.

Shewry, P.R., Tatham, A.S., Forde, J., Kreis, M., and Miflin, B.J., 1986. The classification of wheat gluten proteins. A reassessment. *J. Cereal Sci.* 4 : 97–106.

Shogren, M.D. and Finney, K.F., 1984. Bread-making test for 10 grams of flour. *Cer. Chem.* 61: 418-423.

Shuey, W.C. and Skarsaune, S.K., 1973. The relation between flour mineral content and flour colour reflectance values. *Cereal Science Today* 18(8): 229-230.

Singh, N.K., Donovan, R., and MacRitchie, F., 1990. Use of sonification and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins II. Relative quantity of glutenin as a measure of breadmaking quality. *Cer. Chem.* 67(2): 161–170.

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

Singh, N.K. and Shepherd, K.W., 1985. The structure and genetic control of a new class of disulphide–linked proteins in wheat endosperm. *TAG* 71: 79–92.

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

Spies, R., 1990. Application of rheology in the bread industry. In: Dough rheology and baked product texture. Eds. H. Faridi and J.M. Faubion. Van Nostand Reinhold, New York. pp. 343-361.

Tatham, A.S., Miflin, B.J. and Shewry, P.R., 1985. The beta-turn conformation in wheat gluten proteins: Relationship to gluten elasticity. *Cer. Chem.* 62(2) 405–412.

Tatham, A.S., Shewry, P.R. and Belton, P.S., 1990 Structural studies of cereal prolamins, including wheat gluten. In: Advances in Cereal Science and Technology, Vol. 10. Ed. Y. Pomeranz. American Association of Cereal Chemists, Inc., St. Paul, MN. pp. 1–78.

Van Lill, D., 1992. Environmental effects on yield and bread-baking quality of some South African wheat cultivars. Ph.D. dissertation, University of Stellenbosch, Stellenbosch, South Africa.

Van Lill, D. and Purchase, J.L., 1995. Directions in breeding for winter wheat yield and quality from 1930 to 1990. *Euphytica* 82: 79-87.

Van Lill, D. and Smith, M.F., 1997. A quality strategy for wheat (*Triticum aestivum* L.) where growth environment predominates. *S. Afr. J. Plant Soil* 14: 183-191.

Van Lill, D., Purchase, J.L., Smith, M.F., Agenbag,G.A. and De Villiers, O.T., 1995. Multivariate assessment of environmental effects on hard red winter wheat. I. Principle components analysis on yield and bread-making characteristics. *S. Afr. J. Plant Soil* 12: 158-163.

Van Niekerk, H.A. and Van Lill, D., 1990. Breeding for wheat quality. Proc. of the second National bakery symposium, Eds. M.F. Smith, M.J. Kort, I.R. Clarke and P.B. Bush, National Technikon Printers, pp. 5-13.

Walker, C.E. and Hazelton, J.L., 1996. Dough rheological tests. *Cereal Foods World* 41(1): 23-28.

Wikström, K. and Bohlin, L., 1996. Multivariate analysis as a tool predict volume from mixogram parameters. *Cer. Chem.* 73: 686-690.

Wright, G.M., 1983. Wheat. In: Plant breeding in New Zealand. Eds. Wratt, G.S. and Smith, H.C. Butterworth of New Zealand. pp. 21 – 28.

Wrigley, C.W., 1992. Identification of cereal varieties by gel electrophoresis of the grain proteins. In: Seed Analysis., modern methods of plant analysis, volume 14. Eds. H.F. Linkens and J.F. Jackson. Springer –Verslag, Berlin, Heidelberg. pp. 17-41.

Appendix A

ble 1. The migration distances of the 50 F2 replications and the parents, Tugela (T) and Tugela (Tfg).

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	2	2	12	12	12	2	7	7	2	2	12	7	2	7	2	22	27	2	7	12	2	22	17	17	17
7	17	12	32	22	27	12	27	27	12	7	22	22	22	12	7	42	47	12	22	27	12	42	47	42	32
2	42	27	62	27	47	37	32	37	27	27	32	52	37	17	17	67	62	37	42	42	22	52	57	57	47
7	57	42	72	32	57	52	57	52	32	32	52	72	52	27	42	92	72	52	47	57	37	67	62	72	57
2	82	62	77	52	67	62	72	62	47	42	62	92	57	52	57	107	87	72	57	77	47	82	82	82	67
2	107	72	92	62	72	72	82	77	62	57	77	97	67	67	67	132	97	82	72	87	62	92	97	92	82
7	112	92	102	77	87	87	102	92	72	72	87	122	87	82	82	167	122	97	87	102	77	97	112	112	97
2	122	102	127	92	102	92	122	102	92	92	92	147	97	87	92	192	147	102	97	112	82	112	122	122	112
2	127	127	147	102	122	102	142	122	102	102	102	172	117	117	112		172	127	102	137	92	117	147	142	122
7	137	152	162	127	137	122	167	132	122	112	127	197	147	132	147		182	157	132	162	112	132	162	157	147
7	147	177	172	137	152	147	172	147	132	132	152		162	147	167		197	182	162	187	117	142	167	167	162
	192	197	177	152	167	167	187	167	147	147	172		172	172	192			197	172	197	127	162	197	172	172
			197	167	172	172	197	172	172	172	197		197	192					182		142	172		197	192
				172	197	182		197	197	192				197					187		157	192			197
				187		197				197											167	197			
				192																	177				
				197																	187				
																					197				

7	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	T	TFG
7	2	2	17	47	2	7	32	12	17	22	22	2	2	2	7	7	7	7	7	2	7	2	2	17	17
7	17	22	32	62	12	27	47	27	42	37	37	22	22	22	27	52	12	17	32	17	12	12	22	27	27
2	22	47	47	77	22	42	62	37	57	72	72	42	37	32	52	72	32	32	47	27	32	27	37	37	37
2	32	67	67	82	47	47	77	47	72	87	87	72	47	47	57	87	47	47	62	47	47	42	57	57	57
7	47	82	82	97	62	57	127	52	87	107	107	92	62	57	67	97	57	62	77	62	62	57	67	62	62
2	57	87	97	112	72	72	152	72	102	132	132	107	77	62	77	102	67	67	92	67	67	62	82	62	72
7	67	97	112	117	82	87	177	82	112	142	157	127	87	77	92	107	72	77	102	77	77	72	92	72	77
2	82	112	117	132	92	107	187	92	127	157	162	142	102	92	107	117	87	92	122	92	92	87	107	77	92
2	97	117	132	142	107	137		97	132	162	177	162	112	102	112	127	102	102	132	102	102	102	117	92	97
7	112	132	142	157	117	152		107	157	177	197	182	132	127	137	142	117	117	147	122	122	117	127	97	112
7	122	142	162	167	137	162		117	177	197		192	152	152	152	152	122	122	167	127	127	132	142	112	122
2	147	162	172	192	167	187		132	187				157	157	162	167	132	132	187	137	137	147	162	122	152
2	162	167	192	197	192	197		142					177	167	187	182	147	147	197	152	147	157	192	152	162
2	172	192	197					157					182	177		192	162	162		162	162	172		162	167
7	192	197						177					197	187			167	167		172	172	192		167	187
	197							187									192	182		192	192	197		187	197
																		192		197	197			197	

ble 2. The migration distances of the 50 F2 replications and the parents, Tugela (T) and Letaba (Let).

