THE EFFECTS OF LOW-MOLECULAR-WEIGHT AND HIGH-MOLECULAR-WEIGHT GLUTENIN SUBUNITS ON PHYSICAL DOUGH PROPERTIES OF BREAD WHEAT

by

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1.0mm

Dedicated to my late father,

to whom

I owe everything (in my life)

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SUMMARY

It has long been recognised that the gluten protein fractions (gliadins and glutenins) are the main determinants of the viscoelastic property of doughs made from wheat flours (Dimler, 1965). While the knowledge about the genetic control and genetic effects on physical dough properties of the HMW glutenin loci has increased dramatically during the last decade, there has been less progress in understanding the contribution of the LMW glutenin alleles. Elucidation of the effects of these latter should make it possible to devise more efficient breeding and selection strategies for improving flour properties. In the present work the association between certain *Glu-3* alleles encoding LMW glutenin subunits and the dough properties of wheat flours and the extent of the interaction between *Glu-3* and *Glu-1* (HMW glutenin) alleles in determining dough properties has been demonstrated.

The simplified procedure of Singh *et al.* (1991b) for 1-D separation of glutenin subunits was used to screen segregating progenies. Since a number of LMW glutenin subunit and gliadin bands are controlled by a cluster of tightly linked genes (Singh and Shepherd, 1988), the co-inheritance between these two fractions was first studied using a triple translocation stock produced by Gupta (1989). A F_1 hybrid between bread wheat cultivars [Halberd x (Wl x MMC)], possessing contrasting LMW subunit patterns, was crossed to the triple translocation (TrTr) stock to produce test-cross seeds. In order to study the inheritance of band blocks within a cultivar, the TrTr was used as the recurrent parent in back-crosses with two cultivars Barunga and Suneca. The study showed that there was no recombination between the blocks or cluster of bands produced by *Glu-3/Gli-1* loci, therefore, Recombinant Inbred Lines (RILs) from crosses between these cultivars could be screened easily for their LMW glutenin alleles from their gliadin patterns.

A set of RILs derived from a cross between a breeding line (Wl x MMC) and the cultivar Halberd were studied to confirm the relationships between glutenin subunits at the *Glu-1* and *Glu-3* loci and dough properties, published by Gupta *et al.* (1994a), over years and sites. These lines had been developed by Dr. J.G. Paull for studies of tolerance to soil B and Na, and as the parents of these progenies differed with respect to the alleles present at each of the three HMW and LMW glutenin loci 64 genotypic combinations of these alleles could have been present in their RILs. The 59 different RILs represented only 32 of 64 genotypic combinations. These lines were planted in replicate trials at two and three field sites in 1992 and 1993 respectively and their flour was analysed using SDS-sedimentation and extensograph measurements. Weather damage at Winulta provided an opportunity to evaluate the relationship between genes controlling glutenin subunits with those controlling sprouting resistance. The genes controlling HMW but not the LMW glutenin

subunits were linked with Falling Number values. Other results showed that all the *Glu-3* loci were closely linked to grain hardness genes, whereas, amongst the *Glu-1* loci, the only possible linkage was observed for the *Glu-A1* locus. The alleles 2* (*Glu-A1*), 17+18 (*Glu-B1*), *c* (*Glu-A3*) and *b* (*Glu-B3*) were found to be positive and mostly cumulative on SDSS volume but the usual results of a large effect of glutenin subunits 5+10 compared to 2+12 (*Glu-D1*) on dough strength was not observed. In general, the results for the allelic effects of the glutenin subunits on maximum resistance and extensibility were in agreement with those of Gupta *et al.* (1994a).

A number of different South Australian varieties were used to evaluate the association between two glutenin quality scores (Payne score and local score) and dough properties with the aim of developing predictive equations. These varieties were grown at two field locations and their quality characteristics were compared to those obtained by the South Australian Research and Development Institute from 1985-1993. The results showed that Rmax was significantly described by both the Payne and local scores for all sets of data, whereas neither predicted extensibility.

An important difficulty with breeding for appropriate dough properties is defining the interaction between the environment and genotypes. As a preliminary evaluation of this major concept of quality improvement, two groups of selections from the same cross [(Turkey787 x Warigal) x Warigal] varying at the *Glu-A1*, *Glu-D1* and *Glu-B3* loci were grown at four different field locations in 1992. While there were significant differences between these two groups for dough properties, a significant interaction was also observed between sites and genotypes for dough properties and grain yield.

A cross (Barunga x Suneca) were made to determine which glutenin loci needed to be changed to upgrade South Australian Hard wheats to Prime Hard specifications, and this provided an opportunity to survey the allelic effects at the *Glu-B1*, *Glu-A3*, *Glu-B3* and *Glu-D3* loci. The lines were multiplied and planted as two experiments (replicated and unreplicated) at a number of sites in South Australia. However because of drought in 1994, only the highest yielding site was evaluated on the extensograph and by HPLC analysis. The results showed that selection on the basis of glutenin subunits alone in an early generation does not limit selection for grain yield in later generations. The *Glu-A3* locus consistently showed the largest effect on dough properties compared to other loci and the *Glu-A3d* allele was always associated with higher SDS-sedimentation, higher maximum resistance and greater dough extensibility than *Glu-A3c*. The effect at *Glu-B3* (*b* v *h*) was significant for Rmax in one experiment only whereas allelic variation at *Glu-B1* (17+18 vs 7+8) did not show any significant difference for dough properties.

There was a wide range of variation between lines within each glutenin genotype, indicating that quality differences can originate from genetic differences at unidentified loci other than the glutenin loci.

Epistatic effects (interaction between glutenin loci), were found to have significant effects on dough properties, particularly for SDS-sedimentation volume. These interactions mostly involved the *Glu-B1* and *Glu-D3* loci. For Rmax and extensibility only a few interactions were observed (*Glu-B1 x Glu-A3* for Rmax and *Glu-B1 x Glu-B3* for extensibility).

Surprisingly, the *Glu-B3* alleles, having the larger number of bands, did not show significant effects on extensibility, since earlier workers have shown an association between the number of LMW glutenin bands present and extensibility. Also variation at *Glu-B3* and at *Glu-D3* for extensibility/protein ratio was not significant.

The stronger dough properties associated with *Glu-A3d* compared to *Glu-A3c* were mainly due to its capacity to form large aggregated proteins as measured by HPLC, accompanied by a higher glutenin to gliadin ratio, The subunit bands 7+8 and 17+18 at the *Glu-B1* locus did not have large effects on Rmax. This was mainly due to their compensatory effects on dough quality with the 17+18 genotype having a higher protein concentration and the 7+8 genotype a higher percentage of glutenin.

The results of this study provide information for devising improved strategies for wheat breeders. While it has been shown that incorporation of *Glu-A3d* would result in improved dough properties, the breeder must:

a) test many lines within the genotypes to maximize favourable background effects

b) conduct trials over a number of environments to measure genotype x environment effects

c) examine the data closely to avoid undesirable linkage effects

It is concluded that further investigations are required to identify the genes other than glutenin which are involved in controlling dough extensibility.

STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any other university or other tertiary institution and, to best of my knowledge and belief, contains no material previously published or written by any other person, except where due reference is made in the text.

I give consent to this copy of my thesis, when deposited in the university library, being available for loan and photocopying.

SIGNED. Date 3/7/96

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ABBREVIATION USED IN THE THESIS

Abbreviation	Meaning
*	significant at 5% level
**	significant at 1% level
***	significant at 0.1% level
1-D	one-dimensional
1RS	short arm of group 1 Rye chromosome
Albg%Fl	percentage of albumin-globulin in flour
ANOVA	analysis of variance
ASW	Australian ~~Soft Wheat~~ standard white
BC	backcross
BU	Brabender Unit
DDT	dough development time
E/P	extensibility/protein ratio
Ext	extensibility
F1, F2, F3,....	first, second,... filial generation
FN	falling number
FY	flour yield
Gli%Fl	percentage of gliadin in flour
Gli-1	gliadin loci located on the short arm of group 1 chromosome
Gli-2	gliadin loci located on the short arm of group 6 chromosome
Glu%Fl	percentage of glutenin in flour
Glu-1	HMW glutenin loci located on the long arm of group 1 chromosome
Glu-3	LMW glutenin loci located on the short arm of group 1 chromosome
GY	grain yield
HMW	high molecular weight
LMW	low molecular weight
M	maturity
Mo	monomeric fraction
NIR	near-infrared reflectance spectroscopy

ns non-significant

(pers. comm.) personal communication
P/M polymeric to monomeric ratio
P1 glutenin fraction of HPLC chromatogram
P1/P2 glutenin to gliadin ratio
P2 total gliadin fraction of HPLC chromatogram
P2-1 first gliadin subfraction of HPLC chromatogram
P2-2 second gliadin subfraction of HPLC chromatogram
P3 albumin-globulin fraction of HPLC chromatogram
PC protein concentration
PSI particle size index
PY protein yield

r coefficient of correlation
R^2 coefficient of determination
RC Roseworthy Campus
RILS recombinant inbred lines
Rmax maximum resistance

RP-HPLC Reversed phase-high performance liquid chromatography

SARDI South Australian Research and development Institute
SDS-PAGE sodium dodecyl sulphate polyacrylamide gel electrophoresis
SDSS SDS-sedimentation
SE-HPLC size exclusion high performance liquid chromatography
SEM standard error mean

TC test cross
TI tolerance index
TrTr Triple Translocation

vs versus

(Wl x MMC)/Wl/10 [Warigal x (Site cerros x Mengavi) x Crime)-selection1/10]
WA water absorption
Waite Institute Waite Agricultural Research Institute

CHAPTER 1

GENERAL INTRODUCTION

Wheat is the most important cereal in world agriculture and provides approximately 30% of the world's grain (Orth and Shellenberger, 1988). Bread is one of the most ancient of food staples and is still one of the most common and popular food products. Noodles, biscuit, cakes, pastries, and pasta are other products of wheat flour. Alternatively, flour can be further processed to produce starch, gluten and gluten products.

Wheat is grown in all states of Australia (except the Northern Territory) in an arc from central Queensland, around eastern and southern Australia to the central coast of Western Australia. Most of Australia's wheat-producing country can be described as semi-arid and irrigation is rarely applied. Soil types in the wheat-growing zones vary from heavy, deep clay-loams in northern New South Wales and southern Queensland to light sandy soils in parts of Western Australia and the mallee regions of south-eastern Australia. As a result, different types of wheat are grown in the different climatic and soil zones. High protein hard wheat is grown in northern New South Wales and Queensland while lower protein wheat is grown in the southern states and these are mostly exported to markets in Asia for flat breads, alkaline and white noodles, breads and steam breads.

Australian wheat farmers produce, on average, about 15 million tonnes of wheat, with a domestic usage of approximately 3.5 million tonnes per annum. Therefore about 80 percent of Australian wheat is exported. Australia is the fourth largest wheat exporter in the world exceeded by the USA, EC and Canada. Of the 100 million tonnes of wheat traded globally each year, Australia contributes about 11 percent.

Australia's major markets for wheat exports are China, Japan, Iran, Egypt, Indonesia, Malaysia, South Korea, Iraq, Yemen and the states of the former Soviet Union. Each market has particular buying specifications according to its consumer requirements. In order to tailor properties of wheat varieties to the strict specification required for marketing, a balance in dough making properties and hence in the proteins coded by genes at all principal loci of seed storage proteins (i.e. *Glu-1*, *Glu-3*, *Gli-1* and *Gli-2*) is needed in relation to the protein concentration. These genes controlling protein quality are less subject to environmental influence than those controlling protein concentration.

The storage proteins in the endosperm of wheat constitute more than 80% of the total proteins in the grain (Osborne, 1907) and are the most important determinants of the

functional qualities of the flour (Elton and Ewart, 1967; Kasarda *et al.*, 1976). These proteins can be divided into two main groups, the gliadins and glutenins. Interest in the gliadin/glutenin ratio as an indicator of dough properties has continued since the time of Osborne (1907). It was also shown that the highest molecular weight fractions of glutenin play an important role in the properties of the glutenin (Orth and Bushuk, 1973) and it has become generally accepted that glutenin is built up from subunits, covalently linked through disulfide bonds into hydrophobically-stabilised polymers.

The first clear correlations of particular combinations of glutenin subunits (defined by their SDS-PAGE patterns) with quality were established by Payne *et al.* (1981a) and these correlations have been widely substantiated. This concept has developed to the point that the Payne score has been used for defining wheat breeding strategies (Payne, 1987). Electrophoresis studies have shown that glutenin comprises two groups of proteins:Low and High-molecular weight glutenin subunits (Khan and Bushuk, 1979b). Gupta *et al.* (1990b) found that the quality of a set of wheat varieties was not satisfactorily described by the Payne score, so they suggested that the predictions of quality could be improved if the contributions of the gene products from the LMW glutenin loci were taken into consideration. Gupta and Shepherd (1990a) were the first to study allelic variation of LMW glutenin loci in a collection of more than 200 cultivars of bread wheat and a preliminary approach to ranking LMW glutenin alleles in order of quality has been reported by Gupta *et al.* (1991).

The main aim of the work presented in this thesis has been to study the quality effects of the LMW glutenin loci using the new, simplified 1-dimensional SDS-PAGE procedure developed at the Waite Institute by Singh *et al.* (1991b) for characterising the subunits. This method gave a much improved resolution for both HMW and LMW subunits of glutenin and can be used for fractionating different glutenin subunits from gliadins in segregating progeny. This method provided an opportunity to study the inheritance of the LMW glutenin subunits of different varieties, later used as parents for producing Random Recombinant Inbred Lines (RILs). The co-inheritance of different patterns of LMW glutenins with their associated *Gli-1* alleles were studied using a special genetic stock lacking all the LMW glutenin B subunits (triple translocation) as the third parent in test-crosses (Chapter 3).

The relative contributions to dough properties of HMW and LMW glutenin polypeptides are very important for a plant breeder. It has been reported that the HMW glutenin loci generally make a larger contribution to Rmax value than the LMW glutenin subunits (Gupta *et al.,* 1990b; 1991). To evaluate whether variation in dough properties of Australian wheat varieties can be predicted on the basis of the Payne score (Payne *et al.* 1987a) or the local score (Cornish, 1994), a set of varieties released from the different

wheat programs in South Australia were studied (Chapter 5). These varieties mostly had high LMW quality scores, providing a good opportunity to examine only the HMW scores as predictors of dough properties.

Alleles at the different loci of HMW or LMW glutenins have shown different effects on breadmaking quality in different genetic backgrounds (Kolster and Krechting, 1993) so dough properties may reflect the background genotypes of the chosen varieties or, alternatively, the genetic interaction between the glutenin loci. The major experimental approach used in this thesis was to examine sets of RILs derived from F_2 or F_3 segregants from hybrids between two varieties. Such lines permit the estimation of genetic effects of particular glutenin alleles in numerous lines with the same glutenin genotypes. The mean effects over all lines of a common genotype, in the absence of linkage and pleiotropic effects give a better estimation of the direct effects of major loci coding glutenin subunits than is available from alternative experimental material such as collections of varieties or isogenic lines. For this purpose RILs from two crosses were studied. The lines from cross [Halberd x (Wl x MMC)], examined previously by Gupta *et al.* (1994a) at two sites, were tested over two years and several sites to obtain an estimate of the importance of genotype x environmental interaction in quality characteristics (Chapter 4).

An important difficulty with breeding for appropriate dough properties is defining the interaction between the environment and genotypes. As a preliminary evaluation of this major concept of quality improvement, a set of random lines of [(Turkey787 x Warigal) x Warigal] harvested from different sites in southern Australia were analysed for dough properties (Chapter 6).

The second set of RILs was derived from (Barunga x Suneca) cross to determine the inheritance of factors differentiating the South Australian Hard varieties from Northern NSW Prime Hards. The yield-quality interrelationship was evaluated in relation to preliminary quality tests (SDS-sedimentation), for the *Glu-1* and *Glu-3* allelic constitution (Chapter 7). The conventional dough tests (Chapter 8) ~~was~~ were examined in the F_3 derived lines in the F_6 from grain grown at Winulta to provide estimates of the main effects and interactions of the *Glu-1* and *Glu-3* alleles. The Size-Exclusive High Performance Liquid Chromatography (SE-HPLC) (Batey *et al.*, 1991) was used to measure the proportion of aggregating compared to monomeric ~~gluten~~ proteins (Chapter 9) in an effort to provide some explanation for the differences in dough properties of glutenin subunits encoded by different alleles.

CHAPTER 2

REVIEW OF LITERATURE

2.1 The place of Australian wheats in world

Pan bread, cakes and biscuits are the main end-products of wheat in Australia. The total consumption of bread and related products such as bread rolls amounts to approximately 800,000 tones per annum, equivalent to about 46 kg of bread per head per year. More than 80% of the wheat produced in Australia is exported and in recent years the figure has approach 90% (Simmonds, 1989). Export availability can vary between 8 and 15 million tonnes due to the effects of environmental conditions during growth and harvesting of the crop. Up to 50% of the wheat produced in Australia is exported to West Asian countries like Iraq and Iran and Egypt, where the flour is used for flat bread (sometimes called pita or pocket bread). Also up to 40% is exported to South-East Asian countries for the production of many different types of noodles (Wrigley, 1991). Therefore the different wheats varieties need to be tailored to their particular end use. Condon (1993) has predicted that in the future the wheat segregation process will be extended to include 45 "market-based grades".

2.2 Wheat classes grown in the Australia

Australia is somewhat unique in that it is able to export several classes of wheats. Wheats are referred to as either soft or hard depending on the hardness of kernel, with the degree of hardness in a wheat resulting in different milling properties. There are basically seven major classes, based on its variety, general cleanliness and soundness, and protein content. These classes are then further subdivided in to a number of grades according to protein content, variety and state of origin as shown in Fig 2.1 (Simmonds, 1989).

Brief description of all Australian wheat classes are as follow:

Australian Prime Hard

This is the primary wheat class used to produce of high protein breads and some specialty noodle products. Prime hard wheat has excellent milling characteristics and strong, extensible dough properties. This class is marketed at guaranteed minimum protein levels of 13%, 14% and 15%.

Figure 2.1 Classification of Australian wheats based on marketing (Simmonds, 1989). The ASW grade has recently been split into an Australian Premium White aimed at noodle-type and a residual ASW (Australian Standard White). South Australia has recently become a durum exporter. (See over)

Australian Wheats

Class	Grade	States
PRIME HARD	15% PROTEIN 14% PROTEIN 13% PROTEIN	QLD NSW
HARD	14% PROTEIN 13% PROTEIN No.1 12% PROTEIN No.2* 11.5% PROTEIN	QLD* NSW* VIC* SA WA
ASW	STANDARD WHITE	QLD NSW VIC SA WA
SOFT	SOFT	NSW VIC SA WA
DURUM	No.1 No.2	NSW
GENERAL PURPOSE No.1	LIGHTWEIGHT SCREENINGS WEED SEEDS	QLD NSW VIC SA WA
GENERAL PURPOSE No.2	LIGHTWEIGHT SCREENINGS WEED SEEDS MINOR SPROUTING	QLD NSW VIC SA WA
FEED		

*** Australian No.2**

Australian Hard

Wheat in this class is limited to hard-grained wheat varieties which is used primarily for production of a wide range of volume breads, Arabic style flat breads and Chinese style noodles. The varieties in this class have good milling and dough qualities and marketed at guaranteed minimum proteins levels of 11.5%, 12%, 13% and 14%.

Australian Premium White

This class now includes the better milling and dough property varieties from the Australian Standard White class. It is likely to be deliberately tailored for noodle production in South East and East Asia.

Australian Standard White (ASW)

This is a multi-purpose class of wheat with intermediate grain hardness and protein levels in the range of 9% to 11.5%. Australian Standard White is suitable for markets where there is less emphasis on quality and is made into a wide variety of flour products including European style loaf breads, Indian style flat breads, steamed bread and most types of noodles. The varieties in this class are segregated throughout Australia and therefore buyers have generally become accustomed to purchasing ASW from certain regions of production. Accordingly, attempts are made to ensure that there is some uniformity and continuity in the quality of wheat from these various production regions.

Australian Soft

A low protein content with relatively weak gluten characteristics (weak extensible dough properties) to make it suitable for products such as sweet biscuits, cakes and pastry goods.

Australian General Purpose

Varieties in this class are subdivided in two classes as No.1 and No.2, that have failed to conform to the receival standards of any of the above classes. Wheat in this class has characterised in terms of test weight, weather damage (degree of sprouting), unmillable material or contamination with foreign matter or seeds. General purpose No.1 comprises milling wheats with mild quality defects and is marketed essentially as a second grade milling wheat. General purpose No.2 comprises lower grade milling wheats and may include mildly sprouted grain.

Australian Feed

In general, this class consist of wheat with physical defects such as low test weight, high levels of screenings, foreign material, excessive weed seeds and high levels of sprouting. This class is suitable only for feed-milling.

Australian Durum

This class of wheat grown in Australia is mainly used for production of semolina (pasta manufacturing). It is desirable that durum wheats possessed a protein concentration of about 13%, and values below 11% generally result in poor products (Dick and Matsuo, 1988).

2.3 Quality requirement in Australian wheat

The quality requirement for different classes of Australian wheat can be summarised under the three general headings of protein content, grain hardness and dough strength. The key quality parameters of Australian wheat have been tabulated as guidelines for wheat breeders (Wrigley, 1991) (Table 2.1).

Table 2.1 Quality attributes of Australian wheats for specific products. Adopted from Wrigley (1991).

Product	Grain protein content	Grain hardness	Dough strength
Breads			
Pan bread	>13%	Hard	Strong
Flat bread	11-13%	Hard	Medium
Steamed-Nthn[(A)]	11-13%	Hard	Medium/Strong
Steamed-Sthn[(A)]	10-12%	Soft/medium	Medium
Noodles			
Alkaline	11-13	Hard	Medium
White	10-12%	Medium/Soft	Medium
Instant	11-12%	Medium	Medium
Biscuit/cake	8-10%	Very soft	Weak
Starch/gluten	>13%	Hard (Soft preferred)	Strong

(A) Northern and Southern China.

Quality characteristics such as high milling yield with good flour colour, extensible not sticky doughs, with a high mixing tolerance and not needing excessive mixing time when strong, must be considered by plant breeders when they select for quality type.

2.4 Wheat storage protein

Storage proteins are those proteins which are present in protein bodies of the endosperm and function as a nitrogen store for the growing embryo (Pernollet, 1978). The storage proteins of wheat flour typically consist of 50% gliadins, 10% HMW glutenin subunits, and 40% LMW glutenin subunits (Payne and Corfield, 1979; Payne *et al.*, 1984a). The storage proteins in endosperm of wheat seeds have been shown to be the most important determinants of the functional qualities of the flour (Elton and Ewart, 1967; Kasarda *et al.*, 1976). Gliadins and glutenin have been shown to have a functional role in dough while albumins and glubulins were mainly metabolically active proteins (rewiews by Wall, 1979; Miflin *et al.*, 1983).

It has long been known that gluten, the water and salt-insoluble protein complex of flour, makes an important contribution to dough rheology (Cunningham *et al.*, 1955; Wall, 1979). To form gluten, both gliadin and glutenin together with water and salt are necessary. Glutenin is said to give solidity to the gluten and the gliadin, which has a soft sticky texture, is responsible for binding. The gliadin sticks to glutenin and so prevents it being washed away in the process of washing out a gluten (Kent *et al.*, 1967). It has been also reported that a mixture of these two components is essential to impart to dough the viscoelastic properties that are associated with good breadmaking performance (Wall, 1979; Bushuk and MacRitchie, 1988).

The seed storage proteins are synthesised on rough endoplasmic reticulum (Pernollet, 1978) and first appear in the endosperm 10 days after anthesis (Jenner *et al.*, 1991). At 16 days after anthesis, representing the early to mid maturation stage of the wheat grain, many of the wheat endosperm cells contain a central vacuole (Levanony *et al.*, 1992). In these cells, most of the storage proteins accumulate within membrane bound spherical bodies (Shotwell and Larkins, 1988) referred to as protein bodies. Studies have shown that the golgi ~~apprature~~ apparatus is involved in transport of storage protein to vacuoles (Kim *et al.*, 1988).

In the tribe Triticeae, prolamins (gliadins) in wheat accounted for approximately 40-50 percent of the seed proteins and the glutelins (glutenins) in wheat for about 35-40 percent. The seed storage proteins of wheat are known to associate into aggregates during the formation of gluten (Kasarda, 1980; Bietz and Huebner, 1980).

At the early stages of grain development in wheat the gliadins and glutenins are packaged independently into different protein bodies. Suter *et al.* (1994) concluded that during

grain filling, gliadin synthesis is initially rapid and then levels off, whereas glutenin synthesis increases after an initial lag period. During this synthesis the glutenin polymers go through an aggregation process, increasing in average molecular weight. At the end of grain filling the quantities of gliadins and glutenin are such that there is balance between the polymeric and monomeric components. Panozzo *et al.* (1994) reported that the development of polymeric glutenin is initiated with the synthesis of a backbone structure of HMW glutenin, followed by the synthesis of LMW glutenin which then builds the glutenin molecule. During the final stages of grain filling many protein bodies fuse, forming a continuous, highly compressed protein matrix in which the starch granules are embedded (Jenner *et al.,* 1991).

Bread dough is a viscoelastic material made up of gluten which is filled with starch. As with other polymers (Sperling, 1986), the filler raises the elastic modulus of the system by forming a variety of physical and chemical bonds with the polymer. Therefore dough made from lower protein flour has a thinner polymer matrix (gluten) with a higher ratio of filler (starch). This result in a higher elastic modulus and a dough that expands less and at a slower rate (He and Hoseny, 1992)

2.5 Classification and nomenclature of wheat storage proteins

The earliest classification of cereal proteins was based on a fractionation procedure introduced by Osborne (1907). This uses sequential extraction to separate albumin/globulins (soluble in salt solution), prolamins (70% aqueous ethanol soluble), and glutelins (insoluble in both salt and 70% ethanol). In the case of wheat, prolamins correspond to gliadins and the glutelins to glutenins. The glutenin fraction as defined by Osborne (1907) comprises many different molecules ranging from single polypeptide chains of fairly low molecular weight (perhaps as low as 10,000) to molecules made up of many polypeptide chains, crosslinked by disulphide bonds, resulting in very high molecular weights ($\geq 10^6$ dalton) (MacRitchie, 1987). The term polymeric is used here to indicate that these proteins are polymers formed from a number of different polypeptide chains by intermolecular disulphide bond linkage. Gliadins, as well as most albumin/globulins consist of single polypeptide chains which do not aggregate and thus are termed monomeric.

Four main groups of gliadins are usually distinguished in electrophoresis. The α, β, γ and ω gliadins occur with decreasing order of mobility and therefore increasing molecular size (Woychik *et al.*, 1961). Gliadins do have disulphide bonds, but they are thought to be intramolecular only (Pence and Olcott, 1952; Beckwith *et al.*, 1965). The ω-gliadins contain virtually no sulphur, therefore they possess no internal or external disulphide bonds. A new classification of gliadin into sulphur-rich (α, β, γ) and sulphur-poor (ω) fractions has been suggested (Kreis *et al.*, 1985) based on their amino acid

sequence and sulphur content. This classification agrees with that suggested by Shewry *et al.* (1986). They revised the Osborne classification by noting that, when the disulphide bonds of the polymeric glutelins are broken, their individual chains (usually termed subunits) become soluble in aqueous alcohol. They therefore argued that they should also be considered as prolamins. This similarity, in turn, is though to be a consequence of their common genetic ancestry, another reason for classifying them together (MacRitchie, 1992). All gliadins are known as true prolamines because they are soluble in aqueous ethanol in their native state.

It has been demonstrated that disulphide bonds have a major influence on the structure of native glutenin (Pence and Olcott, 1952; Nielsen *et al.*, 1962). Glutenins in their native state are not resolved by gel electrophoresis. If reducing agents such as mercaptoethanol or dithiothreitol are used, the interchain disulphide bonds are broken and complete solublization can be achieved (Osborne and Vorhees, 1893). When the total reduced proteins are loaded onto a gel, their individual chains (usually termed subunits) are identified by SDS-PAGE. One set of glutenin subunits is clearly separated with components showing lowest mobility. These have been called the High-Molecular Weight (HMW) or A glutenin subunits.

There are two other sets of glutenin subunits which are located below the HMW glutenin subunits in gels and therefore have faster mobility. These have been classified as B and C subunits and are usually referred to as the Low-Molecular Weight (LMW) glutenin subunits. Studies on LMW glutenin subunits were initiated by Jackson *et al.* (1983) in bread wheat and Autran and Berrier (1984) in durum wheat. From SDS-PAGE mobility, the molecular weights of the B subunits are estimated to range from 40,000-55,000 and the C subunits from 30,000-40,000. They are polypeptides which form large polymers linked by disulphide bonds or by noncovalent association between other LMW and HMW glutenin subunits (Khan and Bushuk, 1979b).

SDS-PAGE of unreduced total protein extracts from hexaploid wheat endosperm shows three slow-moving bands (denoted as triplet bands in a zone of heavy background streaking). The proteins associated with these bands have been studied in detail by Singh and Shepherd (1985) and have been shown to be globulins similar to those in legumes, oats and rice and have been renamed triticin.

Because of many contradictory reports in relation to protein fractions and grain protein concentration, which mostly arise from the differences between the fractionation methods based on the solubility of the protein and different quantities of protein, Shewry *et al.*, (1986) proposed a different classification and nomenclature system for gluten proteins. This classification is based on the biochemical and genetical relationship of

individual subunits rather than their aggregating properties. In this system both gliadin and glutenins were refered to as prolamins and divided into three groups:
(1) The S-rich prolamins (α –, β– and γ gliadins as well as LMWglutenin subunits).
(2) The S-poor prolamins (ω-gliadins).
(3) HMW prolamins.

2.6 Relation between proteins and bread making quality

Bread, noodles, pasta, cakes, cookies and pastries are made from different types or classes of wheat. Common wheats (*Triticum aestivum*. L) are preferred for breadmaking (Finney and Barmore, 1948) whereas durum wheats (*Triticum turgidum* L.) are the preferred class of wheat for production of high quality pasta (Irvine, 1971). The preference for a particular class of wheat for a certain type of product is based on several flour characteristics which are summarised below:

2.6.1 Protein content

The protein content of wheat is well documented as the most important criterion for most aspects of processing capability and nutritional value. Wheat crops in Australia in recent times have been characterised by declining grain protein levels. It has been claimed that the main reason for this is the decline in soil N levels and partly due to breeding objectives being concentrated on grain yield, where increased yield usually results in decreased protein level (Austin *et al.*, 1977), but this has not been supported by comparisons between old and new varieties when these were grown in common trials (H. Eagles, pers. comm.). In some instance there are some varieties which deviate favourably from the protein relationship (Mesdag, 1979). Varieties may differ slightly in their capacity to produce grain of high protein content under any given growing conditions. This is related to their capacity to translocate or mobilise nitrogen from the rest of plant, particularly the flag leaf, into the grain.

Genes for high protein concentration have been located on the group 5 chromosomes of bread wheat (review by Garcia-Olmedo *et al.*, 1882). However, Joppa and Cantrell (1990) carried out backcrosses using Langdon D-genome disomic substitution lines and found that the dicoccoides chromosomes 2A, 3A, 6A, 5B and especially 6B, significantly increased the whole grain protein content relative to that of the recurrent parent Langdon, while 2B substitution lines were low in vigour and partially sterile. The plus-and minus-effects on protein content were apparent in each of the environment in which the material was assessed, thought not always significant or of the same magnitude. There was some evidence that regulatory genes on group 2 and 5 chromosomes of wheat influenced the expression of structural genes for gluten proteins (Shepherd, 1968; Law *et al.*, 1978; Brown and Flavell, 1981; Garcia-Olmedo *et al.*, 1982).

The physiological causes of the negative association between grain yield and grain protein concentration is somewhat complicated. The protein content of wheat depends on the relative amounts of carbohydrates and nitrogenous compounds made available to the maturing wheat (Simmonds and O'Brien, 1981). Genes controlling the amount of starch assimilated in the grain will affect the protein percentage just the same as genes controlling the assimilation of protein. Penning de Vries *et al.* (1974) have estimated that 1 g of glucose produced by photosynthesis can be used by the crop to produce 0.83 g of carbohydrates or 0.40 g of protein when nitrate is the N source. This implies that an increase in protein content requires more photosynthate, thus decreasing available photosynthate for starch synthesis and thus grain yield. Johnson *et al.* (1985) believed that the negative correlation between grain yield and protein content seldom exceeded 0.6, therefore much of the variation in protein content is indepedent of yield and that simultaneous advances in both yield and protein content are possible.

Increasing the protein quantity in wheat may have the disadvantage that as protein increases, the increase in protein content is accompanied by disproportionate increase in the gliadin component relative to the glutenin and albumin/globulin fractions (Gupta *et al.*, 1992) and the very high protein lines have poor mixing properties (Bariana *et al.*, 1993). Kovacs *et al.* (1993) found that protein content was correlated negatively with gluten viscoelasticity. For some products such as pan bread, an increase in protein level will produce high sedimentation volume (Axford *et al.,* 1979) and a better loaf of bread (Finney and Barmore, 1948; Fifield *et al.*, 1950; Bushuk *et al.,* 1969).

For flat bread it has been found that varieties with higher water absorption produce the best type of bread and water absorption increases linearly with increasing protein content (Finney, 1979). For biscuit manufacture, wheat flour must have good extensibility, a property conferred by the gliadin fraction, and low dough strength. Extensibility is also correlated to protein level and so increasing protein will give a higher extensibility (Gupta *et al.,* 1989).

As mentioned earlier (Table 2.1), grain hardness is another components of quality and must be taken into consideration along with the protein level. Typically, soft wheat flours are preferred for pastry, cake, or cookie processes whereas hard wheat flours are preferred for bread making (Palmer, 1989). Therefore information on the flour protein level and grain hardness are vital for deciding which varieties to release to wheat farmers. Experience has shown that the market requires wheat grades in which there is a balance between grain hardness and protein content for different end uses. This relationship has been detailed by Moss (1973, 1978) resulting in the production of a diagram (Fig 2.2) which shows the relevant protein and hardness values for each grade for different end uses.

Evaluation of the baking properties of a number of Australian wheat varieties has shown that loaf volume shows a direct linear relationship with protein content. The performance of any particular flour does not depend only on the overall protein content but also a less tangible factor related to "quality" or composition of that protein. If the protein content is particularly low, there may even be insufficient of the storage group of proteins present to form a satisfactory and cohesive dough. As a result, the alternative strategy to breeding for protein quantity is to breed for protein quality. Here, there may be no inverse relationship between yield and protein quality. Breeding for protein quality does not limit selection for grain yield (O'Brien *et al.*, 1989).

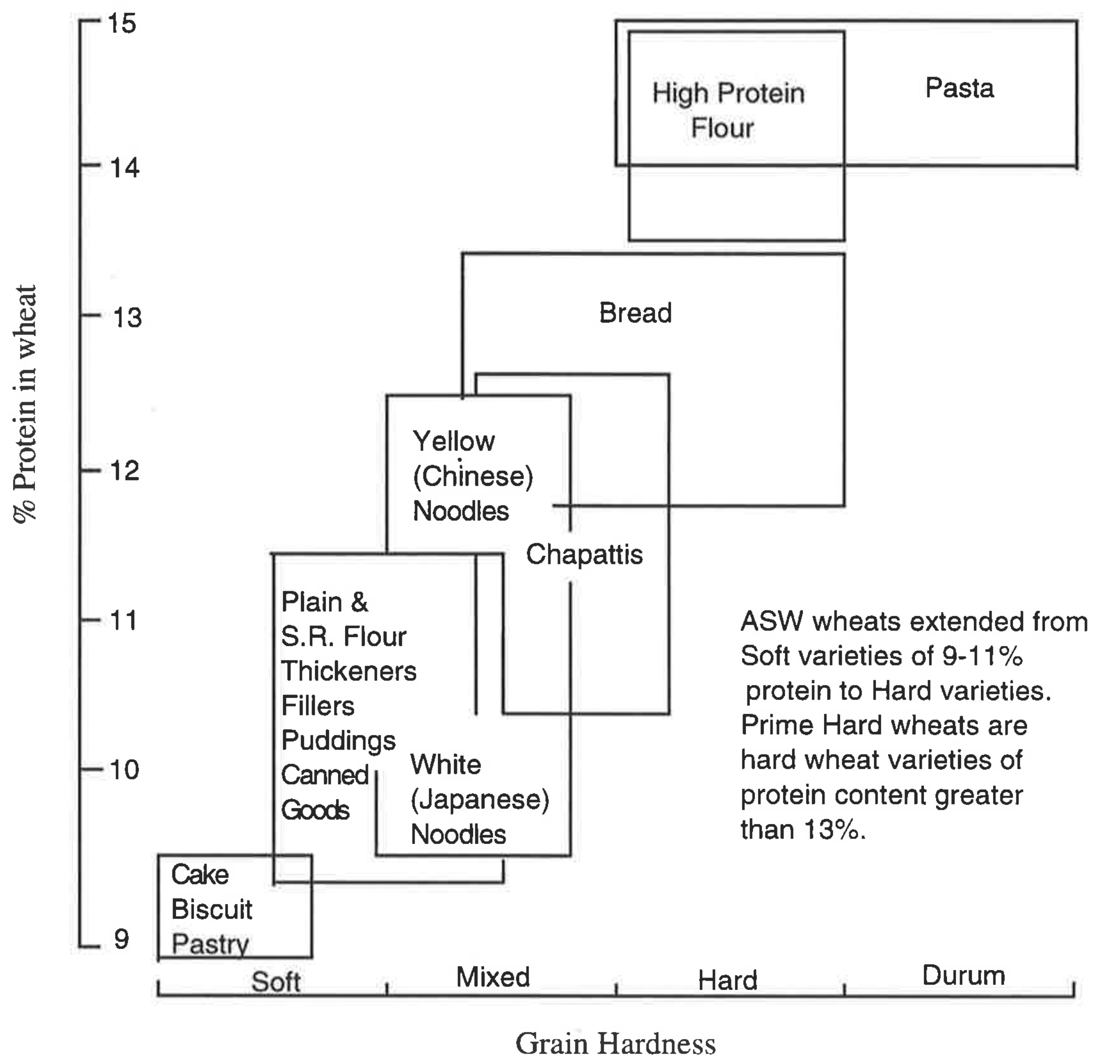


Figure 2.2 Optimal grain hardness and flour protein level for different wheat products, adopted from Moss (1978).

2.6.2 Protein quality

The term "protein quality" has several meanings, depending on which crop is being bred and for what purpose. In food processed from wheat, protein quality is in terms of viscoelasticity for making leavened bread and chapattis, extensibility for biscuit-making and hardness or strength for the manufacture of pasta products, spaghetti, macaroni and noodles.

The first major breakthrough in understanding differences in protein quality came from the work of Pomeranz (1965) and subsequently from Orth and Bushuk (1972).

It is widely accepted that the unique properties of wheat flour, when mixed with water to form a viscoelastic dough, are due to its water-insoluble proteins. Although starch is the major component of the seed, the rheological properties of dough are mainly attributed to protein and lipids, and not to the starch content (Pomeranz, 1980). By removing lipids, starch, and water-soluble carbohydrates from the flour, it has been demonstrated that the remaining gluten, which is 80% protein, still forms a hydrated, rubbery mass (Wall, 1979). The presence of lipids in gluten acts as a lubricant (Kent *et al.*, 1967). Also a synthetic flour made from gliadin and starch shows practically no development properties. Therefore the properties of wheat flours are largely governed by the native glutenin structure. These rheological properties of dough have been extensively investigated by reconstitution techniques (Smith and Mullen, 1965; Hoseney *et al.*, 1969). MacRitchie *et al.* (1990) found a positive correlation between glutenin content of natural and reconstituted flour, as well as the glutenin subunit composition, with the rheological properties of dough. Determination of the way different subunits are linked and the resultant size distributions of glutenins and the ascertainment of the positions of interchain disulphide bonds by amino acid sequencing are very important in explaining the functionality of protein composition.