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
7	37	7	2	12	2	2	27	17	2	2	2	2	47	17	2	12	2	7	12	2	2	2	2	2	2
27	57	12	47	22	22	12	47	27	17	12	22	17	67	22	17	42	12	22	42	7	17	12	27	12	7
2	72	22	62	27	37	17	67	32	27	22	37	47	77	42	22	57	17	37	52	17	52	17	47	22	12
57	107	47	77	37	47	22	82	42	32	32	47	62	82	47	42	72	32	52	82	47	67	22	57	32	22
77	132	62	87	47	67	37	97	47	42	47	62	82	97	67	62	87	37	72	107	62	92	37	72	37	32
92	157	72	117	67	82	52	112	67	47	62	77	92	112	82	72	102	47	92	117	72	97	42	102	47	42
02	177	82	142	77	97	67	127	82	67	77	97	107	117	92	82	107	67	97	132	87	107	47	107	57	57
07	187	92	167	87	112	77	137	102	82	82	102	117	127	112	92	132	77	102	142	92	117	57	127	72	67
32		102	187	97	122	87	152	107	102	92	112	122	142	142	112	147	97	122	182	112	127	67	132	77	97
52		112	192	102	137	97	172	112	107	102	122	142	152	152	122	177	127	132	197	122	142	82	147	97	107
32		117	197	107	152	107	182	122	112	107	137	152	162	167	137	182	147	172		137	157	92	162	102	127
37		127		117	192	117	187	137	122	112	147	192	182	187	152	192	177	177		152	167	102	172	117	132
		137		127		127		147	137	122	157		197	192	162	197	192	192		157	177	112	177	122	142
		152		137		142		157	147	137	167				187			197		172	192	122	182	132	157
		187		152		152		167	157	147	182									182		132	192	142	177
		192		162		187		182	167	162										192		142	197	157	187
		197		172		192		187	177	172												157		172	192
				177				197	182	182												162		192	197
				192					197	187												172		197	
				197						197												177			
																						192			
																						197			

[illegible]7	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	T	LET
2	2	17	2	2	2	2	2	2	2	2	27	2	2	17	2	12	2	2	7	2	12	2	2	17	12
7	17	42	17	17	7	7	12	17	7	32	57	12	7	27	12	22	7	22	27	12	22	17	12	27	27
7	27	52	52	27	22	12	22	27	17	57	72	17	17	32	22	27	17	27	42	22	32	27	22	37	47
2	52	67	72	52	32	22	37	47	22	67	97	22	37	47	27	42	42	42	57	42	47	37	47	57	57
2	67	92	92	72	52	27	47	57	52	87	102	57	52	62	47	57	57	57	77	57	62	47	62	62	67
7	92	102	102	92	72	32	57	67	77	92	112	72	67	87	57	87	77	77	92	77	77	57	77	62	72
2	102	112	122	102	97	57	67	87	102	117	122	87	92	102	82	97	92	92	112	82	87	77	92	72	77
7	122	122	127	112	107	72	82	97	132	127	132	92	102	107	97	117	97	97	122	87	97	82	102	77	92
2	127	132	142	122	122	92	92	112	167	142	152	102	122	122	117	127	107	112	142	97	112	92	112	92	107
2	142	142	157	132	137	107	102	122	172	162	167	122	132	127	127	137	117	117	147	112	122	102	122	97	122
2	157	157	167	142	147	122	122	127	187	182	172	132	157	157	147	147	137	142	167	122	142	112	142	112	132
7	172	167	177	157	162	132	132	152	192	187	192	157	177	187	157	152	142	152	177	137	162	142	152	122	142
2	177	177	187	162	182	147	147	177	197	192		177	187	192	162	162	152	177	187	142	172	167	162	152	167
7	187	187	197	172	192	162	157	192				182	197	197	182	172	162	197	197	162	182	187	177	162	187
7	192	192		177		167	182	197				187			187	187	177			177	192	197	182	167	197
7				192		182	192					192					182			182			197	187	
				197		192											187			197				197	
																	197								

ble 3. The migration distances of the 50 F2 replications and the parents, Tugela (T) and Gariep (Gar).

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
12	12	17	7	7	12	2	7	12	17	2	12	12	12	2	2	17	12	12	17	17	12	2	12	12
22	22	37	12	17	27	12	12	32	32	17	32	27	27	17	12	52	22	27	27	27	27	12	27	27
37	42	42	17	27	42	27	32	42	47	27	47	37	37	27	22	57	37	37	37	47	42	27	37	37
52	52	57	22	47	57	42	37	47	62	32	62	47	42	37	27	72	42	42	42	62	62	37	47	47
62	62	67	27	57	67	57	47	62	72	47	72	62	57	52	42	82	57	57	57	67	72	42	62	62
77	82	87	37	67	82	67	62	72	82	62	82	67	67	72	52	122	67	67	67	82	87	62	72	72
87	92	97	47	72	92	87	67	87	92	72	97	87	87	82	77	142	82	87	87	97	97	67	92	92
97	102	102	57	82	107	92	87	97	97	82	107	97	97	97	87	162	92	92	97	107	107	87	97	97
112	112	117	67	87	122	97	92	107	102	92	127	107	122	112	117	187	107	107	107	127	127	97	107	112
117	122	127	82	92	137	122	107	127	127	102	132	127	132	117	137	197	122	127	127	132	147	107	132	127
127	127	132	87	107	147	142	122	132	132	112	142	137	152	137	157		132	132	152	157	162	132	137	132
142	147	147	97	122	162	152	127	142	142	127	157	152	172	162	172		152	152	167	177	182	147	162	162
162	162	157	102	142	172	172	147	157	157	132	177	162	187	182	182		177	177	192	197	197	162	177	172
182	182	167	122	152	192	177	162	167	177	157	192	172	192	192	192		197	182	197			177	182	182
192	192	177	127	162	197	197	172	177	192	172	197	187	197	197				192				182	197	187
197		192	137	177			192	197		192		197						197				197		197
		197	147	192			197			197														
			157	197																				
			172																					
			187																					
			192																					
			197																					