2.6.3 Functionality of wheat proteins in dough

Functionality of the protein can be explained in terms of qualitative differences in protein composition as well as differences in the quantities of the different classes of protein (Kasarda *et al.*, 1976)

Physical properties of dough depend more on factors such as molecular weight and molecular weight distribution of the proteins. The rheological properties of wheat-flour doughs depend on the following factors:

Amino acid composition

A number of the proposed models of glutenin structure suggest that the glutenin polymers consist of high molecular subunits and low molecular subunits linked via cystein residues (Moonen *et al.*, 1985; Kasarda, 1989). The amino acids residues making up wheat storage protein can be classified according to their capacity for interacting within and between protein chains. The disulphide bond is one of the most important aspects and is formed by the interaction and oxidation of two cysteine residues. The covalent disulphide bond can form inter-and intra-molecular cross-links which on reduction, liberate the individual protein chain subunits carrying free-SH (sulfhydryl) group. Disulphide interchange can occur between a disulphide bond and a second molecule carrying a free sulfhydryl group. Interchain disulphide linkage is a possible mechanism for explaining the rheological properties of wheat flour doughs (Jones *et al.*, 1974; Bloksma, 1975). Lasztity (1984) has reported that several different kinds of forces are involved in the formation of an elastic but resilient matrix which confers unique visco-elastic properties on dough. These forces can be grouped into (a) covalent forces in glutenin aggregation (Ewart, 1968; Graveland *et al.*, 1985), (b) non-covalent interactions in gluten structure (Kasarda *et al.*, 1967; 1976) and (c) involvement of both covalent and non-covalent forces (Dimler, 1965; Ewart, 1979; Khan and Bushuk, 1979a; Wall, 1979; Bushuk *et al.*, 1980; Bietz and Wall, 1980). Glutenins are thought to interact with gliadins to form the matrix. Gliadin is viscous and gives extensibility, allowing the dough to rise during fermentation, whereas glutenin gives elasticity, preventing the dough from being over extended and collapsing either during fermentation or during baking.

Hydrogen bonds, hydrophobic interactions and electrostatic bonds are other possible mechanisms responsible for the elasticity and strength of gluten. The amino acid composition of the protein shows that they contain many amino acid residues capable of promoting association between subunits. For example, there are high concentrations of hydrophobic amino acids such as leucine capable of promoting hydrophobic interactions and of glutamine which is capable of forming hydrogen-bonds. These considerations again support the idea that the glutenin fraction is largely responsible for functionality of wheat flour in bread-making (Bushuk *et al.*, 1980; Bushuk, 1985). Bartels *et al.* (1984) have found that the location of a cysteine residue near the amino terminal of a subunit would make that region of the molecule highly hydrophobic, facilitating strong hydrophobic association with other similar proteins or with non-protein constituents (for example lipids). In this regard it has been inferred that glutenin from high quality wheat varieties has stronger hydrophobic association in aggregation than glutenin from poor quality wheats (Kobrehel, 1984). Comparison of amino acid sequences in different

subunits has indicated differences that could effect the structure and physical properties of gluten (Shewry *et al.*, 1989; Greene *et al.*, 1988)

Molecular weight

It is well established that the molecular size distribution of the polymeric glutenin fraction is an important factor determining dough characteristics. Orth and Bushuk (1972) were first to find that polymers with the largest molecular weight have the greatest effect on dough strength. Wheat gluten consists of two major fractions, gliadin and glutenin. Each fraction has been shown to comprise a mixture of some 50 or more components or subunits linked together through disulphide, hydrogen, hydrophobic and electrostatic bonds (Khan and Bushuk, 1979a; Bushuk, 1985). Gliadins consist of a single polypeptide chain with only intra-molecular disulphide bonds which do not have positive effects on strength properties of wheat flours (Singh *et al.*, 1990; Gupta *et al.*, 1993). On the other hand glutenins have many polypeptide chains cross-linked by inter-molecular disulphide bonds. Polymeric glutenins are fundamentally different from the monomeric gliadins because of their intermolecular disulphide bonding capacity. Therefore glutenins have a much greater capacity for variation because of this polymerising property (Ewart, 1990).

There is evidence that gluten properties are primarily determined by the size distribution of glutenin polymers (MacRitchie, 1992). Many researchers have shown that the glutenin polypeptides contribute directly towards gluten properties, and that some are more effective than others (MacRitchie, 1987; Dhaliwal and MacRitchie, 1990). The loss of cohesive elastic properties of dough or polymeric proteins (native glutenin) (Dimler, 1965) when the disulphide linkages are cleaved, even partially, (Nielsen *et al.*, 1962) clearly supports the positive roles of disulphide-polymers in governing dough strength (Field *et al.*, 1983). The solubility of the glutenin fraction in strongly dissociating solvents indicates that glutenin of better quality wheat varieties has a higher average molecular weight than the glutenins of lower quality wheats (Khan *et al.*, 1979b; Kobrehel, 1984; Huebner, 1970).

The HMW subunits are very important in stabilising the native glutenin structures because of their strong interaction with other smaller subunits (Huebner and Wall, 1976). Bekes *et al.* (1994) have shown that the size of the HMW glutenin subunits has the major effect on the rheological properties of dough. The LMW glutenin subunits are smaller in size and apparently less effective than the HMW subunits, although they account for three times as much of the glutenin.

It has usually been assumed that the molecular weight distribution results from random polymerization of the subunits (Ewart, 1987; Kasarda, 1989; Payne and Corfield, 1979; Bietz and Wall, 1980), but there is some evidence to suggest that there may be a nonrandom incorporation of subunits. Gupta *et al.* (1994b) have reported that specific subunits of HMW glutenin subunit may be responsible for the formation of polymers of different sizes. Also it has been suggested that HMW subunits form the backbone of linear glutenin polymers because of their better polymerizing ability than the LMW subunits (Graveland *et al.*, 1985).

The amount of aggregated protein (proportion of aggregated to monomeric gluten) has been used an indicator of dough properties. A higher proportion of aggregates gives stronger dough properties (Batey *et al.*, 1991). MacRitchie (1992) compared two biotypes of the Australian cultivar Warigal and found that the stronger one of them produced a higher proportion of the larger aggregates of glutenin polypeptide than the other.

The importance of the size distribution of the molecules in governing physical properties of synthetic high polymers (e.g. tensile strength) is well established in polymer science (MacRitchie, 1992; Bersted and Anderson, 1990). It is well established that molecules below a certain size limit (threshold level) do not contribute to strength properties.

2.7 Genetic aspects of protein quality

The relationship between the various functional properties of flour and its chemical composition together with knowledge about genetic control of the chemical constituents, makes it possible to devise more efficient breeding and selection strategies for improving flour properties. The chromosomal location of genes and the allelic variation which exists for the wheat storage proteins is described below. This allelic variation provides a basis for studying the relationship between the different alleles controlling particular gluten proteins and wheat bread-baking properties.

Common wheats (*Triticum aestivum* L.), which are preferred for breadmaking are allo-hexaploids. They have three genomes, each with seven pairs of chromosomes, making a total of 42 chromosomes. The genomes are denoted by the letters A, B, and D and the seven pairs of chromosomes in each genome are numbered from 1 to 7.

Electrophoretic studies of the special wheat cytogenetic stocks available in wheat have proved very useful in the allocation of specific storage proteins genes, including both gliadin and glutenin subunits (Boyd and Lee, 1967; Shepherd, 1968), to particular chromosomes. In these stocks, which have whole or parts of chromosome deleted or

exchanged, electrophoresis of protein subunits allows individual proteins to be visualized as a series of discrete bands in a gel. Therefore when a particular chromosome is absent, this may coincide with the absence of particular band or bands in the gel, thus identifying the chromosome that carries the gene(s) responsible for the synthesis of the missing protein(s). Inter-varietal chromosome substitution lines can also be used as suitable stocks for allocating genes coding particular proteins to chromosomes (Law and Worland, 1973). In these lines a pair of chromosomes from a recipient variety has been replaced by the homologous pair from a donor variety by means of cytogenetical manipulation. Much of the progress in this area has relied on the extensive cytogenetic stocks provided by Sears (1969). The chromosomal location of the genes coding for the gluten proteins and their linkage relationships in hexaploid wheat is presented in Fig 2.3.

The pioneer work in protein separation and genetic studies was conducted independently by Boyd and Lee (1967) and Shepherd (1968) and later by Wrigley and Shepherd (1973). They examined the distribution of α, β, γ and ω gliadins in Chinese Spring aneuploid stocks, particularly the compensating nullisomic-tetrasomic and ditelocentric lines. These early studies revealed that the genes coding for the gliadin proteins are located on the short arms of group 1 and 6 chromosomes (Wrigley and Shepherd, 1973; Brown and Flavell, 1981). However, because of overlap, many of the gliadin bands could not be analysed in 1-dimensional electrophoresis. Therefore attempts were made to improve the resolution of the protein separation method. Ultimately isoelectric focusing (Wrigley, 1970) and non-equilibrium pH gradient electrophoresis (NEPHGE) (Mecham *et al.*, 1978; Lafiandra *et al.*, 1984) combined with SDS-PAGE (Jackson *et al.*, 1983) have been used in the further analyses of the genetics of gliadins. All of these studies have confirmed that the group 1 chromosomes control all the ω gliadins, most of the γ gliadins, and a few of the β gliadins, whereas genes on the group 6 chromosomes code for all the α gliadins, most of the β gliadins and some of the γ gliadins. Genes coding for gliadin proteins are tightly linked to each other and occur as a single complex locus designated as *Gli-A1*, *Gli-B1* and *Gli-D1* in the three genomes. Recombination within these groups of gliadins is rare or absent, and therefore they have remained unchanged through repeated generations (Sozinov and Poperelya, 1980 ; Doekes, 1973). These groups of bands inherited as a unit have been termed "blocks" (Metakovsky *et al.*, 1984a, 1984b). Recently some additional genes have been identified which control the synthesis of minor gliadin components (Galili and Feldman, 1984). These minor gliadin components are coded by a separate locus on the short arm of chromosome 1A and have been studied further by Metakovsky *et al.* (1986).

The High-Molecular Weight (HMW) subunits of wheat glutenin have been the subject of extensive biochemical and genetical studies (Payne *et al.*, 1980, Lawrence and Shepherd, 1980, Payne *et al.*, 1981b). This is due mainly to their important role in determining

glutenin structure. Analyses of intervarietal substitution lines and inheritance studies have revealed that genes controlling the HMW glutenin subunits are located near the centromere on the long arms of chromosomes 1A, 1B and 1D (Bietz *et al.*, 1975; Lawrence and Shepherd, 1980; Payne *et al.*, 1980; Payne *et al.*, 1982; Lawrence and Shepherd, 1981), these loci being designated *Glu-A1*, *Glu-B1*, *Glu-D1*, respectively. At each locus two types of single-copy genes are present (Harberd *et al.*, 1986) encoding subunits which are classified as x-type (~~lower~~ higher molecular weight) or y-type (~~higher~~ lower molecular weight) glutenin subunits (Payne *et al.*, 1981b). The differences between these two genes are reflected by the types of protein coded by each gene (Galili and Feldman, 1985; Payne, 1987). Evolutionary divergence of x- and y-type genes has given rise to multiple forms of the HMW glutenin subunits. The data on the *Glu-1* loci indicated that the ancestral HMW glutenin subunit genes had already duplicated in to x and y genes before the formation of the cultivated tetraploids, and probably they had become physically isolated. No further duplication has occurred, but these two genes diversified into the many existing allelic forms by unequal crossing-over and point mutations to form the present-day x and y genes (review by Payne, 1987). Because of the tight linkage, the x- and y-type genes seldom recombine in crosses so that specific pairs of proteins are usually expressed together. In nature, the subunit pairs related to *Glu-D1* are always expressed together (5+10 or 2+12). However, it is found that the y subunits are never expressed at the *Glu-A1* locus and the x-type gene is only sometimes expressed. Both x and y subunits of *Glu-A1* are present in most accessions of diploid *T. monococcum* (Liu, 1994). It is assumed that the Ay genes must have become inactive in the diploid wild ancestor of the A genome, or at the tetraploid level prior to the formation of durum and bread wheats (Waines and Payne, 1987). The cause of inactivation of prolamin genes (for example y genes at *Glu-A1*) is not clear. Some cultivars which did not produce any *Glu-A1* coded subunit actually possess the structural gene(s). For example, a stop codon has been found in the middle of the coding sequence of a 1Ay gene (Forde *et al.*, 1985). The y subunits are also sometimes not expressed at the *Glu-B1* locus in common hexaploids. Seed lacking HMW glutenin subunits controlled by chromosome 1D in bread wheat were first described by Bietz *et al.* (1975) in Nap Hal. These lines or varieties are valuable for clarifying the relationship between composition and quality (Lafiandra *et al.*, 1991, Novoselskaya *et al.,* 1991). The term null is used for those genes that appear to be present in the genome, but the gene is silent, being expressed as proteins. Null alleles of HMW glutenin subunits have been observed to have deleterious effects on quality, possibly because of the decreased percentage of HMW subunits to total endosperm protein (Lawrence *et al.*, 1988). Even a single null involving the *Glu-D1* locus, as in bread wheats Ottawa and Nap Hal, resulted in a large reduction in quality properties (Lawrence *et al.*, 1988; Payne *et al.*, 1987b).

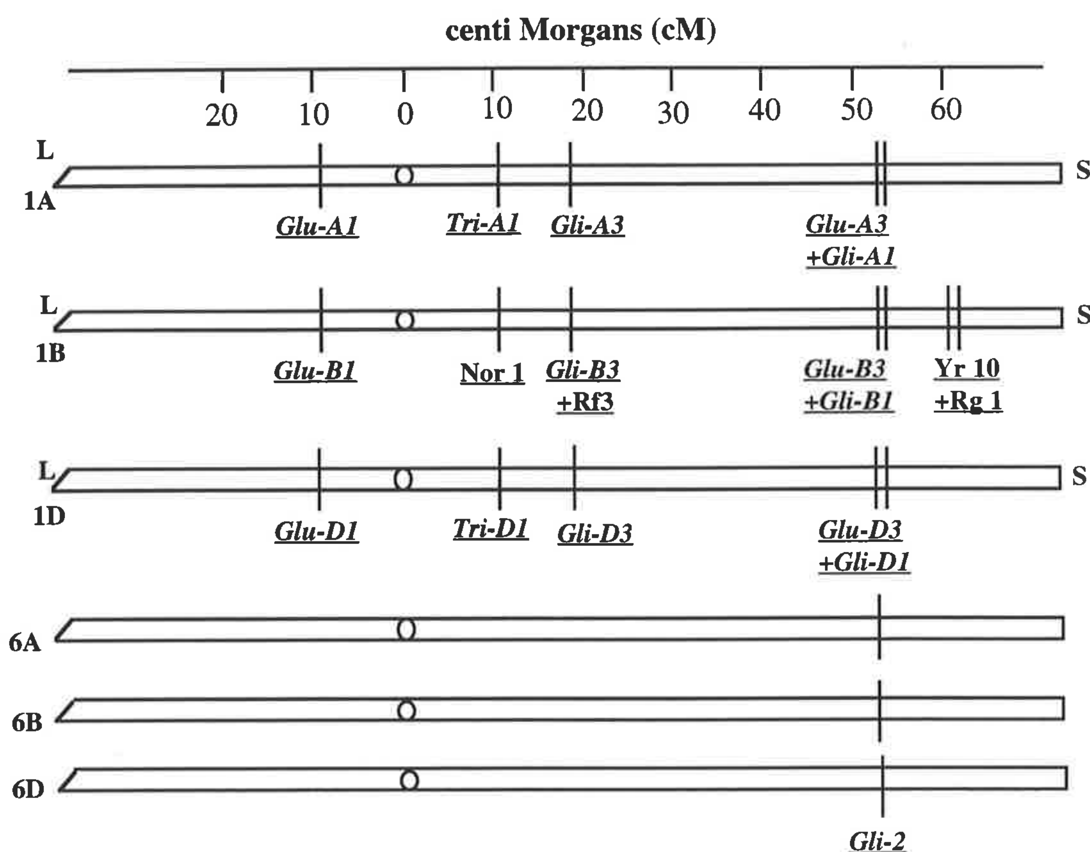


Figure 2.3 Chromosomal location of the genes coding for the gluten proteins and their linkage relationships (adopted from Payne *et al.* 1986 and Payne, 1987). L stands for long arm and S for short arm. Only part of the long arm is shown. Locus Rf3 on chromosome 1B, restores fertility to male sterility conditioned by *Triticum timopheevi* cytoplasm and Nor 1 is the site of the ribosomal RNA genes and the point of the secondary constriction on this chromosome (Snape *et al.*, 1985). Yr10 is a gene conferring resistance to certain races of yellow rust (*Puccinia striiformis*) and Rg1 red-glume colouration (Payne *et al.*, 1986). The *Gli-1* are collective loci controlling three groups of endosperm storage proteins β-, γ- and ω gliadins (Payne *et al.*, 1982). *Gli-2* are collective loci for gliadins for the homoeologous set of group 6 chromosomes (*Gli-A2*, *Gli-B2* and *Gli-D2*) and control mostly α-, β- and γ-gliadins (du Cros *et al.*, 1983; Lafiandra *et al.*, 1983). Likewise *Glu-1* are collective loci of HMW glutenin subunits (*Glu-A1*, *Glu-B1*, *Glu-D1*) located on the long arms of homoeologous group 1 chromosomes (Lawrence and Shepherd, 1980). The symbol *Glu-3* is assigned to the loci controlling LMW glutenin subunits (Singh and Shepherd, 1988). *Tri-A1* and *Tri-D1* are two loci controlling triticins, as a minor group of seed proteins (Singh and Shepherd, 1985).

The low molecular weight (LMW) subunits of glutenin comprise about one-third of the total seed protein and approximately 60% of the total glutenin (Bietz and Wall, 1973; Payne and Corfield, 1979), but until 1982 virtually nothing was known about the genetic control of the LMW components. The delay in obtaining this information has been due to the lack of suitable procedures for separating the LMW subunits from gliadins, which have similar solubility and electrophoretic mobilities. This problem was first overcome by Jackson *et al.* (1983), who showed that 2D separation procedures could be used to subdivide the LMW glutenin subunits into B, C and D subunits. The production of wheat-rye translocation lines (single, double and triple) (Gupta, 1989; Gupta and Shepherd, 1993) was another approach to overcome the problem. In these lines, one, two, or all three of the short arms of group 1 chromosomes have been replaced by the short arms of the homoeologous chromosome 1 of rye. The genetic control of the low-molecular weight (LMW) subunits of glutenin (B and C subunits) has been determined using nullisomic-tetrasomic and ditelocentric stocks of Chinese Spring wheat and it was found that the majority of these subunits are controlled by genes on the short arms of group 1 chromosomes (Payne *et al.*, 1984b; Gupta and Shepherd, 1987). No recombination was detected between the genes controlling different LMW glutenin subunits indicating that the LMW glutenin subunits segregated in linkage blocks like the gliadin components (Sozinov and Poperelya, 1980). Genes controlling LMW subunits are linked with the *Gli-1* loci especially those controlling ω-gliadins (Singh and Shepherd, 1984; 1988; Payne *et al.*, 1984b). This close linkage is particularly useful for identifying the *Glu-B3* alleles and some of the *Glu-D3* alleles in breeding programs since the gliadins are easier to screen than the LMW subunits (Singh *et al.*, 1991b).

Triticins are a minor group of seed proteins which have been referred to as triplet band proteins because of their appearance. The genetic control of this group of proteins was determined by Singh and Shepherd (1985) using nullisomic-tetrasomic and ditelocentric lines of the variety Chinese Spring. Fractionation of these stocks by SDS-PAGE has shown that two loci (designed *Tri-A1* and *Tri-D1*) located on the short arms of the 1A and 1D chromosomes encode these proteins. *Tri-A1* is positioned nearer *Glu-A1* on the long arm than to *Gli-A1* on the short arm, and so must lie close to the centromere on chromosome 1A (Singh and Shepherd, 1984)

2.7.1 Allelic variation of protein composition and association of variants with rheological properties of dough

Considerable allelic variation exists for the wheat storage proteins (Payne, 1987). This allelic variation has been the basis for studying the relationship between particular gluten protein and wheat bread baking properties. Furthermore, this variation has been useful for wheat cultivar identification and in some case has even proved valuable in

establishing phylogenetic relationships (Lafiandra *et al.*, 1991). The relationship between amount of "gluten" protein and quality is very dependent on the efficiency of extraction and, where only poor extraction is achieved, a negative correlation may be obtained (Orth and Bushuk, 1972).

The number of genes present at the complex loci controlling gliadins is not known but a high degree of polymorphism for the *Gli-1* loci has been reported by several researchers (Payne, 1987; Metakovskey *et al.*, 1985; Kasarda, 1980). This complex locus codes for ω, γ and β gliadins and the genes are very tightly linked with those controlling LMW glutenin subunits and usually show no recombinant (Payne *et al.*, 1984b). For this reason it is difficult to determine which of these groups are responsible for any difference in quality associated with different alleles of the *Glu-3* and *Gli-1* loci. Pogna *et al.* (1988) detected a null-gliadin recombinant line in durum wheat and showed that the gluten elasticity of this naturally occurring recombinant line is equal to its high quality near isogenic line which possessed the same gliadin bands, thus providing evidence for a casual relationship between the LMW glutenin bands and the gluten elasticity. These observations suggest that the associations found between certain gliadins and bread wheat quality by other groups (Sozinov and Poperelya, 1980; Wrigley *et al.*, 1981; Branlard and Dardevet, 1985a; Payne *et al.*, 1987c) may be due to the linked LMW glutenin subunits. This finding is consistent with those of Singh *et al.* (1990) and Gupta *et al.* (1992) and confirmed that the gliadin fractions from different flours behave similarly in regard to functionality.

Detection of bread wheat lines nulls, lacking certain storage protein components, has provided an alternative approach to establishing the relationship between protein components and quality (Lafiandra *et al.*, 1987; 1988; D'Ovidio *et al.*, 1991).

The HMW glutenin subunits show large intraspecific variation resulting from the existence of multiple allelism at each complex locus. Use of different genetic backgrounds and genetic variants in near isogenic lines are the two main approaches which can provided information on a broader scale about the effects of allelic variability. Payne *et al.* (1987b) have used near-isogenic lines (NILs) in which some HMW glutenin subunits genes have been transferred into the genetic background of a common donor variety by repeated back crossing. Also the HMW line null at the *Glu-B1* locus and isogenic lines null at the *Glu-A1* and *Glu-D1* loci have been produced by Lawrence *et al.* (1988). By crossing the triple HMW null line with a triple rye translocation line (Gupta *et al.* 1993), tetranulls, pentanulls and hexanulls for glutenin subunit can be produced. Lawrence *et al.* (1987) have reported several HMW glutenin subunit biotypes for a number of Australian cultivars. Pioneering work by Payne *et al.*, (1981a) with correlation studies reported that certain HMW glutenin subunits are associated with

negative effects. Some examples of the allelic variation in HMW subunits of hexaploid wheats are shown in Fig.2.4.

Based on the analyses of a large number of varieties, a quality rating system for HMW subunits has been formulated (Payne *et al.,* 1987a). In this system individual subunits are graded with numbers based on the quality evaluation. The majority of these quality evaluations have involved indices such as Zeleny, SDS sedimentation, loaf volume, alveograph, farinograph and extensograph measurements. Three main types of genetic material have been used in studies of this sort:

1) segregating populations with varying degrees of inbreeding, F_2-F_8, obtained through self fertilization of a cross of two cultivars usually of contrasting qualities (Payne *et al.*, 1981a; 1984a; 1987c; Moonen *et al.*, 1982; 1983; Lorenzo *et al.*, 1987; Gupta and Shepherd, 1987; Lagudah *et al.*, 1988; Carrillo *et al.*, 1990).

2) collections of variable size of homozygous and homogeneous cultivars (Payne *et al.*, 1979; Burnof and Bouriquet, 1980; Moonen *et al.*, 1982; 1983; Branlard and Dardevet 1985a, b; Lawrence *et al.*, 1987).

3) populations obtained through more sophisticated techniques such as random mating, or else near-isogenic lines, chromosomal substitution lines, or interspecific crosses (Lagudah *et al.*, 1987; Payne *et al.*, 1987c; Zemetra *et al.*, 1987; Dong *et al.*, 1991).

The results of these studies have all revealed that at the *Glu-D1* locus, strong dough strength is specifically associated with a pair of subunits 1Dx5+1Dy10 (Payne *et al.*, 1981a, 1984a; Moonen *et al.*, 1982, 1983; Branlard and Dardevet, 1985b ; Lagudah *et al.*, 1987; 1988; Lawrence *et al.*, 1987; Lorenzo *et al.*, 1987; Carrillo *et al.*, 1990). However for alleles of other loci (*Glu-A1*, *Glu-B1*) inconsistent rankings have been reported which are:

1) *Glu-A1* locus. 1>Null (Payne *et al.*, 1981a; 1987c ; Lagudah *et al.*, 1988; Carrillo *et al.*, 1990); 2*>1>Null (Moonen *et al.*, 1982; 1983); 2*=1>Null (Payne *et al.*, 1984a; Branlard and Dardevet, 1985b); 1>2*(Lorenzo *et al.*, 1987); 2*=1=Null (Dong *et al.*, 1991).

2) *Glu-B1* locus. 7+8=17+18=13+16>7+9>7>6+8 (Payne *et al.*, 1984a); 7+8>17+18 (Lorenzo *et al.*, 1987; Lagudah *et al.*, 1988; Carrillo *et al.*, 1990); 7+8>13+19 (Lorenzo *et al.*, 1987); 17+18>7+8 (Metakovsky *et al.*, 1990; Shahriari *et al.*, 1994a).

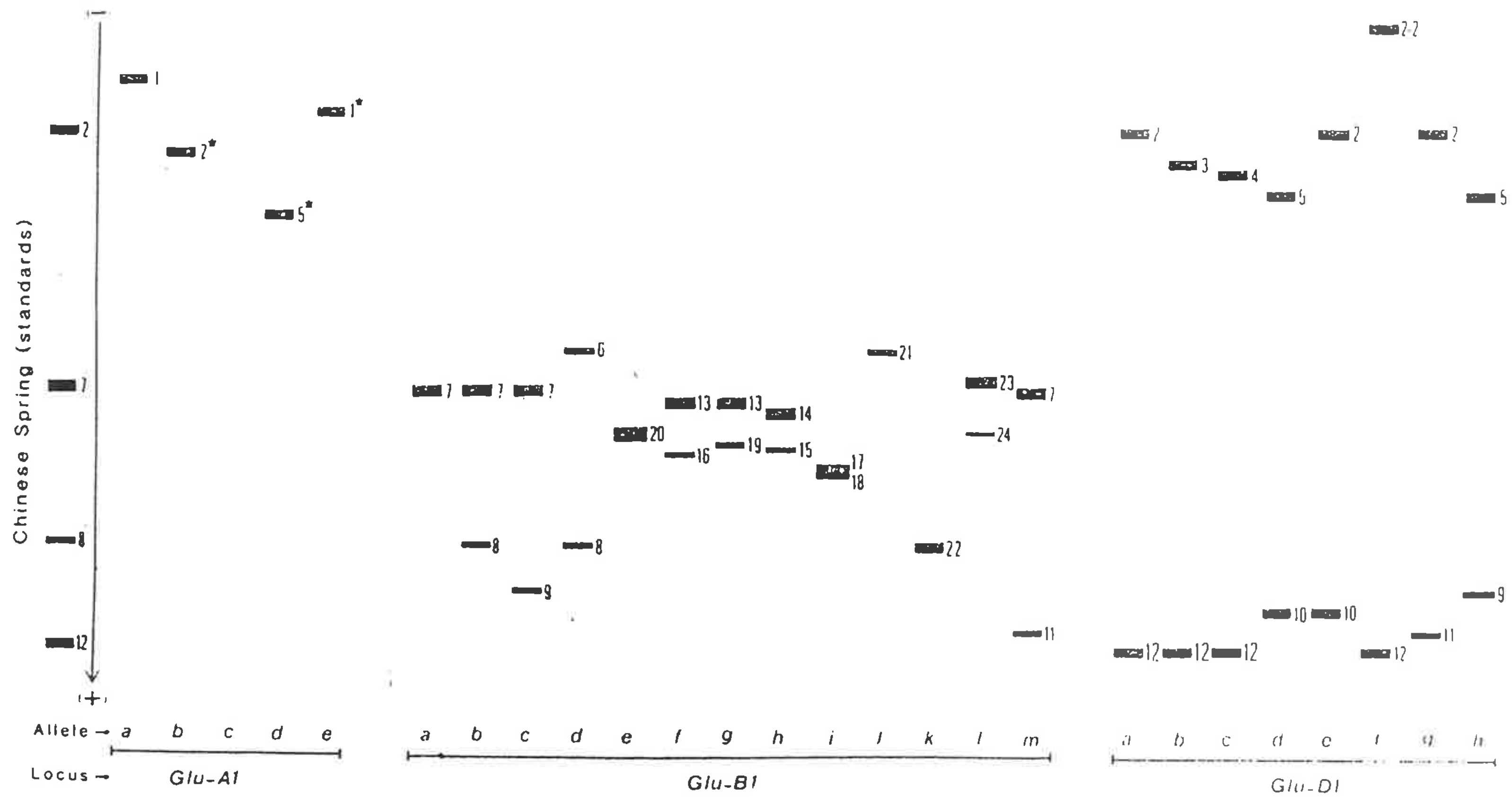


Figure 2.4 Allelic variation in HMW subunits of glutenin at the three gene loci (*Glu-A1*, *Glu-B1* and *Glu-D1*) (adopted from Payne *et al.*, 1984a). Subunits were fractionated by SDS-PAGE, with the direction of migration from top to bottom of the figure. The subunit pattern of the control Chinese Spring has been shown on the left to allow comparison of relative mobilities. Lower case letters refer to the allele designation of Payne *et al.* (1984a).

The inconsistencies between the rankings reported are most likely due to either different genetic backgrounds, epistasis, genotype x environment interactions or simply to statistical errors associated with small differences. Therefore the quality evaluation attemted by the 'Payne score' cannot be completed unless contributions of other gene products such as LMW (Gupta *et al.*, 1990b) and gliadin genes (Metakowsky *et al.*, 1990) are taken in to consideration. Furthermore interactions between the HMW glutenin alleles are also important in determining bread-making quality (Lorenzo *et al.*, 1987; Odenbach and Mahgoub, 1988; Carrillo *et al.*, 1990).

In contrast to the HMW, the allelic variation for LMW glutenin subunits are not well characterized mainly because of the lack of, until recently, a rapid method for analysing a large number of samples in a single gel in a gliadin-free background. A new procedure, which is based on sequential extraction of Marchylo *et al.* (1989), was developed by Singh *et al.* (1991b). This method gives a much improved resolution for both HMW and LMW subunits of glutenin and can be used for detecting different glutenin subunits in segregating progeny. Gupta and Shepherd (1990a) were the first to study allelic variation for more than 200 cultivars of bread wheat. In this survey 20 band patterns were detected which classified into six, nine and five alleles at the *Glu-A3*, *Glu-B3* and *Glu-D3* loci respectively (Fig 2.5).

Recently more studies have revealed that allelic variation at these loci has been associated with significant difference in dough quality in bread (Gupta and Shepherd, 1988; Gupta *et al.*, 1989) and durum wheat (Pogna *et al.*, 1990). The LMW glutenin subunits, with their ability to form large aggregates, were more likely to be the causal factors of increased gluten strength rather than the gliadin component (Autran and Berrier, 1984; Payne *et al.*, 1984c), since the genes controlling them were known to be closely linked (Payne *et al.*, 1984b). Payne *et al.* (1987c) were the first workers to associate LMW glutenin subunits with quality characters of hexaploid wheat by normal SDS-PAGE techniques. A preliminary approach to ranking LMW glutenin alleles in order of quality also has been reported by Gupta *et al.* (1991). However, it has been suggested that the effect of these alleles on quality will become more accurately assessed if they are considered in conjunction with the HMW glutenin subunits (Gupta *et al.*, 1989).

There are some instances which suggest that selection based on gluten profiles may lead to improvements in other traits of interest with particular variant alleles being associated with agronomic or end-use characteristics. Carrillo *et al.* (1990) have reported an association between several HMW glutenin subunits and grain yield. The results suggest

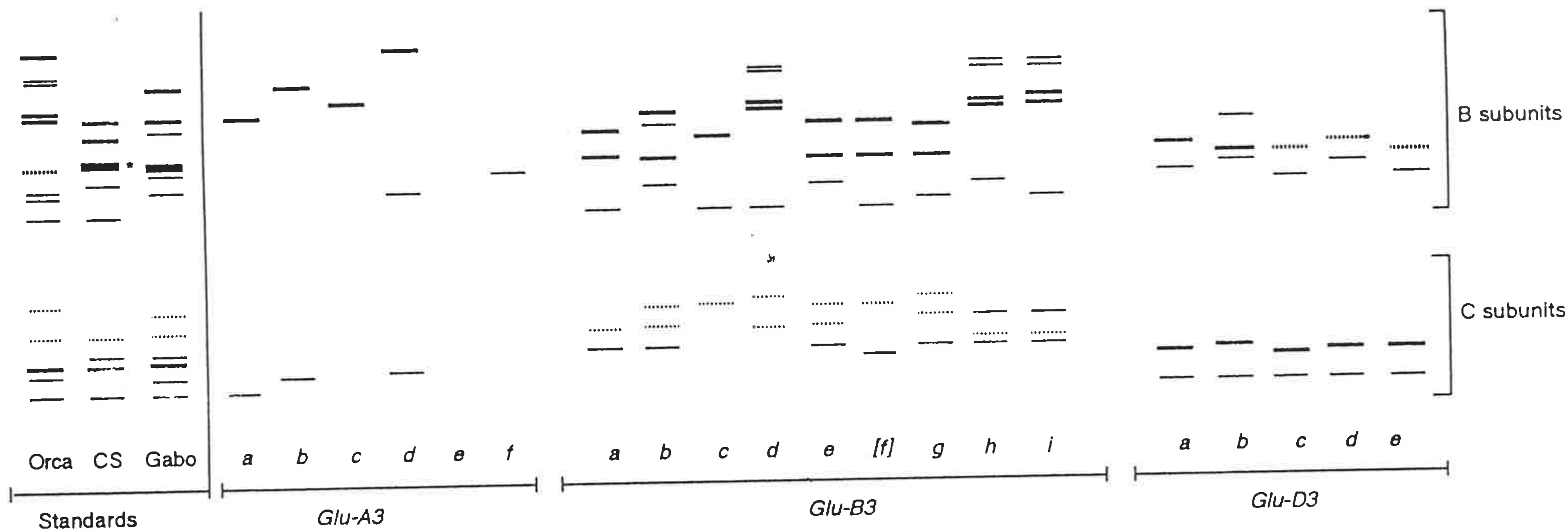


Figure 2.5 The three groups (from the A, B, and D genomes, left to right) of B and C LMW glutenin subunit combinations identified by two-step SDS-PAGE analysis of over 200 bread wheat varieties, together with the patterns for three references varieties, Orca, Chinese Spring (CS) and Gabo. Dotted lines represent faint bands. Patterns *a* and *b* in each group are from Chinese Spring and Gabo, respectively (Gupta and Shepherd 1990a).

that higher yielding lines tended to have particular HMW glutenin alleles with the strongest association involving alleles at the *Glu-D1* locus.

2.7.2 Biochemical basis of the allelic variation of protein composition on dough properties

It has become clear that both high molecular and low molecular weight glutenin subunits are important in determining dough strength of hexaploid wheats. There is evidence to suggest that HMW subunits form the backbone of linear glutenin polymers and have better polymerizing ability than LMW subunits (Graveland *et al.*, 1985). Gupta *et al.* (1994b) reported that high molecular glutenin subunits 5+10 has greater capacity to form larger sized polymers than subunits 2+12. Comparison of amino acid sequence of these two different subunits has indicated that subunits 5 have one more cystein residue than subunit 2 (Greene *et al.*, 1988). Also subunit 5 has a greater molecular weight than subunit 2 as deduced from cDNA clones. Halford *et al.* (1992) have also reported that improvement in breadmaking quality associated with the presence of 1Ax subunit 2* or 1 may result from an increase in the total proportion of HMW glutenin subunits which can in turn result in a higher amount of high molecular gluten polymers.

It has been known that the LMW subunits form disulphide-linked aggregates and make up a large proportion (about 30%) of the total endosperm proteins (MacRitchie, 1992). The relative effects of HMW and LMW glutenin subunits on the size distribution of the polymeric protein is a more important determinant of dough strength than the amount of total polymeric proteins (MacRitchie, 1992; Gupta *et al.*, 1993). Lafiandra (1993) found that the presence of the LMW subunits encoded at the *Glu-D3* locus has a negative effect on gluten properties when compared with the null allele. This negative effect was attributed to the presence of two D-subunits (Chinese Spring type allele) that could interact with other subunits, determining a reduction in the size of glutenin polymers and thus a reduction in the amount of the highly insoluble aggregated proteins.

2.8 Effects of environmental conditions on rheological properties of dough

Wheat is grown in Australia as a winter crop. Its production is largely confined to areas bounded by the 300 and 650 mm rainfall isohyets (Simmonds, 1989). The several different soil types found in the wheatbelt which, together with seasonal conditions, contribute to the variability encountered throughout the crop in any one season and from season to season. There are six critical stages for the wheat plant that occur after sowing. These stages along with an approximate time scale is given in Fig 2.6. This time was estimated for areas such as Western Australia, Eyre Peninsula in SA, northern NSW and southern Queensland.

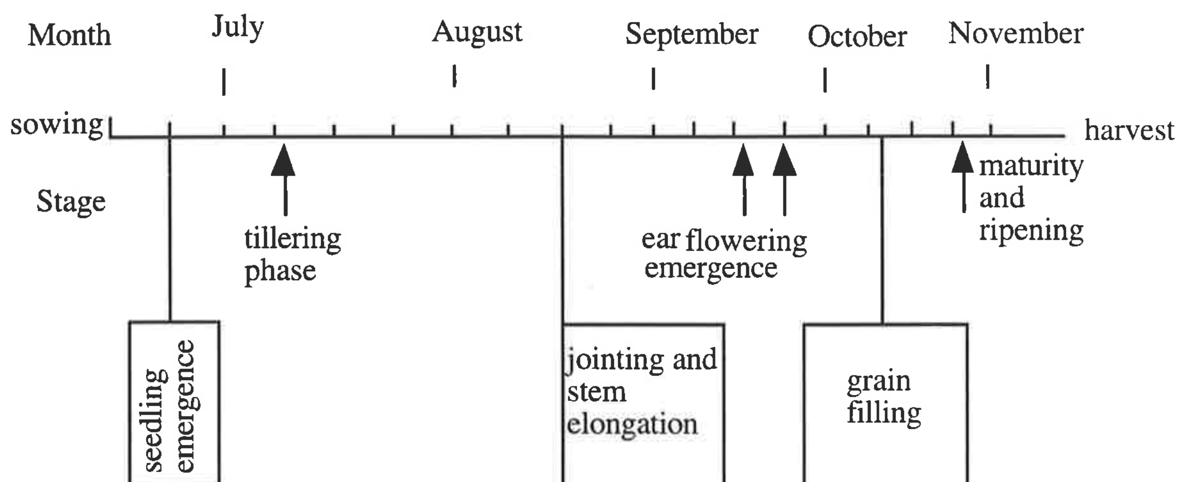


Figure 2.6 An approximate time scale for normal growth stages of wheat plant in Australia (adopted from wheat user's guide 1992-1993, Bread Research Institute of Australia)

The success of breeding for high technological quality depends on improvements in both the potential level of genotypes and stability of quality expression. For breeders and end users, such as millers and bakers, the ideal cultivar is one having an optimal mean value with a low variance in parameters when measured across environments. A stable cultivar being preferred to a high quality, but unstable cultivar. Several recent reports suggested that environmental conditions quantitatively affect storage protein components (Bietz, 1983; Kruger and Marchylo, 1985; Huebner and Bietz, 1988; Marchylo *et al.*, 1990). Some reports has indicated that under different conditions, protein composition measured by SDS-PAGE can be changed (Fullington *et al.*, 1987). Environmental conditions can impose their effect during growth of plant and sometimes during the post harvest period as summarised below:

2.8.1 Growing conditions

Branlard and Dardevet (1985a) have reported that within a variety, different growing condition and soil types may affect protein composition. There is also evidence that shows the S nutrition, particularly when considered in relation to N supply, can affect the dough properties (Randall and Wrigley, 1986). The element sulfur is required specifically for the synthesis of the amino acids cysteine/cystine and methionine, so severe deficiency in this element is reflected in a change in the electrophoretic pattern:

low-sulphur wheats have decreased quantities of albumins and gliadins of high electrophoretic mobility, because these are richer in sulfur (Wrigley *et al.*, 1981; 1984; Moss *et al.*, 1981, 1983).