28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	T	GAR
12	17	17	17	12	12	7	2	2	22	17	17	17	12	12	12	12	12	7	22	2	2	22	17	2
27	32	32	22	27	27	22	12	22	37	37	32	32	27	27	27	32	32	17	37	22	22	37	27	52
42	42	37	32	42	42	37	17	37	52	52	47	37	37	37	37	47	37	32	52	37	37	47	37	67
47	47	62	42	57	57	52	37	57	72	67	67	47	47	42	47	62	47	42	72	57	52	72	57	82
57	67	92	47	67	67	67	52	72	82	82	77	62	62	62	62	72	67	52	82	72	72	77	62	102
67	72	102	67	82	82	82	72	82	92	97	97	67	72	67	77	92	77	72	102	82	82	102	62	117
87	82	137	72	107	107	92	82	107	102	112	112	92	82	82	92	102	92	82	107	107	102	112	72	127
92	97	162	97	127	127	102	92	117	112	137	122	107	92	92	97	107	102	102	112	117	117	122	77	137
97	112	177	102	152	157	117	107	142	137	152	137	112	102	102	112	112	107	112	142	147	142	147	92	147
107	132	182	112	182	172	122	122	157	147	167	142	127	127	107	132	137	137	142	157	172	157	167	97	157
132	147	197	132	197	177	147	137	172	157	182	147	132	137	127	142	152	142	147	172	187	177	182	112	182
157	162		157		192	157	142	187	172	187	162	137	142	137	152	167	152	157	187	192	187	192	122	192
167	177		172		197	172	157	192	187		182	147	157	147	162	182	172	172	192		192		152	197
177	182		182			197	172		192		187	162	172	157	187	187	182	187					162	
197	192		187				187					182	182	177	197	197	192	192					167	
	197		197				192					197	197	182									187	
														197									197	

le 4. The migration distances of the 50 F2 replications and the parents, Tugela (T) and Palmiet (Pal).

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
12	12	2	7	17	17	12	12	2	2	22	2	2	7	12	12	12	12	2	2	2	2	7	2	2
22	22	17	27	22	27	17	27	17	17	32	22	17	22	27	22	32	47	17	12	7	12	27	7	22
32	37	42	42	32	52	27	42	32	32	47	32	27	37	32	37	52	62	32	27	27	27	37	22	37
57	57	52	62	47	67	42	52	42	47	57	47	42	52	52	52	62	72	57	37	37	42	47	37	47
72	77	67	77	67	82	52	62	57	67	72	62	57	62	67	62	92	102	72	47	52	52	62	47	62
82	87	77	117	82	112	67	82	67	82	87	72	67	72	87	72	107	107	87	62	62	67	72	62	72
107	107	92	132	87	117	82	97	77	102	102	82	82	87	102	87	122	122	107	82	77	77	87	72	87
132	137	117	147	97	147	92	122	82	122	127	97	97	102	122	102	162	132	127	102	82	82	97	87	102
177	162	137	162	122	167	117	137	97	137	137	127	122	127	127	122	177	147	137	117	97	102	117	102	127
187	177	167	167	132	172	132	147	122	147	147	147	142	147	142	137	192	172	157	122	122	127	122	122	142
	197	187	192	147	187	142	167	132	167	152	157	147	157	152	147		182	182	132	127	147	147	142	147
		197	197	167	192	147	172	142	177	177	172	157	172	157	167			192	142	147	152	152	157	157
				177		157	187	152	192	192	177	172	177	172	172				162	152	162	172	177	167
				197		167	192	167	197	197	192	177	192	177	192				167	172	167	187	197	177
						172		177			197	192	197	197	197				187	187	177	192		182
						187		192				197							192	197	187			197
						197		197											197		197			

29	27	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	T	PAL
2	12	7	7	2	2	2	2	2	2	7	2	2	22	2	2	12	2	7	7	2	2	7	17	12
12	22	12	22	12	7	17	17	7	22	27	7	12	67	22	22	17	27	17	27	12	12	27	27	27
22	37	27	37	27	27	27	32	22	32	37	27	17	77	47	27	22	32	22	37	32	22	42	37	37
37	47	37	52	42	37	42	42	42	42	47	37	27	97	72	42	32	42	37	47	42	32	52	57	47
47	62	47	62	47	47	52	57	67	67	67	47	37	112	82	57	47	62	47	62	52	42	72	62	57
62	72	62	72	67	62	62	72	82	82	82	67	47	137	92	72	57	72	57	77	72	52	87	62	62
72	87	72	87	82	77	72	82	97	92	97	77	62	147	112	82	77	82	77	87	82	72	97	72	77
87	97	82	97	102	82	77	92	112	112	117	82	67	152	142	92	82	97	97	97	87	87	112	77	87
97	117	97	117	122	97	92	112	127	127	122	97	77	162	162	117	97	112	122	117	102	102	122	92	97
127	147	122	127	132	117	102	127	147	142	137	112	82	167	167	142	112	127	132	122	117	112	137	97	117
147	157	132	147	162	127	112	132	152	152	147	127	102	177	182	152	127	142	142	142	122	127	162	112	162
152	167	147	152	172	142	122	142	187	167	167	137	112	197	192	162	142	152	147	152	142	142	167	122	182
167	177	157	167	182	162	137	147	192	192	172	147	122			167	162	162	157	162	162	162	182	152	
172	192	167	172	192	167	162	192		197	197	162	142			192	167	167	162	167	177	172	187	162	
177	197	177	192		182	167					167	162			197	192	177	167	177	187	182	197	167	
187		192	197		187	182					187	167				197	182	187	187	197	187		187	
197		197			197	187					197	182					192	192	192		197		197	
						197						197												

le 5. The migration distances of the 50 F2 replications and the parents, Tugela (T) and Tugela DN
N).

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	2	7	7	7	7	2	7	2	7	7	7	2	7	7	7	2	12	2	2	2	7	7	2	7
22	27	27	27	27	12	27	27	27	27	27	27	17	12	17	27	17	37	7	12	7	27	12	12	17
42	32	37	37	37	27	32	37	37	37	52	42	32	32	32	37	22	57	22	27	27	32	27	27	27
62	47	47	47	47	32	42	47	47	47	67	52	42	42	42	47	42	72	32	32	37	47	32	32	32
82	62	62	62	62	47	57	62	62	62	87	67	52	52	52	62	57	102	42	47	47	62	47	47	47
92	87	87	87	82	62	82	82	82	82	97	87	67	67	67	87	77	132	57	62	62	82	62	62	62
112	92	92	97	97	82	92	92	97	97	112	97	87	87	87	97	97	157	82	82	82	97	82	82	72
132	117	117	112	112	92	112	112	112	112	127	112	102	102	102	112	117	182	92	97	97	112	97	97	82
147	137	132	137	137	112	127	122	122	142	147	122	112	117	117	127	127		107	112	112	122	112	112	97
162	152	142	147	147	137	142	137	142	157	167	142	122	127	127	147	137		117	122	122	142	122	122	112
182	167	152	162	162	147	147	142	162	162	192	162	142	147	147	162	142		137	142	142	162	142	142	117
192	187	162	167	167	167	157	157	182	182		167	157	162	162	187	167		167	162	167	167	162	157	137
	197	167	187	182	182	162	162	187	192		187	167	172	172	192	182		172	167	187	177	167	167	157
		187	197	192	187	182	182	192			197	177	192	197	197	192		177	192	197	187	182	182	167
		197				192	192					187	197					192	197		197	187	187	172
												192										197	192	187
												197											197	192
																								197