Nitrogen variability affects mainly flour protein content that causes variation in extensibility. With increasing nitrogen, extensibility is increased. A demonstration of this has been reported in a study by Gupta *et al.* (1989) and similar results have been obtained by Wooding *et al.* (1994). A deficiency of sulfur fertiliser, coupled with excessive nitrogen fertiliser has been shown to modify dough properties by providing a ~~disportionate~~ disproportionate supply of nitrogen versus sulfur and a change in gluten composition to give a higher proportion of gliadin (Randall and Wrigley, 1986), whereas increasing sulfur and nitrogen together causes a reduction in resistance to extension (Wooding *et al.*, 1994). Several studies have been shown that grain quality and dough rheological characteristics can be affected by sulfur-nitrogen fertilizer treatments (Moss *et al.*, 1981; 1983; Wrigley *et al.*, 1984; Fullington *et al.*, 1987; Martin *et al.*, 1992).

The proportion of aggregating protein compared to monomeric gluten can be an indication of dough properties, a higher proportion corresponding to stronger dough properties. Quantitative changes of gliadins (Huebner and Bietz, 1988) and glutenin (Marchylo *et al.*, 1990) from different environmenal conditions have been reported. Amounts of nitrogen and water available during the growing season, especially during the period of grain filling, can interact in a quite complex manner: high moisture levels in the environment favour the synthesis of greater amounts of starch, while high nitrogen fertilizer level influences both protein content and grain yield. As the starch content of grain rises the amount of protein present decreases as a percentage of total grain weight.

2.8.2 Heat stress

Heat stress can be the main seasonal factor modifying dough properties (Blumenthal *et al.*, 1991). High temperatures (about 40°C) can occur in the Australian wheat belt during grain filling. Under heat stress conditions the synthesis of gliadin and low-molecular-weight (LMW) is continued, but the high-molecular-weight glutenins are generally not synthesised (Blumental *et al.*, 1991). An altered balance in the glutenin subunit composition (HMW:LMW) can lead to unacceptably long dough mixing requirements (Gupta *et al.*, 1992). Blumental *et al.* (1993) reported that a higher proportion of gliadin:glutenin in the mature grain produces weaker dough properties.

2.8.3 Rainfall and moisture

In the northern wheat belt of Australia, where a summer dominant rainfall prevails, quality reductions from rain on mature crops can cause significantly economical losses. The southern cropping area of Australia can also suffer severe crop damage because of too much rain over the harvest period (1992/1993 season, Chapter 4). Therefore any delay between maturity and harvest may result in yield and quality losses. If the ripe crop is exposed to excessive rain and high relative humidity, an increase in α-amylase activity leads to sprouting of grain which can have disastrous effects on quality (Buchanan and Nicholas, 1980). A number of rheological tests have been suggested to measure such changes (Kulp *et al.*, 1983; Lukow and Bushuk, 1984a; 1984b).

An important difficulty with breeding for appropriate dough properties is in defining the interaction between the environment and genotype. Cultivars must exhibit acceptable quality characteristic in all the intended environments and therefore must exhibit minimal cultivar by environment interactions. For some of quality tests such as sedimentation values, cultivar and environment are relatively unimportant (Barker and Kosmolak, 1977).

2.9 Quality assessment of wheat and flour for bread-making

There is no one single parameter that can be relied on to determine quality; rather there are a number of different factors. Therefore measurement of wheat quality for a particular end product requires a range of tests. Table 2.2 lists the various stages and levels of testing. In general quality can be related to five different areas; quality of the wheat kernel itself, milling quality, flour quality, physical dough quality, and end-use quality. Wheat kernel quality parameters include factors such as test weight, kernel size, kernel damage, moisture, and protein content. Milling parameters include conditioning properties, ease of milling, and flour extraction. Factors measured on the flour include flour ash, flour colour, protein content, amylase activity, wet gluten content, and starch damage. Measurements of the characteristics of the dough when flour and water are mixed together are most important for particular products (review by MacRitchie, 1984). The following is a brief review of some of the most routine quality assessments which can be used with various sample sizes and stages of variety development in breeding programs.

Table 2.2 Evaluation of wheat and flour quality at various scales of sample size and stages of variety development (adopted from MacRitchie *et al.*, 1990)

Scale of Testing	Sample Size	Test Methods
Commercial production	Kilotonnes	Commercial products
Pilot scale	1 t	Mill, make products
Lab scale	1 kg	Dough tests, pup-loaf bake, noodle-test, small flat bread bake
Small scale	20 g	Hardness tests, mixograph, micro-bake, Rapid Visco-Analyser, sedimentation tests, near infrared spectroscopy
Micro	3 g	Micromixograph, sedimentation tests
Molecular	<1/2 grain	Gel electrophoresis, high-performance liquid chromatography, antibody and nucleotide probe.

2.9.1 Small-scale tests for measuring protein quality

Over a long period plant breeders and cereal chemists have endeavoured to find reliable small scale methods for screening out weak gluten lines in the early generations of a breeding program. Electrophoretic fractionation techniques (Bietz and Wall, 1972), sodium dodecyl sulphate (SDS) sedimentation test (McDermott and Redman, 1977) and near infrared reflectance (NIR) analysis (Williams, 1979) are three major testing methods that have become available to breeders for genetic analysis of bread-making quality.

Electrophoretic fractionation techniques based mainly on gel electrophoresis (Bietz and Wall, 1972), gel isoelectric focusing (Wigley, 1968), sodium dodecyl sulfate (SDS) gel electrophoresis (Payne *et al.*, 1979) and two-dimensional fractionation by gel electrophoresis (Wrigley and Shepherd, 1973; Payne *et al.*, 1985) are the basis for evaluation of gluten composition in early generation of breeding programs (review by Shewry and Miflin, 1985). The advantages of electrophoretic separation techniques are that they require only a very small amount of material (as little as half kernel or less) and a large number of samples can be analysed in a brief period. The technique is essentially non-destructive because brush halves of kernels may be analysed and germ ends grown. Electrophoretic patterns of wheat cultivars or breeding lines can be subjected to statistical examination, to determine which of these protein components are related to flour quality attributes (Orth and Bushuk, 1972; Payne *et al.*, 1979; Kosmolak *et al.*, 1980; du Cros *et*

al., 1982). The major advantage of electrophoretic separation is that it is a direct measurement of the genotype i.e. not confounded by environmental factors.

The SDS-sedimentation test was first introduced by McDermott and Redman (1977) and used as an indirect measurement of the elasticity of gluten in bread wheat (Axford *et al.*, 1978, 1979) and durum wheat (Dexter *et al.*, 1980; Dick and Quick, 1983). In early stages of a breeding program, there is seldom enough grain for quality testing and some of the other bread-making tests are labour intensive and time consuming. Using this simple test, bread-making quality can be assessed on small samples and in a short period of time. This test measures the amount of SDS-insoluble glutenin protein; a parameter which has been shown to be related to bread-making quality. SDS-sedimentation volumes have been shown to be positively correlated at a high level of significance with loaf volume (Axford *et al.*, 1978; Blackman and Gill, 1980; Boggini and Pogna, 1989), dough development time (DDT), mixing consistency and the area under curve determined in the mixograph (Dexter *et al.*, 1980), or the farinograph (Taha and Sagi, 1987). Payne *et al.* (1979) were the first to associate HMW glutenin subunits with quality characters of wheat by using SDS-PAGE and SDS-sedimentation tests. Such an association also was established between LMW glutenin subunits and SDS-sedimentation volumes (Payne *et al.* 1987c)

Near-Infrared Reflectance (NIR) spectrophotometers are rapidly becoming common in cereal quality laboratories. Most chemical constituents of cereals like all agricultural products absorb radiation in the near-infrared range (780-2600 nm) and NIR instruments have been developed and successfully used to measure cereal constituents including protein (William, 1979) and kernel hardness (Rubenthaler and Bruinsma, 1978; Williams and Thampson, 1978). Williams *et al.* (1988) found a satisfactory relationship between protein strength as measured by NIR and overall testing in different classes of wheat.

Protein extracted with various solvents from small wheat flour samples can be analysed by reversed-phase high-performance liquid chromatography (RP-HPLC), size-exclusion HPLC (SE-HPLC) and ion-exchange HPLC (Bietz, 1985; 1986). These techniques can be applied for wheat varietal identification (Bietz, 1984; 1986), and for durum wheat, can rapidly determine quality (Burnouf and Bietz, 1987). After low molecular weight glutenin proteins have benn extracted, high molecular weight glutenin proteins can be reduced, alkylated, isolated and analysed by RP- or SE-HPLC. RP-HPLC, giving accurate measurements of quantitative variation in different gluten fractions (Bietz, 1983), might help in understanding the effects of environmental variation on wheat technological properties. The concentration of aggregating protein (proportion of aggregating compared to monomeric gluten) can be an indication of dough properties. This can be obtained by sonication of a suspension in SDS-containing buffer and

analysed by SE-HPLC (Batey *et al.*, 1991), with a higher proportion corresponding to stronger dough properties. The procedure is sensitive and can be carried out on a single kernel, but, since some variability can be caused by environmental conditions, analysis of composite flour samples (only micrograms of protein) is generally preferable.

The Mixograph is a useful instrument for estimating important physical dough properties in early-generation progenies and can also be used to predict bread loaf volume, mixing requirement, dough oxidation requirement, and water absorption (Finney and Shogren, 1972). The value of this approach in early generations has been demonstrated (Gras and O'Brien, 1992). A small sample size (2 g flour) permits the testing of single-plant selections (Gras and Wrigley, 1991)

2.9.2 Large-scale tests for quality assessment

The most obvious means of improving the quality of harvested grain is to concentrate on developing varieties that have the qualities required by customers. Selecting the most suitable varieties involves evaluation of advanced breeding lines using relevant types of milling, processing and baking. The main aim of a quality evaluation on a large-scale sample is to determine, as far as possible, the heritable millability or end use potential of a strain or line. To minimize the intrusion of environmental factors that might lead to erroneous conclusions, the performance of test lines is compared with those of standard varieties grown under identical conditions. In Australia, the international market requirements have encouraged the development of Australian varieties of wheat whose flours exhibit desired dough strength for several different type of products. A number of rheological tests have been used to measure dough strength. The following provides some details about the instruments which are used for quality assessment.

Brabender Farinograph and Extensograph

In Australia, dough strength is a specific term describing the balance between viscous and elastic properties of a dough (review by MacRitchie *et al.*, 1990). The capacity of a developed dough to be stretched without breaking (i.e.extensibility) is another desirable property. The dough development time (DDT), the time in minutes required for the curve to reach a peak, maximum consistency, the viscosity measured in Brabender Units (BU) at the peak of the curve; tolerance index (TI), the difference in consistency between the values at the peak and four minutes past the peak (AACC, 1983a); and the amount of water required to produce ideal dough (water absorption) can be measured from the farinograph. Weak gluten cultivars gives a short DDT and a high TI, whereas strong-gluten cultivars usually have a longer DDT and very low TI. The farinograph is now

generally used to measure the water absorption capacity of a flours and as a preliminary to extensograph testing of bread wheats.

The extensograph is an instrument that has been widely used for assessing dough properties. In principle, its measurement approximates to a tensile strength test. For measuring the extensibility, the dough must be mixed in farinograph for a fixed time to a fixed consistency to produce a viscosity that give 500 BU resistance (AACC, 1983b). The cylinder of dough is stretched at a fixed rate until it breaks, while the force of resistance to stretching is continuously monitored. The maximum resistance to extension (Rmax) in BU is obtained by measuring the maximum height of the extensograph curve (extensogram) while the extensibility (E) in cm obtained by the length of the extensogram. The area (cm^2) under the curve reflects both dough resistance and extensibility and is a measure of dough strength (energy). Since the extensibility (E) is correlated to protein level (P) (Gupta *et al.*, 1992) interpretation of extensibility data requires knowledge of the protein level. For this reason the extensibility is interpreted by the E/P ratio. This is becoming regarded as one of the more reliable tests for predicting end use quality (A.J. Rathjen, pers. comm.).

Falling number apparatus

Another complementary method for measuring of dough strength is the Falling Number test (FN). This test is based on α-amylase activity on starch in grains and flours, measured as time in seconds for a stirrer to fall a measured distance through a hot starch suspension (Stone *et al.,* 1994). If the ripe crop is exposed to excessive rain and high relative humidity, an increase in α-amylase activity would result in a rapid decline in the grain Falling Number reflecting the beginning of sprouting. Sprouting of grain can have disastrous effects on quality (Buchanan and Nicholas, 1980) and grain quality can be severely reduced well before visible sprouting occurs. Studies have shown that during dough mixing and fermentation, excess α-amylase causes the starch to be degraded and when this occurs, water normally bound by the starch is released, resulting in a slack, sticky dough (Ibrahim and D'Appolonia, 1979; Kulp *et al.*, 1983).

2.10 Breeding strategies for protein quality

The principal long-term objective of relating protein composition to quality is to be able to devise strategies for breeding varieties with specific functionality. Strategies which has been useful in these area as are:

1) all wheat products require highly extensible dough. In this case the Australian breeders have been advised to select the LMW glutenin alleles with a high number of

bands and adopt this as a general strategy as this leads to high levels of extensibility (Cornish, 1994). High extensibility also can be achieved by enhancing flour glutenin content. Therefore selecting for inherently high protein content can be a useful strategy in quality improvement.

2) appropriate dough strength is required for each type of product and selection on the basis of high quality score suggested by Payne *et al.* (1987a) can increase dough strength. A scoring system for HMW glutenin has recently been devised by South Australian breeders based on the effects of HMW glutenin alleles on the dough quality of local varieties (Cornish, 1994).

3) research has shown consistently that certain alleles of HMW glutenin subunits are related to differences in quality (for example subunit bands 5+10 vs 2+12 at *Glu-D1* locus). As a consequence, by using alleles at *Glu-D1* breeders are able to improve breadmaking quality in predictable ways. By contrast some glutenin subunits are not conducive to good quality in any wheat class, therefore Australian wheat breeders purge such undesirable alleles in early generations (Hollamby *et al.,* 1994).

4) since both HMW and LMW subunits are important in determining the suitability of flour for breadmaking, wheat breeders have been advised to cross varieties that have complementary, favourable quality glutenin subunits and then screen progeny which have the best combination of the LMW and HMW subunits i.e the highest glutenin score.

This research work reported in this thesis has been directed of providing more information on the main effects of specific alleles at the *Glu-1* and *Glu-3* loci and their interactions to assist wheat breeders in selecting for suitable dough properties.

CHAPTER 3

CO-INHERITANCE OF LMW GLUTENIN SUBUNITS AND GLIADINS IN BREAD WHEATS

3.1 Introduction

It has long been known that LMW subunits of glutenin make up approximately 60% of the total glutenin fraction in wheat (Bietz and Wall, 1973). However much less is known about the allelic variation, chromosomal location of genes controlling them and their influence on the functional properties of flour. The delay in obtaining this information was due mainly to lack of suitable procedures for separating the LMW subunits from those other subunits which have similar solubility and electrophoretic mobilities. This problem was first overcome by Jackson *et al.* (1983) who applied 2-dimensional electrophoretic techniques to separate these components. However, this technique is complicated and slow, allowing only one or two samples to be analysed on each gel, and therefore it is unsuitable for rapid screening of a large number of breeders' samples or varietal surveys. Singh *et al.* (1991b) have developed a much simplified procedure for 1-D separation of glutenin subunits based on information obtained by Marchylo *et al.* (1989). This procedure can now be used for large scale surveys of cultivar variation or inheritance studies and allows separation of glutenin subunits with little contamination from other protein types.

The next significant development in genetic studies of LMW glutenin was provided by Gupta (1989) who produced a common wheat genotype suitable for use in test-cross analysis. This triple wheat-rye translocation (TrTr) has proved to be useful in inheritance studies and in investigating the influence of LMW glutenin subunits as a group on glutenin and dough structure (Gupta and Shepherd, 1987). The triple translocation stock serves as a suitable third parent in producing test cross seeds for studying the co-inheritance of LMW glutenin subunits and gliadins in different wheat varieties. This parent has all the short arms of group 1 wheat chromosomes replaced with the short arm of 1RS of Imperial rye and therefore lacks all or most of the LMW glutenin subunit bands (Gupta and Shepherd, 1993). Such a parent allows the segregation of contrasting banding patterns in the F_1 to be studied with minimum ambiguity from band overlap in the test cross progeny.

Considerable allelic variation exist for the HMW (Payne, 1987) and LMW (Gupta and Shepherd, 1988) glutenin subunits. This allelic variation has been the basis for studying the relationship between particular glutenin subunits and breadmaking quality. These studies are very dependent on the efficiency of the technique used for band separation and,

where poor resolution is achieved, a misleading results may be obtained. Unfortunately, because LMW glutenin subunits have proved to be difficult to analyse several contrasting types of results have been reported about the allelic effects on quality of alleles at *Glu-3* loci (Redaelli *et al.,* 1995). Since LMW glutenin subunits and gliadin bands are controlled by a cluster of very tightly linked genes, the more easily screened gliadin bands can be used as an indicator for the presence of specific LMW subunit alleles (Singh *et al.,* 1991b; Shahriari *et al.,* 1995). This approach is very useful for analysing segregating progenies from crosses between cultivars of known LMW subunit/gliadin patterns, because the level of recombination between these two protein groups is very low (Singh and Shepherd, 1984; 1988). Gupta *et al.* (1994a) in a survey of segregating progenies from [Halberd x (Wl x MMC)/Wl/10] concluded that the low molecular weight glutenin subunits alleles *Glu-A3c*, *Glu-B3b* and *Glu-D3b* of breeding line (Wl x MMC)/Wl/10 were always co-inherited with their respective gliadin alleles *Gli-A1c*, *Gli-B1b* and *Gli-D1b* and thus they defined the terms *Glu-A3c/Gli-A1c, Glu-B3b/Gli-B1b* and *Glu-D3b/Gli-D1b* to describe these linkage combinations. However their study was based on a segregating population with a high level of overlapping bands. The study described in this Chapter was designed to overcome this problem of overlap bands and to obtain evidence on the co-inheritance of the different patterns of LMW subunits detected in these parents and their associated *Gli-1* alleles, using simplified SDS-PAGE and the triple translocation. The parents used in the main experiments (Chapters 7, 8 and 9) were also included in this study to determine the relationship between their LMW subunits and dough properties later.

3.2 Materials and methods

3.2.1 Parents and F_1 combination

Four hexaploid wheats Halberd, (Wl x MMC)/Wl/10, Barunga and Suneca, having contrasting alleles at each of the three loci of LMW glutenin subunit (*Glu-A3*, *Glu-B3*, *Glu-D3*) were analysed in this inheritance study. The parantage for breeding line (Wl x MMC)/Wl/10 were: [Warigal x (Siete Cerros x Mengavi) x Crime)-selection 1/10].

Test crosses

In order to identify unambiguously all the LMW glutenin subunit present in these parents, the F_1 combinations [Halberd x (Wl x MMC)/Wl/10] and (Barunga x Suneca) were first produced

These F_1 hybrids were then test crossed with the triple translocation line Chinese Spring-Gabo 1AL.1BL.1DL/1RS (Gupta and Shepherd, 1993) obtained from seed stocks held in the Department of Plant Science, Waite Institute.

Back-crosses

In order to study the inheritance of band blocks within a cultivar, the TrTr was used as the recurrent parent in back-crosses. These crosses were as follows:
Barunga x [TrTr]^2
Suneca x [TrTr]^2

3.2.2 Protein extraction and electrophoresis

The procedure used was based on the sequential extraction method described earlier by Marchylo *et al.* (1989) and simplified by Singh *et al.* (1991b).

Gel preparation

The gel system used to visualise these proteins requires the formation of two gel layers, made up of the main (resolving) gel, in which band separation takes place, and the short upper (stacking) gel, to which samples are applied and in which the protein zones are concentrated to give very thin starting zones.

The resolving gel, which occupied most of the gel slab, contained a gradient of gel acrylamide concentrations. An 8.1-12.5% linear acrylamide gradient gel with 1% cross-linker concentration (bisacrylamide/acrylamide ratio) and 180 x 160 x 1 mm dimensions (using Hoefer gel electrophoretic apparatus SE 600) gave the best results for most HMW and LMW glutenin subunits. The main separating gel layer consisted of buffer [(45.412 gms Tris, plus 1 gm SDS and 460 mls H_20, made to 500 ml), adjusted with HCl to pH 8.8], acrylamide solution (0.75 gms bisacrylamide+ 75 gms acrylamide dissolved in water to a volume of 250 mls), 10% Ammonium persulphate solution (0.2 gms Ammonium persulphate dissolved in 1.9 mls H_2O) and N, N, N', N'-tetramethyl-ethylenediamine (TEMED) as the polymerization agent. The stacking gel consisted of buffer (6.06 gms Tris + 0.4 gms SDS and 190 mls H_2O, made to 200 mls, adjusted with HCl to pH 6.8), 87.5 gms acrylamide + 1.32 gms bisacrylamide dissolved in water to a volume of 250 mls which makes 1.1% crosslinker). Ammonium persulphate solution and TEMED were also added to these solutions for polymerization.

Sample preparation

The endosperm half of a single wheat kernel was crushed into a fine powder. The embryo portion was kept and stored at 4°C and depending on the endosperm pattern was later germinated for seed multiplication. The extraction of unreduced prolamin and glutenins for one step SDS-PAGE was carried out as follows:

Extraction of unreduced prolamins

Gliadins were extracted in 200 µl of 70% (v/v) aqueous ethanol from one third of the kernel for at least one hour at 60°C or 3 to 18 hours at room temperature. After centrifugation in a Beckman Microfuge-11 for 5 minutes at speed 1000g, 100µl of the supernatant was transferred to another Eppendorf tube and evaporated at 60-65°C for at least 1 hour. The residue was re-dissolved with 100 µl of sample buffer [containing 80 mM Tris-HCl, 40% glycerol (w/v), 2% SDS(w/v) and 0.02% bromophenol blue]. After a brief vortexing and incubation for 30 min at 60°C, 14 µl was loaded per well onto the SDS-PAGE slab gel.

Extraction of reduced prolamins

The procedure of Singh *et al.* (1991b) for separating total glutenins was slightly modified in this study. The above residue samples were further sequentially extracted with propanol because only portion of the gliadins had been extracted in ethanol. First, the remaining supernatant from the first extraction was removed by aspiration and then the residue was extracted twice in 1.0 ml of 50% (v/v) n-propanol at 60°C by repeated vortexing (at least twice) for 30 minutes. Suspension of flour inside of the tube using a fine spatula assisted in the complete extraction of gliadins at this stage and prevented contamination of glutenin preparations later. After 2 min centrifugation the supernatant was discarded both times. The residue was washed with 0.5 ml of 50% n-propanol, centrifuged for 5 min, and then all the liquid was removed by aspiration.

Glutenin was extracted from the residue by adding 100 µl of 80 mM Tris-HCl (pH 8.0) containing 1% dithiothreitol (DTT) in 50% n-propanol. After a brief initial vortexing the sample was incubated for 30 min at 60°C. Reduced glutenin (including HMW and LMW subunits ~~linked to each other by SH group~~s) was then extracted by 2 min centrifugation and alkylated with 100 µl of 80 mM Tris-HCl (pH 8.0) plus 0.14 M 4-vinylpyridine (4-VP) in 50% n-propanol. Alkylation improves the resolution of the LMW bands, because it prevents reassociation between intramolecular disulphide bands. The samples were further incubated for 15 min at 60°C. After centrifugation for 2 min, 50 µl of the supernatant was transferred to another tube containing 100 µl of sample buffer [containing 80 mM Tris-HCl (pH 8.0), 40% glycerol (w/v), 2% SDS (w/v) and 0.02% bromophenol blue]. After a brief initial vortexing the residue was left to stand for 15 min at 60°C to allow complexing of SDS with the reduced and alkylated glutenin polypeptides. After centrifugation for 2 min, 10-15 µl of the supernatant was loaded into a sample well of the gel for SDS-PAGE separation of the glutenin subunits.

After complete reduction, alkylation and SDS-complexing, each subunit was expected to have a single rod-like conformation (Reynold and Tanford, 1970), giving a single sharp band.

Electrophoresis

Hoefer gel electrophoretic apparatus was used. The electrode buffer, for both upper (cathodal) and lower (anodal) tanks, contained 303.3 gms Tris, 144.2 gms glycine, 10 gms SDS, dissolved in 883 mls H_2O made to 1 litre and adjusted to pH 8.3 with glycine. The samples were loaded at the cathodal end of the gel and electrophoresed at a constant current of 45 mA/gel for 130 min, and the electrode buffer was cooled by circulating water at a constant temperature of 25°C.

3.2.3 Staining and destaining

Gels were stained for at least 3 hours in a solution consisting of one part of 1% (w/v) Coomassie Brilliant Blue R mixed with 40 parts of 6% (w/v) trichloroacetic acid in water:methanol:glacial acetic acid (80:20:7) as described by Lawrence and Shepherd (1980). The stained gel was placed in deionized water for one day with several changes of water for destaining.

3.2.4 Drying

After destaining , gels were immersed for about 30 min in fixing solution (methanol-glacial acetic acid, glycerol 40:10:3, by volume) and then were placed between two layers of cellophane sheet on a glass plate and all sides of the plate were sealed using a strip spacer and clips. After 24 hours at room temperature, gels were removed and stored in a plastic bag.

3.3 Results

3.3.1 [Halberd x (W1 x MMC)/W1/10] test cross

A total of 94 seeds from the test cross [{(Halberd x (W1 x MMC)/W1/10)} x TrTr] were screened by one-step SDS-PAGE. The first two parents in this complex cross differ with respect to the alleles carried on each of the 1A, 1B and 1D chromosomes (Table 3.1).

Table 3.1 Allelic variants at the loci controlling glutenin subunits (HMW† and LMW‡ subunits) at the six loci in Halberd and (W1 x MMC)/W1/10 parents

Gluten subunits	Locus	Allele in parent	
		Halberd	(W1 x MMC)/W1/10
	Glu-A1	1	2*
HMW	*Glu-B1*	20	17+18
	Glu-D1	5+10	2+12
	Glu-A3	*e*	*c*
LMW	*Glu-B3*	*c*	*b*
	Glu-D3	*c*	*b*

† Nomenclature of Payne and Lawrence (1983)
‡ Nomenclature of Gupta and Shepherd (1988)

The LMW glutenin and gliadin patterns of the parents and F_1 hybrids are shown in Figs 1A and 1B. The TrTr stock used as the third parent in the testcross has no bands in the B subunit zone (Fig 1A, P3), therefore it facilitated scoring of the joint segregation of the B subunit blocks in the F_1 parent. The test-cross seeds could be identified easily by the presence of the strong secalin bands below the ω-gliadins (Fig 1B, P3).

The progeny and parents were analysed by one-step SDS-PAGE for both gliadin and glutenin for the co-inheritance study of the bands controlled by *Glu-A3*, *Glu-B3* and *Glu-D3* and which were assumed to be closely linked with the *Gli-1* genes (Singh and Shepherd 1984; 1988). The LMW glutenin subunits of the test-cross progeny were classified into three inheritance groups (1AS, 1BS, 1DS) as reported by Gupta and Shepherd (1993). The band patterns corresponding to three groups in Halberd and (W1 x MMC)/W1/10 are shown diagrammatically in Figs 1A′ and 1B′. Diagnostic LMW glutenin subunits controlled by chromosome arms 1AS, 1BS and 1DS are marked by symbols → , ● and ▶ respectively.

The *e* allele (null) of *Glu-A3* locus did not produce any noticeable band, whereas its counterpart from (W1 x MMC)/W1/10 gave a normal single band. Although the *Glu-A3c* allele can be easily identified without any guidance from the gliadin patterns, some γ gliadins co-segregate with this allele but not with the null *e* allele (Fig 1B, marked by →).

Figure 3.1 Banding patterns of [Halberd x (Wl x MMC)/Wl/10] test cross progeny and parents:

(**A**) SDS-PAGE patterns of glutenin subunits.

(**A'**) Interpretation of segregation patterns shown by B and C subunits.

(**B**) SDS-PAGE patterns of gliadin bands.

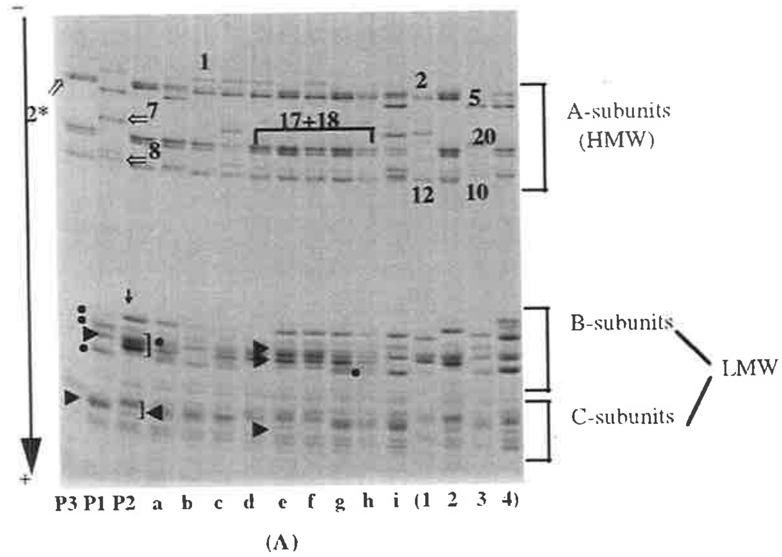
(**B'**) Interpretation of segregation of ω- and γ-gliadins.

Symbols used in the Figures;

P1 (Halberd); **P2** (Wl x MMC)/Wl/10; **P3** (Triple wheat-rye 1RS Translocation (TrTr); a-i (Test-cross seeds from [(Halberd x (Wl x MMC)/Wl/10]; **1, 2, 3, 4** (The wheat standards cultivars: Chinese Spring, Gabo, Jabiru, Wilgoyne); → (Bands controlled by 1AS); ● (Bands controlled by 1BS); ► (Bands controlled by 1DS).

····· faint bands

—— normal bands



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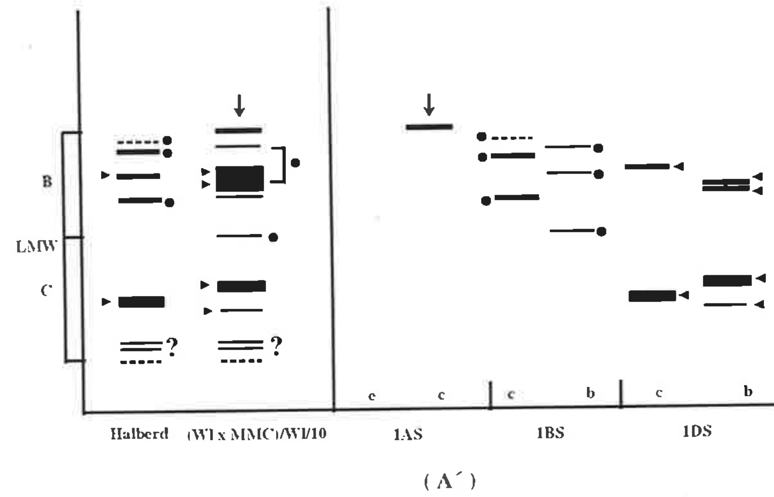

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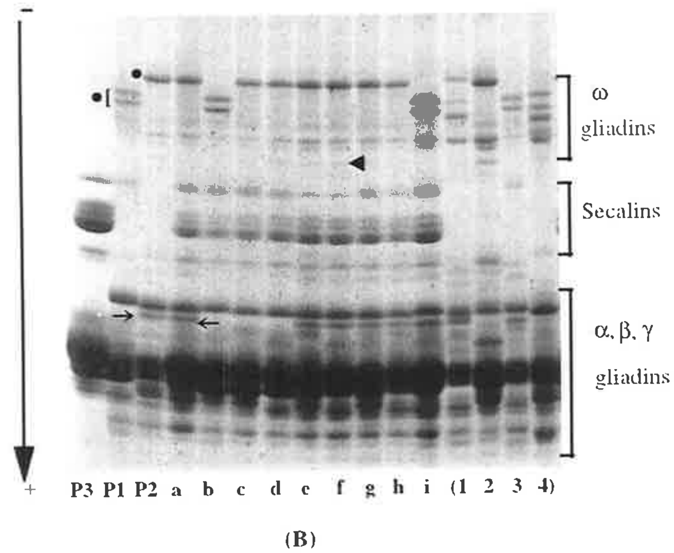

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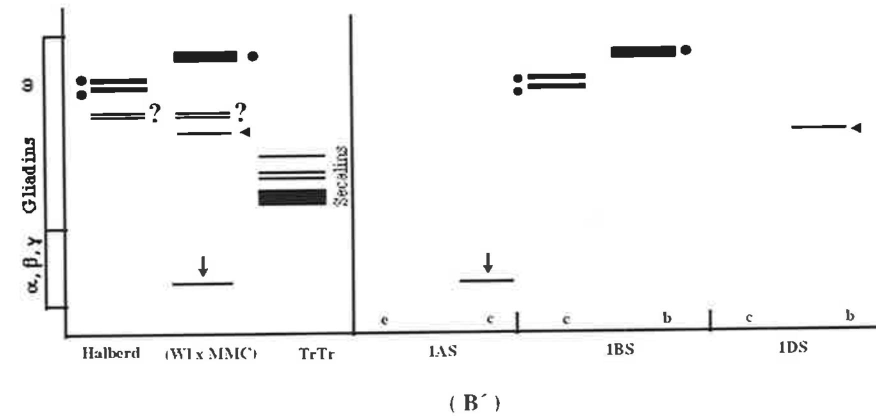


(B´)

Halberd and (W1 x MMC)/W1/10 exhibit major differences in pattern with respect to the B subunits of glutenin. The two bands of Halberd (P1) in the B subunit region (Fig 1A, marked by ●) segregate as alternatives to the three bands of breeding line (Wl x MMC)W1/10 (P2, marked with same symbol), as reported by Gupta *et al.* (1994a). Diagnotic cosegregating gliadin markers were also found for these glutenin blocks (Fig 1B, marked by ●). It was found that these group of 2 and 3 LMW bands are inherited as units and that the controlling genes are allelic or very closely linked at the *Glu-B3* locus. These genes are also closely linked to those controlling ω gliadins as no recombination between the glutenin and gliadin patterns was observed (Table 3.2).

It was noted that two C subunits in the (W1 x MMC)W1/10 parent co-segregated with a single gliadin band between the ω-gliadin and secalin regions (Fig 1B marked by ►), whereas no associated gliadin band was observed with Halberd. This alternative segregation was quite consistent in all of the progenies studied, although, it is not well represented in Fig 1B. This band is assumed to be controlled by *Glu-D3* locus. The absence of recombinant in this region also suggests that the genes controlling C subunits are controlled by tightly linked genes.

Table 3.2 Segregation ratios of B subunit blocks of bands in test cross progeny from Halberd x (Wl x MMC)/Wl/10 F_1 hybrids.

LMW glutenin blocks (B subunits)	observed frequency		χ^2 (P1:P2=1:1)	probability (df=1)
	Parental type (P1:P2)	Non-parental types		
1AS block (*e*:*c*)	45:49	0	0.17	0.68
1BS block (*c*:*b*)	53:41	0	1.53	0.22
1DS block (*c*:*b*)	51:43	0	0.68	0.40

3.3.2 Segregation of LMW glutenin subunits and gliadin bands in Suneca and Barunga backcross progeny

[Suneca x TrTr^2]

A total of twenty BC1 seeds were produced with Suneca and analysed for the the glutenin subunit patterns (Fig 3.2A). Lane 4 shows a backcross seed with the parental Suneca pattern showing seven B subunits including two faintly stained bands. In study here, attribution of bands to each groups of 1A, 1B and 1D was based on the finding of Gupta *et al.* (1993) who specified relative LMW bands using novel translocation stocks. Therefore,

the number and mobility of the bands were considered as a base for their relative group of chromosome. For example, segregation in the BC1 progeny clearly showed that four out of the seven bands (denoted as ● lane 1) segregate together and are controlled by 1BS. Also examination of the gliadin band patterns in the progeny using 1-D SDS-PAGE (Fig 3.2B) showed that a cluster of four bands (lane 1 denoted by ●) were controlled by the 1BS. With respect to the assumed bands group 1AS, although, there was no lane carrying the 1A allele only, comparison of two samples (lanes 2 and 3) clearly differentiated the pair of 1AS bands (➙) from the prominent band controlled by 1DS (▲). The corresponding gliadin patterns for these two samples showed that one band associated with 1AS (lane 2 denoted as ➙) and two bands for 1DS (lane 2 denoted as ▶).

[Barunga x TrTr^2]

A total of twenty BC1 seeds were produced and analysed with Barunga. Barunga has five darkly stained bands in the B subunit region. The same assumption was also made here to assigned relative LMW bands to groups 1A, 1B and 1D as considered for the previous cross. The segregating LMW glutenin subunit patterns for the BC1 progeny showed that some of the darkly stained bands actually consist of two overlapping bands. Four bands segregated as a 1BS-controlled block (Fig 3.3A, lanes 1, 2 and 3 denoted by ●). The corresponding gliadin patterns for these samples (Fig 3.3B) indicated that only one gliadin band (in ω gliadin region) could be associated with the 1BS group of glutenin bands (denoted by ●). It was possible to deduce the 1AS and 1DS blocks of B bands by comparing lanes 4 (1AS+1DS) and 6 (1AS alone). Two bands segregated for 1AS (denoted by ➙ for 1AS, lane 6) and two others for 1DS denoted by ▶ lane 4. Comparison of the gliadin patterns for the corresponding lanes (4 and 6) showed two faint bands in the middle of ω gliadin associated with 1DS and two bands with 1AS (Fig 3.3B, lane 6 denoted by ➙).

Figure 3.2 Electrophoretic banding patterns of four backcross progeny [Suneca x $TrTr^2$].

(A) SDS-PAGE pattern of glutenin subunits;

(A') Interpretation of segregation patterns shown by B and C subunits.

(B) SDS-PAGE patterns of gliadin subunits;

(B') Interpretation of segregation of ω- and γ-gliadin.

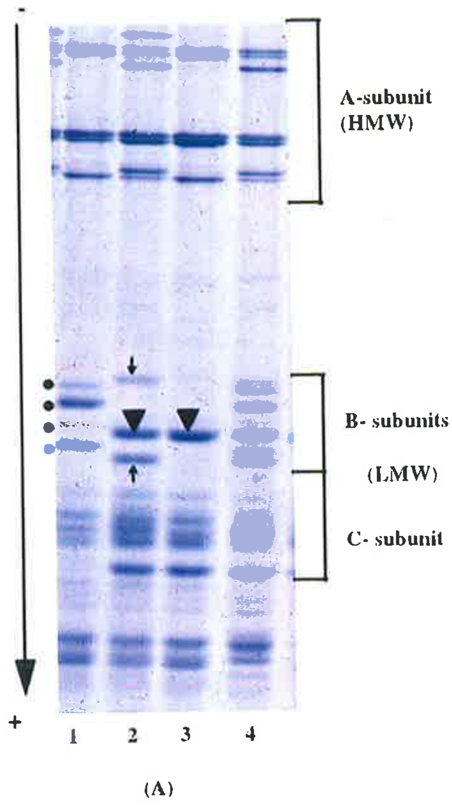
(1, 2, 3, 4) backcross progeny; (4 has all of the Suneca parent LMW and gliadin components).

Symbol used in Figures:

→ = bands controlled by 1AS;

●= bands controlled by 1BS

► = bands controlled by 1DS



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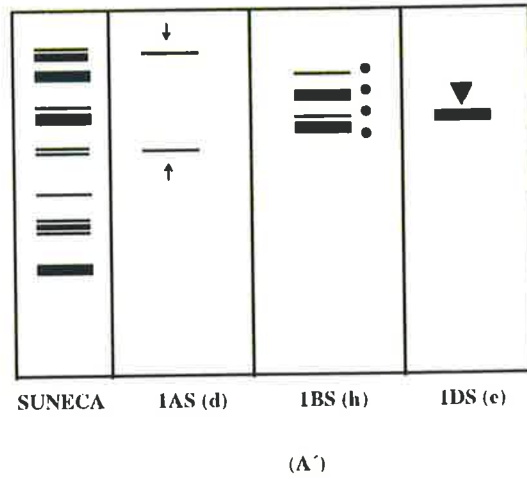

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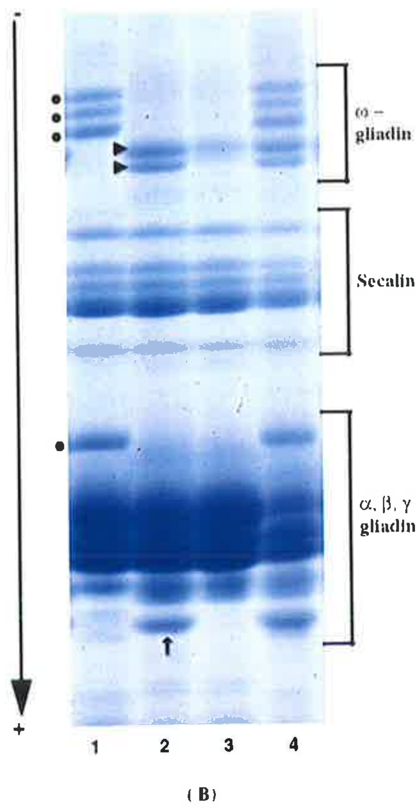
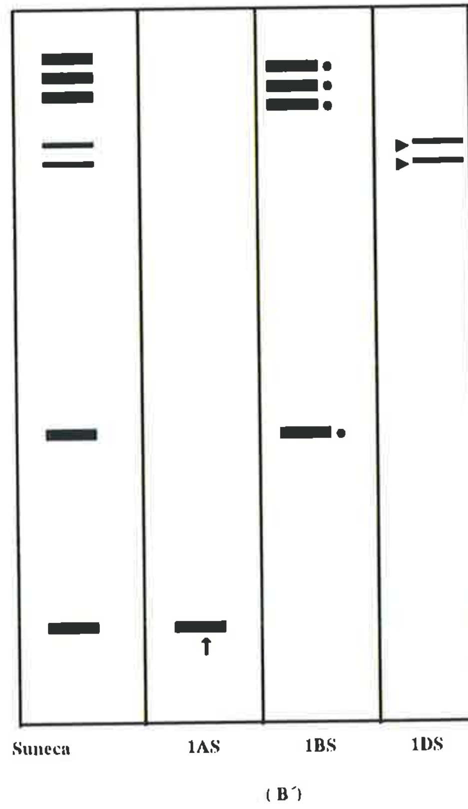


(B)



(B´)

Figure 3.3 Electrophoretic banding patterns of backcross progeny [Barunga x $TrTr^2$].

(A) SDS-PAGE pattern of glutenin subunits;

(A') Interpretation of segregation patterns shown by B and C subunits.

(B) SDS-PAGE patterns of gliadin subunits;

(B') Interpretation of segregation of ω- and γ-gliadin.

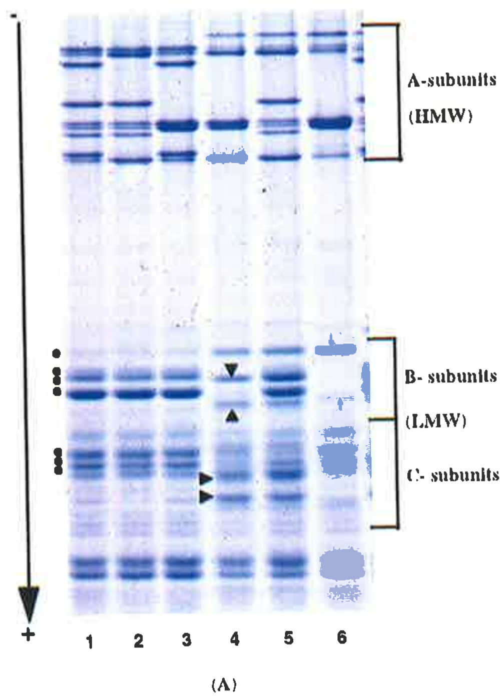
(1, 2, 3, 4, 5, 6) backcross progeny.

Symbol used in Figures:

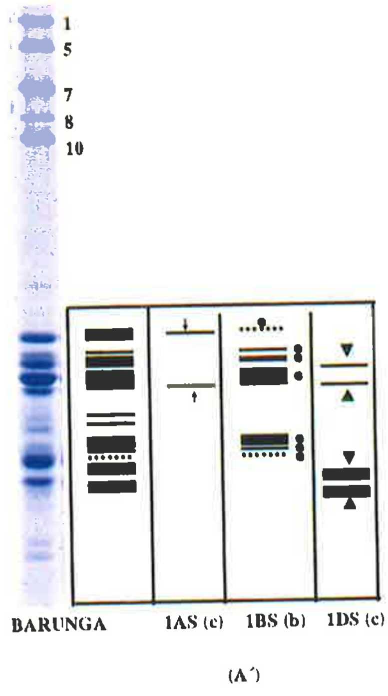
→ = bands controlled by 1AS;

●= bands controlled by 1BS

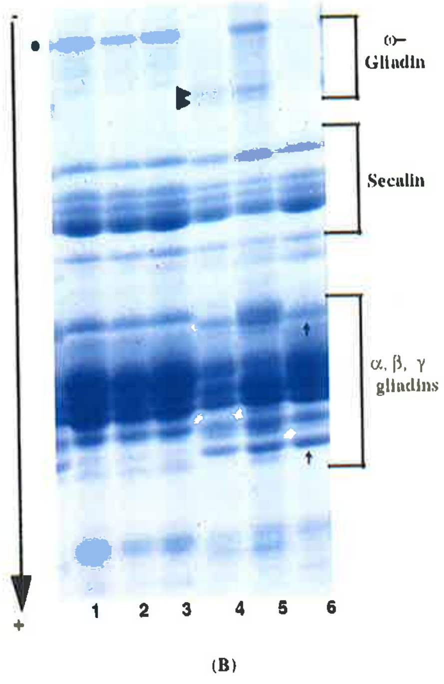
► = bands controlled by 1DS



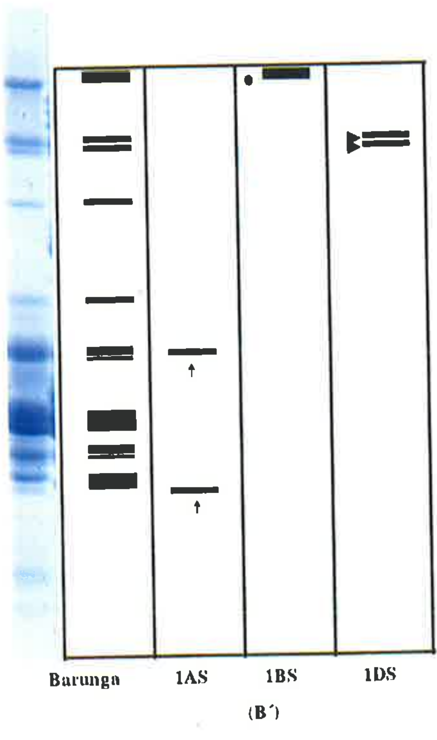
(A)



(A´)



(B)



(B´)

3.4 Discussion

The modified SDS-PAGE method of Singh *et al.* (1991b) allows excellent resolution of LMW subunits and provides a much simpler procedure for studying the genetic control of the LMW glutenin bands than those previously available. This facilitates the analysis of segregating progenies being routinely screened for glutenin subunit composition in breeding programs.

Since a number of LMW glutenin subunits and gliadin bands are controlled by a cluster of very tightly linked genes, as shown here and reported by several other workers (Singh and Shepherd 1984; 1988; Payne *et al.*, 1984b), these co-inheritance studies can be useful to identify the number of bands associated with the individual alleles at these loci, especially for the *Glu-B3* and *Glu-D3* loci. Segregation in the test cross progeny of (Halberd x (W1 x MMC)) W1/10 showed that there was no recombination between the *Gli-1* and *Glu-3* loci, therefore for this specific cross the segregating gliadin pattern can be used to identify the LMW glutenin pattern present in progeny.

It is a very simple procedure to screen for gliadin patterns since only one extraction is required. Thus it is an attractive option in a breeding program to score for LMW types by selecting for the associated gliadin bands. However, the inherent danger with this approach is that rare recombination or point mutation could separate the LMW pattern from this association with glutenin bands and possibly generate a new LMW subunit combination, especially with *Glu-B3* alleles, because this complex locus has shown the greatest polymorphism (Gupta and Shepherd, 1990a). This finding is quite consistent with the hypothesis that the B genome in polyploid wheat is polyphyletic (Sarkar and Stebbins, 1956; Athwal and Kimber, 1972).

In general this study has demonstrated clearly that all three groups of LMW glutenin subunits, as identified by Gupta and Shepherd (1990a), are tightly linked to *Gli-1* loci. It is generally accepted that glutenin rather than gliadin is associated with dough strength (Wall, 1979). However a few researchers (Wrigley *et al.*, 1981; Sozinov and Poperelya, 1980) have shown that allelic variation in gliadins also influence some aspects of breadmaking quality.

The triple translocation stock was used as the recurrent parent in backcrosses to identify inheritance of the band components making up the various blocks within a cultivar. Because of the virtual absence of major B subunit bands in the TrTr stock, the structure of the actual band blocks could be easily determined. The identification of all the LMW glutenin subunits bands in bread wheats by studying the segregation of these bands in progeny of F_1 hybrid is not always possible, because of overlapping mobilities of the

bands for the two parents in SDS-PAGE gels as reported by Gupta and Shepherd (1988). Therefore this back cross procedure can provide an opportunity to study all of the LMW subunits of glutenin in different wheat cultivars without the complication of overlap between bands. The LMW subunits controlled by genes on the short arms of group 1 chromosomes will always be inherited as blocks in the backcrosses because recombination is not expected to occur due to the lack of pairing between these wheat chromosomes arms and the homoeologous rye arm 1RS (Koebner and Shepherd, 1988; Singh and Shepherd, 1988). This raises a potential problem with analysis of 1AS and 1BS controlled patterns because it is known that there are two loci on these chromosomes controlling B bands which show 20% recombination (Pogna *et al.*, 1993). In the BC progeny bands controlled by both of these loci will segregate as a block and hence we cannot identify which bands are controlled by each of these loci.

CHAPTER 4

ANALYSIS OF RELATIONSHIP BETWEEN FLOUR QUALITY AND PROTEIN FRACTIONS IN THE F7 RECOMBINANT INBRED LINES FROM A BREAD WHEAT CROSS

4.1 Introduction

The physical dough properties and bread making quality of wheat flours are primarily determined by their proteins. In general there is a positive relationship between proteins and dough strength properties (Fowler and DelaRoche, 1975). At present, most evidence suggests that physical dough properties, especially those associated with dough strength are mainly determined by the qualitative and quantitative properties of glutenin proteins. In most studies, the proportion or absolute quantity of glutenin, and in particular, the proportion of High Molecular Weight (HMW) or insoluble glutenin was found to be positively related to dough properties (MacRitchie, 1987; Orth and O'Brien 1976; Singh *et al.*, 1990; Huebner and Bietz, 1985). The qualitative relationship between various glutenin subunits and dough strength properties has also been demonstrated by electrophoresis techniques (Payne *et al.*, 1979; 1981a). This work has resulted in the development of a glutenin subunit scoring system (*Glu-1* score) to predict these quality parameters (Payne *et al.*, 1987a). Gupta *et al.* (1990a) reported that the proportion of the variation of dough properties explained by the *Glu-1* score for Australian wheats compared to the world-wheat set was very low (15% and 42% respectively). This indicates that additional aspects of gluten composition (particularly LMW subunits of glutenin) must be examined to provide a more complete explanation. Other protein factors (gliadins, albumins and globulins) (Gupta *et al.*, 1994b) and non-protein factors (lipids and starch) (MacRitchie, 1984; Chung, 1986; He and Hoseny, 1992) could also be responsible for some of the variation in the dough properties of cultivars.

The relationship between glutenin subunits and breadmaking quality is very dependent on the kind of plant materials which have been used for these studies. Many studies have involved sets of cultivars from different wheat breeding programs. In this case it is quite possible that the apparent allelic associations of the six loci of glutenin subunits (*Glu-1* and *Glu-3* loci) with dough properties have been affected by number of factors such as pleiotropy, genetic linkage or chance association through common ancestry.

The objective of the present study was to determine the interrelationships among and between flour quality parameters (with emphasis on physical dough properties) and protein fractions while minimising the effects of the pleiotropy, genetic linkage and

chance association. To meet this objective a set of Recombinant Inbred Lines (RILs) were selected. These materials provide opportunity for looking at the causal relationships more closely than what is possible with cultivar sets, as the background differences are averaged over several selections with the same *Glu-1* and *Glu-3* genotype.

4.2 Materials and methods

4.2.1 Genetic materials

The material for this study were a set of F4-derived lines developed by Dr J.G. Paull for studies on tolerance to B and Na from the wheat-breeding program conducted at the Waite Agricultural Research Institute (South Australia) by A.J. Rathjen. These random RILs (F_2-derived F_7 progeny) were derived from crosses between a breeding line (Wl x MMC)/Wl/10 and the cultivar Halberd. Gupta *et al.* (1994a) reported that Halberd and the breeding line, differed with respect to alleles present at each of three HMW and three LMW glutenin loci and that 64 genotypic combinations of these alleles could be present in the RILs from this hybrid (based on segregating loci $2^n=2^6=64$).

Crosses were made between a breeding line (Wl x MMC)/Wl/10={Warigal x (Siete Cerros x Mengavi) x Crim)- selection 1/10} and the Australian bread wheat cultivar Halberd at the Waite Agricultural Research Institute (South Australia) and the F_1s were grown in a glasshouse to generate F_2 seeds. Thirty five individual F_2 seeds were selected randomly and planted to produce 35 F_3 lines. Subsequently, eight single plants from each of the F_3 lines, thus a total of 280 plants, were multiplied separately in further generations to obtain F_6 seeds (Fig 4.1).

4.2.2 Field experiments

Experimental sites

Field experiments were conducted at two sites in 1992 and three sites in 1993. The location of these are shown in Fig 4.2. These sites were chosen as they represent physical and biological conditions typical of large areas of the cereal growing zone in South Australia and are used as selection sites by the Waite Agricultural Research Institute wheat breeding program. The Palmer site is located approximately 65 km east of Adelaide, on Mr. J. Krause's property. The soil was sandy loam over a clay loam at

Figure 4.1 Procedure for the development of the random lines of [Halberd x (Wl x MMC)/Wl/10]. The F_4 and later generations were based on individual F_2, F_3 derived lines (adapted from Paull, 1990)

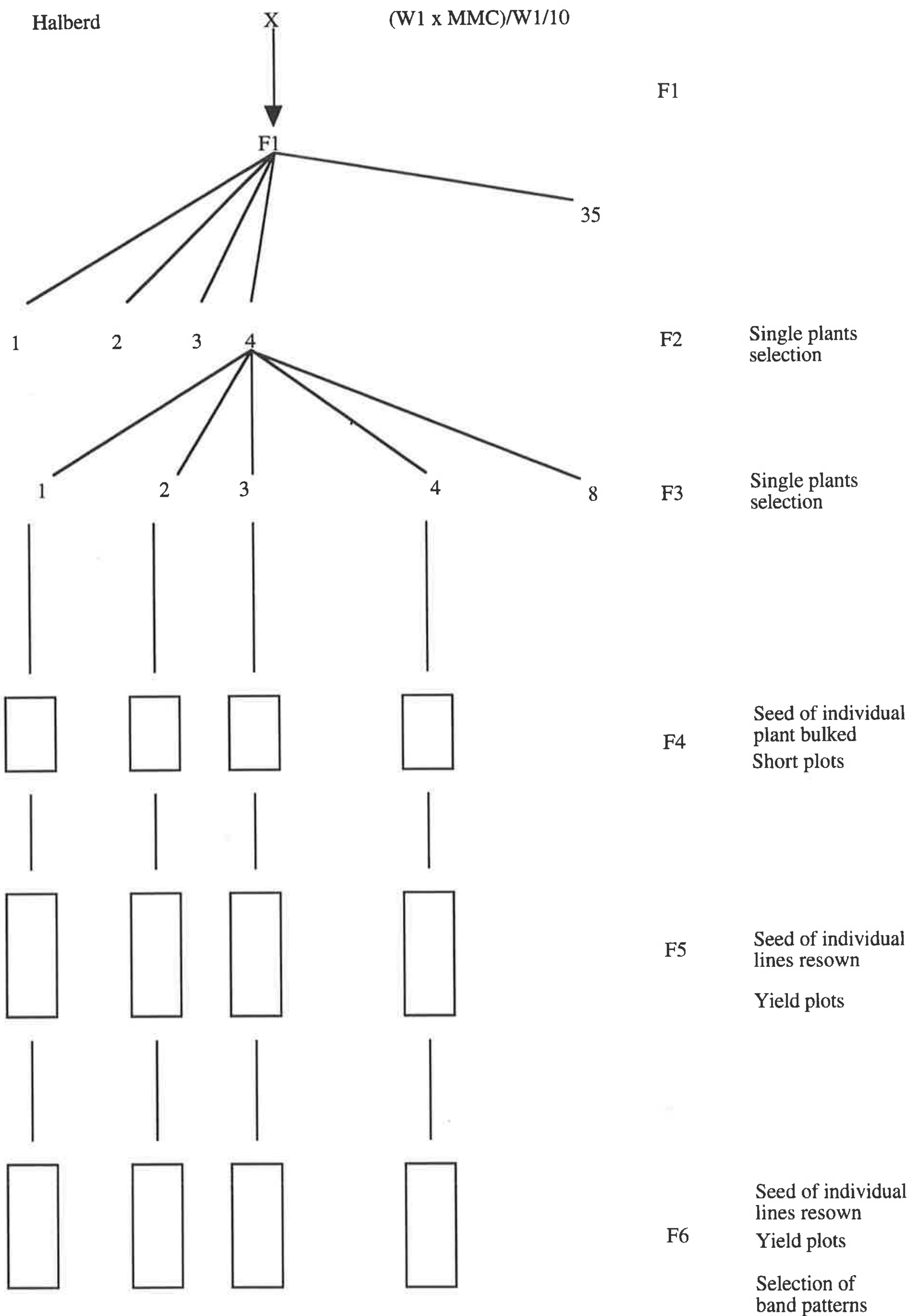
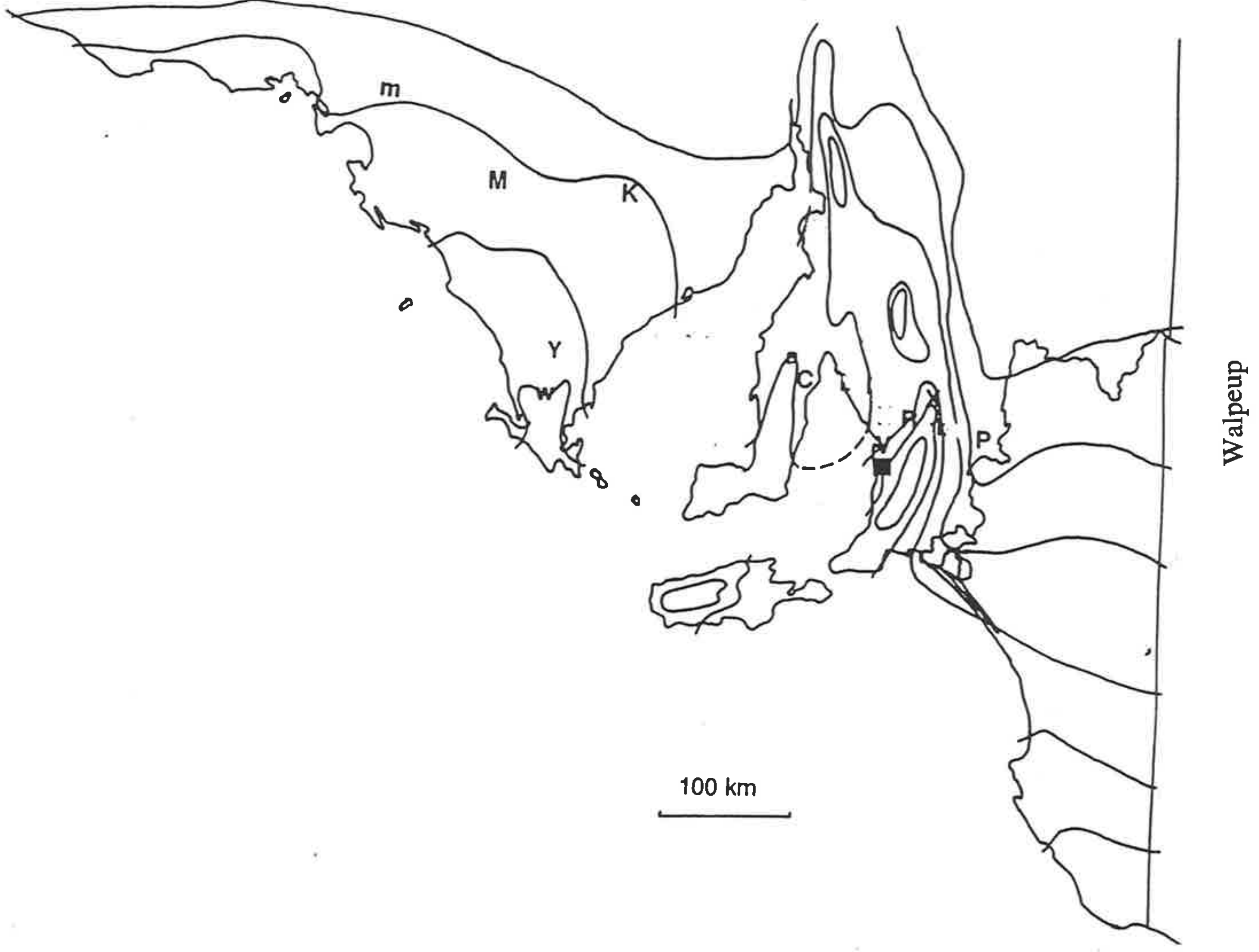

Halberd
X
(W1 x MMC)/W1/10
F1
F1
35
1
2
3
4
F2
Single plants
selection
1
2
3
4
8
F3
Single plants
selection
F4
Seed of individual
plant bulked
Short plots
F5
Seed of individual
lines resown
Yield plots
F6
Seed of individual
lines resown
Yield plots
Selection of
band patterns

Figure 4.2 Location of field sites in the regions of South Australia. Latitude, longitude and height above sea level of the location are as follows:

Location	Code in map	Latitude	Longitude	Elevation
Winulta	C	34° 25´28˝S	137° 54´56˝E	22 m
Minnipa (a)	M	-	-	-
Palmer	P	34° 51´16˝S	139° 09´34˝E	190 m
Yeelanna	Y	34° 08´31˝S	135° 43´42˝E	88 m
Roseworthy	R	34° 31´36˝S	138° 41´18˝E	114 m
Walpeup (b)	-	-	-	-
Lowbanks	-	34° 10´40˝S	139° 58´50˝E	25 m
Kimba	K	33° 08´36˝S	136° 25´03˝E	263 m
Kapunda	X	34° 20´34˝S	138° 54´58˝E	245 m
Mudamuckla	m	34° 07´55˝S	133° 42´30˝E	15 m

(a) located on Eyre Peninsula
(b) located in Victoria


m
M
K
Y
W
C
P
100 km
Walpeup

about 20 cms. The Roseworthy site was on the Roseworthy campus of the University of Adelaide located 6 km west of the Roseworthy with a loamy soil over a clay loam subsoil. The Winulta site is located 100 km north west of the Adelaide on Mr. B. Correll's property. The soil was a shallow red loam over an alkaline clay B horizon. The Minnipa site is located 500 km north west of Adelaide, on the Minnipa Research Station of Primary Industries South Australia. The soil was red sand loam over a clay loam B horizon. The Yeelanna site is located 200 km west of Adelaide, on the late Mr A.Glover's property. The soil was shallow grey sandy loam over a yellow sodic clay.

Field experiments were conducted in conjunction with experiments of the Waite Agricultural Research Institute wheat program and therefore were sown to the same layout and at the same time.

The standard field arrangement (Fig 4.3) consisted of 15 bays, each 6m from the midpoint of adjacent path ways so that the experimental site was 90m wide and entries in experiments were arranged by multiples of 15. Pathways of 1.8m were sprayed out between bays to allow for automatic cleaning of harvesters between plots to prevent contamination of samples. Individual plots were arranged within bays and consisted of four drill rows 15 cm apart. Plots were separated by one missing row, or 30 cm. The total length of each plot was 4.2m and the sown area was 4.2m x 60 cm or 2.52 m^2. Plots were sown at the value of 30 g $plot^{-1}$ (sown over the 6m length) or approximately 60 kg/ha^{-1}. The plots were sown by a modified 14 row combine with three plots being sown simultaneously.

Management of field experiments, including cultivation methods, date of sowing and fertilizer and herbicide application, was in accordance with the local district practices. Normally plots were sown after the opening rain and a short period for ground preparation and weed control. The plants were watered naturally by rainfall with no artificial irrigation. Phosphatic fertilizer only was applied at a rate of approximately 14Kg P/ha. Grain of individual plots was harvested at maturity, using harvesters designed and built at the Waite Agricultural Research Institute.

Rainfall

Monthly and average rainfalls of each location at which field experiments were conducted in 1992 and 1993 are presented in Table 4.1. These show that 1992 was a year of markedly above average rainfall, especially during the late spring and early summer from anthesis to harvest.

Figure 4.3 Experimental field plot layout. The area of each individual plot was 2.52 m^2 (4.2 x 0.6 m), each plot consisted of four drill rows 15 cm apart, with 30 cm between plots. A seeding rate of approximately 60 kg ha^{-1} (30 g per plot) was sown in each plot. This diagram was extracted from Paull (1990).

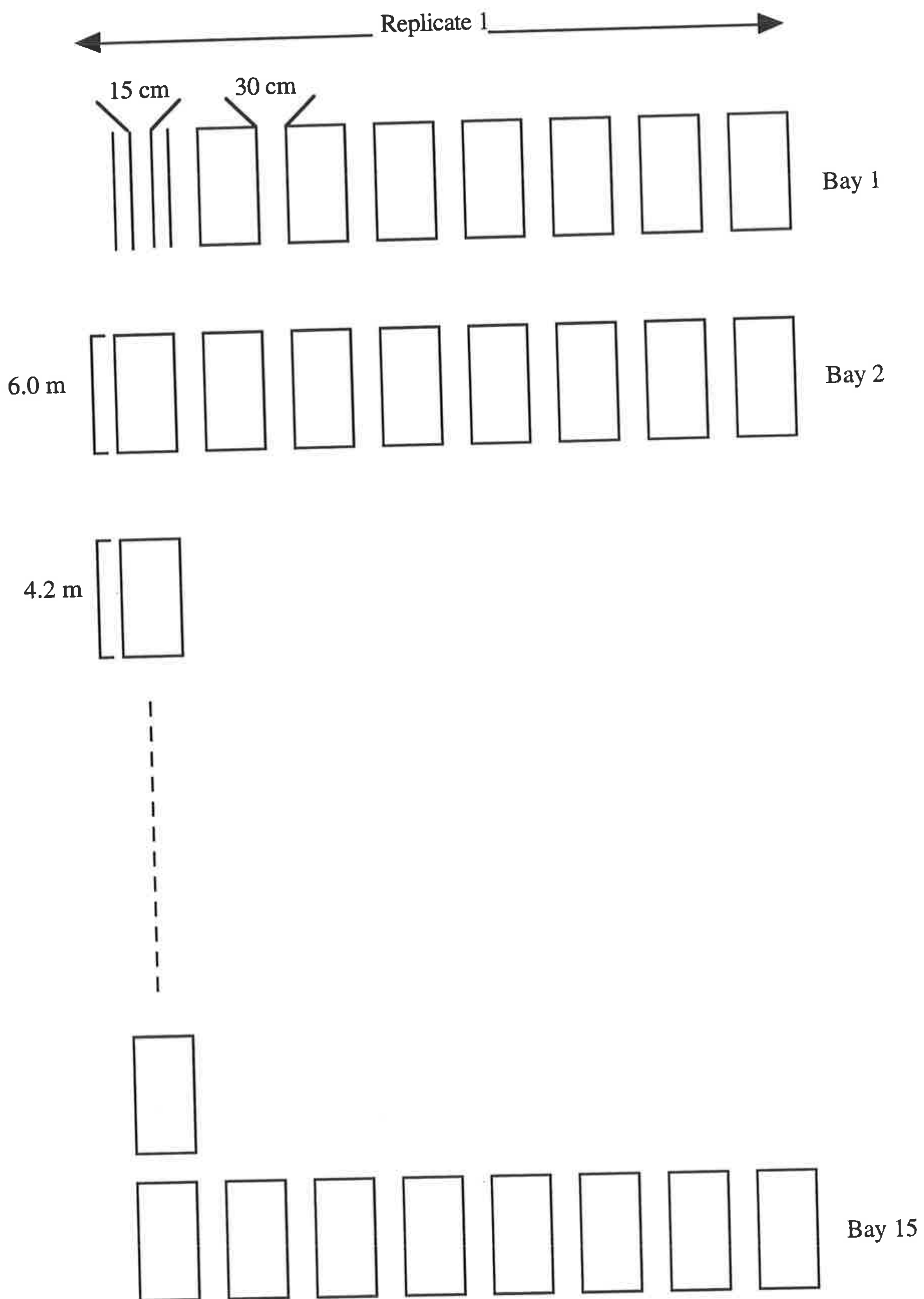

Replicate 1
15 cm
30 cm
Bay 1
6.0 m
Bay 2
4.2 m
Bay 15

Table 4.1 Monthly and total rainfall (mm) recorded at Winulta, Minnipa, Palmer, Roseworthy and Yeelanna. Source: Bureau of Meteorology, Kent Town, South Australia

Months	Winulta				Minnipa				Palmer				Yeelanna				Roseworthy			
	1991	1992	1993	1994	1991	1992	1993	1994	1991	1992	1993	1994	1991	1992	1993	1994	1991	1992	1993	1994
January	38.8	4.0	17.4	52.4	4.3	0.4	77.2	1.5	13.8	4.2	37.2	3.4	3.0	0.6	29.0	8.4	23.2	0.0	52.8	24.2
February	0.0	7.0	4.4	0.0	0.0	18.6	5.6	11.4	0.0	34.4	5.0	10.8	0.0	3.4	3.4	7.0	0.0	9.8	10.4	12.0
March	9.8	52.8	0.0	?	5.4	41.1	3.2	0.0	0.6	54.4	1.0	0.0	3.8	41.8	3.6	0.0	5.8	57.2	11.4	0.0
April	31.8	30.0	3.0	2.0	30.2	39.8	0.4	0.4	30.2	52.0	0.0	4.8	22.2	77.8	2.8	1.7	39.8	29.0	3.6	12.2
May	15.6	40.4	21.4	16.4	13.9	38.8	21.9	12.5	8.8	38.4	15.8	32.0	17.6	50.4	20.8	31.2	10.6	55.6	24.4	21.8
June	86.0	42.8	79.2	31.4	97.6	27.2	30.8	36.6	75.2	20.4	25.2	?	54.0	57.8	43.6	66.0	118.2	56.0	44.2	77.8
July	25.6	21.2	8.2	40.0	35.1	22.4	27.6	34.7	60.2	38.4	56.8	?	49.0	45.7	57.2	27.0	60.2	23.8	36.8	34.2
August	54.2	60.0	?	39.0	55.6	73.0	31.5	9.0	75.4	118.4	29.0	?	64.2	110.5	65.6	20.0	74.0	102.1	35.2	15.6
September	30.8	86.8	?	55.8	58.0	88.4	33.8	14.8	48.0	101.8	31.8	34.0	33.6	94.9	48.0	9.4	54.0	142.4	77.4	15.2
October	5.6	67.2	12.8	51.6	1.8	70.0	72.6	20.7	1.6	71.2	28.0	28.2	4.2	67.3	72.4	27.3	4.0	81.2	93.0	27.8
November	27.4	38.4	13.4	18.8	34.9	48.0	15.4	11.4	18.4	77.0	13.4	15.4	13.4	52.2	27.4	12.4	35.0	72.2	21.4	44.6
December	1.4	63.0	8.0	35.8	0.0	124.5	21.0	7.2	4.0	208.8	22.0	7.4	0.0	139.9	10.6	12.0	0.8	75.8	39.4	8.8
Total	327.0	513.6	?	?	336.8	592.2	341.0	160.4	336.2	819.4	265.2	450.0	265.0	742.3	384.4	222.4	425.6	705.0	450.0	294.2

Experimental design

Experimental material could be grouped into 32 different band patterns with 54 lines in 1992 and 59 in 1993 after seed multiplication of five additional lines where there was insufficient seed available for the 1992 experiments. A randomised complete block design was used with five and three replicates for 1992 and 1993 respectively. There were different number of Recombinant Inbred Lines (RILs) within each band pattern designated here as genotypes. These materials are listed in Table 4.2.

4.2.3 Field observations

Maturity

Since the work of Schlehuber *et al.* (1967) has shown a correlated inheritance between maturity and quality parameters, attention was paid to this aspect. For this purpose the decimal code for the growth stages of cereals compiled by Tottman *et al.* (1979) was used to record the time of anthesis.

Weather damage

The Falling Number test has been accepted world-wide for the determination of α-amylase activity and sprouting damage of cereals. It has been shown that only 5% heavily sprouted grain mixed with 95% sound grain can make the grain unacceptable for bread baking (Perten, 1985). Since the material grown at Winulta in 1992 was exposed to excessive rain during harvest, to avoid any mistakes in quality testing it was decided to determine Falling Number values and use the data as a covariate factor for interpretation of quality results. The Falling Number was measured as time in seconds for a stirrer to fall a measured distance through a hot starch suspension (Stone *et al.,* 1994).

A representative sample of 30g ~~were~~ was taken from each line and ground in a Falling Number Laboratory Mill 3100 and mixed thoroughly. Subsequently 7g of ground sample was placed into a viscometer tube containing 25 ml distilled water and shaken vigorously for 30 sec to obtain a uniform suspension. The viscometer tube together with the viscometer plunger was placed into the Falling Number apparatus model 1600 containing a boiling water bath. The hot starch suspension was stirred for 1 m and then the stirrer was automatically released from its top position and allowed to sink in the heated flour/water suspension. The time taken for the plunger to fall the set distance (dependent on the viscosity of a suspension) was recorded as the Falling Number.

Table 4.2 The genetic constitution of the field experimental material from the [Halberd x (Wl x MMC)/Wl/10] cross grown at different sites in two years (1992-1993).

Genotype	Glutenin subunit loci						Number of lines	
	HMW (Glu-1)			LMW (Glu-D3)				
	1A	1B	1D	A3	B3	D3	1992	1993
1	1	17+18	5+10	c	c	c	2	2
2	1	17+18	5+10	c	b	b	4	4 †
3	1	17+18	2+12	c	c	c	1	1
4	1	17+18	2+12	c	b	c	4	5 †
5	1	20	5+10	e	c	c	3	3
6	1	20	5+10	c	c	c	1	1
7	1	20	5+10	c	c	b	2	2
8	1	20	2+12	e	c	c	1	1
9	1	20	2+12	e	c	b	2	2
10	1	20	2+12	e	b	c	2	2
11	1	20	2+12	e	b	b	2	2
12	1	20	2+12	c	c	c	3	3 †
13	1	20	2+12	c	c	b	3	3
14	1	20	2+12	c	b	b	1	1
15	2*	17+18	5+10	e	c	c	1	1
16	2*	17+18	5+10	c	b	c	1	1
17	2*	17+18	2+12	e	c	c	1	1
18	2*	17+18	2+12	e	c	b	1	1
19	2*	17+18	2+12	c	c	c	1	1
20	2*	17+18	2+12	c	c	b	1	1
21	2*	17+18	2+12	c	b	c	1	4 †
22	2*	17+18	2+12	c	b	b	1	1
23	2*	20	5+10	e	c	b	2	2
24	2*	20	5+10	c	c	c	2	2
25	2*	20	5+10	c	c	b	2	2
26	2*	20	5+10	c	b	c	1	1
27	2*	20	5+10	c	b	b	1	1
28	2*	20	2+12	e	c	c	2	2
29	2*	20	2+12	e	b	b	1	1
30	2*	20	2+12	c	c	c	3	3 †
31	2*	20	2+12	c	c	b	1	1
32	2*	20	2+12	c	b	b	0	1
Total							**54**	**59**

† These lines were chosen for extensograph testing (see section 4.3.4 for more details) in 1993.

4.2.4 Quality testing

Milling for extensograph tests

Tempering or adding water to wheat kernels to achieve a particular moisture content aids in the milling process and prevents the bran of the wheat kernel from becoming friable, resulting in excessive bran specks in the flour. The amount of water added is determined by the hardness of the wheat. The water (Ha in ml) required for tempering was calculated according to the following formula:

$$Ha = M0 \times \frac{Tm - Gm}{100}$$

The grain moisture content of the sample (Gm) was measured using a Moisture Meter (TF933A, Marconi Instruments Ltd, England) on a grain sample ground in a coffee grinder (ESM DIE KRONE). Cleaned sub-samples of each plot (M0=200-300g) were conditioned to 14% (Tm=14%) in a sealed plastic bag overnight before being milled on a Brabender Quadrumat Junior mill with 1.0mm screen. The flour extraction averaged 65% with a range of 60-70%.

Milling for SDS sedimentation and Falling Number test

Untempered wheat samples (11-12% moisture content) of approximately 30 g were milled on a hammer mill (Falling Number 3100) equipped with a 0.8 mm screen and wholemeal flour was obtained.

NIR measurements

Total protein concentration (%) and grain hardness as Particle Size Index (PSI) were determined by Near-Infrared Reflectance Spectroscopy-NIRS (Technicon Infra-Alyser 450) at the Cereals Laboratory of the South Australian Research and Development Institute. NIR analysis was calibrated by a Kjeldahl analysis for a range of protein concentrations.

SDS-sedimentation test

The SDS-sedimentation test was performed to measure the gluten strength of the samples and followed the procedure outlined by Axford *et al.* (1979). The solutions used (all prepared with deionized water) were:

(1) Indicator: 10 mg bromophenol blue added to 1 L of water to give visual differentiation between the sediment and liquid layers.

(**2**) SDS reagent: 20 ml of stock lactic acid solution (one part 88% analar grade lactic acid plus eight parts water) added to 1 L of a 2% (wt/vol) aqueous solution of sodium dodecyl sulphate.

Six gram flour samples were mixed with 50 ml of water in 100 ml measuring cylinders. The material was dispersed by rapid shaking for 15 seconds at 0 min, 2 mins and 4 mins after mixing. Immediately after the last shaking, 50 mls of the SDS-lactic acid reagent was added and mixed by inverting the cylinder four times again at 2, 4 and 6 mins. Each test batch of ten samples include a sample from a standard stock flour. This procedure enabled minor variations in the test procedure, e.g. temperature and the vigour of shaking to be compensated for.

Extensograph tests

The extensograph tests was performed according to the procedure described by Gupta *et al.* (1989). Dough was prepared by mixing 50 g of flour in Brabender Farinograph with the required amount of distilled water (A preliminary farinogram had been conducted to determine the amount of water required for the Extensograph test) at 30°C containing 1 g NaCl, to give a final consistency of 500 Brabender Units (BU) after 5 m of mixing.

A measured amount (75 g) of this dough was mechanically formed into a ball, then rolled and stored in a dough fermentation cabinet for 45 m at 30°C. The dough was then stretched on the extensograph with a constant speed until the breaking point was reached. The maximum resistance to extension (Rmax) in BU was obtained by measuring the maximum height of the Extensograph curve (extensogram) while the extensibility in cm was given by the length of the extensogram.

4.2.5 Extraction and electrophoretic fractionation of proteins

At the time of this experiment the glutenin subunit and gliadin banding patterns of the parents and derived progeny had been identified by Gupta (pers. comm.). However as reported elsewhere (Gupta *et al.,* 1994a), there were low levels of heterogeneity in the protein composition of the derived lines, therefore 10 seeds of each line from the field harvested material were analysed for gliadin and glutenin subunits by electrophoretic procedures (1D SDS-PAGE) as described previously (see Chapter 3).