28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	T	TDN
17	2	2	2	7	7	7	2	7	2	2	2	12	12	22	22	17	7	7	7	2	22	22	17	12
22	17	7	7	27	27	27	12	27	22	22	12	27	27	32	27	27	17	12	12	7	32	32	27	32
37	27	12	27	32	37	47	22	47	27	32	17	32	32	47	42	42	27	22	22	22	42	42	37	42
57	37	22	32	47	47	62	42	67	42	47	27	47	47	62	57	57	42	32	32	32	57	62	57	52
77	57	32	47	62	62	82	57	102	57	62	47	62	62	82	77	77	57	42	42	42	82	82	62	67
87	77	42	62	87	87	97	82	117	77	72	52	77	82	97	92	92	77	52	57	57	92	92	62	77
107	87	57	82	97	97	117	92	132	87	82	62	82	97	107	107	107	92	72	77	82	117	107	72	87
132	107	77	97	117	117	137	112	142	92	97	72	97	112	112	122	117	107	92	92	92	122	127	77	97
157	122	92	112	122	132	152	132	187	107	112	82	112	122	147	142	137	117	107	107	107	142	137	92	112
177	137	107	117	127	142	167	137		117	122	92	122	147	157	157	162	132	117	117	122	157	157	97	122
192	162	117	142	137	152	197	147		137	142	102	147	162	167	167	182	152	137	137	137	167	167	112	127
	182	132	157	142	167		182		162	167	112	167	172	187	192	192	182	157	162	157	177	187	122	142
	192	142	167	162	187		192		167	172	122		192	197	197	197	197	162	177	167	187	197	152	152
		157	192	167	197				172	182	142		197					177	187	172	197		162	187
		162		187					177	197	167							182	192	182			167	192
		187		192					182		177							197		192			187	
		192		197					192		182									197			197	
									197		187													
											197													

le 6. The migration distances of the 50 F2 replications and the parents, Tugela (T) and Flamink (FL).

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
17	2	7	2	7	2	2	17	2	2	2	2	2	7	2	7	2	2	7	2	2	17	7	2	22
22	7	22	17	22	7	17	27	7	17	17	7	17	22	7	22	7	17	12	17	12	22	17	12	37
37	12	32	27	27	17	27	37	17	27	27	22	27	32	22	32	12	27	17	27	17	37	27	17	52
57	22	42	37	37	27	37	57	22	37	42	27	42	42	32	42	22	37	32	37	27	62	37	37	67
67	32	57	52	57	37	52	62	37	52	52	42	57	57	42	57	32	52	57	62	42	77	62	67	77
77	42	77	77	77	62	77	77	62	57	67	52	62	77	57	72	42	57	67	72	62	82	77	77	97
92	57	87	107	107	77	82	92	72	72	77	77	77	87	72	77	57	72	82	92	72	92	92	92	112
112	77	107	122	122	87	107	102	87	87	92	92	87	112	77	97	62	87	102	107	82	117	117	117	137
117	82	122	147	142	107	122	122	102	102	107	107	107	122	97	107	77	92	127	137	92	137	137	137	157
132	92	142	192	157	132	142	127	112	117	117	117	122	142	107	122	92	102	147	152	112	147	147	157	167
172	102	167		182	157	162	142	132	132	122	122	137	162	122	127	107	132	177	162	137	157	152	162	177
182	117	192		187	162	187	152	142	152	137	142	142	187	137	137	122	147		192	152	162	157	172	192
187	132			192	182	197	167	162	177	162	157	157	192	157	167	137	162			162	167	172	177	197
197	172			197	187		172	187	187	172	162	182	197	167	172	152	167			167	192	192	192	
	182				197		182	197	192	187	192	192		177	197	167	177			177				
	187						187		197	197		197		187		197	187			197				
	197						197							197			192							
																	197							

28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	T	FL
2	7	2	7	7	7	7	12	12	7	12	12	7	17	2	7	7	2	12	7	2	2	2	17	12
22	22	7	12	27	12	12	22	32	12	22	22	12	27	17	17	22	12	32	12	12	17	12	27	27
37	32	12	27	32	27	17	27	52	17	37	37	27	37	22	27	32	22	42	17	22	22	22	37	37
62	42	27	42	47	37	27	42	62	37	52	52	37	52	37	37	47	27	62	22	32	32	32	57	47
72	62	42	67	62	47	32	62	82	62	67	67	52	57	57	57	57	37	82	32	42	42	42	62	62
82	77	62	82	77	62	47	67	87	72	72	72	62	72	77	67	67	52	122	42	52	57	57	62	77
92	82	77	87	87	77	62	77	97	97	87	87	72	87	87	72	77	62	132	52	62	67	67	72	87
117	97	82	102	97	87	72	92	107	102	107	107	87	107	107	92	92	67	142	67	82	72	72	77	112
137	112	97	122	117	97	82	107	112	127	132	132	107	122	127	107	107	77	162	72	92	77	87	92	142
147	142	117	142	142	117	97	132	122	157	142	142	127	137	132	117	117	87	192	82	102	82	92	97	152
152	162	142	157	147	132	112	147	157	182	187	187	142	142	142	122	137	102	197	87	117	92	102	112	197
172	172	157	172	152	147	127	157	162	197			157	162	157	132	157	107		102	132	102	122	122	
197	182	167	192	157	162	142	162	167				187	182	182	162	187	122		117	147	117	127	152	
	187	172	197	167	172	162	182	177							172	197	142		127	162	127	147	162	
	197	182		172	192	167	192	182							187		157		147	167	132	157	167	
		192		182	197	187	197	192							197		167		162	187	147	167	187	
		197		192		197		197									187		167	192	162	172	197	
				197															192		172	197		
																					192			

le 7. The migration distances of the 50 F2 replications and the patents, Tugela (T) and Inia (IN).

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	17	2	7	12	12	2	22	7	22	2	7	7	7	2	2	2	17	7	7	12	7	12	12	12
7	32	17	17	22	27	7	57	22	37	12	12	22	17	7	22	22	22	12	17	22	17	27	32	32
12	47	32	32	32	37	12	77	32	52	22	22	37	22	22	37	32	32	22	27	32	27	37	37	42
32	57	57	47	52	52	22	97	47	62	32	32	62	37	37	52	47	47	47	37	37	52	52	47	52
42	67	67	57	67	72	27	112	62	72	47	47	77	47	52	62	62	57	62	47	52	62	62	62	67
52	77	92	72	87	77	42	127	77	82	62	62	102	62	62	72	72	67	72	67	62	72	72	72	72
62	107	122	82	107	92	52	142	82	102	77	72	117	72	72	87	87	82	87	82	72	82	82	82	87
67	117	152	102	117	107	72	152	102	112	97	82	127	77	87	102	102	97	112	97	87	92	97	97	97
87	132	177	112	127	117	107	167	112	122	117	102	132	107	112	117	117	112	132	112	97	112	112	112	112
102	162	197	117	142	122	117	187	127	142	127	117	147	112	127	127	122	122	142	127	112	127	127	132	127
112	182		132	152	137	137	192	142	152	132	127	152	117	147	147	147	142	162	142	127	137	142	142	142
122	187		182	187	147	157		147	162	147	147	167	127	152	157	152	167	192	152	147	152	162	152	147
142	192				162	172		162	167	162	152	172	132	162	167	162	172		162	162	162	167	162	162
162	197				187	182		167	192	172	162	197	152	172	172	167	182		167	167	167	187	167	167
167						187		192			172		167	197	197	177	197		187	187	187	192	182	182
177						192					197		172			187			197	197	192	197	192	192
187						197							182			192								
197													197			197								

28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	T	IN
2	2	2	2	2	2	2	2	2	12	2	2	2	7	7	7	7	7	2	17	17	7	2	17	7
17	7	22	7	22	7	12	7	22	17	12	22	27	12	27	12	12	17	7	37	27	12	22	27	27
32	22	37	12	37	12	22	22	37	32	22	42	42	27	42	27	27	27	17	42	37	22	42	37	52
42	27	47	22	47	22	42	42	47	42	27	62	57	32	57	32	32	47	37	52	47	42	47	57	67
52	37	57	42	57	42	52	52	57	52	42	77	77	42	82	42	42	67	47	67	57	47	57	62	92
67	47	72	47	72	52	62	62	72	62	57	92	92	62	92	62	62	72	57	72	72	62	72	62	107
72	57	77	57	77	62	72	72	77	72	62	107	107	77	112	82	82	82	72	87	92	72	77	72	122
87	72	92	72	92	72	82	77	92	82	77	132	127	92	127	97	87	97	87	102	97	77	92	77	132
102	77	102	77	102	77	92	82	102	97	92	137	137	112	137	127	97	112	112	112	102	92	97	92	182
117	92	117	92	117	92	107	92	117	107	102	147	147	132	147	137	107	132	127	132	117	102	107	97	
127	97	127	102	142	107	117	102	132	122	122	157	162	147	157	142	127	142	142	142	132	107	117	112	
142	102	142	107	162	117	132	117	147	132	132	177	182	157	162	147	132	152	147	147	142	117	132	122	
152	117	147	117	167	127	152	132	167	142	147	187		167	177	157	142	167	162	162	152	132	142	152	
162	132	152	132	177	147	167	142	182	157	157	197		182	182	162	147	187	167	167	162	142	152	162	
167	142	162	142	182	162	187	147	192	162	177			192	192	187	162	192	187	187	172	147	157	167	
187	157	167	152	192	167	192	157	197	172	187						182		197	197	187	157	172	187	
192	167	192	162	197	187		167		192	197						187				197	167	177	197	
197	182		167		192		182														172	192		
	192		177				192														192	197		
			182																		197			
			192																					

le 8. The migration distances of the 50 F2 replication and the parents, Tugela (T) and Gamtoos DN (GT

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	2	2	2	2	7	12	2	2	2	2	22	17	22	22	2	2	2	7	2	2	12	2	2	2
17	12	12	7	7	27	32	12	7	12	22	42	27	27	27	22	17	12	27	12	12	32	17	12	12
22	22	22	17	22	42	37	17	27	22	42	47	37	37	37	27	27	22	42	22	32	42	22	32	17
37	32	32	22	37	62	52	37	37	37	47	57	47	47	47	37	37	32	47	42	47	52	37	42	37
52	47	42	37	57	67	62	42	47	47	57	72	72	57	57	47	47	42	62	57	67	67	42	52	42
72	52	57	52	67	82	67	57	57	62	72	77	82	72	72	72	57	52	67	62	77	77	52	62	52
82	67	72	57	77	92	82	67	67	72	77	82	92	77	77	77	72	67	72	77	87	92	62	72	72
92	82	77	62	92	112	97	72	77	77	92	92	102	92	92	87	77	77	82	102	102	102	72	87	77
102	87	92	72	107	127	112	87	92	92	107	102	117	102	102	97	87	87	92	117	117	117	87	97	87
117	112	107	87	127	137	127	97	107	102	117	117	132	107	117	107	97	102	107	157	132	132	102	117	102
132	127	122	102	132	152	137	107	117	117	132	132	147	117	132	117	107	112	122	177	142	147	117	132	112
142	142	132	122	142	157	167	112	132	132	142	142	162	132	142	132	117	127	132	182	147	152	132	142	132
147	162	137	127	157	167	187	127	142	147	152	152	167	142	157	142	132	142	137	197	162	162	142	147	142
157	172	142	142	162	182	197	142	162	162	162	162	177	152	162	152	142	162	162		172	172	157	157	152
167	182	157	157	172	192		162	172	172	172	172	187	162	167	162	147	167	177		187	187	162	167	167
172	192	162	177	182			197	192	192	192	197	197	167	177	172	152	182	187		197	197	167	182	182
192	197	177	182	187				197	197	197			182	182	182	162	187					187	192	192
197		182											187	192	187	172	192					192		
		192											197	197	197	182								
		197														187								
																197								