4.2.6 Statistical analysis

To relate the different quality variables with the glutenin subunits, an unbalanced analysis was carried out which takes into account the numerical difference in occurrence of glutenin subunit combination (non-orthogonality of the genotypes). That is, all six loci were fitted in the regression model which allowed the detection and measurement of

interloci interactions. It was assumed that effects at different loci are additive, and if there was some interaction, that effects were between pairs of loci, rather than of greater complexity. This form of analysis of variance, which was advised by Dr. J. Wood from CSIRO, Canberra has also been reported by several other researchers (Gupta *et al.*, 1994a; Kolster *et al.*, 1991; Schepers *et al.*, 1993).

Since the extensograph test is very time consuming, only two replications of 19 lines (designated by † in Table 4.2) harvested from the 1993 experiment were chosen from the results of SDS-sedimentation analysis for extensograph tests and further analysis. The same statistical method and package (Genstat 5) were employed to analyse this experimental data. Analyses were carried out on data from different sites separately as well as together (Pooled).

To evaluate, the linkage between genes controlling different aspects of bread making quality, a chi-square test was used. The linear correlation coefficient were also calculated between parameters to find relationships between them.

4.3 Results

4.3.1 Genotypic constitution

The parents showed allelic variation at all six glutenin loci and the three linked gliadin groups located on group 1 chromosomes, as summarised in Table 4.3.

Table 4.3 Allelic differences between parents for gluten proteins and the location of these genes.

Protein class	locus	Allelic variants for proteins in parents	
		(Wl x MMC)/Wl/10	Halberd
LMW glutenin	*Glu A3*	*c*	*e*
	Glu-B3	*b*	*c*
	Glu-D3	*b*	*c*
HMW glutenin	*Glu-A1*	2*	1
	Glu-B1	17+18	~~20~~ 7+9 or 20
	Glu-D1	2+12	5+10
Gliadins †	*Gli-A1*	*c*	*e*
	Gli-B1	*b*	*c*
	Gli-D1	*b*	*c*

†=The gliadin bands controlled by *Gli-A1*, *Gli-B1* and *Gli-D1* have been designated with the same symbols as the *Glu-A3*, *Glu-B3* and *Glu-D3* loci to show their tight genetic linkages.

Electrophoretic profiles of glutenin subunits and gliadins for each parent are shown in Fig 4.4. Based on the segregation behaviour of the low molecular glutenin subunits and gliadins in the lines analysed in this study and elsewhere (Chapter 3), it was concluded that LMW glutenin loci *Glu-A3*, *Glu-B3* and *Glu-D3* could be more easily screened by their linked gliadin bands. Based on these linkage combinations, only a few seeds especially for low molecular glutenin alleles showed anomalies between this study and the identification by Gupta *et al.* (1994a). It seems that the occasional differences observed in the band patterns could be accounted for by a low level of impurity rather than by the improved method of separation by 1D-SDS-PAGE as described before (Chapter 3). Consequently the same band patterns as those of Gupta *et al.* (1994a) were accepted as being correct. Based on this analysis 32 different homozygous genotypes out of the 64 different allelic combination expected from six segregation loci of glutenin subunit (*Glu-1*, *Glu-3*) were identified (Table 4.2).

4.3.2 Weather damage analysis

The unfavourable weather conditions at Winulta, resulting in rain damage in 1992, provided an opportunity to evaluate the relationship between Falling Number value with glutenin subunits. To test this hypothesis, the mean Falling Number values of RILs were determined for five replications. Two distinct Falling Number classes were identified, based on the description of Lukow *et al.* (1989). As shown in Fig 4.5 the bimodal frequency distribution had a low point at a Falling Number value of 420 therefore the random lines were grouped into two classes:

1) lines with a mean Falling Number value less than 420.

2) lines with Falling Number value greater than 420.

The chi-square value of 32.9 (11 degrees of freedom, and significant at the 5 percent level) from the 2 x 12 contingency table (Table 4.4) indicated that Falling Number and glutenin subunit composition were related. The mean Falling Number value for Halberd and (Wl x MMC)/Wl/10 showed that Halberd had a higher Falling Number than the other parent (394 sec vs 347 sec). Therefore it appeared that one or more of the bands of Halberd (1, 20, 5+10, *e*, *c*, *c*) could be linked with a gene conferring a higher Falling Number value than the other parent. To test for this association, lines with the Halberd pattern type were grouped for analysis. The total chi-square value of 11.3 (5 d.f) obtained from this analysis was significant at the 5 percent level of significance.

Figure 4.4 A and B. SDS-PAGE patterns of parents (P1) Halberd and (P2) (Wl x MMC)/Wl/10.

A. 1D-SDS-PAGE patterns of high molecular and low molecular weight glutenin subunits. The alleles under study have been labelled and the arrow indicates the direction of protein migration.

B. 1D-SDS-PAGE patterns of unreduced protein of the same seeds extracted by using 70% (v/v) ethanol.

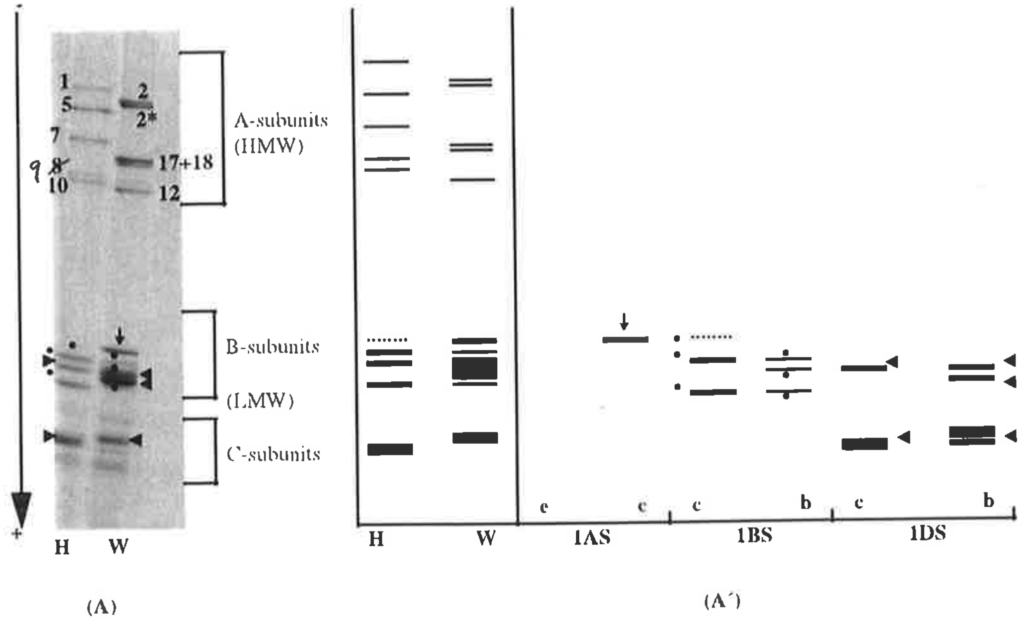
Symbols used in the Figures

→ Bands controlled by 1AS

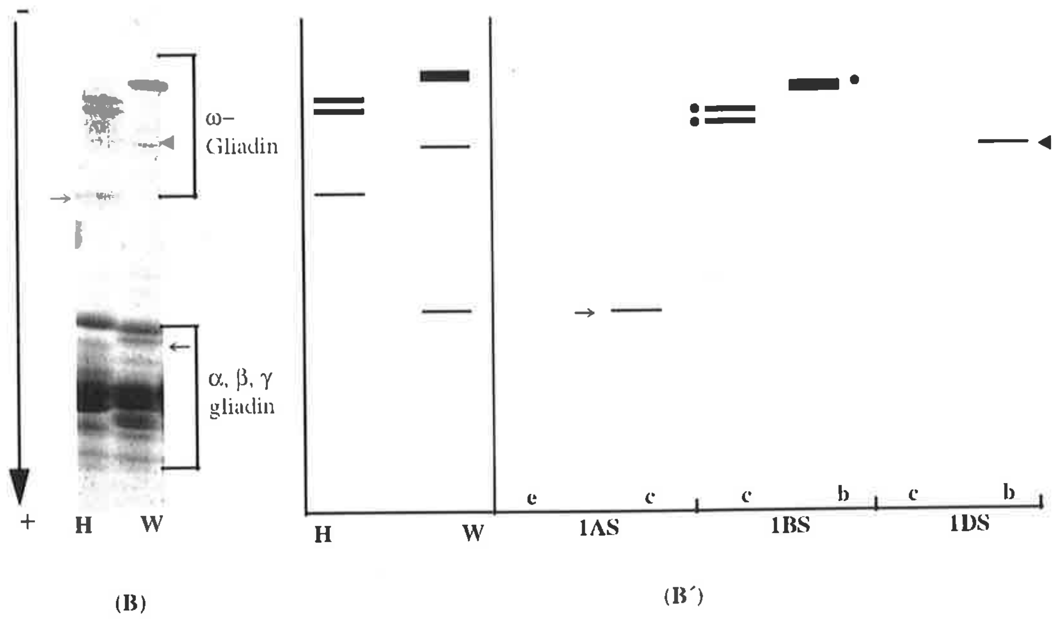
● Bands controlled by 1BS

► Bands controlled by 1DS

The gliadin bands (B) have also been marked with the same symbols as the *Glu-A3*, *Glu-B3* and *Glu-D3* loci to show their tight genetic kinkages (see Chapter 3, section 3.3.1)



(A) (A´)



(B) (B´)

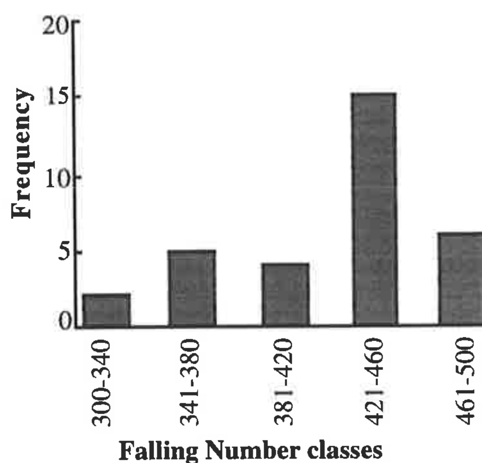


Figure 4.5 Frequency distribution of Falling Number for RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at Winulta with five replications in 1992. These lines were grouped to 31 genotypes and classified into a bimodal distribution (<420 and >420) according to their mean Falling Number values.

To determine which glutenin subunit loci were more closely associated with Falling Number value, the chi-square value for each of the glutenin loci were calculated separately (Table 4.4). The results showed that all the HMW glutenin loci were significantly associated with Falling Number values but none of the *Glu-3* loci were associated.

Relationship between allelic variation of glutenin subunits and Falling Number value

The 32 different genotypes that were rain damaged at Winulta in 1992 were subjected to analysis of variance to test whether the variation in glutenin subunits was associated with variation in Falling Number value. This test showed a highly significant difference between genotypes (P<0.001).

The Falling Number values ranged between 306 and 484 among the genotypes. Significant differences were observed between alleles at the *Glu-1* loci, but there were no significant differences between alleles of LMW glutenin subunits. As shown in Fig 4.6, progeny with HMW glutenin subunit band 20 had a higher Falling Number value than the 17+18 type (443 vs 414). Similarly, HMW glutenin subunit band 1 at *Glu-A1* showed a higher mean Falling Number value than 2* (434 vs 405). This was also observed for glutenin subunit bands 5+10 at *Glu-D1* compared to its counterpart allele (i.e 2+12) (444 vs 413). These two latter subunit bands were also present in Halberd.

Table 4.4 Frequency distribution and χ^2 analysis of Falling Numbers for RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at Winulta with five replications in 1992. Data classified on the basis of individual alleles at *Glu-1* and *Glu-3* loci.

Allele at glutenin loci	Observed frequency		Expected frequency		Chi-square
	<420	>420	<420	>420	
Glu-A1					
1	45	108	54.3	98.6	**5.3 ***
2*	57	77	47.6	86.4	
Glu-B1					
17+18	47	55	32.7	69.3	**14.3 ***
20	45	140	59.3	125.7	
Glu-D1					
5+10	27	79	38.4	67.6	**7.3 ***
2+12	77	104	65.6	115.4	
Glu-A3					
c	74	129	72.1	130.8	**0.2**
e	28	56	29.8	54.1	
Glu-B3					
c	54	118	61.7	110.3	**3.2**
b	49	66	41.3	73.7	
Glu-D3					
c	63	96	56.5	102.5	**2.6**
b	39	89	45.5	82.5	

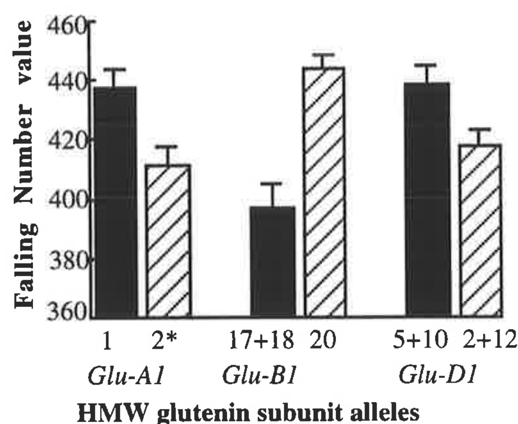


Figure 4.6 Mean value of Falling Number for RILs derived from [Halberd x (Wl x MMC)/Wl/10] carrying specific HMW glutenin alleles. Data from the field experiment with five replications at Winulta in 1992 which were subjected to rain damage before harvesting.

The high Falling Numbers values related to subunit bands 1 and 20, were associated with weaker dough strength (see section 4.3.4), shown by the negative correlation calculated between Falling Number and SDS-sedimentation test (r=-0.14)

The interloci interactions (*Glu-B1* x *Glu-B3*, *Glu-A3* x *Glu-B3* and *Glu-A1* x *Glu-D3*) showed significant effects on Falling Number values (Table 4.5). Subunit bands 17+18 in the presence of allele *c* at *Glu-B3* gave the lowest Falling Number values (Table 4.6). Likewise subunit band 2* at *Glu-A1* in the presence of allele *b* at *Glu-D3* gave a lower Falling Number than when the *c* allele was present. With respect to the allelic combinations at *Glu-A3* and *Glu-B3*, the lowest Falling Number value was with the combination of *c* and *b*, both associated with high dough strength (see section 4.3.4).

Table 4.5 Mean square and statistical significance for Falling Number value of 59 RILs derived from [Halberd x (Wl x MMC)/W1/10] grown at Winulta with five replicates in 1992. These lines were subjected to rain damage before harvesting.

Source of variance	d.f	Mean square
block	4	29478 ***(a)
Glu-A1	1	46584 ***
Glu-B1	1	140562 ***
Glu-D1	1	24530 *
Glu-A3	1	492 ns
Glu-B3	1	6175 ns
Glu-D3	1	2115 ns
Glu-A1 x Glu-B1	1	2111 ns
Glu-A1 x Glu-D1	1	7632 ns
Glu-B1 x Glu-D1	1	131 ns
Glu-A1 x Glu-A3	1	11297 ns
Glu-B1 x Glu-A3	1	7841 ns
Glu-D1 x Glu-A3	1	1078 ns
Glu-A1 x Glu-B3	1	1 ns
Glu-B1 x Glu-B3	1	17524 *
Glu-D1 x Glu-B3	1	9013 ns
Glu-A3 x Glu-B3	1	15851 *
Glu-A1 x Glu-D3	1	30417 ***
Glu-B1 x Glu-D3	1	5286 ns
Glu-D1 x Glu-D3	1	81 ns
Glu-A3 x Glu-D3	1	2134 ns
Glu-B3 x Glu-D3	1	2447 ns
Residual	261	3933
Total	286	

a=see abreviation list

Table 4.6 Interaction effects of alleles at different loci of glutenin subunits on Falling Number values of RILs from [Halberd x (Wl x MMC)/Wl/10] at Winulta subjected to rain damage in 1992.

Loci combination		Number of samples	FN	SEM†
Glu-B1x Glu-B3				
17+18	*c*	40	414.2	12.2
17+18	*b*	72	387.6	9.7
20	*c*	131	443.6	5.5
20	*b*	44	445.1	6.2
Glu-A1 x Glu-D3				
1	*c*	83	431.3	9.1
1	*b*	70	445.6	6.0
2*	*c*	79	414.9	7.7
2*	*b*	55	407.2	10.3
Glu-A3 x Glu-B3				
c	*c*	109	439.9	6.2
c	*b*	92	398.2	8.1
e	*c*	62	431.1	9.2
e	*b*	24	452.3	8.5

† Standard error of mean

4.3.3 Hardness survey

Hardness is a very important parameter which can be used to categorise a wheat cultivar for a specific end-use purpose (Williams, 1986), so particle size index (PSI) has been widely used to measure grain hardness. Grain hardness is related to other parameters such as starch damage and water absorption (Williams, 1967). In this survey the correlation between the PSI data from Winulta in 1992 and quality attributes of dough were calculated. Although both parents are hard, the rain damage made them somewhat softer so that the Halberd parent had a PSI of 23.2 and (Wl x MMC)/Wl/10 22.7. The derived RILs following the guidelines of Williams and Sobering (1986) were classified into two groups:

1) lines with range from 20-25.

2) lines with range from 26-30 .

Frequency distribution of PSI values for each of the lines based on their glutenin subunits was analysed with chi-square test. The chi-square value of 54.1 from the 2 x12

contingency table (11 degrees of freedom, at 5 percent level) indicated that PSI and glutenin subunits are linked to each other.

To test which of the glutenin loci were more closely associated to grain hardness, the chi-square for each glutenin locus was calculated (Table 4.7). The results showed that among the *Glu-1* loci, the only significant association was observed for *Glu-A1*, while all the *Glu-3* loci were significantly associated with hardness. The significant association of two non-homoelogous loci *Glu-A1* and *Glu-A3* with grain hardness indicate that a gene for grain hardness may be located on chromosome 1A, in agreement with Carrillo *et al.* (1990) who reported that group 1 wheat chromosomes affect hardness.

4.3.4 Genetic and environmental contribution to rheological properties of dough

Effect of field site on dough properties

The flour protein concentrations of the RILs were significantly different ($P<0.05$) at two of the sites with average values 12.6 at Winulta and 12.0 at Minnipa (Table 4.8). Similarly, the SDS-sedimentation volumes differed ($P<0.05$) with a mean of 46.7 at Winulta and 49.9 at Minnipa. It does not appear that the differences in the SDS-sedimentation can be assigned to differences in the protein content, because no correlation was found between them (see section 4.3.6, Table 4.17). The PSI between the RILs was significantly different at the two sites ($P<0.001$) with mean values for Winulta and Minnipa being 24.8 and 23.1 respectively.

Because of the rain damage at Winulta, separate analysis of variances were also carried out for each of these sites separately (Table 4.9). There were highly significant differences in the SDS-sedimentation and PSI values amongst F_7 families at both locations. However the differences between families were not significant for protein concentration at Winulta. The absolute values of variance (F value) for SDSS and PSI were higher at Winulta than the other sites and this can be accounted for by the rain damage. This shows that the SDS-sedimentation test has an ability to give useful information about protein quality even under conditions of rain damage as shown by Axford *et al.* (1979). The larger differences between genotypes for PSI at Winulta can also be attributed to rain damage, as reported by Nemeth *et al.* (1994). They suggested that when wheat becomes damaged during a wet harvest, α-amylase activity is increased and grains become softer.

Table 4.7 Frequency distribution of grain hardness for RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at Winulta with five replications in 1992. Data classified on the basis of individual alleles at the *Glu-1* and *Glu-3* loci.

Allele at glutenin loci	Particle Size Index				Chi-square
	Observed frequency		Expected frequency		
	<25	>25	<25	>25	
Glu-A1					
1	112	40	96.9	55.1	**13.9 ***
2*	69	63	84.1	47.9	
Glu-B1					
17+18	78	33	70.7	40.2	**3.3**
20	103	70	110.2	62.7	
Glu-D1					
5+10	62	43	66.9	38.1	**1.5**
2+12	119	60	114.1	64.9	
Glu-A3					
c	120	80	128.2	71.8	**4.9 ***
e	62	22	53.8	30.2	
Glu-B3					
c	88	82	107.2	61.6	**25.9 ***
b	93	21	72.6	41.3	
Glu-D3					
c	94	67	102.6	58.4	**4.6 ***
b	87	36	78.4	44.6	

Table 4.8 Mean square and statistical significance of the quality parameters for RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at Winulta and Minnipa in 1992.

Source of variation	d.f	Mean square value for quality parameters[a]		
		SDSS	PC	PSI
Site	1	1086.9 *	38.6 *	299.1 ***
Residual (1)	6	120.3	4.1	6.7
Genotype	30	188.3 ***	0.6 **	18.3 ***
Genotype x site	30	8.9 ns	0.3 ns	2.8 ns
Residual (2)	364	10.0	0.3	2.7
Total	431			

a=see abbreviation list

Table 4.9 Mean square and statistical significance of quality parameters for 54 RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at two sites (Winulta and Minnipa) in 1992.

quality parameters	Winulta		Minnipa	
	F value	P	F value	P
Protein concentration	1.2	>0.05	1.9	0.005
SDS-sedimentation	13.4	<0.001	8.0	<0.001
Particle Size Index	5.3	<0.001	3.3	<0.001

The 54 different RILs harvested in 1992 with five additional lines (total of 59 lines) were sown again at three sites (Palmer, Roseworthy Campus and Yeelanna) in 1993 to evaluate whether the results were consistent between locations over different years. The analysis of variance of pooled data showed that differences between sites were not significant for SDS sedimentation, whereas a highly significant difference was observed for protein concentration (Table 4.10). In this analysis it was also observed that the interaction effects between site and genotypes were insignificant for SDSS and protein concentration. These similar trends observed for both years indicate that environmental factors are relatively unimportant for SDS-sedimentation so, a single SDSS value determination can predict dough strength for a number of growing environments.

Table 4.10 Mean square and statistical significance of the quality data for the 59 RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at three sites (Palmer, Roseworthy Campus and Yeelanna) in 1993.

Source of variance	d.f	SDS-sedimentation volume	Protein concentration
Site	2	678.4 ns (a)	1048.2 ***
Residual (1)	6	987.6	5.4
Genotype	31	778.5***	1**
Site x Genotype	62	79.1 ns	0.4 ns
Residual (2)	429	70.5	0.4
Total	530		

a=see abbreviation list

Glutenin subunits vs SDS-sedimentation volume

The effect of each of the six loci of glutenin subunits was first considered for each site to clarify the significance of the *Glu-1* and *Glu-3* loci on SDS-sedimentation volume (Table 4.11).

Genotypic differences in HMW subunit composition of glutenin

Allelic variation at the *Glu-A1* locus gave highly significantly different values for SDSS volume at both sites in 1992, whereas the difference between alleles at this locus was only significant at Palmer in 1993 with the HMW glutenin subunit 2* giving a higher SDSS volume than 1 (Fig 4.7). The alleles at *Glu-B1* consistently showed highly significant differences for all sites in both years of experiment. Thus the progeny possessing subunit bands 17+18 had a higher sedimentation value than those with subunit 20. With *Glu-D1*, a significant difference was only observed for data in 1992 when lines with subunit bands 5+10 gave a higher sedimentation value than those with subunits 2+12. However this allelic effect was quite different in 1993 (Fig 4.7) when subunit bands 2+12 showed a slightly higher SDSS value than 5+10.

The effects of variation in HMW glutenin allele composition on variation in SDS-sedimentation volume were mostly additive, however some interaction effects were also observed. Interactions between alleles at the *Glu-B1* and *Glu-D1* loci contributed significantly ($P<0.001$) to the variation in SDSS at Winulta (Table 4.11). When *Glu-B1* subunits 17+18 are present, *Glu-D1* subunit 2+12 had a higher sedimentation value than the other *Glu-D1* subunit. The *Glu-D1* allele encoding the subunits 5+10 had a significantly higher SDS-sedimentation compared to its allelic counterparts only in the presence of *Glu-B1* subunits 20 (Fig 4.8). This epistatic effect may be the result of the

Table 4.11 Variance ratio for SDS-sedimentation value of RILs derived from [Halberd x (WI x MMC)/WI/10] grown at two sites in 1992 and three sites in 1993. Analysis of variance was performed on the basis of six glutenin subunit loci fitted into the regression model to detect main and interloci effects.

Source	1992		1993		
	Winulta	Minnipa	Palmer	Roseworthy	Yeelanna
Glu-A1	25.4 ***(a)	20.5 ***	6.6*	1.0 ns	0.9 ns
Glu-B1	67.0***	22.5 ***	28.6***	14.6 ***	34.4 ***
Glu-D1	7.2***	6.3 *	2.2 ns	0.9 ns	0.4 ns
Glu-A3	105.7***	74.7 ***	27.7**	30.2 ***	41.1 ***
Glu-B3	58.8***	22.4 ***	38.2***	18.2 ***	26.4 ***
Glu-D3	2.7 ns	5.3 *	0.4 ns	2.5 ns	0.9 ns
Glu-A1 x Glu-B1	0.5 ns	0.4 ns	0.3 ns	0.0 ns	0.0 ns
Glu-A1 x Glu-D1	0.0 ns	0.4 ns	0.5 ns	0.9 ns	0.0 ns
Glu-B1 x Glu-D1	14.7 ***	1.3 ns	1.9 ns	0.0 ns	0.5 ns
Glu-A1 x Glu-A3	1.1 ns	0.6 ns	2.2 ns	0.5 ns	2.7 ns
Glu-B1 x Glu-A3	0.4 ns	0.5 ns	0.3 ns	0.3 ns	0.0 ns
Glu-D1 x Glu-A3	0.0 ns	0.4 ns	0.2 ns	1.7 ns	0.8 ns
Glu-A1 x Glu-B3	12.3 ***	11.3 ***	0.4 ns	0.5 ns	0.2 ns
Glu-B1 x Glu-B3	0.5 ns	3.7 ns	6.4 *	0.3 ns	1.6 ns
Glu-D1 x Glu-B3	12.9 ***	14.5 ***	1.2 ns	14.5 ***	9.0 ***
Glu-A3 x Glu-B3	19.8 ***	0.0 ns	0.3 ns	0.9 ns	0.2 ns
Glu-A1 x Glu-D3	4.5 *	8.3 ***	0.0 ns	0.3 ns	1.8 ns
Glu-B1 x Glu-D3	10.9 ***	4.1 *	0.0 ns	0.6 ns	3.4 ns
Glu-D1 x Glu-D3	2.7 ns	1.2 ns	1.5 ns	0.8 ns	1.4 ns
Glu-A3 x Glu-D3	2.4 ns	5.6 *	0.0 ns	0.5 ns	4.8 *
Glu-B3 x Glu-D3	0.2 ns	0.6 ns	1.4 ns	1.9 ns	3.0 ns

a=see abbreviation list

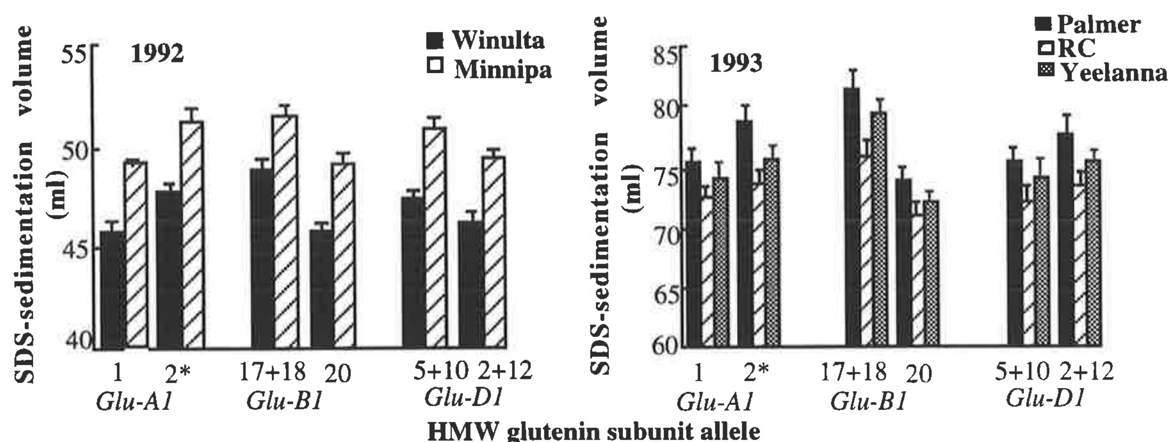


Figure 4.7 Mean effects of the three *Glu-1* loci for SDS-sedimentation in the RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at two sites in 1992 and three sites in 1993.

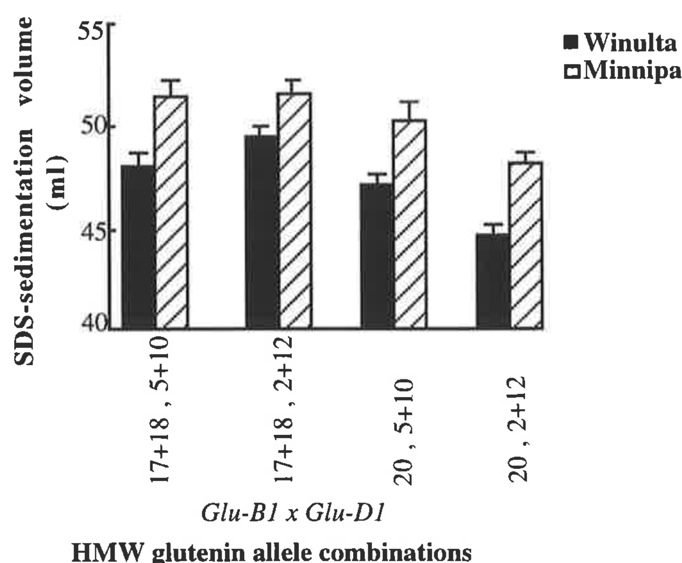


Figure 4.8 Mean value of SDS-sedimentation for RILs derived from [Halberd x (Wl x MMC)/Wl/10] carrying specific combinations of *Glu-B1* and *Glu-D1* alleles. The data is from Winulta and Minnipa in 1992.

rain damage. An additive effect between these two loci were also noted at Minnipa (Fig 4.8). The progeny with bands 17+18, 5+10, expected to have highest SDS-sedimentation volume, were lower than that observed in the combination of 17+18, 2+12, however this difference was not significant.

Genotypic differences in LMW subunit composition of glutenin

The alleles at *Glu-A3* and *Glu-B3* showed highly significant differences on SDSS at all sites in 1992 and 1993. However the effect of alleles at *Glu-D3* was only significant at

Minnipa (Table 4.11). It was also observed that the variance ratio for *Glu-A3* locus was mostly higher than the other two loci, indicating that the difference between alleles at *Glu-A3* was more pronounced than the others. Progeny with the *c* allele had a higher SDS-sedimentation volume than those with the null allele *e* (Fig 4.9). Comparison of the allelic effect at *Glu-A3* with that at *Glu-A1* showed that the effect of *Glu-A3c* on SDS-sedimentation volume was larger than that of 2* at *Glu-A1*, indicating that the LMW glutenin subunit has a substantial role in controlling dough strength.

Regarding the *Glu-B3* locus, the *b* allele gave a larger SDS-sedimentation volume than the *c* allele, in agreement with the results of Gupta *et al.* (1994a).

Although allelic variation at *Glu-D3* had a significant influence on SDS-sedimentation under normal environment conditions at Minnipa in 1992, their effects were not significant under the rain damage conditions of Winulta in 1992 and in the dry season of 1993.

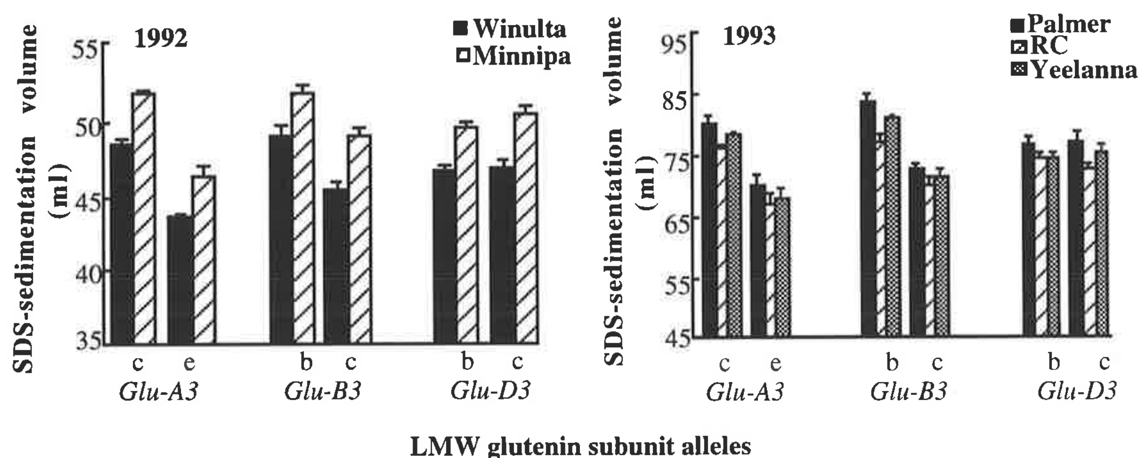


Figure 4.9 Mean value for alleles at the *Glu-3* loci for SDS-sedimentation in RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at two sites in 1992 and three sites in 1993.

The interaction effects between different loci of LMW glutenin subunits were only significant between *Glu-A3* and *Glu-B3* at Winulta, and *Glu-A3* and *Glu-D3* at Minnipa and Yeelanna (Table 4.11). These data suggest that their effects were mostly additive. It was observed that progeny carrying alleles (*c*, *b*) from both *Glu-A3* and *Glu-B3* loci gave the highest SDSS volumes, while those with the alleles (*e*, *c*) gave the lowest volume (Fig 4.10).

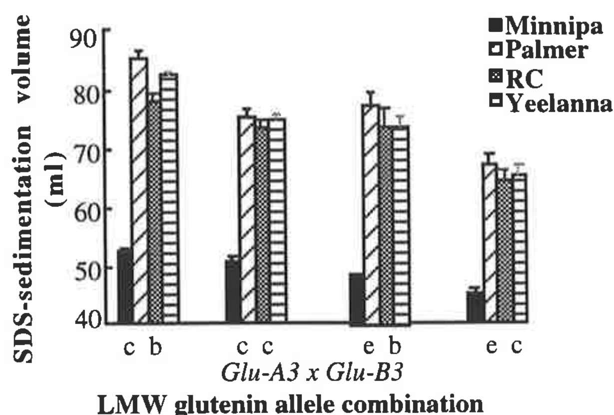


Figure 4.10 Mean value for interactions between alleles at the *Glu-A3* and *Glu-B3* loci for SDS-sedimentation in RILs derived from [Halberd x (Wl x MMC)/Wl/10]. Data from Minnipa in 1992 and three sites (Palmer, Roseworthy Campus and Yeelanna) in 1993.

HMW and LMW glutenin subunit interaction for SDS-sedimentation

The effect of allelic variation at *Glu-1* and *Glu-3* loci on SDS-sedimentation volume was not solely additive and some interactions were quite noted.

An additive effect between two non-homeologous loci *Glu-A1* and *Glu-A3* was noted for all sites in both years. As shown in Fig 4.11 the highest SDS-sedimentation was observed for the combination of 2* and *c* for all sites in 1993.

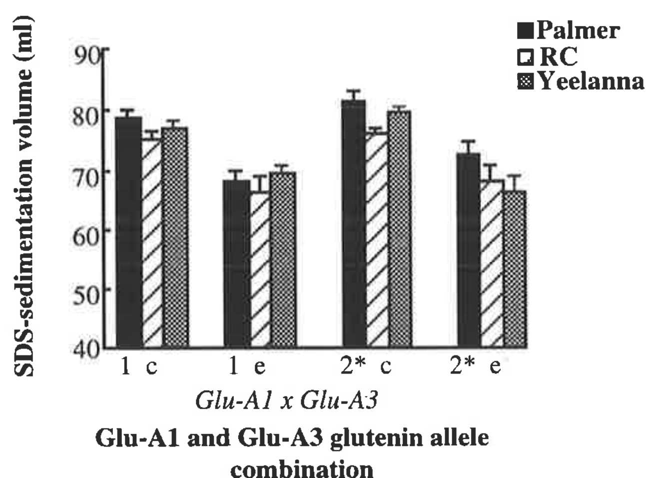


Figure 4.11 Mean value for alleles at the *Glu-A1* and *Glu-3* loci for SDS-sedimentation in RILs derived from [Halberd x (Wl x MMC)/Wl/10]. Data from the field experiment in 1993 at three locations.

Although there was an additive effect between the two non-homoelogous loci of *Glu-B1* and *Glu-B3* at most of the sites in both years (Table 4.11), however their interaction at Palmer was highly significant in 1993 (Fig 4.12).

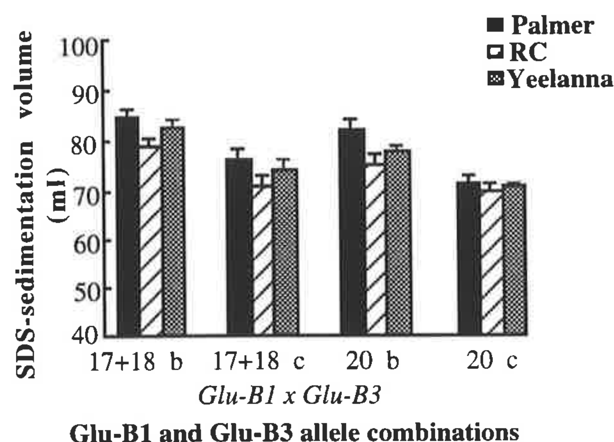


Figure 4.12 Mean value for alleles at *Glu-B1* and *Glu-B3* loci for SDS-sedimentation for RILs derived from [Halberd x (Wl x MMC)/Wl/10]. Data from field experiment in 1993 grown at three locations.

Glutenin subunits vs protein

A significant difference between alleles at the *Glu-B1* and *Glu-D3* loci on protein concentration was observed at two sites in 1993 (Table 4.12) but not in 1992. The comparison of alleles at *Glu-B1* showed that lines with subunits 17+18 consistently had a higher protein concentration than those with subunit 20 at Palmer (12.9 vs 12.7) and Yeelanna (10.6 vs 10.5) whereas for *Glu-D3*, there was no consistent effect between alleles *b* and *c*. At Roseworthy Campus with high level of protein concentration, the *b* allele gave higher protein than *c* (15.5 vs 15.3), whereas at Palmer the trend was the opposite (12.9 vs 12.6). These latter associations are most likely due to chance effects.

Combined analysis of variance for glutenin subunits vs SDS-sedimentation

The data from two sites in 1992 and three sites in 1993 were pooled to evaluate the effects of the glutenin subunits on SDS-sedimentation volume. Analysis of variance for pooled data showed similar effects to those separately observed in the two different years. As shown in Table 4.13, the allelic effect at *Glu-1* loci was only significant at the *Glu-A1* and *Glu-B1* loci. The subunit bands 2*, 17+18 had a higher SDS sedimentation values than their counterparts (i.e. 1, 20) (Fig 4.13).

Table 4.12 Variance ratio for protein concentration and particle size index value for RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at two sites in 1992 and three sites in 1993. Analysis of variance was performed on the basis of six glutenin subunit loci fitted into a regression model to detect main and interloci effects.

Source	1992 measurements				1993 measurement		
	Protein concentration		Pearling Size Index		Protein concentration		
	Winulta	Minnipa	Winulta	Minnipa	Palmer	RAC	Yeelanna
Glu-A1	3.2 ns [(a)]	0.0 ns	19.3 ***	12.3 ***	1.0 ns	0.7 ns	3.0 ns
Glu-B1	0.4 ns	2.0 ns	1.4 ns	0.9 ns	4.9 *	0.4 ns	4.9 *
Glu-D1	3.2 ns	0.8 ns	0.3 ns	1.2 ns	0.4 ns	0.1 ns	1.9 ns
Glu-A3	5.1 *	0.1 ns	7.4 ***	2.4 ns	1.8 ns	0.7 ns	2.9 ns
Glu-B3	0.0 ns	0.2 ns	37.7 ***	30.5 ***	0.5 ns	2.5 ns	0.3 ns
Glu-D3	0.5 ns	0.0 ns	9.8 ***	0.6 ns	5.1 **	4.0 *	3.2 ns
Glu-A1 x Glu-B1	0.6 ns	1.4 ns	0.0 ns	0.8 ns	3.3 ns	5.1 *	3.3 ns
Glu-A1 x Glu-D1	0.6 ns	4.6 *	4.1 *	5.3 *	0.2 ns	1.6 ns	2.3 ns
Glu-B1 x Glu-D1	1.1 ns	0.0 ns	1.2 ns	6.0 *	5.2 *	1.9 ns	12.4 ***
Glu-A1 x Glu-A3	0.0 ns	0.1 ns	2.8 ns	2.5 ns	0.0 ns	0.2 ns	4.6 *
Glu-B1 x Glu-A3	3.1 ns	4.4 *	0.2 ns	2.5 ns	0.1 ns	0.7 ns	0.3 ns
Glu-D1 x Glu-A3	0.0 ns	0.9 ns	3.4 ns	1.7 ns	0.3 ns	7.7 ***	0.0 ns
Glu-A1 x Glu-B3	1.2 ns	0.1 ns	0.1 ns	0.0 ns	0.0 ns	2.0 ns	1.0 ns
Glu-B1 x Glu-B3	1.9 ns	4.3 *	1.8 ns	0.1 ns	0.6 ns	1.4 ns	1.1 ns
Glu-D1 x Glu-B3	0.1 ns	7.5 ***	1.8 ns	3.4 ns	0.1 ns	0.0 ns	0.0 ns
Glu-A3 x Glu-B3	3.0 ns	5.9 *	0.0 ns	0.0 ns	0.1 ns	0.0 ns	4.8 *
Glu-A1 x Glu-D3	0.1 ns	0.3 ns	4.0 *	1.3 ns	1.3 ns	1.2 ns	2.0 ns
Glu-B1 x Glu-D3	0.2 ns	0.5 ns	4.2 *	1.5 ns	0.0 ns	1.8 ns	4.0 *
Glu-D1 x Glu-D3	1.7 ns	0.1 ns	12.8 ***	1.3 ns	0.7 ns	0.0 ns	0.0 ns
Glu-A3 x Glu-D3	0.1 ns	0.6 ns	4.8 *	2.9 ns	0.0 ns	9.2 ***	1.0 ns
Glu-B3 x Glu-D3	3.0 ns	2.8 ns	10.3 ***	2.0 ns	3.9 *	2.6 ns	2.4 ns

a=see abbreviation list

Table 4.13 Variance ratio for main effects and interactions for the *Glu-1* and *Glu-3* loci for SDS-sedimentation and protein concentration value of RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at two sites in 1992 and three sites in 1993. The analysis of variance was performed over the combined sites for both years.

Source of variance	SDSS		Protein	
	Fvalue	P	F value	P
Glu-A1	8.2	<0.001	2.3	ns
Glu-B1	79.52	<0.001	2.8	ns
Glu-D1	0.0	ns	0.7	ns
Glu-A3	154.5	<0.001	2.2	ns
Glu-B3	94.4	<0.001	0.2	ns
Glu-D3	0.3	ns	0.3	ns
Glu-A1 x Glu-B1	1.2	ns	4.3	<0.05
Glu-A1 x Glu-D1	0.4	ns	0.0	ns
Glu-B1 x Glu-D1	3.5	ns	1.1	ns
Glu-A1 x Glu-A3	0.2	ns	4.1	<0.05
Glu-B1 x Glu-A3	0.2	ns	1.2	ns
Glu-D1 x Glu-A3	0.1	ns	0.2	ns
Glu-A1 x Glu-B3	5.8	<0.05	2.1	ns
Glu-B1 x Glu-B3	3.9	<0.05	1.4	ns
Glu-D1 x Glu-B3	29.7	<0.001	0.7	ns
Glu-A3 x Glu-B3	0.6	ns	5.0	<0.05
Glu-A1 x Glu-D3	5.0	<0.05	0.1	ns
Glu-B1 x Glu-D3	0.0	ns	4.0	<0.05
Glu-D1 x Glu-D3	0.8	ns	1.7	ns
Glu-A3 x Glu-D3	1.1	ns	3.5	ns
Glu-B3 x Glu-D3	0.1	ns	13.1	<0.001

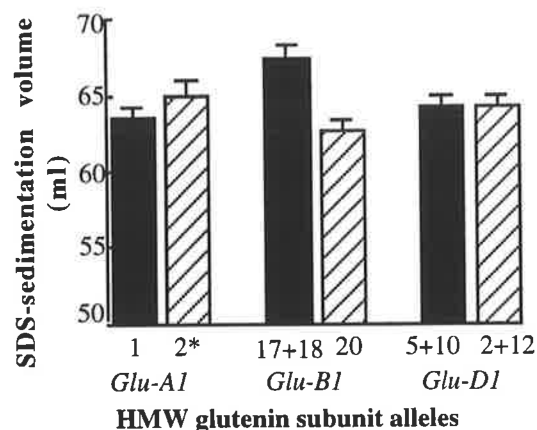


Figure 4.13 Mean value for alleles at the *Glu-1* loci for SDS-sedimentation in RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at two sites in 1992 and three sites in 1993.

Among the *Glu-3* loci, there were highly significant difference between alleles on SDSS except at *Glu-D3* (Fig 4.14). Allele *c* at *Glu-A3* and allele *b* at *Glu-B3* gave higher SDSS volumes than their counterpart alleles at the different sites over both years. The highest variance ratio for SDS-sedimentation was observed for the *Glu-A3* locus, followed by *Glu-B3*. The ranking for these loci was the same as those reported for separate sites in 1992 and 1993.

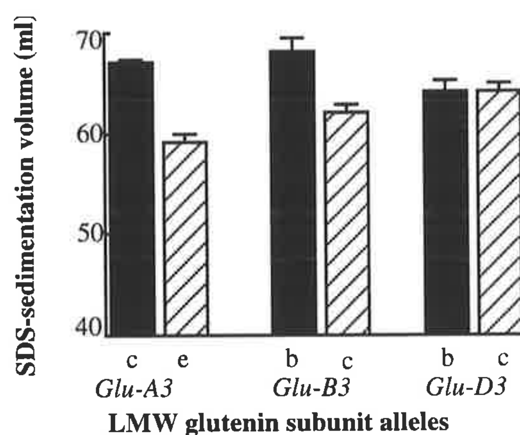


Figure 4.14 Mean value for alleles at the *Glu-3* loci for SDS-sedimentation volume in RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at two sites in 1992 and three sites in 1993.

In the analysis of the pooled data for SDS-sedimentation volume, an interaction between *Glu-D1* and *Glu-B3* was apparent (Table 4.13), mainly because of its consistent effect at most of the sites in both years of the experiment (see Table 4.11). Although the highest SDS-sedimentation value was observed with the combination of 2+12 and *b*, its effect was not significantly different from the 5+10 and *b* combination (Fig 4.15).

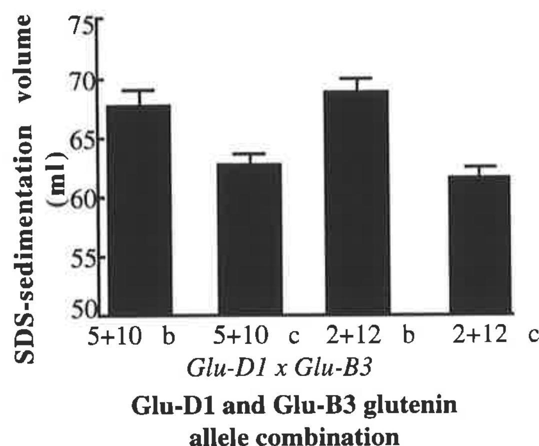


Figure 4.15 Mean value for interactions between alleles at *Glu-D1* and *Glu-B3* loci over two sites in 1992 and three sites in 1993 for SDS-sedimentation in RILs derived from [Halberd x (Wl x MMC)/Wl/10].

Combined analysis of variance for glutenin subunits vs protein concentration and PSI

There were no differences between alleles at the different glutenin loci for protein concentration (Table 4.13). However some interactions were quite large (*Glu-A1 x Glu-B1, Glu-A1 x Glu-A3, Glu-A3 x Glu-B3, Glu-B1 x Glu-D3* and *Glu-B3 x Glu-D3*). The largest of these was the *Glu-B3* x *Glu-D3* where the combination of *b* and *b* had the higher protein concentration than others. It was observed that these significant interactions were always accompanied by non-significant of the same interactions on SDS-sedimentation.

Significant differences were observed between alleles at the *Glu-A1*, *Glu-A3*, *Glu-B3* and *Glu-D3* loci for PSI in 1992 (Fig 4.16). Inspection of the data over sites showed that progeny with subunit band 2* tend to be softer than those with 1 (Fig 4.16). Similarly at *Glu-A3* , progeny carrying ~~subunit~~ allele *c* were softer than progeny with ~~subunit~~ allele *e*. It does not seem that these hardness values show any correlation with dough strength. Although the *b* allele at *Glu-B3* gave a higher SDSS volume than its counterpart (Fig 4.9), the progeny with this allele tend to have harder grain than progeny with allele *c*. Likewise progeny possessing allele *c* at *Glu-D3* were softer than those having *b*.

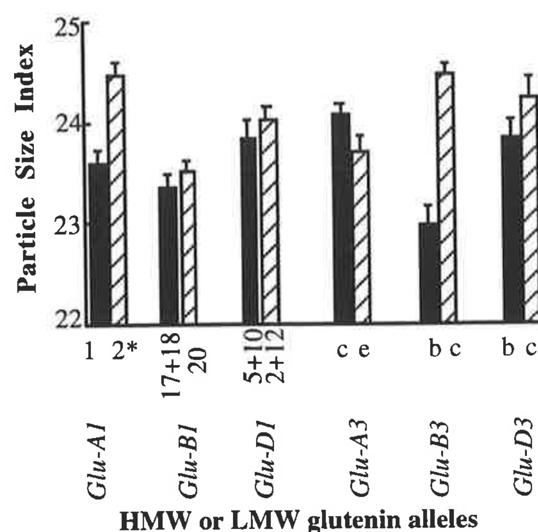


Figure 4.16 Mean value of main effects of the *Glu-1* and *Glu-3* loci on particle size index in RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at two sites in 1992.

Extensograph evaluation

Nineteen different lines were chosen for extensograph evaluation (Table 4.2). The selection of these lines was based on the allelic effects of different loci on SDS-sedimentation volume and priority was given to those lines which provide comparisons between glutenin subunits. Two replicates of each of these lines were evaluated from two sites (Palmer, Roseworthy Campus) in 1993. The 19 different lines could be grouped into five different genotypes and were subjected to analysis of variance (Table 4.14). There were significant differences between sites for maximum resistance, extensibility and protein concentration. The non-significant difference between sites for water absorption, flour yield and PSI, indicate that different growing locations did not affect these quality parameters in these trials.

The most highly significant differences were observed between genotypes for Rmax and water absorption. The genotype and site interaction was significant for Rmax, protein concentration and water absorption, indicating that these quality parameters differed amongst genotypes in different growing locations (Table 4.15).

Table 4.14 Mean square and statistical significance of the quality parameters of 19 RILs, selected from 59 RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at two sites (Palmer and Roseworthy Campus) in 1993 and analysed over the sites.

source of variation	d.f	Quality parameters [a]						
		Rmax	Ext	PC	E/P	WA	FY	PSI
Site	1	49317.1**	123.5*	105.6*	0.007 ns	1.0 ns	97.3 ns	0.0 ns
Residual (1)	2	219.1	1.4	1.6	0.003	0.7	48.0	12.0
Genotype	4	59749.9 ***	1.8 ns	0.4 ns	0.01 ns	2.7 ***	30.0 ns	8.3 ns
Genotype x site	4	1545.4 *	1.3 ns	1.8 *	0.01 ns	0.8 *	26.7 ns	1.6 ns
Residual (2)	64	590.9	1.8	0.6	0.01	0.3	22.2	3.9
Total	75							

a=see abbreviation list

Table 4.15 Variance ratios for different quality parameters of 19 F_7 RILs, selected amongst 59 RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at two sites (Palmer, Roseworthy Campus) in 1993.

Character	Palmer		RC	
	F value	P	F value	P
Maximum resistance	46.6	<0.001	55.3	<0.001
Water absorption	9.0	<0.001	3.4	<0.05
Flour yield	2.0	0.12	0.6	0.7
Extensibility/protein ratio	1.2	0.32	0.9	0.49
Extensibility	0.8	0.53	0.1	0.43
Protein concentration	2.8	<0.05	0.1	0.44
Particle Size Index	2.4	0.07	0.7	0.63

Since Rmax and water absorption showed significant differences at the two sites, these were also analysed as separate sites as described below:

Individual glutenin alleles vs dough resistance (Rmax) and water absorption

Because of the nature of the selected RILs, the individual effects of LMW glutenin loci could not be calculated. However, analysis of variance for the HMW glutenin subunit loci showed that the alleles at *Glu-B1* and *Glu-D1* had significantly different Rmax values for both sites (Table 4.16).

The absolute value of variance ratios (F value) for the alleles at *Glu-B1* were much larger than that for *Glu-D1*, indicating that the effects of alleles at *Glu-B1* on Rmax are more pronounced than the *Glu-D1*alleles. As shown in Fig 4.17 the progeny with HMW glutenin subunit bands 17+18 had higher Rmax values than the 20 type. Similarly, bands with HMW glutenin bands 5+10 were stronger than those with subunit bands 2+12.

Table 4.16 Variance ratios for maximum resistance and water absorption of 19 RILs, selected amongst 59 RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at two sites (Palmer, Roseworthy Campus) in 1993.

Source of variance	Palmer		RC	
	Rmax(a)	WA	Rmax	WA
Glu-A1	0.7 ns	0.2 ns	0.4 ns	1.6 ns
Glu-B1	113.9 ***	1.6 ns	124.0 ***	2.6 ns
Glu-D1	53.7 ***	11.4 ***	70.6 ***	17.1 ***
Glu-A1 x Glu-B1	18.2 ***	0.2 ns	26.1 ***	11.6 ***

a=see abbreviation list

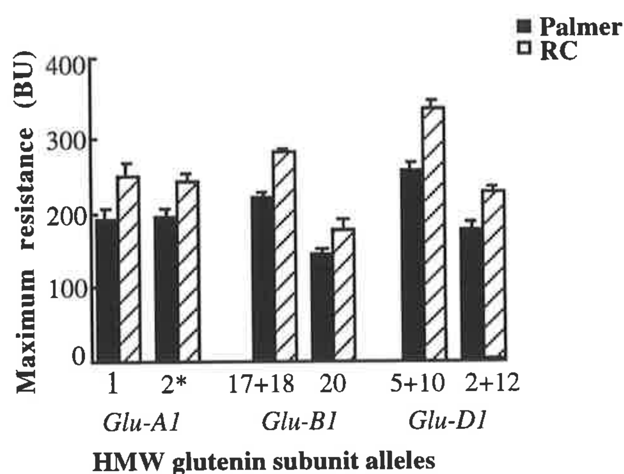


Figure 4.17 Mean value for specific alleles at the three *Glu-1* loci from two sites (Palmer and Roseworthy Campus) in 1993 for maximum resistance in 19 RILs derived from [Halberd x (Wl x MMC)/Wl/10].

A highly significant difference for water absorption was observed between alleles at *Glu-D1*. Progeny with glutenin subunit bands 2+12 had a higher water absorption than those having 5+10 (Fig 4.18)

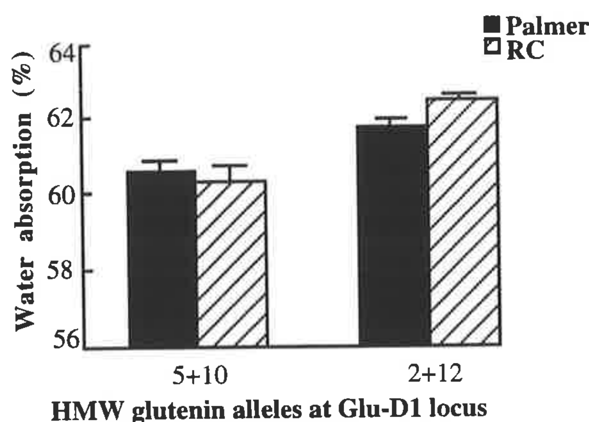


Figure 4.18 Mean value for different alleles at the *Glu-D1* locus from two sites (Palmer and Roseworthy Campus) in 1993 for water absorption in 19 RILs derived from [Halberd x (Wl x MMC)/Wl/10] for water absorption

As shown in Table 4.16, variation in Rmax was affected by interaction between alleles of *Glu-A1* and *Glu-B1* at both sites. *Glu-A1* subunit 1 gave a higher Rmax than 2* only in combination with *Glu-B1* subunits 17+18 (Fig 4.19 a). This interaction was also observed for water absorption at Roseworthy Campus. The highest water absorption was observed in the combination of 1 at *Glu-A1* and 20 at *Glu-B1* (Fig 4.19 b).

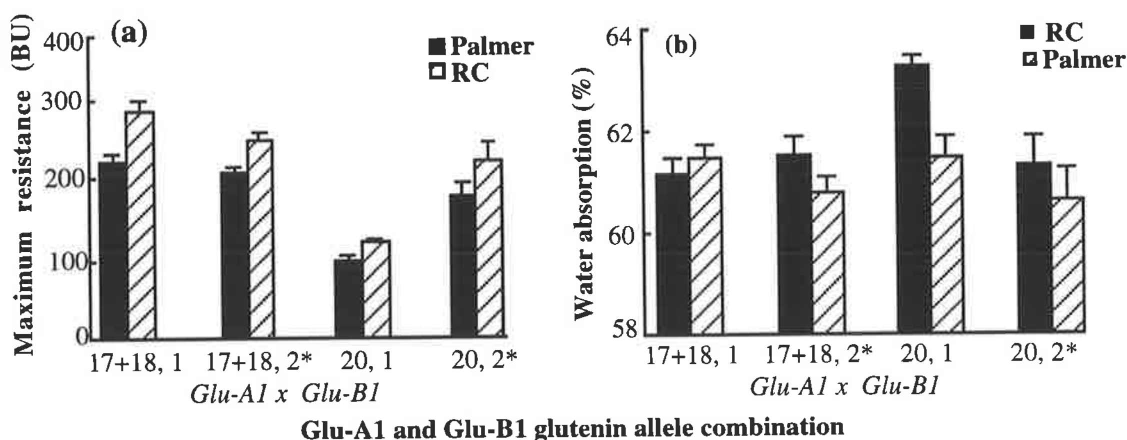


Figure 4.19 Mean value for combinations of alleles at *Glu-A1 and Glu-B1* from two sites (Palmer and Roseworthy Campus) in 1993 for **(a)** maximum resistance and **(b)** water absorption in 19 RILs derived from [Halberd x (Wl x MMC)/Wl/10].

4.3.5 Relationship between glutenin subunits and maturity

The decimal code for growth stages of cereals devised by Zadoks, Chang and Konza (1974), reproduced by Tottman *et al.* (1979) was used to score the RILs at three sites in 1993. At Palmer, the scoring was performed at the anthesis stages (60-69), whereas for Roseworthy Campus and Winulta plots were scored at the milk stage of development (70-79). Analysis of variance showed that there was a significant difference between sites for maturity of genotypes. The significant differences between genotypes for maturity were not associated with any interaction between site and genotypes. The only significant difference between genotypes was observed at Palmer.

It was thought that there may be some linkage or pleiotropy between genes controlling of glutenin subunits and those controlling maturity. To test this, the maturity of the 59 different lines at Palmer, which ranged between 59-66, were divided into eight distinct classes and analysed on the basis of the alleles present at the glutenin loci. The total chi-square value of 63.5 obtained from a χ^2 analysis (77 d.f) was non-significant at the 5 percent level indicating that maturity and glutenin subunits were independent attributes.

4.3.6 Relationship between maturity and dough properties

Correlations between maturity and quality characteristics were calculated for the 19 lines over two sites (Palmer and Roseworthy Campus) in 1993. Simple correlations (Table 4.17) showed that there was a highly significant relationship between maturity and quality parameters such as SDSS, Rmax, extensibility and protein concentration, with the relationship being negative with SDSS. A similar negative correlation was also reported by Schlehuber *et al.* (1967). The strongest correlation was between maturity and protein concentration (r=0.78).

Although there was a significant correlation between maximum resistance and SDSS (r=0.24), its low value indicated that SDSS can not be used reliably as an indicator of dough strength. The high correlation between Rmax and extensibility (r=0.43) suggested that lines with high dough strength also tended to have good extensibility. Negative correlations between Rmax and water absorption and between flour yield and extensibility (r= -0.31) were significant.

Table 4.17 Correlation matrix between flour properties and maturity of 19 RILs derived from [Halberd x (Wl x MMC)/Wl/10] grown at two sites (Palmer and Roseworthy Campus) in 1993.

		Correlation matrix (a)								
		1	2	3	4	5	6	7	8	9
Maturity	1	1.00								
Maximum resistance	2	0.37 **	1.00							
SDS-sedimentation	3	-0.33 **	0.24 *	1.00						
Water absorption	4	0.13	-0.41 **	-0.06	1.00					
Flour yield	5	-0.19	-0.05	0.08	-0.11	1.00				
Extensibility/protein	6	-0.09	0.08	0.21	-0.18	-0.09	1.00			
Extensibility	7	0.65 **	0.43 **	0.06	-0.00	-0.31 *	0.50 **	1.00		
Protein concentration	8	0.78 **	0.37 **	-0.14	0.18	-0.24 *	-0.39 **	0.59 **	1.00	
Particle Size Index	9	0.01	-0.09	0.05	-0.08	0.06	0.00	-0.02	-0.03	1.00

a=see abbreviation list

4.4 Discussion

Halberd and the breeding line (Wl x MMC)/Wl/10 differed with respect to the alleles present at each of the three HMW and three LMW glutenin loci so that 64 genotypic combinations of these alleles could have been present in the sample of Recombinant Inbred Lines (RILs) derived from the hybrid of these cultivars. This would have permitted partitioning of the genetic variance associated with the six HMW and LMW glutenin loci into additive and epistatic components. However in the study here there were a limited number of lines within each of the genotypes and only 33 out of 64 combinations were present. Therefore the mean effects over the restricted number of lines were not particularly reliable estimates of the direct effects of the major loci of glutenin subunits. Gupta *et al.* (1994a) evaluated the individual and combined effects of alleles at these loci using Brabender Extensograph data. Therefore the results here, using SDS-sedimentation test can be compared with those of Gupta *et al.* (1994a).

The association of HMW glutenin subunits with SDSS volume showed that progenies with subunit bands 2* at *Glu-A1* had higher SDSS volumes than their counterparts alleles (Fig 4.7) as found by other researchers (Moonen *et al.*, 1982; 1983; Cornish, 1994), although some inconsistencies in ranking have been reported for these alleles (Payne *et al.*, 1984a; Lorenzo *et al.*, 1987). However, the present results (Fig 4.17) were consistent with those of Gupta *et al.* (1994a) when both studies were compared on the basis of extensograph evaluation, resulting that alleles 2* and 1 at *Glu-A1* do not differ significantly for Rmax values.

With respect to the *Glu-B1* locus the current results (Fig 4.17) were again consistent with the results of Gupta *et al.* (1994a) who found that the subunit 17+18 was stronger than its counterpart allele 20 for maximum resistance. The consistent effect of the alleles at *Glu-B1* on Rmax in both studies indicated that this effect is likely to be consistent over years and locations. The difference between subunit bands 17+18 and 20 may be due to the number and position of cysteine residues as demonstrated by other researchers (Tatham *et al.*, 1991; Margiotta *et al.*, 1993). A significant difference was also observed by SDS-sedimentation test with the subunits 17+18 being associated with a larger SDS sedimentation volume (Fig 4.7) as found by other researchers (Payne *et al.*, 1984a; Branlard and Dardevet, 1985b).

With *Glu-D1*, a significant difference was only observed for data in 1992 when lines with subunit bands 5+10 had a higher sedimentation value than those with subunits 2+12 (Fig 4.7). This difference was also observed for maximum resistance (Fig 4.17), agreeing with the results of Gupta *et al.* (1994a). Although nearly all the research regarding the allelic effects at *Glu-D1* has shown that the subunit bands 5+10 are

stronger than 2+12 (Payne *et al.*, 1981a; 1984a; 1987a; Branlard and Dardavet, 1985a), Gupta *et al.* (1991) reported that these alleles did not give different Rmax values in a study of Australian wheat cultivars. The results here also showed some inconsistencies in the ranking of the alleles at this locus in 1992 and 1993 (Fig 4.7). That is, the subunit bands 2+12 were stronger than 5+10 in 1993. The inconsistencies of ranking over all trials indicates some form of epistasis or genotype x environment interaction effects, as was reported by Shahriari *et al.* (1994b).

The positive synergistic effects associated with the LMW glutenin allele *c* at the *Glu-A3* locus was most likely due to the greater number and or intensity of bands as shown in Fig 4.4 and as reported by Gupta and Shepherd (1990a). The null allele was unlikely to have positive synergistic effects on the elastic properties of glutenin due to the lack of any protein products. Hence its counterpart allele (i.e. *Glu-A3c*) with a dark single band (Chapter 3 section 3.3.1) showed a large increase in dough strength compared to the *e* allele (Fig 4.9). The inferiority of the null allele *e* at the *Glu-A3* locus on dough quality (Fig 4.9) has been also reported by Payne *et al.* (1987c) and Gupta *et al.* (1989, 1994a). Gupta *et al.* (1989) also showed that the *Glu-A3e* was associated with reduced dough resistance and extensibility compared to its counterparts. Allelic variation at *Glu-A3* had a larger effect on gluten quality than variation at *Glu-A1*.

In the present study the effects of alleles at *Glu-B3* on SDSS volume were consistent over sites in both years of the experiment with allele *b* always being associated with a higher SDSS volume (Fig 4.9). Although it was impossible to analyse 19 RILs for the effects of LMW subunits on extensograph parameters, the trend of the allelic effects at the *Glu-B3* locus on SDSS volume was consistent with that of Gupta *et al.* (1994a). With both *Glu-B1* and *Glu-B3* loci having large effects in the present study, chromosome 1B had a particularly important role in influencing SDSS volume.

The effects of alleles *b* and *c* at *Glu-D3* on SDS-sedimentation (Fig 4.9) were similar to those recorded by Gupta *et al.* (1994a), with neither having any significant influence on Rmax. Nieto *et al.* (1994) also reported that allelic variation at *Glu-D3* loci had no significant effect on dough strength.

Based on the results of *Glu-3* analysis (pooled and separate data), the LMW glutenin subunits were ranked as *Glu-B3* [*b* vs *c*]>*Glu-A3* [*c* vs *e*]>*Glu-D3*[*b* vs *c*]. This ranking was consistent with those reported by Gupta *et al.* (1994a).

The effects of *Glu-A1* and *Glu-A3* were additive on SDSS volume at two sites (Fig 4.11). Payne *et al.* (1987c) also found additive effects from alleles at the *Glu-A1* and *Glu-A3*

loci. Gupta *et al.* (1989) also reported a cumulative effect of the allelic variation at these same loci on dough resistance and extensibility on this same F_6 population.

A comparison of SDS-sedimentation volume as an indicator of dough strength with maximum resistance showed that the effects of the growing conditions were more pronounced on Rmax. This was also observed for the interaction between site and genotypes (Table 4.14). The significant correlation between Rmax and protein concentration (r=0.37) may be the main reason for this interaction, as there was no correlation between SDS-sedimentation and protein concentration (Table 4.17).

The SDS-sedimentation test can give useful information about protein quality. Although in general, the average of SDSS volume for each of the alleles at loci investigated was higher for a site which had not been rain damaged (Figs 4.7 and 4.9), there was no change in ranking at the rain damaged site. Here the SDS-sedimentation test has ability to give useful information about protein quality even under rain damaged condition as found by Axford *et al.* (1979). This, together with the similar trends of allelic effects for both years of experimentation, indicated that environmental factors were relatively unimportant for the SDS-sedimentation test.

The present results and those of Gupta *et al.* (1994a) show that effects of individual *Glu-3*, *Glu-1* or *Glu-3* plus *Glu-1* loci (allelic effects) were largely cumulative. This finding was in agreement with other research (Gupta *et al.*, 1989; Payne, 1987c; Pogna *et al.*, 1990). But the current results also showed that variation in SDS-sedimentation volumes did not only depend on additive effects, but that epistasis also may have a significant effect (Table 4.11). The relative importance of these epistatic effects suggested that they should be included in predictive models when breeding for bread making quality. Thus, when establishing the contribution of an allele in breeding for quality, one should take into account interactions with alleles at other loci as these might affect the quality of the dough.

This study showed that sprouting resistance was significantly associated with alleles controlling HMW glutenin subunits but not with the LMW subunits (Table 4.4). A similar association was also reported by Lukow *et al.* (1989) but in this case between gliadin proteins and Falling Number. It is worth investigating these associations further because these effects can be due to linkage between genes controlling sprouting resistance and those controlling HMW glutenin subunits. This linkage could provide an opportunity in selecting for sprouting resistance in early generations. Pre-screening of early generation lines for specific HMW glutenin subunits would increase the frequency of lines with improved sprouting resistance. In the cross of [Halberd x (Wl x MMC)/Wl/10], selecting lines with HMW glutenin subunit bands 1, 20 and 5+10 would increase the FN value of lines up to 380 (similar to Halberd parent). This possible

linkage between HMW glutenin subunit and sprouting resistance loci would be useful, providing it accompanies good breadmaking quality. Unfortunately, in the current study the alleles giving higher Falling Number did not give higher dough strength. While bands 1 and 5+10 were associated with stronger dough, band 20 was associated with weakness.

It was observed that *Glu-A1* and *Glu-3* loci were associated with Particle Size Index (PSI) suggesting genetic linkage, particularly on chromosome 1A (Table 4.7). Therefore in a plant breeding program, manipulation of PSI could possibly be achieved by selecting appropriate glutenin alleles.

CHAPTER 5

THE RELATIONSHIP BETWEEN GLUTENIN SUBUNIT COMPOSITION AND THE BREADMAKING QUALITY OF SOUTH AUSTRALIAN WHEAT VARIETIES

5.1 Introduction

A cause-and-effect relationship has been established between the glutenin fraction and the rheological and baking properties of wheat flour (Dimler, 1965; Orth and Bushuk, 1972; MacRitchie, 1989). Glutenin represents a heterogeneous mixture of polypeptides linked together by intermolecular disulfide bonds, with the polymeric nature of glutenin giving it its viscoelastic properties. Much effort has been directed toward relating qualitative variation in the polypeptide composition of glutenin, particularly the high molecular weight (HMW) subunits, with variability in flour quality.

The accurate identification of HMW glutenin subunits became applicable when the Laemmli (1970) method of SDS-PAGE was adapted for the analysis of wheat proteins independently by Payne in Cambridge and Lawrence in Adelaide. This enabled both workers to identify with precision the many electrophoretically different subunits present in a wide range of hexaploid wheat cultivars (Lawrence and Shepherd, 1980; Payne *et al.*, 1980). The first clear correlations of particular combinations of HMW glutenin subunits with quality were established by Payne *et al.* (1981a) and these correlations have since been widely substantiated (Carrillo *et al.*, 1990). The first important breeding outcome of this in Australia was the contribution of HMW glutenin screening to the release of Schomburgk in 1986 (A.J. Rathjen, pers.comm.). The proportion of variation in quality that has been attributed to variation in the HMW glutenin subunits among varieties or lines has ranged from 60% (Payne *et al.*, 1987a) to 13% (Rousset *et al.*, 1992) for different sets of materials.

Selecting for alleles of HMW glutenin subunits associated with high breadmaking quality in breeding lines was accelerated from 1987, when Payne *et al.* (1987a) devised a score based on the effects of individual HMW glutenin subunits to predict varietal mixing and baking quality. This approach has been successful in selecting genotypes in Britain, Spain and Canada (Payne *et al.*, 1987a; MacRitchie *et al.*, 1990), whereas Metakovsky *et al.* (1990) in a survey of Australian bread wheats found that only a small proportion of the variation in dough quality can be accounted for by differences in HMW glutenins and many Australian wheat breeders have seemed reluctant to adopt this form of screening technology. In contrast, Gupta *et al.* (1991) in a survey of Australian wheats showed that

the LMW and HMW subunits of glutenin together could account for about 70% of the variation in extensograph resistance.

Detailed study of the LMW glutenin subunits only become possible when the method of electrophoretic fractionation was modified by Singh and Shepherd (1988). This provided the techniques needed to permit screening of a large number of genotypes to establish the genetics and quality relationship of the LMW subunits as undertaken by Mr G. Cornish and Mr G. Palmer and their colleagues at South Australian Research and Development Institute (SARDI) (Cornish *et al.*, 1993). There is now a widespread interest among Australian breeders with a consensus that the *b*, *b*, *b* allelic combination at the three loci of *Glu-A3*, *Glu-B3* and *Glu-D3* is associated with good dough properties (Gupta *et al.*, 1991). Also it has been suggested that for those end-products which require highly extensible dough, the LMW glutenin alleles responsible for a high number of bands should be selected. Australian wheat breeders have been advised to adopt this as a general strategy (G. Cornish, pers. comm.) as most wheat products require doughs with high levels of extensibility.

In the current study the electrophoretic patterns of the wheat varieties most commonly grown in South Australia have been analysed and their HMW glutenin subunits scored using the system of Payne *et al.* (1987a) and as modified by Cornish (1994) for local varieties. These were correlated with dough strength. The LMW glutenin alleles have been scored based on the number of bands, as suggested by Gupta *et al.* (1990a, 1990b), and this has been correlated with extensibility. Two sets of data have been used to develop predictive equations to estimate Rmax and extensibility, based on allocating a weighting factor for each of the three HMW glutenin loci (*Glu-A3*, *Glu-B3* and *Glu-D3*). This was undertaken by multiple regression analyses.

5.2 Materials and methods

5.2.1 Wheat varieties

Fifteen wheat varieties, from two field trials series and commonly grown in South Australia were studied (Table 5.1) using data both from quality analyses conducted as part of this project and provided by the SARDI . The wheat variety Halberd was included in this study because its high *Glu-1* quality score contrasted with its comparatively poor extensibility and low resistance.

Table 5.1 Classification of 15 South Australian wheat varieties based on the marketing classification, date of release, pedigree and their rheological properties.

Variety	market type	Date of release	Pedigree	Comments
Angas	ASW	1991	Schomburgk 3*///Aroona/Moro	Weak dough strength.
Barunga	Hard	1993	(Halberd*Schomburgk≠4)*Molineux#)/8/3	Good dough strength and extensibility
Bindawarra	Soft	1980	((Mexico-120*Koda)*Raven)/122/16	Weak dough strength but very extensible, excellent biscuit wheat.
Excalibur	ASW	1991	RAC177(Sr26)/Uniculm492//RAC311S	Unbalanced dough properties, strong but poor extensibility, poor flour color.
Halberd	ASW	1969	Scimitar/Kenya 6042/Bobin/Insignia 49	Poor extensibility and weak dough strength.
Janz	Hard	1989	3AG3/4*Condor//Cook	Weak dough for a Hard variety.
Machete	Hard	1985	(Son64//TZPP/Y54)/2*Gabo//Madden	Good dough properties.
Meering	Hard	1984	Condor selection	Good extensibility, low dough strength.
Molineux	Hard	1988	((Pitic 62*Festiguay)*Warigal**2)/21/7/16	Excellent dough strength and extensibility.
Schomburgk	Hard	1986	(W3589*Oxley)*Warigal**2)*Aroona**2	Good dough properties.
BT-Schomburgk	Hard	1992	(Halberd*Schomburgk#4)/3/27	Good dough strength and extensibility.
Spear	ASW	1984	Sabre/Sonora 64//Tezanos Pintos Precoz/Yaqui 54//Insignia.	Moderate dough strength and moderate extensibility.
Tatiara	Soft	1987	((Mexico 120*Koda)*Raven)*(Mengavi*Siette Cerros)W18/18	Acceptable biscuit wheat with weak dough strength and moderate extensibility.
Wyuna	Soft	1984	Sonora64*Knott^{2}*11-8156-100Y-Rojo/Olympic	Biscuit wheat with excessive dough strength for this purpose and low extensibility.
Yarralinka	ASW	1992	((Mengavi*Siette Cerros)*((Mengavi*Siette Cerros)*Crim)*(CombinationIII*Warigal#))/13/9	Strong with moderate extensibility and poor flour extraction

5.2.2 Field experiments

Field trials were grown at Palmer on the property of Mr J. Krause and Roseworthy Campus (RC) of the University of Adelaide (see Chapter 4 for their details) in a completely randomised block with two replications. The field arrangement was the same as those described in Chapter 4.

The decimal code for the growth stages, similar to those reported in Chapter 4, was used to record the time of anthesis at Roseworthy Campus in 1992.

Data from the SARDI secondary trials conducted by Mr S. Jefferies and his colleagues and assessment for quality by Mr G. Palmer and his colleagues of SARDI for the 15 varieties were included to provide a long term reference in this study. Data for each variety were extracted using the dBase program available at SARDI and kindly provided by Mr G. Cornish. Since these trials had been grown during the period from 1985 to 1993 at a number of sites (up to ten sites) per year, to allow comparison between varieties not grown in the same trial series, the means of individual varieties relative to Spear were calculated by:

$$\text{Adjusted value for variety A} = \frac{\text{mean of variety A x Grand mean of Spear}}{\text{mean of Spear in the common trials with variety A}}$$

5.2.3 Electrophoresis band patterns and quality assessment

The electrophoresis patterns were analysed by Mr. G. Cornish of SARDI and kindly made available through a dBase program (Cornish *et al.*, 1993).

The quality assessments were conducted as described in Chapter 4, including milling, NIR measurements for determining protein concentration, SDS-sedimentation and the extensograph test. The extensograph data supplied by SARDI was measured on 150 g doughs samples

5.2.4 Quality scores

HMW-scoring

The scoring system of Payne *et al.* (1987a) was based on the relationship between individual HMW glutenin subunits and quality, as determined by the SDS-sedimentation test (Payne *et al.*, 1984a).

This score was modified on the basis of the allelic effects at the *Glu-1* loci on breadmaking quality of Australian varieties as reported by Cornish *et al.* (1991, 1994). The highest score for a subunit pair was 4 assigned to 5+10, the score of 3 was given to subunits 2* and 17+18 to conform with reports by several researchers (Cornish, 1994; Metakovsky *et al.*, 1990) that these alleles were associated with greater dough strength than other alleles. A score of 2 was given to the pairs 2+12, 7+8, 7+9 and 1. Finally, a score of 1 was assigned to the chromosome 1A null allele, and the single 1B coded subunit 20. The total score for each variety was summed on the basis of the score assigned to each subunit or subunit pair in a variety. For example, the wheat variety Yarralinka with subunits 2*, 5+10, and 17+18 receives a quality score of 10, the highest ranking. The scores assigned to each HMW glutenin allele for the Payne and local systems are given in Table 5.2

Table 5.2 Allelic variants amongst 15 South Australian wheat varieties at the *Glu-1* loci and two quality scores (Payne and local) assigned to individual or pairs of HMW glutenin subunits.

Locus	Allele	Payne score	Local Score
	null	1	1
Glu-A1	1	3	2
	2*	3	3
	7+8	3	2
	17+18	3	3
Glu-B1	7+9	2	2
	20	1	1
	5+10	4	4
Glu-D1	2+12	2	2

LMW scoring

Quality scoring for LMW glutenin alleles was based on the number of bands present which is positively correlated to extensibility (Gupta and Shepherd, 1990a, 1990b) and was first used by Cornish (1994). The score assigned to each LMW glutenin allele along with their relative number of bands is given in Table 5.3.

Table 5.3 Allelic variants at *Glu-3* loci in 15 South Australian varieties, showing number of bands present and quality scores assigned (Cornish, 1994) to each variants.

Locus	Allele	Number of bands	Score
	b	2	2
Glu-A3	c	1	1
	e	0	0
	j	8	8
	h	8	8
Glu-B3	b	7	7
	c	3	3
	b	5	5
Glu-D3	c	5	5
	a	4	4

5.2.5 Statistical analyses

The analyses of variance (ANOVA) were performed on both individual site data and the pooled data. The effects of allelic variation at *Glu-1* loci on dough parameters were studied by regression analysis using the Genstat 5 statistical package. That is the alleles at the three loci were fitted into a regression model due to the non-orthogonality of the glutenin band patterns attributed to different varieties. A multiple linear regression was calculated for Rmax and extensibility using the actual means of each variety to estimate the allelic effects for each of the *Glu-1* loci. The Rmax and extensibility were then estimated by solving a series of simultaneous equations obtained for the alleles of the *Glu-1* loci. The relationships between actual and estimated quality data were determined by linear correlation coefficients. Percentage of variance (adjusted R^2) in quality measurements that was accounted for by HMW glutenin subunit loci was determined using multiple linear regression.