28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	T	GT DN
2	2	2	2	2	2	2	2	2	17	2	2	7	7	7	12	12	7	12	17	2	12	12	17	12
17	17	7	22	22	17	12	7	27	37	7	17	17	22	27	27	27	12	32	27	17	32	32	27	27
32	37	22	27	42	27	27	27	42	52	27	27	32	37	37	32	42	27	37	47	32	47	42	37	52
47	42	37	42	47	42	32	32	52	67	42	42	47	47	47	37	57	37	42	62	47	57	52	57	72
57	52	47	47	57	52	42	42	62	82	47	57	57	62	62	52	62	42	52	67	57	67	67	62	87
67	62	57	57	72	62	52	57	72	87	62	67	67	77	77	62	72	57	67	77	67	77	77	62	107
77	67	72	72	77	72	62	72	82	112	67	72	82	82	87	72	87	62	87	92	82	92	82	72	112
87	82	77	82	82	82	77	87	87	127	77	82	97	87	97	77	102	77	97	107	92	97	92	77	122
102	92	92	92	92	92	82	97	92	137	92	92	112	97	117	87	117	92	107	122	102	107	102	92	137
112	102	107	97	102	107	87	102	107	162	107	112	132	112	137	102	122	107	127	127	122	122	122	97	152
127	112	117	107	117	117	92	107	122	187	122	137	137	142	147	117	132	122	142	137	137	137	142	112	162
137	127	132	117	132	132	102	122	137	192	132	147	147	157	152	132	147	137	172	147	152	152	147	122	177
147	142	142	132	142	142	112	137	147	197	157	167	162	167	167	152	157	152	177	157	157	167	157	152	192
152	162	152	142	157	157	122	147	167		182	182	167	177	172	167	167	172	187	162	177	182	172	162	
167	167	162	147	162	162	132	152	172			187	177	187	187	172	172	187	197	192	187	187	177	167	
182	187	167	152	167	167	147	157	192			197	182	192	192	187	187	192		197	197	192	187	187	
192	192	187	162	187	187	157	167	197				192	197		192	192						192	197	
		192	167	192	197	167	172					197												
		197	187			172	182																	
			192			187	187																	
						197	197																	

Appendix B

The different LMW subunit combinations for parent cultivars and F2 replications.

Table 1. The different LMW subunit combinations for the parent cultivars and F2 replications.

Parents and replications	A genome	B genome	D genome
TUGELA	a/c/f	B	d/e
TUGELA fg	a	a/c	a
1	f	c	e
2	c	a	-
3	b/f	a	e
4	b/f	-	e
5	a/b/f	b	d/e
6	a/b/f	c	-
7	b/f	c	d/e
8	a	-	e
9	f	a	c*
10	b/f	b	c*
11	a	a	-
12	b/f	a*/b*/c*	c*
13	e	c	-
14	f	c	a*
15	b/f	a*	-
16	c/f	c	-
17	f	c	e
18	a/f	c	e*
19	b	a*/c*	a*/d*/e*
20	a	c	a
21	a	a/c	e
22	a/b/f	a/c	d/e
23	a/f	a/c	a/e
24	c/f	c	d*
25	c	a/c	-
26	c/f	c	a*
27	c/f	c	a*
28	c/f	c	a*
29	f	c	-
30	c/f	c	-
31	f	c	e*
32	f	c	e*
33	a	a	a*/d*/e*
34	f	-	e*
35	a/b	a/c	a*/d*/e*
36	c	a/c	a*/d*/e*
37	e	-	-
38	a	-	-
39	a	a	a*/d*/e*
40	a/f	a/c	d/e
41	a/f	b	d/e
42	a/f	-	a/e
43	e	a*/c*	e
44	f	a*/c*	a/e
45	c/f	c*	e
46	f	c*	e
47	c/f	c*	e*
48	b/f	c*	e*
49	b/f	a	d*/e*
50	f	a/c	a

* - one of the combination's bands is absent.

Table 2. The different LMW subunit combinations for parent cultivars and F2 replications.

Parents and replications	A genome	B genome	D genome
TUGELA	a/c/f	b	d/e
LETABA	a	c	c/e
1	a/c	g	-
2	e	c	c*/d*/e*
3	a/f	c	c
4	d/f	c	c/e
5	b/f	c	c
6	f	c	-
7	a/c/f	c	a*
8	a/f	c	c
9	a/f	c	e
10	a/f	c	d
11	a/f	c	c
12	a/f	c	c
13	c/f	c	-
14	f	c	c
15	a/c	c	e
16	ac/f	c	c
17	e	g	c*
18	c/f	c	c
19	a	b	c
20	b	a	-
21	b/f	c	c
22	c/f	-	a
23	a/c/f	a/c	a
24	a/d	-	c
25	a/d	b	c*/d*/e*
26	a/b/d	b*/c*	c
27	a/c/d	-	a*/d*/e*
28	a/c	-	a
29	c/f	c*	a
30	c/d	-	c*/d*/e*
31	a/c/d	b*	c*/d*/e*
32	a	b	e
33	a/d	h	e
34	a/f	c	a
35	a/c	c	-
36	a/c	-	-
37	f	c*	a
38	a	b	-
39	a/c	c	c*/d*/e*
40	b/c/f	-	a
41	a/c/f	c	d
42	a	c	d
43	a/b	c	d
44	c/d	c	d
45	d	c	-
46	a	b*	c*/d*/e*
47	a	a/c	c*/d*/e*
48	a	c	c/e
49	a/c	b/c	-
50	a/b	c	c/e

Table 3. The different LMW subunit combinations for patent cultivars tested and the F2 replications.

Parents and replications	A genome	B genome	D genome
TUGELA	a/c/f	b	d/e
GARIEP	f	a/c	a
1	c	e	a
2	a/f	a/b	e
3	a/f	a/c	d
4	c/f	a/c	a
5	a/c/f	c	a/d
6	c/f	c	a
7	b/f	c	a
8	b/f	a/c	a/d
9	b/f	c	b
10	b/f	c	c/e
11	c/f	c	c/e
12	a/b/c/d/f	c	c/e
13	b/f	c	c/e
14	a/b/f	a/c	a
15	a/b/f	c	a
16	a/c/d	e	a*/d*/e*
17	a/b/d	a	d
18	c	-	a
19	b/f	c	d
20	a/b/f	a/c	a
21	b/c/f	c	a
22	b/c/f	c	a*/d*/e*
23	a/f	c	e
24	a/b/f	c	b
25	a/b/f	c	c/e
26	a/f	b*/c*	e
27	a/b/f	b*/c*	e
28	b/f	a/c	a
29	b/c/f	c	e
30	b/c/f	-	d*/e*
31	a/b/f	b	e
32	a/f	a	a
33	b/f	c	a
34	a	a	a
35	b/c/d	c	a*/d*/e*
36	a/d	a	a
37	a	b*	e
38	c/f	a	a
39	c/f	e*	e
40	c/f	-	b
41	a/b/f	a/c	c/e
42	a/b/f	c	b
43	a/f	c	e
44	f	-	e
45	b/f	-	e
46	c/d	a/c	a
47	a	a	a
48	a	a	-
49	a	a	a
50	a/f	-	e

Table 4. The different LMW subunit combinations for parent cultivars and F2 replications.