5.3 Results

5.3.1 Varieties and locations

There was a wide range of variation for most of the measurements among the South Australian wheat varieties (Table 5.4). Much of this variation was expected because the trial included varieties selected for a range of end uses from the low dough strength

required for biscuits to the high strength for blending and breadmaking. Since all varieties, except Halberd, had similar LMW quality scores (see Table 5.7), it was excluded from many of the analyses of variance.

Analysis of variance showed that the differences between the varieties at the two sites, Palmer and Roseworthy Campus, were highly significant for most of the quality parameters but there were no significant differences for maturity (Table 5.5). Significant differences were observed between sites for maximum resistance (Rmax), extensibility, protein concentration and maturity suggesting that these parameters are to some extent environmentally dependent. By contrast, the non-significant differences between sites for SDS-sedimentation, water absorption and flour yield indicate that these were mainly genotype dependent, at least within these two trial sites.

Although the data presented in Table 5.5 indicated that the site x variety interaction was insignificant for most of the measurements and that the pooled ANOVA was adequate, the sites were analysed separately due to the large differences in grain protein concentrations between the two sites. The analyses showed that there were highly significant differences between varieties for all quality parameters at Roseworthy Campus (Table 5.6), but at Palmer, significant differences were observed only for Rmax, water absorption and flour yield. The non-significant difference between varieties for protein concentration at Palmer indicated that the significant differences for other quality parameters observed at that site cannot be due to quantitative variation in flour protein concentration.

The value of SDS-sedimentation for most of the varieties (11 of 15) from Palmer was generally higher than those from Roseworthy Campus, whereas the Rmax values from Roseworthy Campus were generally higher than those from Palmer (Table 5.4).

5.3.2 Correlation of HMW and LMW glutenin subunits with quality

The HMW and LMW glutenin subunits composition for the fifteen South Australian varieties are given in Table 5.7. Of the fifteen varieties studied, ten were homogeneous for HMW glutenin composition and five had more than one biotype (shaded area in Table 5.7).

The varieties classified to the Hard class had high quality scores, predicting strong doughs suitable for breadmaking but not biscuits. Quality scores varied from 5.5 to 10 (local score) and 6 to 10 (Payne score). Although the quality score was set up to range from 3 to 10 (with 10 representing highest quality), almost all Australian wheat varieties intended for breadmaking scored at least 7. The allele most strongly associated with good quality,

Table 5.4 Mean values of quality parameters and maturity measurements of 15 South Australian wheat varieties grown at two sites (Palmer and Roseworthy Campus) in 1993. The adjusted mean data obtained by South Australian Research and Development Institute (SARDI) in the period of 1985-1993 over several locations per year has been included.

Variety	Palmer								RC								SARDI†					
	SDSS[a]	Rmax	Ext	PC	E/P	WA	FY	Maturity	SDSS	Rmax	Ext	PC	E/P	WA	FY	Maturity	Rmax	Ext	PC	E/P	WA	FY
Janz	95.5	255.0	16.1	11.7	1.38	59.6	71.2	63.5	88.5	303.5	21.2	14.8	1.43	59.2	72.2	74.0	394.2	18.8	10.2	1.84	60.5	73.5
Meering	86.0	227.0	16.2	12.3	1.32	58.2	69.2	63.5	80.0	250.5	18.0	15.0	1.20	58.6	70.0	74.0	331.8	19.7	10.6	1.85	59.6	73.4
Barunga	91.5	265.0	16.5	12.5	1.32	58.6	71.3	65.0	89.5	313.5	19.5	14.4	1.35	57.2	71.7	73.0	506.6	19.5	10.3	1.89	60.1	72.9
Molineux	90.5	250.0	18.6	12.5	1.49	58.0	70.3	64.5	85.5	345.0	23.1	15.0	1.54	57.6	73.2	74.0	444.4	21.0	10.6	1.98	60.2	73.6
Machete	85.0	254.0	15.8	13.5	1.17	62.2	68.3	64.0	90.0	299.5	20.7	14.3	1.45	59.8	69.5	73.0	419.5	18.6	10.3	1.80	62.4	72.2
Spear	80.0	169.0	14.0	11.3	1.24	61.0	71.3	64.0	83.0	254.0	16.5	14.2	1.16	60.8	73.5	72.0	331.8	17.0	10.1	1.68	61.6	72.7
Schomburgk	87.5	194.5	15.3	12.3	1.24	60.0	72.7	64.0	89.0	296.0	22.4	14.3	1.57	57.4	71.8	75.0	383.5	19.0	10.4	1.82	60.4	75.0
Excalibur	84.5	246.0	15.0	12.9	1.16	61.4	70.5	64.5	77.5	309.5	18.8	15.0	1.25	62.0	69.7	74.0	448.6	18.1	10.5	1.72	61.2	72.7
BT-Schomburgk	88.5	207.5	17.5	12.0	1.46	59.0	68.2	65.0	81.0	283.5	19.2	13.7	1.40	55.8	71.7	74.0	308.5	20.0	10.8	1.85	61.7	74.3
Angas	88.0	204.0	16.8	12.4	1.35	60.0	71.3	63.5	85.5	252.5	19.9	14.9	1.33	60.8	73.7	74.0	368.1	19.6	10.7	1.83	61.2	74.8
Yarralinka	91.0	275.0	18.2	13.4	1.36	57.6	66.8	64.0	83.0	315.5	22.7	16.4	1.38	59.0	71.2	72.0	496.8	19.7	11.4	1.73	59.8	72.5
Tatiara	95.5	155.0	15.9	11.6	1.37	56.2	58.8	65.5	86.0	195.0	19.2	13.1	1.46	55.4	63.5	72.0	171.7	18.1	9.4	1.92	55.2	72.5
Wyuna	73.0	228.0	14.0	10.9	1.28	52.2	64.8	64.5	65.0	278.0	18.4	13.6	1.35	55.0	64.8	75.0	-	-	-	-	-	-
Bindawarra	84.0	108.5	17.3	11.6	1.49	56.8	63.5	65.0	70.0	204.0	18.7	12.8	1.46	57.0	64.0	73.0	204.1	19.1	10.2	1.87	53.7	75.7
LSD (5%)	**ns**	**59.2**	**ns**	**ns**	**ns**	**1.2**	**5.1**	**ns**	**7.6**	**46.2**	**3.2**	**1.5**	**ns**	**2.2**	**3.5**	**ns**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
Halberd	63.5	160.0	14.1	13.3	1.06	59.0	69.3	62.0	67.0	225.0	17.5	14.1	1.24	29.9	71.3	74.0	279.9	16.0	10.4	1.53	60.3	73.3

a=See abbreviation list
† Relative to Spear which was the common check (see Material and methods).
NA=not available.

Table 5.5 Mean square and statistical significance of the quality parameters and maturity measurements of the 14 South Australian wheat varieties (Halberd excluded) grown at two sites (Palmer and Roseworthy Campus) in 1993.

Source of variance	d.f	Rmax(a)	Ext	PC	E/P ratio	SDSS	WA	FY	Maturity
Site	1	52951.5 *	185.8 **	67.3 *	0.03 ns	320.6 ns	0.5 ns	311.1 ns	1179.4 **
Residual (1)	2	858.6	0.4	0.8 ns	0.002	198.3	0.5	21.1	3.6
Variety	13	7588.1 ***	8.8 **	2.4 *	0.03	152.9 **	4.8 **	452.1 ***	1.1 ns
Site*variety	13	609.7 ns	2.5 ns	0.5 ns	0.01 ns	27.3 ns	0.6 *	28.5 ns	1.8 ns
Residual (2)	26	605.6	2.3	0.9	0.02	25.8	0.2	37.0	1.7

(a)=see abbreviation list.

Table 5.6 Variance ratios (F values) for quality parameters and maturity measurements among 14 South Australian varieties (Halberd excluded) at two sites (Palmer and Roseworthy Campus) in 1993.

Characters	Palmer	RC
Flour protein%	0.8 ns	3.6 **[a]
Maximum resistance (B.U)	6.0 ***	8.0 ***
Extensibility (cm)	1.8 ns	3.1 **
Extensibility/protein ratio	0.7 ns	2.0 ns
SDS-sedimentation volume (ml)	1.8 ns	8.9 ***
Water absorption (%)	36.8 ***	9.3***
Flour yield (%)	5.3 ***	9.1 ***
Maturity score	0.9 ns	0.8 ns

a=see abbreviation list

Glu-D1 subunits 5+10, was present in seven of the varieties. At *Glu-A1*, all of the varieties possessed subunit 1 or 2* and the only null allele was observed in a biotype of Bindawarra. The three varieties Tatiara, Wyuna and Bindawarra classified as soft wheats generally had lower *Glu-1* scores than the Hard wheats. Wyuna, a poor quality biscuit wheat, had a high score of 8, confirmation of the negative relationship between *Glu-1* quality score and biscuit quality as proved by several researches (Payne *et al.*, 1987a; Graybosch, 1992; Lookhart *et al.*, 1993).

Since all varieties were similar in their LMW quality scores, there was no point in using the LMW quality score in the correlation study, and they were surveyed for their *Glu-1* quality score alone. To evaluate whether the quality of South Australian varieties can be better described by the Payne score or the local score, correlation coefficients were computed between the quality parameters and both of these two scores (Table 5.8). The results showed that maximum resistance is significantly associated with both quality scores for all sets of data, whereas for extensibility neither of them was relevant.

In contrast to Rmax, the correlations between HMW glutenin scores and SDSS volumes (Table 5.8) were not significant.

The varieties were grouped into ten different band patterns and subjected to an analysis of variance (Table 5.9). Identification of the primary loci of HMW glutenin subunits

Table 5.7 HMW and LMW glutenin composition and two quality scores for HMW glutenin subunits (Payne and local) and a LMW score (Cornish, 1994) for 15 wheat varieties grown in South Australia.

Variety	HMW glutenin subunit loci (Glu-1)			Score		LMW glutenin subunit loci (Glu-3)			score
	1A	1B	1D	Payne	local	A3	B3	D3	
Janz	1	7+8	2+12	8	6	b	b	b	14
Meering	2*	7+8	2+12	8	7	b	b	b	14
Schomburgk	1	7+8 7+9	2+12 5+10	8.5	7	c	b	c	13
Barunga	1	7+8	5+10	10	8	c	b	c	13
Molineux	1	7+8	5+10	10	8	c	b	c	13
Excalibur	2* 1	7+8 17+18	2+12	8	7	c	b	a	12
Machete	2*	17+18	2+12	8	8	b	b	b	14
Spear	1	7+9	5+10	10	8	c	h	c	13
BT-Schomburgk	1	7+8 7+9	2+12 5+10	8.5	7	c	b	c	13
Yarralinka	2*	17+18	5+10	10	10	c	b	b	13
Tatiara	1	7+9	2+12	7	6	c	b	c	13
Bindawarra	1 null	7+9	2+12	6	5.5	b	b	c	14
Wyuna	2*	17+18	2+12	8	8	c	h	b	14
Halberd	1	7+9 20	5+10	8.5	7.5	e	c	c	8
Angas	1	7+8	2+12	8	6	c	j	c	14

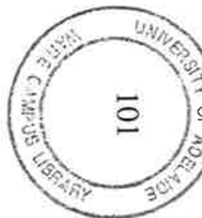


Table 5.8 Correlation matrix between two quality scores (Payne score and local score) and dough properties of 14 (Halberd excluded) and 15 (Halberd included) South Australian varieties at two sites (Roseworthy Campus and Palmer) in 1993 and SARDI data in the period 1985-1993.

parameters	Maximum resistance			Extensibility			E/P ratio			SDS-sedimentation	
	RC	Palmer	SARDI	RC	Palmer	SARDI	RC	Palmer	SARDI	RC	Palmer
Halberd excluded											
Payne score	0.71 *	0.58 *	0.70*	0.29	0.11	0.22	-0.13	-0.12	-0.19	0.46	0.01
Local score	0.57 *	0.57 *	0.70*	0.34	0.27	0.21	-0.19	-0.15	-0.37	0.23	-0.13
Halberd included											
Payne score	0.70 *	0.56 *	0.68 *	0.28	0.10	0.18	-0.08	-0.11	-0.13	0.36	0.01
Local score	0.55 *	0.54 *	0.66 *	0.30	0.22	0.13	-0.18	-0.15	-0.29	0.15	-0.11

Table 5.9 Mean squares and statistical significance of the quality parameters of 14 South Australian varieties (Halberd was excluded) on the basis of HMW glutenin subunit loci conducted as randomised complete block with two replications at two sites (Palmer and Roseworthy Campus) and over two sites (pooled) in 1993

Source of variation	d.f	Palmer						RC						Pooled					
		Rmax[a]	Ext	E/P	WA	SDSS	FY	Rmax	Ext	E/P	WA	SDSS	FY	Rmax	Ext	E/P	WA	SDSS	FY
Glu-A1	2	15757 **	1.7 ns	0.05 ns	1.6 ns	63.2 ns	323.6 ns	5849 **	2.8 ns	0.05 ns	4.3 *	218.0 **	430.2 **	19194 ***	1.6 ns	0.10 *	3.5 ns	236.4 ***	749.4 ***
Glu-B1	2	12054 **	1.6 ns	0.03 ns	0.4 ns	41.0 ns	251.7 ns	9875.5 **	1.8 ns	0.00 ns	0.5 ns	60.8 ns	218.0 ns	21865 ***	1.7 ns	0.02 ns	0.8 ns	100.6 ns	445.8 ***
Glu-D1	1	2037 ns	3.4 ns	0.11 ns	0.3 ns	4.4 ns	78.1 ns	6174.3 **	7.5 ns	0.01 ns	3.0 ns	77.8 ns	285.0 *	7652 ***	10.6 *	0.02 ns	2.6 ns	59.7 ns	330.7 *
Residual	47	1116	2.8	0.02	1.1	33.1	109.6	896.9	3.5	0.02	1.3 ns	37.6	69.5	1007	3.1	0.02	1.2	35.4	89.5

(a)=see abbreviation list.

involved in Rmax and extensibility characters was attempted through a stepwise addition of *Glu-A1*, *Glu-B1* and *Glu-D1* as variables in a multiple regression analyses.

Although, an analysis of variance for SARDI data was not possible, due to the lack of replication, the mean values of the quality parameters over sites were used to compare the allelic effects at *Glu-A1*, *Glu-B1* and *Glu-D1* with those of Palmer and Roseworthy Campus, particularly for those quality parameters which showed significant effects.

There were highly significant differences between the alleles at the *Glu-1* loci for Rmax except for *Glu-D1*, where there was no significant difference at Palmer. In general glutenin subunit bands 2*, 17+18 and 5+10 were associated with greater dough strength than their counterpart alleles (Fig 5.1). As shown in Fig 5.1, the trend of allelic effects for the SARDI data was similar to that at Palmer and Roseworthy Campus.

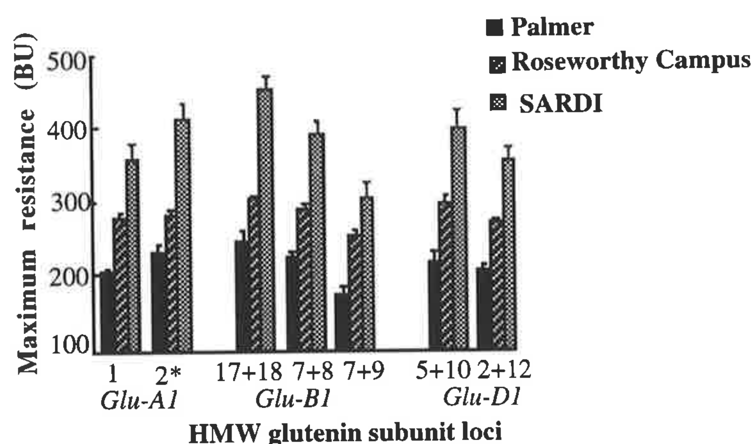


Figure 5.1 Mean maximum resistance for varieties carrying specific alleles of HMW glutenin subunits. Data from two separate sites (Palmer and Roseworthy Campus) in 1993 and SARDI data in the period of 1985 to 1993.

The *Glu-1* alleles generally did not show any significant differences in extensibility. However in study of the pooled data, a significant difference was observed between the alleles at *Glu-D1*. Varieties having glutenin subunit bands 5+10 were more extensible than those possessing 2+12 (18.6 cm vs 17.8 cm). This is in contrast to the results of Gupta *et al.* (1991) who reported higher dough extensibility for Australian varieties having subunit bands 2+12.

SDS sedimentation volumes of varieties were only significant for the *Glu-A1* at Roseworthy Campus and over sites (Pooled data). The highest SDSS volume was observed for varieties carrying subunit 1, similar to the results of Lorenzo *et al.* (1987).

The differences between water absorption of varieties were only significantly related to *Glu-A1* in Roseworthy Campus (Table 5.9). Varieties having subunit band 2* had a higher water absorption than the others (59.8% vs 58.2%).

In general, varieties having alleles for higher dough strength had higher flour yield, (Fig 5.2), with significant associations with the *Glu-A1* and *Glu-D1* loci at Roseworthy Campus for flour yield. It is notable that varieties carrying allele 2* with greater effect on dough strength (Fig 5.1) had lower flour yield than those possessing 1. Similarly the flour yield of the 17+18 varieties was lower than those for the 7+8 group. These trends were also observed for the SARDI data.

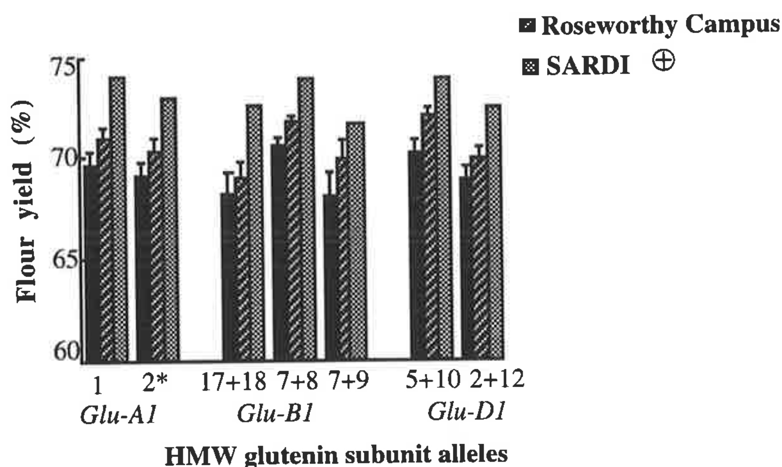


Figure 5.2 Mean flour yield for varieties carrying specific alleles of HMW glutenin subunit alleles. Data from two sites (Palmer and Roseworthy Campus) in 1993 and SARDI data in the period 1985 to 1993.

⊕ This data was not analysed for glutenin subunit alleles.

Predictability of dough properties using Glu-1 alleles

Since the effects of alleles at *Glu-1* loci showed significant difference amongst varieties, the Rmax data for the Palmer, Roseworthy Campus, pooled data over these two sites and SARDI data were subjected to multiple regression analyses with the aim of estimating allelic effects and hence dough properties (measured by Rmax and extensibility).

The equations took the form

$$Rmax_{est} = a_{\circ} + \sum (a_i \times \overline{Rmax}),$$

where $a_{\circ}$ is the intercept, a_i is the slope for each individual locus and $\overline{Rmax}$, is the mean value of Rmax for the individual alleles (Table 5.10). Rmax and extensibility effects were estimated for each allele of the *Glu-A1*, *Glu-B1* and *Glu-D1* loci. The Rmax and

Table 5.10 The mean effect for the individual alleles of *Glu-1* loci relative to 1 at *Glu-A1*, 17+18 at *Glu-B1* and 5+10 at *Glu-D1* for the 15 South Australian varieties for extensibility and maximum resistance. Data from two sites (Roseworthy Campus and Palmer) in 1993 and SARDI secondary trials in the period of 1985-1993.

Locus	Allele	Quality measurements at different sites											
		extensibility						maximum resistance					
		RC		Palmer		SARDI		RC		Palmer		SARDI	
		H.I(a)	H.E(b)	H.I	H.E	H.I	H.E	H.I	H.E	H.I	H.E	H.I	H.E
Glu-A1	null	-0.64	-0.81	+1.44	+1.29	+0.41	+0.21	-46.6	-43.7	-68.3	-66.5	-90.5	-94.0
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2*	-0.43	-0.42	+0.15	+0.17	+0.19	+0.21	11.6	-11.9	+11.0	+10.8	+19.9	+20.2
Glu-B1	17+18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	7+8	+0.04	+0.01	+0.81	+0.78	+0.83	+0.81	-20.7	-20.2	-20.5	-20.2	-55.2	-55.5
	7+9	-0.78	-0.54	+0.46	+0.67	+0.23	+0.52	-56.2	-64.0	-61.4	-64.0	-135.0	-129.7
	20	-3.18	0.0	-1.7	0.0	-2.53	0.0	0.0	-102.4	0.0	-89.9	-185.8	0.0
Glu-D1	5+10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2+12	-0.56	-0.86	-0.32	-0.58	-0.06	-0.41	-24.5	-19.3	-14.1	-10.8	-35.2	-41.5
Intercept		**20.7**	**20.9**	**15.8**	**16.0**	**18.5**	**18.7**	**331.3**	**327.4**	**252.3**	**249.9**	**464.8**	**469.3**

a=Halberd included
b=Halberd excluded

extensibility estimates were then subjected to simple correlation analysis to find the correlation coefficients between the actual dough test-data with those estimated by the multiple regression equations. To evaluate the *Glu-3* score, the cultivar Halberd which has a high *Glu-1* score, but carries *Glu-3* alleles with low ranking, was included. The results showed that there were generally high correlation coefficients between the actual and the estimated values of Rmax for all sets of data (Fig 5.3). It is notable that there were no marked changes in the correlation coefficients when Halberd was included.

A similar series of calculation were applied to extensibility. The correlation coefficient between the estimated and the actual extensibility was not significant, indicating that the HMW subunit composition cannot predict dough extensibility. However, the correlation between actual and estimated data was dramatically increased when Halberd was included (Fig 5.4). This indicates that some of the variation in extensibility can be accounted for by LMW glutenin subunits.

To evaluate whether the data from one set can be predicted by other sets of data, the predicted value from one set of data were plotted against the actual values for the other two sets for maximum resistance and extensibility. Since, the predicted value for quality measurements at Roseworthy Campus was relatively high when compared with their actual value, this relationship was used as a standard for the comparisons. For Rmax the reciprocal correlation between actual and predicted values was significant between the three sets of data (Fig 5.3). The highest correlation was observed between predicted Rmax at Roseworthy Campus with actual data at Palmer (Fig 5.3c). It is notable that the correlations were generally higher when Halberd was included.

With respect to extensibility, a high correlation was only observed between the predicted extensibility at Palmer vs actual extensibility from the SARDI data when Halberd was included (Fig 5.4b).

There were a number of interesting correlations which are given in Table 5.11. In general there was a high correlation between the actual measurements of the different sets of data for both Rmax and extensibility, indicating that the data obtained in this study were relatively accurate compared to SARDI data as a long term reference. Comparison between different sets of data showed that actual Rmax at Palmer was highly correlated with the predicted Rmax at SARDI (r=0.94). Likewise actual Rmax at SARDI was highly correlated with predicted Rmax at Roseworthy Campus (r=0.92). This highly reciprocal correlation between different sets of data, particularly with the long term reference data (SARDI), indicates that Rmax can be estimated on the basis of the alleles at the three HMW glutenin loci (*Glu-A1*, *Glu-B1* and *Glu-D1*). By contrast the correlation between actual extensibility with predicted of another set was low, however, when Halberd was

Figure 5.3 Relationship between predicted and actual Rmax for different sets of data. In all cases the relationship between actual value of Rmax at Roseworthy Campus against its predicted value (grey) is included for comparison as actual vs predicted at the same site could be expected to be highly correlated.

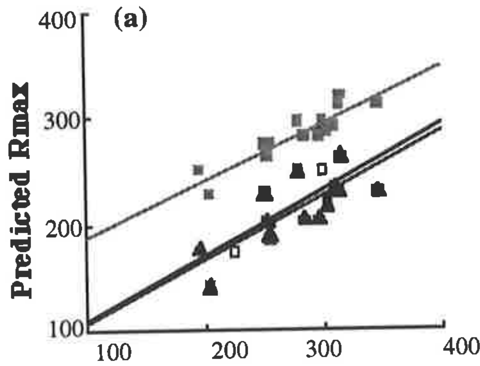
Description of Figure:

(a) Predicted by Palmer vs actual at Roseworthy Campus.
(b) Predicted by Palmer vs actual at SARDI.
(c) Predicted by Roseworthy Campus vs actual at Palmer.
(d) Predicted by Roseworthy Campus vs actual at SARDI.
(e) Predicted by SARDI vs actual at Palmer.
(f) Predicted by SARDI vs actual at Roseworthy Campus.

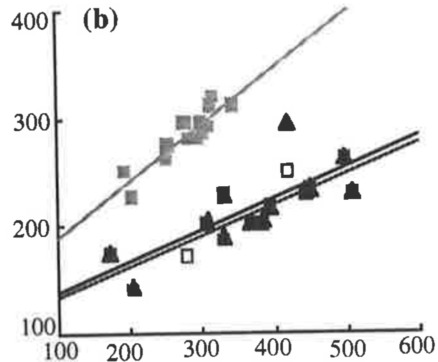
Symbols used in Figure:

P= Palmer
RC=Roseworthy Campus
S=SARDI
St=standard
(-H)=Halberd excluded
(+H)=Halberd included

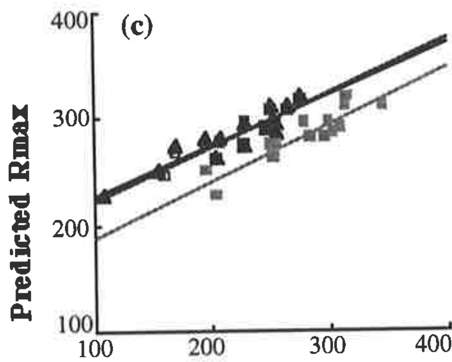
■ y = 137.6 + 0.52x R^2 = 0.82 | RC vs RC (St)
▲ y = 49.2 + 0.61x R^2 = 0.49 | P vs RC (-Halberd)
□ y = 48.7 + 0.59x R^2 = 0.61 | P vs RC (+Halberd)



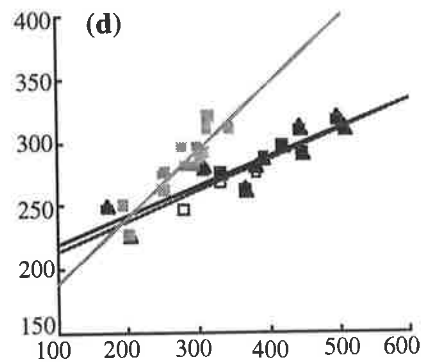
y = 137.6 + 0.52x R^2 = 0.82 | RC vs RC (St)
y = 110.2 + 0.29x R^2 = 0.59 | P vs S (-Halberd)
y = 107.3 + 0.28x R^2 = 0.74 | P vs S (+Halberd)



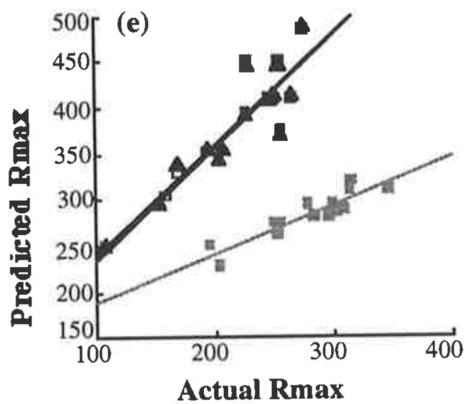
▲ y = 179.3 + 0.48x R^2 = 0.84 | RC vs P (-Halberd)
□ y = 170.5 + 0.51x R^2 = 0.91 | RC vs P (+Halberd)



y = 197.2 + 0.23x R^2 = 0.83 | RC vs S (-Halberd)
y = 190.7 + 0.24x R^2 = 0.84 | RC vs S (+Halberd)



▲ y = 119.8 + 1.20x R^2 = 0.82 | S vs P (-Halberd)
□ y = 111.2 + 1.23x R^2 = 0.84 | S vs P (+Halberd)



y = 66.4 + 1.13x R^2 = 0.59 | S vs RC (-Halberd)
y = 55.2 + 1.16x R^2 = 0.61 | S vs RC (+Halberd)

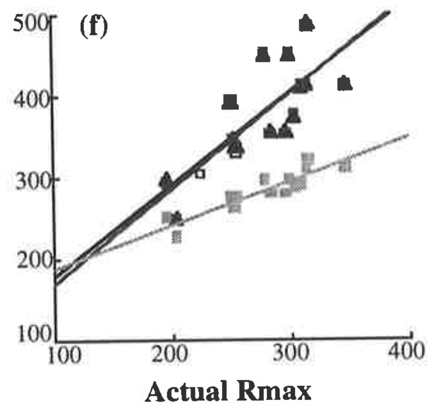


Figure 5.4 Relationship between predicted and actual extensibility for different sets of data. In all cases the relationship between actual value of extensibility at Roseworthy Campus against its predicted value has been plotted (grey) for comparison as actual vs predicted at the same site could be expected to be highly correlated.

Description of Figure:

(a) Predicted by Palmer vs actual at Roseworthy Campus .

(b) Predicted by Palmer vs actual at SARDI.

(c) Predicted by Roseworthy Campus vs actual at Palmer.

(d) Predicted by Roseworthy Campus vs actual at SARDI.

(e) Predicted by SARDI vs actual at Palmer.

(f) Predicted by SARDI vs actual at Roseworthy Campus .

Symbols used in Figure:

P= Palmer

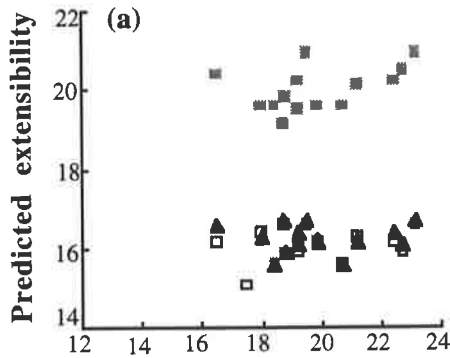
RC=Roseworthy Campus

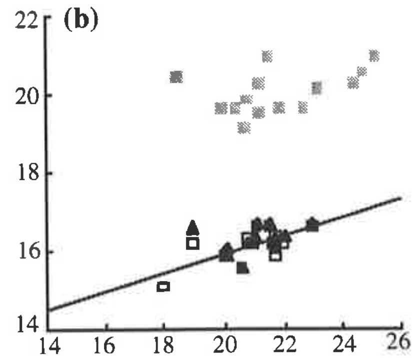
S=SARDI

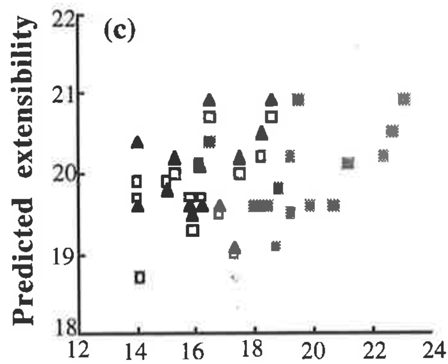
St=standard

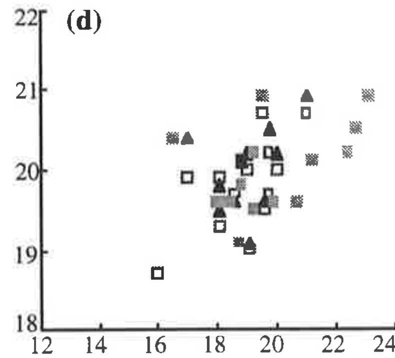
(-H)=Halberd excluded

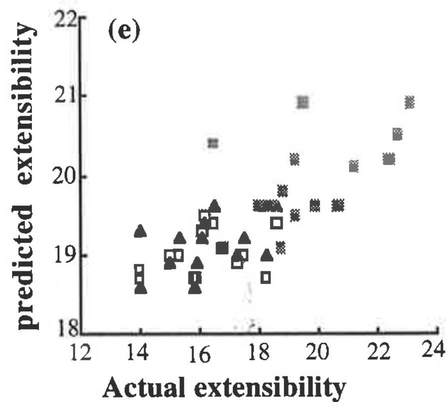
(+H)=Halberd included


y = 17.6 + 0.12x R^2 = 0.18 RC vs RC (St)
y = 16.2 + 3.40x R^2 = 0.00 P vs RC (-Halberd)
y = 15.0 + 5.35x R^2 = 0.06 P vs RC (+Halberd)
(a)
Predicted extensibility


y = 17.6 + 0.12x R^2 = 0.18 RC vs RC (St)
y = 14.5 + 9.58x R^2 = 0.08 P vs S (-Halberd)
y = 11.8 + 0.23x R^2 = 0.49 P vs S (+Halberd)
(b)


y = 18.2 + 0.11x R^2 = 0.08 RC vs P (-Halberd)
y = 17.3 + 0.16x R^2 = 0.17 RC vs P (+Halberd)
(c)
Predicted extensibility


y = 16.7 + 0.17x R^2 = 0.01 RC vs S (-Halberd)
y = 14.6 + 0.28x R^2 = 0.39 RC vs S (+Halberd)
(d)


y = 17.7 + 8.80x R^2 = 0.16 S vs P (-Halberd)
y = 16.2 + 0.17x R^2 = 0.23 S vs P (+Halberd)
(e)
predicted extensibility
Actual extensibility

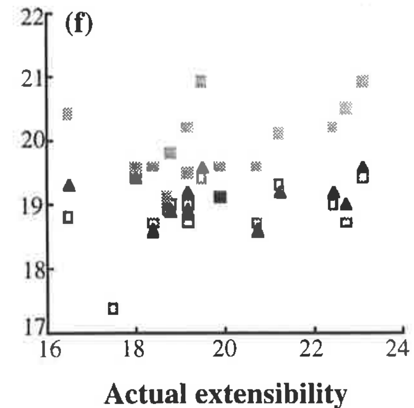

y = 18.7 + 2.10x R^2 = 0.02 S vs RC (-Halberd)
y = 17.2 + 8.52x R^2 = 0.11 S vs RC (+Halberd)
(f)
Actual extensibility

Table 5.11 Correlation coefficients between different combinations of data sets for maximum resistance and extensibility (actual or predicted) of 14 or 15 (including Halberd) South Australian varieties grown at two sites (Palmer and Roseworthy Campus) in 1993. The data obtained by SARDI in the period of 1985-1993 at several locations was included as long term reference. Halberd was included as a variety with low ranking glutenin subunits.

Quality parameters	Comparison		-Halberd		+Halberd	
	predicted	actual	predicted	actual	predicted	actual
Maximum resistance						
actual by RC	Palmer	Palmer	0.79	0.86	0.80	0.86
actual by RC	SARDI	SARDI	0.80	0.91	0.81	0.91
actual by Palmer	RC	RC	0.95	0.86	0.96	0.86
actual by Palmer	SARDI	SARDI	0.94	0.89	0.94	0.90
actual in SARDI	RC	RC	0.92	0.91	0.92	0.91
actual in SARDI	Palmer	Palmer	0.85	0.89	0.86	0.90
extensibility						
actual by RC	Palmer	Palmer	-0.05	0.56	0.20	0.62
actual by RC	SARDI	SARDI	0.07	0.58	0.32	0.63
actual by Palmer	RC	RC	0.26	0.56	0.44	0.62
actual by Palmer	SARDI	SARDI	0.22	0.88	0.48	0.89
actual in SARDI	RC	RC	0.42	0.58	0.63	0.63
actual in SARDI	Palmer	Palmer	0.46	0.88	0.70	0.89

included, the correlation was increased. In this case the highest correlation was observed between the actual extensibility on the SARDI data with predicted extensibility at Palmer (r=0.70).

Prediction based on the *Glu-1* alleles showed that about 25% of the variation (Pooled data) in Rmax could be accounted for by *Glu-1* loci (Table 5.12). This was higher when the data from different sites were taken into consideration (about 40%). Extensibility was poorly predicted resulting in a negative value of percentage variance (R^2-adjusted). This was not markedly improved when Halberd was included, although, for the SARDI data 13% of variation in extensibility could be accounted for by the *Glu-1* loci. The amount of variation accounted for by the *Glu-1* loci for the SARDI data for the extensibility/protein ratio was higher than the other sets, particularly when the Halberd was included in the prediction model.

Table 5.12 The percentage variance (R^2-adjusted) of dough properties of varieties accounted by *Glu-1* loci. Data from two sites (Palmer and Roseworthy Campus), over sites (pooled) in 1993 and SARDI secondary trials in the period of 1985-1993 over several locations. Halberd was included as a variety with low ranking glutenin subunits.

Quality parameters	R^2-adjusted (%)			
	Palmer	RC	Pooled	SARDI
Halberd excluded				
Maximum resistance	47.4	40.3	25.0	42.4
Extensibility	Negative	Negative	Negative	Negative
E/P ratio [a]	4.3	4.1	6.8	9.0
Halberd included				
Maximum resistance	49.6	41.0	26.5	43.1
Extensibility	Negative	2.3	Negative	12.6
E/P ratio	7.2	3.4	8.3	21.9

a=see abbreviation list

5.3.3 Relationship between quality parameters of South Australian varieties

A correlation matrix was computed between quality parameters of 14 South Australian varieties, grown at two different sites (Palmer and Roseworthy Campus), the pooled data and data obtained by SARDI over several years. The correlation coefficients between Rmax and protein concentration were significant for Roseworthy Campus, the pooled and SARDI data (Table 5.13). Extensibility and protein concentration were both interrelated

Table 5.13 Correlation matrix between the rheological properties of doughs of 15 South Australian varieties grown at two different sites (Palmer and Roseworthy Campus) and over sites (pooled) in 1993 and the mean value of these varieties measured by South Australian Research and Development Institute (SARDI) from the secondary trials data in the period 1985-1993 over several locations.

Quality (a) parameters		Correlation matrix															
		Palmer				RC				Pooled				SARDI			
		Rmax	Ext	WA	PC	Rmax	Ext	WA	PC	Rmax	Ext	WA	PC	Rmax	Ext	WA	PC
Rmax	1	1.00				1.00				1.00				1.00			
Ext	2	0.35	1.00			0.56 *	1.00			0.59 *	1.00			0.29	1.00		
WA	3	0.33	-0.11	1.00		0.35	0.17	1.00		0.22	0.31	1.00		0.67 *	0.03	1.00	
PC	4	0.51	0.29	0.36	1.00	0.62 *	0.43	0.66 *	1.00	0.68 *	0.66 *	0.22	1.00	0.60 *	0.58 *	0.44	1.00

(a)=See abbreviation list

to maximum resistance for Roseworthy Campus and pooled data, indicating that under growing conditions favourable for the production of high levels of protein, varieties have relatively good dough strength. There was also a high correlation between protein concentration and water absorption which was in agreement with the results of Finney (1979). Amongst the data from SARDI, it was observed that there were high correlations between Rmax and water absorption, and extensibility and protein concentration.

5.4 Discussion

Studies of the allelic variation amongst Australian varieties have shown that there are three, eight and four variants at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively (Lawrence, 1986). The high frequency of alleles 17+18 and 7+8 at *Glu-B1* was again evident in the South Australian wheats, alleles which are rare amongst varieties in a world collection as reported by Payne and Lawrence (1983). It is of interest that those alleles assessed as low scoring are generally rare amongst Australian varieties. This can be explained to a large extent by the common parentage and selection pressure towards high breadmaking quality. This is in contrast to varieties grown in UK, Germany and Spain which are characterised by the frequent occurrence of the null allele at the *Glu-A1* locus (Payne *et al.*, 1987a; 1988; Rogers *et al.*, 1989).

The above results showed that the *Glu-1* quality score of the most of the varieties studied here was relatively high, however the score 8 (Table 5.7) observed in Wyuna, a soft wheat, was somewhat surprising. Wyuna has been known as a soft wheat which is too strong and lacks good extensibility and is therefore a poor quality biscuit wheat with relatively high dough strength under conditions which produce a high level of protein (Roseworthy Campus compared to Palmer) (Table 5.4). Perhaps some of such soft wheats with high scores for HMW glutenin could be made into acceptable bread. Lookhart *et al.* (1993) suggested that a protein concentration at 11% or greater would be required.