Parents and replications	A genome	B genome	D genome
TUGELA	a/c/f	b	d/e
PALMIET	a/b/f	c	d/e
1	b/c/f	c	d*/e*
2	a/b	-	-
3	b	c	e
4	c/f	c	e
5	d/f	-	e
6	a/b/c/f	c	e
7	a/c/f	-	a*
8	a/b/c/f	a/c	a
9	a/b/f	c	d*
10	c/f	c	a/e
11	c/f	c	-
12	a	c	-
13	a/f	c	e
14	b/c	c	a
15	f	c	d/e
16	b/f	c	a
17	b/f	c	e
18	c/f	-	d
19	f	-	c
20	c/d	-	d*/e*
21	a/f	c	d*/e*
22	a/f	c	d*/e*
23	a/c/d	c	a/e
24	a/f	c	c
25	d/f	c	e
26	a/f	c	e
27	b/f	c	c/e
28	b/f	c	-
29	a/b/f	c	c/e
30	b/d/f	c	e
31	f	a/b	c
32	a/b/f	c	a
33	d/f	c	e
34	b/c/d/f	c	d*/e*
35	c	c	-
36	a/f	c	e
37	b/c/f	c	e
38	f	c	-
39	a/d/f	c	e
40	a/c/f	c	b/e
41	a/f	-	e
42	a/d	c	e
43	a/d	c	e
44	a/c	b/c	e
45	a/d/f	c	e
46	a/c/d	c	e
47	a/f	c	c/e
48	b/d	c	d*/e*
49	a/b/d	a	d*/e*
50	a/d	c	d*/e*

*-one of the combination's bands is absent.

Table 5. The different subunit combinations for parent cultivars and F2 replications

Parents and replications	A genome	B genome	D genome
TUGELA	a/c/f	b	a/e
TUGELA DN	f	a/c	a/e
1	a/f	b	c/e
2	a/f	a/c	e
3	a/f	c	d*/e*
4	a/f	a/c	d*/e*
5	a/f	c	d*/e*
6	a/f	c	d*/e*
7	a/f	c	d*/e*
8	a	a/c	a*/d*
9	a/f	c	d*/e*
10	a/f	c	d*/e*
11	a/f	c	d*/e*
12	a/f	c	a
13	a/f	a/c	a
14	c/f	a/c	a
15	b/f	a/c	a
16	c/f	a/c	a
17	a/f	a/c	d*/e*
18	a/c	a/b	-
19	c	-	a/e
20	a	a/c	c
21	a/f	c	d*/e*
22	a/f	c	d*/e*
23	a/f	c	d*/e*
24	a/f	c	d*/e*
25	a/f	c	d*/e*
26	a/c/f	c	e
27	a	c	a*/d*/e*
28	a/c	a	a*/d*/e*
29	a/c	a	e
30	a	a/c	e
31	a/f	c	d*/e*
32	a/f	c	d*/e*
33	a/f	c	d*/e*
34	f	c	-
35	a	a/c	-
36	a/f	-	-
37	a/d	a/c	a*/d*/e*
38	a/f	c	e
39	a/f	c	e
40	a/f	c	e
41	a/b/f	c	d*/e**
42	a/f	c	d*/e**
43	a	c	d*/e**
44	a/c	c	d*/e**
45	a/c	a	-
46	a	b	a*/d*/e*
47	a	a	a*/d*/e*
48	a	c	a*/d*
49	a	a/c	a*
50	a/f	c	d*/e*

.*- one of the combination's bands is absent.

Table 6. The different LMW subunit combinations for parent combinations and F2 replications.

Parents and replications	A	B	D
TUGELA	a/c/f	b	d/e
FLAMINK	f	c	e
1	c/f	e	e
2	a/c/f	b	a/e
3	a/b/d	a/b/c	-
4	a	b	-
5	a/c	-	-
6	a/d	-	d*/e*
7	a/d/f	b	e
8	a/c/d	a	d*/e*
9	a/c/f	b	d/e
10	a/c/f	a	e
11	a/c	a	c*
12	a/c/d/f	c	a/e
13	a/d	c	a*/d*/e*
14	a/c/d/f	c	d/e
15	a/d	a	a*/d*/e*
16	a/d	e	d/e
17	a	b/e	-
18	f	c	d/e
19	a/c/d	f	d/e
20	c/f	-	a*
21	a/c/d/f	-	e
22	a/c/d/f	c	e
23	a/c/f	c	e
24	a/c/d/f	c	e
25	b/c/f	-	e
26	a/f	b	a/e
27	a/f	b	e
28	f	c	-
29	a/d/f	b/c	e
30	a/c/d/f	c	e
31	a/b/f	c	e
32	a/d/f	c	d/e
33	a/b/d/f	c	d/e
34	a/c/d/f	c	e
35	a/f	c	e
36	b/f	c	d
37	c/d/f	-	e
38	a/f	-	a/e
39	a/f	b	a*/e*
40	a/d/f	-	d/e
41	a/c	b	e
42	a/c/d	a	d*/e*
43	a/c/d/f	b/e	a/e
44	a/d/f	b	a/e
45	a/d/f	-	d/e
46	f	-	e
47	a/d/f	a/c	a/e
48	a/f	a/c	d
49	a/c/d/f	a/b/c	a/e
50	b/d/f	c	a*/e*

* - one of the combination's bands is absent.

Table 7. The different LMW subunit combinations for parent cultivars and F2 replications.

Parents and replications	A genome	B genome	D genome
TUGELA	a/c/f	b	d/e
INIA	a/f	a	a
1	b/f	a/c	a/e
2	b/f	a	a/b
3	c/f	e	a/e
4	c/f	-	a
5	c	a/e	d*/e*
6	a/f	b	a
7	a	b	a/e
8	a/b/d	b	a
9	a	a	e
10	a/d/f	b	e
11	a/f	a/b	d/e
12	b/d/f	b	e*
13	b/d/f	b	e*
14	b/f	b	e*
15	a/c/d/f	a	e
16	d/f	a	d*/e*
17	d/f	a	d*/e*
18	a/d/f	b	e
19	a/c/f	c	a
20	a/d/f	-	e
21	a/c/f	c	d
22	a/d/f	a/b	d/e
23	a/c/d/f	a/b	d/e
24	a/f	a/b	d/e
25	b/f	c	e
26	b/f	a	a/e
27	a/b/f	a/b	a/e
28	c/d/f	a	a/e
29	a/d	b	a/e
30	d	b	a/e
31	a/b/d	b	a/e
32	a/d	b	a/e
33	a/d/f	b	d/e
34	a/d/f	a/b/c	d/e
35	a/d/f	a/c	d/e
36	b	b	a/e
37	b/c/f	a/c	a/e
38	a/b/d/f	c	d/e
39	a/d/f	-	e
40	a/d	a	e
41	a/d/f	b	e
42	a	a/c	-
43	a/f	c	e
44	a/f	a	e
45	a/c/d/f	c	e
46	c/d	a	a/e
47	c/f	a	a/e
48	a/c	a/b	e
49	b/d/f	c	e
50	d	a/c	e*

o - one of the combination's bands is absent.

Table 8. the different LMW combinations for parent combinations and F2 replications.

Parents and replications	A	B	D
TUGELA	a/c/f	b	d/e
GAMTOOS DN	a/b	a	a
1	c/d	a	a*
2	c/d	a/b	e*
3	a/b/f	a	a
4	a/b/d	b	a
5	a/c/d/f	a	d/e
6	a/d/f	b	a/e
7	a/f	c	a
8	b/f	a	a
9	d/f	a	a*/d*/e*
10	a/d/f	b	a/e
11	a/b/d/f	b	e
12	a/d	a	e
13	b	a	-
14	a/c	c	e
15	a	b	d*
16	a	b	d*
17	a/d	a/b/c	d*
18	a/c/d	a/b/c	d*
19	a/d/f	a	a/e
20	a/d/f	c	e
21	a/b/d/f	a	d/e
22	b/d/f	a	a/e
23	b/f	a	a
24	a/c/d/f	a	d/e
25	d/f	a/c	d/e
26	c	a	d*
27	a/c/d/f	a/b/c	a/e
28	c/f	a	a/e
29	c/f	a/c	a/b
30	a/d	b	d*
31	a/d	a/b/c	a*/d*/e*
32	a/d	a/b/c	d*
33	c/d/f	a/c	d/e
34	a/b/d/f	a/b/c	d/e
35	a/d	a/b/c	d*
36	a/f	c	d/e
37	c/f	a	a
38	a/d/f	b	a/d/e
39	a/c/f	c	a/e
40	c/f	c	a
41	a/d/f	c	d/e
42	a/f	a/b/c	d/e
43	a/b/f	a	d/e
44	a/b/f	a	d/e
45	a/b/f	b	d/e
46	b/f	c	a
47	c/f	-	d/e
48	c/f	c	a
49	b/f	c	a/e
50	b/f	-	a/e

* - one of the combination's bands is absent.

Appendix C. Table of the all the quality data for three replications for each genotype.

Genotype	VK	BFLY(%)	FLY(%)	FPC(12%MB)	SDS(ml)	FCL(C76)	FLN(s)	MDT(min)
Kariega	82	24.4	79.6	11.6	90	-0.9	368	2.3
	80	23.9	79.0	12.9	91	-1.3	410	2.4
	86	24.5	79.2	12.0	91	-.0.9	408	2.3
Tugela	94	19.0	76.4	12.9	89	2.2	410	3.6
	84	19.2	76.9	14.9	92	1.8	367	3.7
	84	19.2	76.4	14.3	89	1.2	410	3.6
TugxFlamink	82	21.0	77.2	15.4	84	0.4	410	3.2
	88	21.0	77.4	15.7	80	1.8	410	3.1
	88	21.8	77.3	17.9	83	2.5	410	3.3
TugxPalmiet	90	21.8	80.7	16.0	85	1.2	410	2.8
	84	21.1	77.8	14.6	86	2.3	410	2.8
	84	21.2	77.1	13.1	86	2.2	410	2.9
TugxInia	84	21.5	77.4	12.7	90	4.5	410	2.9
	84	21.9	78.2	13.0	89	3.0	410	2.8
	88	22.0	78.1	14.1	87	3.4	410	2.5
TugxGariep	92	23.6	76.5	16.5	85	3.7	410	2.5
	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
TugxGamtDn	88	21.9	77.1	15.5	88	1.1	410	2.6
	90	21.8	77.2	13.7	88	1.8	410	2.5
	86	21.6	77.3	16.2	90	2.0	410	3.0
TugxTug fg	92	20.6	76.3	12.9	89	3.8	408	3.2
	96	20.3	76.5	13.5	90	5.8	410	2.9
	-	-	-	-	-	-	-	-
TugxTug Dn	90	20.5	78.1	13.5	90	2.5	410	3.0
	84	20.9	77.9	16.5	91	2.0	410	3.2
	88	20.8	77.5	13.8	92	2.1	410	3.1
TugxLetaba	92	22.9	78.5	17.2	86	1.8	375	2.2
	76	23.3	78.5	17.1	87	3.3	410	2.5

Appendix C: Table continue

Genotype	FABS(%)	P/L	STRENGTH	LFV12%(cm³)	LVF(cm³)	HLM(kg/ha)	Gluten 12%
Kariega	58.60	0.30	33.03	931	915	80.85	33.86
	59.75	0.32	38.84	859	895	81.04	32.63
	60.75	0.35	37.00	875	875	79.84	33.38
Tugela	69.00	1.03	86.70	769	805	79.84	35.22
	69.70	1.02	92.20	694	810	79.74	34.16
	69.70	1.26	91.90	683	775	79.91	36.48
TugxFlamink	69.20	0.64	75.38	839	975	78.38	38.77
	69.20	0.56	76.15	837	985	78.49	39.95
	67.45	0.60	79.66	674	910	77.71	40.71
TugxPalmiet	67.00	0.46	62.54	840	1000	79.38	39.24
	66.30	0.52	58.72	901	1005	78.82	39.19
	67.05	0.48	54.28	951	995	77.72	39.04
TugxInia	65.60	0.55	72.32	792	820	78.32	34.76
	67.45	0.53	74.62	775	815	78.12	38.47
	68.05	0.59	72.32	811	895	79.84	38.21
TugxGariep	67.80	0.82	75.54	-	-	78.96	43.60
	-	-	-	-	-	-	-
	-	-	-	-	-	-	-
TugxGamtDn	68.05	0.61	65.44	860	1000	79.35	38.30
	66.65	0.50	63.15	882	950	80.51	36.94
	68.80	0.49	64.22	827	995	80.64	37.49
TugxTug fg	74.70	0.86	92.97	-	-	78.72	39.11
	73.95	1.04	94.50	-	-	78.86	39.30
	-	-		-	-	-	-
TugxTug Dn	69.10	0.71	89.30	810	870	80.42	38.26
	70.05	0.92	90.67	815	995	80.32	39.05
	71.80	0.78	99.39	833	905	80.93	37.80
TugxLetaba	69.80	0.56	69.72	-	-	81.18	42.05
	70.10	0.52	66.97	796	1000	80.45	41.16

Missing plot values are indicated with a (-), missing plots were caused by bird damage.
Abbreviations used in Appendix C

VK – vitreous kernels, BFLY – breakflour yield, FLY – flour yield, FPC – flour protein content, SDS – SDS-sedimentation volume, FCL – flour colour, FLN – falling number, MDT – mixograph dough development time, FABS – farinograph waterabsorption, P/L – alveograph P/L-value, Strength – Alveograph dough strength, LFV12% - loaf volume at 12% protein content, LFV – loaf volume and HLM – hectoliter mass

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