Five cultivars Schomburgk, Excalibur, BT-Schomburgk, Angas and Wyuna had two biotypes with different band patterns for some of the HMW loci (Table 5.7). From the viewpoint of a breeding program, selection for increasing breadmaking quality by choosing the biotype with a glutenin composition associated with better quality could be applied (Lawrence *et al.*, 1987).

The general superiority of subunits 17+18 and 7+8 to 7+9 as reported by several researchers (e.g. Grama *et al.*, 1987; Payne *et al.*, 1984a) was also observed in this study (Fig 5.1). The glutenin subunit bands 17+18 are prominent among Australian wheat being in 34% of the 106 cultivars examined by Lawrence (1986). These bands have been found to have a higher Rmax than 7+8 (Metakovsky *et al.*, 1990). However Gupta *et al.*, (1991) reported that the effects of the subunit bands 17+18 and 7+8 on Rmax were equal in Australian wheats. This inconsistencey of ranking would be due to different genetic backgrounds or different growing conditions.

Estimation of Rmax on the basis of the three loci for the HMW glutenin subunits (*Glu-A1*, *Glu-B1* and *Glu-D1*) showed that there was a high correlation coefficient between actual Rmax and estimated value for all sets of data (Fig 5.3). This indicated that HMW glutenin

subunits have a relatively large contribution to controlling Rmax. Results obtained from analysing genetic lines deficient in varying numbers or amounts of the LMW or HMW glutenin subunits has indicated that the HMW glutenin subunits, on a constant weight basis, have more pronounced effects on dough resistance than the LMW subunits of glutenin (Gupta *et al.,* 1990b).

The HMW quality score showed a lower correlation with extensibility than with Rmax (Fig 5.4), however when Halberd was included, approximately 13% of variation in SARDI data could be accounted for by the *Glu-1* loci (Table 5.12). However the quality scores on HMW glutenin (Payne and local) were not developed to predict extensibility. Gupta *et al.* (1991) reported that the extensibility of Australian wheat varieties was better correlated to the LMW glutenin subunits than to the HMW glutenins. In the study here, the LMW glutenin scores of the other varieties were similar so it was difficult to assess these relationships. The low correlation between the HMW quality score and extensibility may be due to the molecular size distribution. Selection of lines based on a high level of expression of HMW glutenin subunits (high quality score) could cause the molecular weight distribution to be shifted to the high molecular weight group and consequently reduced extensibility. Therefore the best strategy may be maintain the LMW:HMW glutenin subunits ratio using specific subunits that do not contribute to excessive dough strength (e.g. HMW subunits 2+12 rather than 5+10).

It has been also reported that certain combinations of HMW glutenin subunits can cause larger effects than could be assumed from the size effect only (Bekes *et al.,* 1994). Scoring on the basis of HMW alone may be insufficient to account for differences in quality. This point was also demonstrated in a set of 32 cultivars from North America (Khan *et al.,* 1989). These cultivars, despite the uniformity of their high molecular glutenin subunit compositions varied in their dough properties. Several research projects have indicated that HMW and LMW loci interact with each other in the manifestation of dough strength. Therefore a given allele may not necessarily show its superior effect in all genetic backgrounds.

Metakovsky *et al.* (1990) in a survey of 28 Australian bread wheats found that the LMW subunits of glutenin had a much stronger correlation with dough quality (as determined by extensigraph) than the HMW subunits. This was in contrast to genotypes from other countries, e.g., Britain, Spain, and Canada, where the HMW glutenin subunits had very high correlations with wheat quality (Payne *et al.,* 1987a; MacRitchie *et al.,* 1990). The results here showed that the HMW glutenin subunits as reflected by the *Glu-1* quality scores were positively associated with dough strength with an average of 38% of the variation for Rmax (Table 5.12) being accounted for by the HMW glutenins. This amount compared to those reported by Payne *et al.* (1987a) was relatively low, indicating either

that, for Australian varieties, additional aspects of gluten composition must be involved in controlling dough strength or that there was comparatively restricted genetic variability within the group.

The LMW subunits are quantitatively the major group of glutenin subunits, contributing considerably more than the HMW subunits to total glutenin. A part of the explanation for the higher extensibility of varieties at Roseworthy Campus than Palmer could lie in the balance of protein composition (glutenin/gliadin). Studies have shown that an increase in protein content is accompanied by a disproportionate increase in gliadin component relative to the other components such as glutenin and albumin/globulin (Bariana *et al.*, 1993; Gupta *et al.*, 1992) and high levels of gliadins have been associated with high extensibility.

Although SDS-sedimentation volume and maximum resistance have both been used for the estimation and measurement of dough strength, the HMW quality score was only significantly correlated to Rmax (Table 5.8). These results suggest that the strength properties of wheat flour were measured differently by the SDSS and extensograph tests. 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 polymers by forming intermolecular disulphide linkages. In the SDS sedimentation test, sodium dodecyl sulphate (SDS) is used as a solvent for the gluten proteins. This synthetic ionic detergent as a denaturing agent change the native glutenin structure, whereas with the dough strength measurements using the extensograph, the nature of glutenin is comparatively unchanged, and therefore the disulphide linked polymers can show their positive effects on dough strength. Evidence supporting the positive role of disulphide polymers in governing dough strength has been widely available (Nielsen *et al.*, 1962; Dimler, 1965; Field *et al.*, 1983 MacRitchie, 1987).

An explanation for the higher SDSS (Table 5.4) values at Palmer than Roseworthy Campus could be related to the higher protein concentration at the latter site, similar to the results reported by Lorenzo *et al.* (1987). In the study here, sodium dodecyl sulphate (SDS) was used at a 2% concentration (20 gL^{-1}). This concentration may not have been sufficient to dissolve the greater amount of protein present in the flour sample from Roseworthy Campus. Also there is a possibility that the additional protein synthesised at the higher protein site may not contribute to the viscoelastic properties of the dough, resulting in lower SDSS values.

The non-significant correlation between HMW glutenin score and SDSS indicates that for Australian varieties, SDSS cannot fully explain the variation in dough strength and would not seem to be a suitable test for Australian breeding programs.

CHAPTER 6

VARIATION OF WHEAT SEED STORAGE PROTEINS WITHIN RANDOMLY SELECTED BREEDING LINES AND EFFECT ON DOUGH RHEOLOGICAL PROPERTIES

6.1 Introduction

There are several approaches which can be used to study the effect of protein components on end-use products. Reconstitution experiments are one of the most reliable methods and have been used widely for correlation studies. The success of the method depends on carrying out the extraction and reconstitution without altering the functional properties of the components (Finney, 1943; MacRitchie, 1985). By using this approach it was found very early on that the differences in baking quality between varieties are due to differences in protein composition (Finney, 1943). Once it was established that gluten is largely responsible for differences in functionality, the contribution of specific fractions of gluten were examined by the addition of varied amounts of each fraction to a control flour (MacRitchie, 1992). The results showed that gliadins reduced dough strength, whereas glutenins improved dough quality.

Gel electrophoresis has been an approach that has been widely used to define the qualitative relationship between various glutenin subunits and dough strength properties. The pioneering work of electrophoretic studies undertaken with wheat cytogenetic stocks (Boyd and Lee, 1967; Shepherd, 1968; Wrigley and Shepherd, 1973; Lawrence and Shepherd, 1980, 1981) has revealed that the genes controlling seed storage proteins were mostly located on group 1 and group 6 chromosomes. Payne *et al.*, (1981a), established that certain HMW glutenin subunits were associated with positive effects on flour properties whereas others were associated with negative effects. Subsequently several other groups (Moonen *et al.*, 1983; Branlard and Dardevet, 1985b; Lawrence *et al.*, 1987) have confirmed and extended these correlations.

Identification of wheat lines lacking certain gliadin or glutenin components encoded by genes on a particular chromosome has offered another approach for investigating the relationship between quality and protein composition (Rogers *et al.*, 1991; Lawrence *et al.*, 1988; Benedettelli *et al.*, 1992). Whole chromosome substitution lines (Zemetra *et al.*, 1987; Mansur *et al.*, 1990) and homozygous back-cross derived lines, isogenic for glutenin subunit alleles (Payne *et al.*, 1987b) provide direct comparisons. By using these methods different loci of HMW glutenin subunits have been shown to have differential effects on bread baking quality (Branlard and Dardevet, 1985b; Lawrence *et al.*, 1987; Khan *et al.*,

1989). An analysis of the effect of glutenin alleles on breadmaking quality can suggest breeding strategies that could be undertaken to produce new wheats with appropriate dough properties.

Studies involving a group of cultivars have the problem that pleiotropy and linkage can not be distinguished from chance associations as a result of common ancestry. Random Recombinant Inbred Lines (RILs) from a hybrid between two inbred cultivars (Brunori *et al.*, 1989; Gupta *et al.*, 1989; Carrillo *et al.*, 1990; Rousset *et al.*, 1992; see also Chapter 7) largely allow these effects to be recognised, but genetic linkage (which is not so important in wheat with its 21 chromosomes) can complicate interpretation. However, this sort of study may produce results which are specific to the particular cross involved.

The success of breeding for high technological quality depends on improvements in both the potential of the genotypes and the stability of quality expression. It is desirable for a variety to have optimal mean values with low variances in these parameters when measured across environments. In most of the breadmaking quality studies this latter concept has not been adequately considered. Therefore the experiment reported here was performed using lines, selected for grain yield, but unselected for protein quality to determine whether the genotypes with high grain yields also have stability for quality parameters in response to a range of environments.

6.2 Materials and methods

6.2.1 Genetic material

This study involved randomly selected lines from a population with a broad genetic base. Nine F_7 breeding lines from the cross [(Turkey787 x Warigal) x Warigal] which had been subjected to yield but not quality selection were harvested at four locations (Yeelanna, Walpeup, Mudamuckla and Palmer) in southern Australia and analysed for their LMW and HMW subunits at the *Glu-3* and *Glu-1* loci respectively.

It is notable that the selection had been conducted in two steps. The first selection was two plants in the F_2 generation, simply indicated by /5/ and /2/. The second step of selection was actually a reselection of the above two selected lines, indicated by the /2 to /10 and /1 to /7 (see Table 6.1 for more details). Therefore two groups of F_2 selected lines were included in the experiment.

6.2.2 Experimental sites

The selected lines were grown at four sites (Yeelanna, Walpeup, Mudamuckla and Palmer) in 1992. The Walpeup site was located in the malle district of Victoria at the Mallee Research Station of the Victorian Department of Agriculture and the Mudamuckla site was on the property of Mr. C. Martin, on upper Eyre Peninsula, South Australia. The soil type at both sites was an alkaline sandy loam over clay loam B horizon with a high level of $CaCo_3$. The annual rainfall of both sites was approximately 300 mm. Details of the Yeelanna and Palmer sites have been given in Chapter 4.

6.2.3 Electrophoresis

Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis was used in accordance with the newly developed sequential extraction technique (Singh *et al.*, 1991b) (Chapter 3, Materials and methods).

6.2.4 Quality testing

Near-Infrared Reflectance (NIR) and Brabender Farinograph and Extensograph properties (maximum resistance, extensibility) were measured on dough samples using the same methods as those reported in Chapter 4.

6.2.5 Statistical analysis

The F_7 lines were not strictly a randomly selected population because they had previously been selected for boron tolerance in the BC_1F_2 and for grain yield and were derived from only two F_2 plants. Therefore, two groups of selected lines (/5/ and /2/) were considered as the first factor in a split block design and the analysis of variance was performed over sites as a factorial.

6.3 Results

6.3.1 Glutenin subunit composition of lines

Two different groups of genotypes could be identified based on their HMW and LMW glutenin subunits; variation at the *Glu-A1*, *Glu-D1* and *Glu-B3* loci resulted in one group having band patterns 1, 5+10, j and the other 2*, 2+12, b (Table 6.1). There was no allelic variation at the *Glu-B1*, *Glu-A3* and *Glu-D3* loci. For convenience the terms /5/ and /2/ are used for these two groups.

Table 6.1 HMW and LMW glutenin subunit composition of nine lines selected from the cross [(Turkey787 x Warigal) x Warigal]

Lines	Glutenin subunits					
	Glu-1(HMW)			*Glu-3* (LMW)		
	A1	B1	D1	A3	B3	D3
[(Turkey x Warigal) x Warigal-A2] 5/2	1	7+8	5+10	c	j	a
[(Turkey x Warigal) x Warigal-A2] 5/3	1	7+8	5+10	c	j	a
[(Turkey x Warigal) x Warigal-A2] 5/4	1	7+8	5+10	c	j	a
[(Turkey x Warigal) x Warigal-A2] 5/5	1	7+8	5+10	c	j	a
[(Turkey x Warigal) x Warigal-A2] 5/7	1	7+8	5+10	c	j	a
[(Turkey x Warigal) x Warigal-A2] 5/8	1	7+8	5+10	c	j	a
[(Turkey x Warigal) x Warigal-A2] 5/10	1	7+8	5+10	c	j	a
[(Turkey x Warigal) x Warigal-A2] 2/1	2*	7+8	2+12	c	b	a
[(Turkey x Warigal) x Warigal-A2] 2/3	2*	7+8	2+12	c	b	a
[(Turkey x Warigal) x Warigal-A2] 2/4	2*	7+8	2+12	c	b	a
[(Turkey x Warigal) x Warigal-A2] 2/5	2*	7+8	2+12	c	b	a
[(Turkey x Warigal) x Warigal-A2] 2/7	2*	7+8	2+12	c	b	a

6.3.2 Variation of quality parameters between groups of lines

The analysis of variance for quality parameters of the two groups of lines over four sites (Yeelanna, Walpeup, Mudamuckla and Palmer) showed that there were highly significant differences between sites for all quality parameters (Table 6.2). The highly significant difference between sites for protein concentration provided an opportunity to evaluate the quality parameters of these two groups of lines at a range of protein concentrations.

Table 6.2 Mean square and statistical significance for quality parameters of two groups of random lines (/5/ and /2/) from the cross [(Turkey x Warigal) x Warigal] over four sites in southern Australia.

source of variation	d.f	Mean square				
		Rmax[(a)]	Ext	PC	E/P	WA
Site	3	8250.2 ***	54.9 ***	21.4 ***	0.07 ***	4.5 ***
Genotype	1	38629.9 ***	5.1 **	0.5 ns	0.09 ***	3.2 ***
Genotype x site	3	5313***	17.9 ***	4.9 ***	0.01 *	0.4 ns
Residual	24	618.5	0.9	0.2	0.004	0.3

a=see abbreviation list

Among the quality parameters, maximum resistance, extensibility and water absorption were highly significantly different between the two groups of genotypes. Since extensibility is correlated to protein level (Gupta *et al.*, 1992), the extensibility/protein concentration (E/P) ratio was used to indicate the presence of factors other than protein concentration affecting extensibility. Although, there was no significant difference between the two group of lines for protein concentration, the E/P ratio showed a highly significant difference. The non-significant difference between the two groups of random lines for protein concentration indicated that the differences between most of the quality parameters quoted above is most likely due to their protein quality, perhaps reflected by the different banding patterns of glutenin subunits. By contrast, the highly significant site mean square and interaction between genotype and sites for protein concentration indicated that environmental factors were relatively important for protein concentration.

It is obvious that lines within each genotype could show the effect of genetic background other than glutenin subunits on quality parameters. To evaluate how much of the variation in quality parameters could be accounted for by genotypes, lines within genotypes and sites; the percentage of variance component for each of these was calculated (Table 6.3).

Table 6.3 Percentage of variance attributed to genotypes (glutenin subunits), lines within genotypes (same glutenin bands) and sites (growing conditions) for different quality parameters, for the [(Turkey787 x Warigal) Warigal] random lines.

Quality parameters	Percentage of variance component		
	genotypes	lines	site
Maximum resistance	84	16	- †
Extensibility	35	10	55
Protein concentration	1	90	9
Extensibility/protein ratio	40	38	22
Water absorption	20	38	42

† Negative variance component, so the value has been excluded when calculating the percentage of the variance component.

The high percent of variation in Rmax accounted for by genotypes indicates that glutenin subunit alleles have a central role in these lines for controlling dough strength. By contrast, the lower proportion of the variation of extensibility accounted for by genotypes and lines indicates that environmental factors have a large effect on dough extensibility, confirmed by the high percentage of variance accounted for by site. The variation for

protein concentration accounted for by lines, possibly indicates that there are different genes which affect the protein concentration at different sites and that environmental factors affect the protein content as reported by Joppa and Cantrell (1990).

The lines within selection /5/ with specific band patterns previously associated with strong doughs, were consistently associated with a higher maximum resistance than those lines within selection /2/ at three sites (Fig 6.1a). However, this effect was not observed at Palmer. The highly significant interaction between genotype and site for this quality parameter can be clearly seen in the results from Walpeup and Yeelanna. The value of Rmax associated with the /5/ group of lines was relatively high at Walpeup whereas at Yeelanna the /2/ group was relatively weak. This was in contrast with the results of Gupta *et al.* (1994a) who reported that the effects of alleles coding for both LMW and HMW glutenins on dough resistance were consistent over locations.

With respect to the E/P ratio, a higher ratio was observed for selection /2/ at the most of the sites, however, at Yeelanna the two groups of selections were about the same. (Fig 6.1b). This, compared to the relatively high value of E/P ratio at the other three sites, was reflected in a significant interaction between site and genotype.

Slightly higher water absorption was observed for the /5/ group lines at Walpeup, Yeelanna and Mudamuckla, but at Palmer there was no difference between the two groups of lines (Fig 6.2). The interaction between site and genotype for this quality parameter was not significant, indicating that environmental conditions were of little importance in controlling water absorption for the genotypes in this trial.

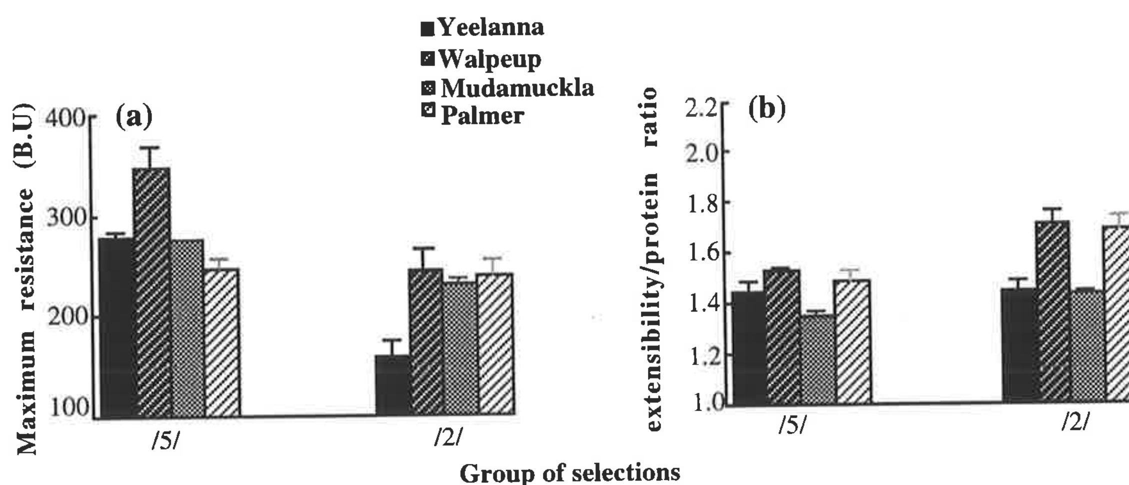


Figure 6.1 Mean **(a)** maximum resistance and **(b)** extensibility/protein ratio of two group of random lines from the cross [(Turkey787 x Warigal) x Warigal] grown at four locations.

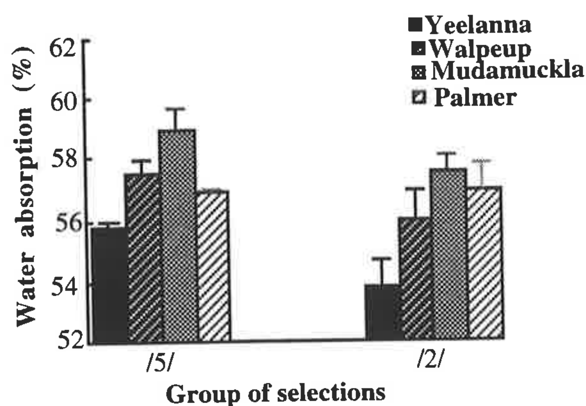


Figure 6.2 Mean water absorption (%) of two group of random lines from the cross [(Turkey787 x Warigal) Warigal] grown at four locations.

The interaction between genotype and site was more pronounced for extensibility than maximum resistance when compared by their variance ratio. The higher variance ratio attributed to extensibility was most likely due to its close relationship to protein concentration. In the present study, there was a highly significant positive correlation between extensibility and protein concentration (r=0.87) (Table 6.4 and Fig 6.3a).

A high positive correlation was also observed between water absorption and protein concentration (r=0.70) (Fig 6.3b). There was also a significant correlation between extensibility and water absorption (r=0.41) (Fig 6.3c). In these diagrams, the results of the individual selections within the two groups have been differentiated to emphasise genotype x environmental interactions.

The clustering of points in Figs 6.3b and 6.3c of the random lines originating from the F_2 plant selections 5/2, 5/5, 5/7, 5/10 (see Table 6.1) indicating the high water absorption of this group at Mudamuckla, is illustrative of this interaction. Although these overall correlations were significant, the relationships of the two groups of selections varied across sites (e.g. compare the /5/ with /2/ groups for protein concentration and extensibility at Yeelanna and Mudamuckla). Extensibility and maximum resistance showed a highly significant correlation (r=0.50).

Figure 6.3 Relationship between **(a)** extensibility and protein concentration, **(b)** protein and water absorption and **(c)** extensibility and water absorption for two group of random lines from the cross [(Turkey787 x Warigal) x Warigal] over four sites in 1992.

Symbols used in the Figure:

□ group /5/ at Yeelanna

○ group /5/ at Walpeup

◇ group /5/ at Mudamuckla

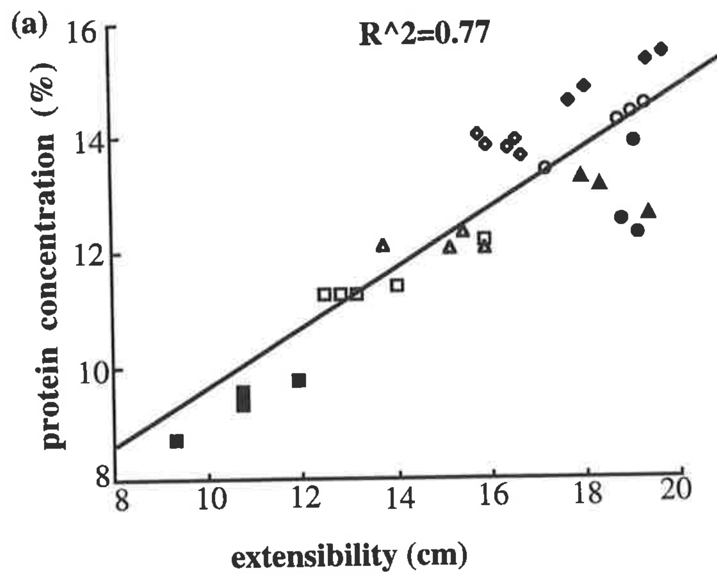
△ group /5/ at Palmer

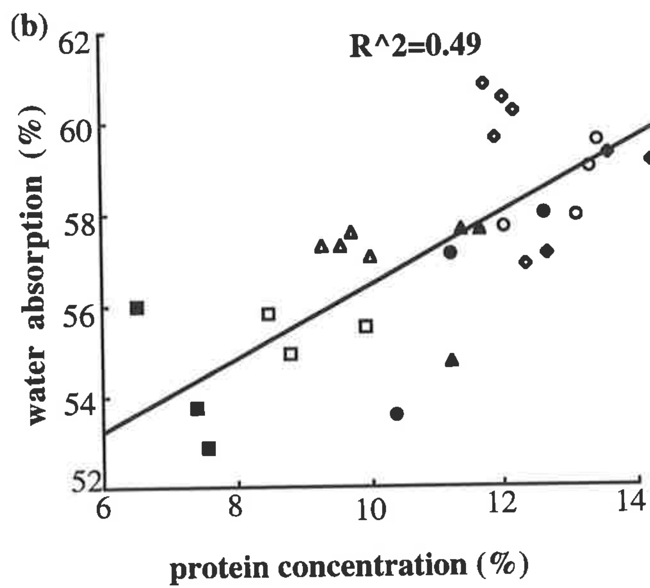
■ group /2/ at Yeelanna

● group /2/ at Walpeup

◆ group /2/ at Mudamuckla

▲ group /2/ at Palmer


(a)
R^2=0.77
protein concentration (%)
extensibility (cm)
16
14
12
10
8
8
10
12
14
16
18
20


(b)
R^2=0.49
water absorption (%)
protein concentration (%)
62
60
58
56
54
52
6
8
10
12
14

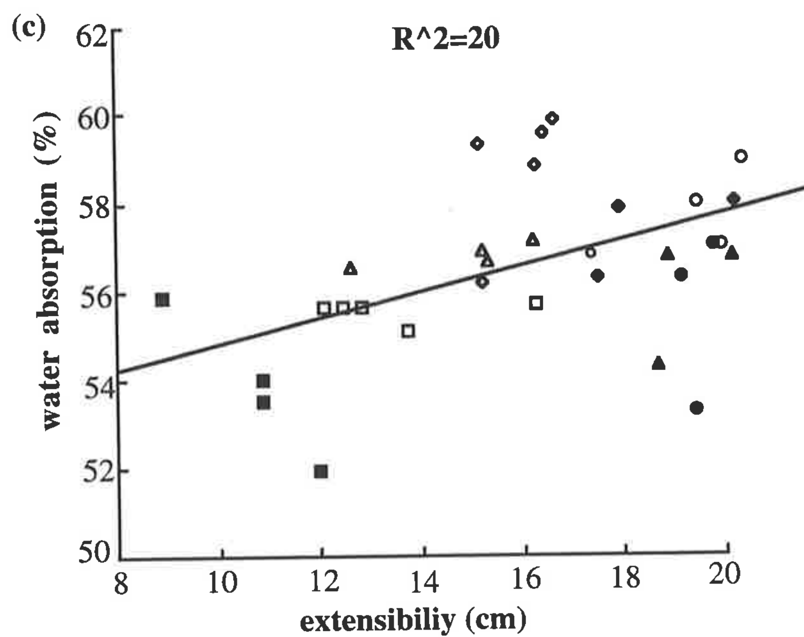

(c)
R^2=20
water absorption (%)
extensibiliy (cm)
62
60
58
56
54
52
50
8
10
12
14
16
18
20

Table 6.4 Correlation matrix between flour properties of lines from [(Turkey787 x Warigal) x Warigal] grown at four sites in southern Australia.

quality parameters (a)	Correlation matrix				
	Ext	PC	E/P	Rmax	WA
Ext	1.00				
PC	0.87 **	1.00			
E/P	0.57 **	0.09	1.00		
Rmax	0.50 **	0.58 **	0.02	1.00	
WA	0.41*	0.70 **	-0.35	0.54 **	1.00

(a)=see abbreviation list.

Although there was a highly significant correlation between Rmax and protein concentration (r=0.58), the correlation coefficient was lower than that for the extensibility-protein relationship, indicating that maximum resistance could be improved by the incorporation of different types of glutenin subunits as well as by increasing grain protein levels.

6.3.3 Association between glutenin alleles and grain yield

To evaluate the relationship between the band patterns and grain yield, the two groups of selected lines were analysed over the four sites. The results of analysis of variance showed that there was a highly significant difference between sites and genotypes for grain yield. As shown in Fig 6.4, the random lines within the /5/ group had a higher mean grain yield than those within the /2/ group

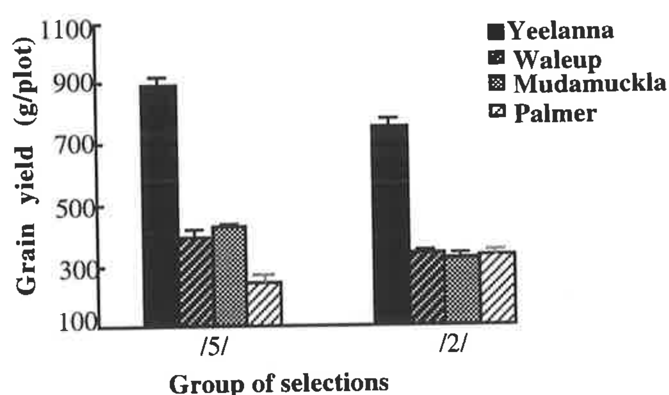


Figure 6.4 Mean grain yields (g/plot) of two groups of random lines from the cross [(Turkey787 x Warigal) x Warigal] selected for grain yield

There was also clear evidence of a significant interaction between genotypes and sites with the lower grain yield of the /5/ group of lines compared to the /2/ group at Palmer responsible for this.

Since only eight percent of variation for grain yield was accounted for by genotypes, it was most likely that there was no linkage between genes coding glutenin subunits with those controlling grain yield.

6.4 Discussion

A comparison between different genotypes within a set of random inbred lines offers one of the most convincing demonstration of genetic effects. Ideally this requires that there be replication of both the individual lines within the genotypes and a sufficient number of lines for each genotype to calculate an accurate mean effect of the genotype. Unfortunately the set of lines chosen for this experiment did not meet these criteria as most of the variation was traceable to the two F_2 selections. For this experiment to provide good estimates of the effects of the three glutenin loci, the segregation should have occurred within the F_2 lines i.e. all eight genotypes would have been present within each of the F_2 selections. Hence it is not possible to draw any conclusions on the effects of individual glutenin loci on quality parameters.

Maximum resistance is affected by both the amount and the composition of the glutenin, whereas extensibility largely depends on the amount (Gupta *et al.*, 1990b, Singh *et al.*, 1991a). Although in study here it was not possible to partition the effects of the three loci (*Glu-A1*, *Glu-D1* and *Glu-B3*), the mean results for the three loci showed that the presence of HMW glutenin subunits 5+10 were associated with higher maximum resistance compared with their allelic alternatives 2+12, whereas higher extensibility was observed in the presence of 2+12. The positive synergistic effects associated with the inclusion of the glutenin subunit bands 5+10 at *Glu-D1* were most likely due to a greater capacity of these subunit bands to form larger sized polymers than subunits 2+12, or their having greater molecular weight as reported by Gupta *et al.* (1994b). Either situation can lead to a higher amount of high molecular weight gluten polymers.

Regarding the alleles at *Glu-A1*, it has been reported that both alleles 1 and 2* can be involved in increasing the total proportion of HMW glutenin subunits which in turn results in a higher amount of high molecular gluten polymers (Halford *et al.*, 1992). In the South Australian environment it is thought that the 2* allele is generally associated with greater dough strength (Cornish, 1994).

Almost no evidence is available on the effect of the alleles at *Glu-B3*, although comparisons of the dough properties of the backcross derivative Yr10Bindawarra with its recurrent parent Bindawarra suggest that the former with the *j* allele gives more extensible doughs than the latter with a *b* allele (H. Wallwork, pers. comm.). Visual observations in gels has shown that the LMW *j* allele has a greater number of bands than its counterpart *b* allele and this may be related to an increase in either or both dough strength and extensibility (G. Cornish and H. Wallwork pers. comm.).

The interaction between genotypes and sites showed that the dough properties were markedly affected by environmental conditions. There is little known about the possible cause of this variability. Randall and Moss (1990) noted that high temperatures up to 30°C during crop development in northern Australia give greater dough strength than those developed in southern regions. As reported by Blumenthal *et al.* (1993) heat stress, prolonged gliadin synthesis and reduced glutenin synthesis produced a higher gliadin:glutenin ratio in the mature grain and consequently weaker dough properties. It has been hypothesised that a high level of water availability after anthesis is associated with weak doughs (P.C. Williams, pers. comm.). In the experiment reported here it is particularly noticeable that the normally large genotypic differences in resistance were almost absent at Palmer, but replaced by unusually large genotypic differences in the extensibility/protein ratio. Although the group /5/ lines generally had a higher grain yield, at Palmer they were lower yielding than the group /2/ lines. This suggests that there is little or no genetic association of grain yield with quality characteristics and that the association observed here merely reflects the F_2 ancestry.

CHAPTER 7

EFFICIENCY OF SMALL SCALE TESTS AS SELECTION CRITERIA FOR BREAD MAKING QUALITY IN AN EARLY GENERATION AND ITS EFFECTS ON GRAIN YIELD IN LATER GENERATION

7.1 Introduction

Grain yield and end-use quality are the major objectives of southern Australian wheat breeding programs. In this area many diverse types of wheats are produced. The higher protein content hard-grained Australian Hard, Australian Premium White (APW) and Australian Standard White (ASW) are used for bread, flat breads and noodles, while softer blends are used for a range of soft wheat uses including biscuit and cakes. Quality evaluation in early generations using the standard measuring devices (Farinograph and Extensograph) is not practical owing to limitations of seed supply and the large number of lines that need to be assessed.

Wheat breeders and cereal chemists have long sought reliable small scale methods for screening weak gluten lines in the early generations of a breeding program and some wheat breeders now use the SDSS volume procedure for estimating dough strength in early generation progenies. This small scale test is usually applied to F_3, F_4, or F_5 lines. Preston *et al.* (1982) showed that SDSS volume appears to be a good predictor of dough strength parameters. Correlations between the SDS-sedimentation test and dough strength parameters have also been demonstrated in bread wheats (Blackman and Gill, 1979) and in durum wheats (Quick and Donnelly, 1980). It has also been reported that SDSS volume is highly correlated with the amount of SDS-insoluble proteins of a wheat variety (Danno and Hoseney, 1982; Moonen *et al.* 1982; Bietz, 1984; Dachkevitch and Autran, 1989). Since the SDS-insoluble proteins consist largely of glutenin proteins, this provides an explanation for the predictive value of the SDSS volume as bread making quality is largely governed by both the amount and composition of glutenin proteins (Moonen *et al.*, 1982).

Glutenin consists of large molecules from two different groups of subunits that are linked by disulphide bonds (Wall, 1979), creating a large variability in the size of the polymers. Treatment of glutenin with reducing agents causes breakage of the disulphide bonds. These groups of subunits have been classified as High Molecular Weight (HMW) and Low Molecular Weight (LMW) subunits (Payne *et al.*, 1979).

The positive influence of the HMW glutenin subunits on gluten strength in bread wheat have been shown by several researchers. Payne *et al.* (1981a) were first to relate allelic differences at the *Glu-1* loci among wheat genotypes to differences in dough strength and devised a scoring system for ranking the alleles at the three *Glu-1* loci (HMW) for their contribution to bread-making. Unfortunately this system has not always been applicable. Gupta *et al.* (1990a) have shown that the variation explained for maximum resistance on the basis of the *Glu-1* score (Payne *et al.*, 1987a) for Australian wheats compared to the world-wheat set is very low (15% vs. 42%) and therefore additional aspects of gluten composition, particularly the LMW subunits of glutenin, must be examined to provide a more complete explanation. It has been suggested that the effects of individual *Glu-3* alleles which code the LMW subunits are largely additive to those of the *Glu-1* alleles which code the HMW subunits (Gupta *et al.*, 1989; 1994a).

The efficiency of screening for grain quality in early rather than later generations has been questioned by Brennan and O'Brien (1991). They point out that selection for yield should be the predominant aim early in a breeding program even though O'Brien *et al.* (1989) have shown that breeding for protein quality does not limit selection for grain yield. The main aim of the present study was to investigate the interrelationship between grain yield, and flour quality measured by small-scale tests by using groups of F_4 lines generated from the cross between Barunga and Suneca.

Barunga was released at the University of Adelaide (AWCC, 1994) and has similar quality characteristics to other good quality South Australian hard wheats, having good, not exceptional, dough strength and extensibility. Suneca was released by the University of Sydney as a Prime Hard variety (AWCC, 1983), but has been not widely grown in South Australian trials as it has low grain yields under South Australian conditions. Some aspects of the quality characteristics of these two parents are given in Table 7.1. As Suneca and Barunga have rarely been grown in the same trials, the comparison between them was performed in relation to the common standard variety Halberd. Suneca as a Prime Hard wheat has 19% higher protein concentration than Halberd whereas for Barunga there was no difference between them. The dough strength of Barunga was 85% higher than Halberd, whereas Suneca was only 39% higher. By contrast the extensibility of Suneca was 42% higher than Halberd, but Barunga was only 22% higher.

These parents possess several different HMW and LMW glutenin subunit components (Table 7.2) with Barunga having the LMW pattern *c*, *b*, *c* common in breeding lines released from the Waite Institute.

Table 7.1 A comparison between Suneca and Barunga for different quality parameters using Halberd as the common reference variety. Data obtained from Interstate Wheat Variety Trials (1981 and 1982) at 8 sites analysed by the Bread Research Institute, Sydney and the South Australian secondary trial series analysed by the South Australian Research and Development Institute (SARDI) in 1989-1993 over 35 trials.

Varieties	quality parameters[(a)]									
	P.S.I.	Flour Yield (%)	Colour Grade (KJ)	Water abs (%)	Rmax (B.U.)	Ext (cm)	Protein (%)	Loaf Volume (mls)	Loaf Score (%)	Dev. (mins)
Suneca	13	78	-0.7	58.8	382	27.9	13.2	735	74	6.5
Halberd	13	77	-0.8	60.1	275	19.7	11.1	660	67	3.5
Suneca (% difference from Halberd)	**(0.0%)**	**(+1.3%)**	**(-12.5%)**	**(-2.2%)**	**(+38.9%)**	**(+41.6%)**	**(+19.0%)**	**(+11.4%)**	**(+10.4%)**	**(+85.7%)**
Barunga	15	72.3	-2.5	59.3	519	19.6	10.2	602	67	5.7
Halberd	16	72.6	-2.1	60.2	281	16.1	10.3	533	55	3.9
Barunga (% difference from Halberd)	**(-6.2%)**	**(-0.4%)**	**(+19.0%)**	**(-1.5%)**	**(+84.7%)**	**(+21.7%)**	**(-0.1%)**	**(+12.9%)**	**(+21.8%)**	**(+46.1%)**

(a)= see abbreviation list

The cross was made mainly to determine which glutenin loci needed to be changed to upgrade South Australian wheats (Barunga) to the Prime Hard level (Suneca) to find whether the background genotype appreciably affects the physical dough qualities. Since little research has been carried out on the association between LMW glutenin subunits and dough strength in bread wheat, the effects of the LMW subunits themselves and the allelic combinations of HMW and LMW glutenin genes on dough strength received special attention. This cross provided an opportunity to survey the effect of different alleles at the *Glu-B1*, *Glu-A3, Glu-B3* and *Glu-D3* loci, in the absence of variation in the alleles at *Glu-A1* and *Glu-D1* whose effects are well known.

Table 7.2 HMW and LMW alleles (band pattern) at the six glutenin loci of Barunga and Suneca

Gluten subunits	Locus	Alleles (band pattern)	
		Barunga	Suneca
HMW	*Glu-A1*	1	1
	Glu-B1	7+8	17+18
	Glu-D1	5+10	5+10
LMW	*Glu-A3*	*c*	*d*
	Glu-B3	*b*	*h*
	Glu-D3	*c*	*e*

7.2 Materials and methods

7.2.1 Experimental materials

Barunga and Suneca differ at four loci coding for glutenin subunits, so 16 different homozygous genotypic classes, representing all combinations of specific bands at *Glu-B1* (7+8, 17+18), *Glu-A3* (*c*, *d*), *Glu-B3* (*b*, *h*) and *Glu-D3* (*c*, *e*) were possible in the F_2 generation. However at the F_2 generation the level of heterozygosity for loci would have been high, therefore selection for band pattern was performed in the F_3 generation when a greater proportion of the progeny were homozygous.

The F_3 seeds from the cross between Barunga and Suneca was kindly provided by Dr A.J. Rathjen of the Plant Science Department, Waite Agricultural Research Institute. The F_3 seeds had been analysed electrophoretically for their HMW and LMW glutenin composition previously and the results were kindly provided by Mr Geoff Cornish at the Cereal Laboratory of the South Australian Research and Development Institute (SARDI). The procedure for the development of the RILs is presented in Fig 7.1.

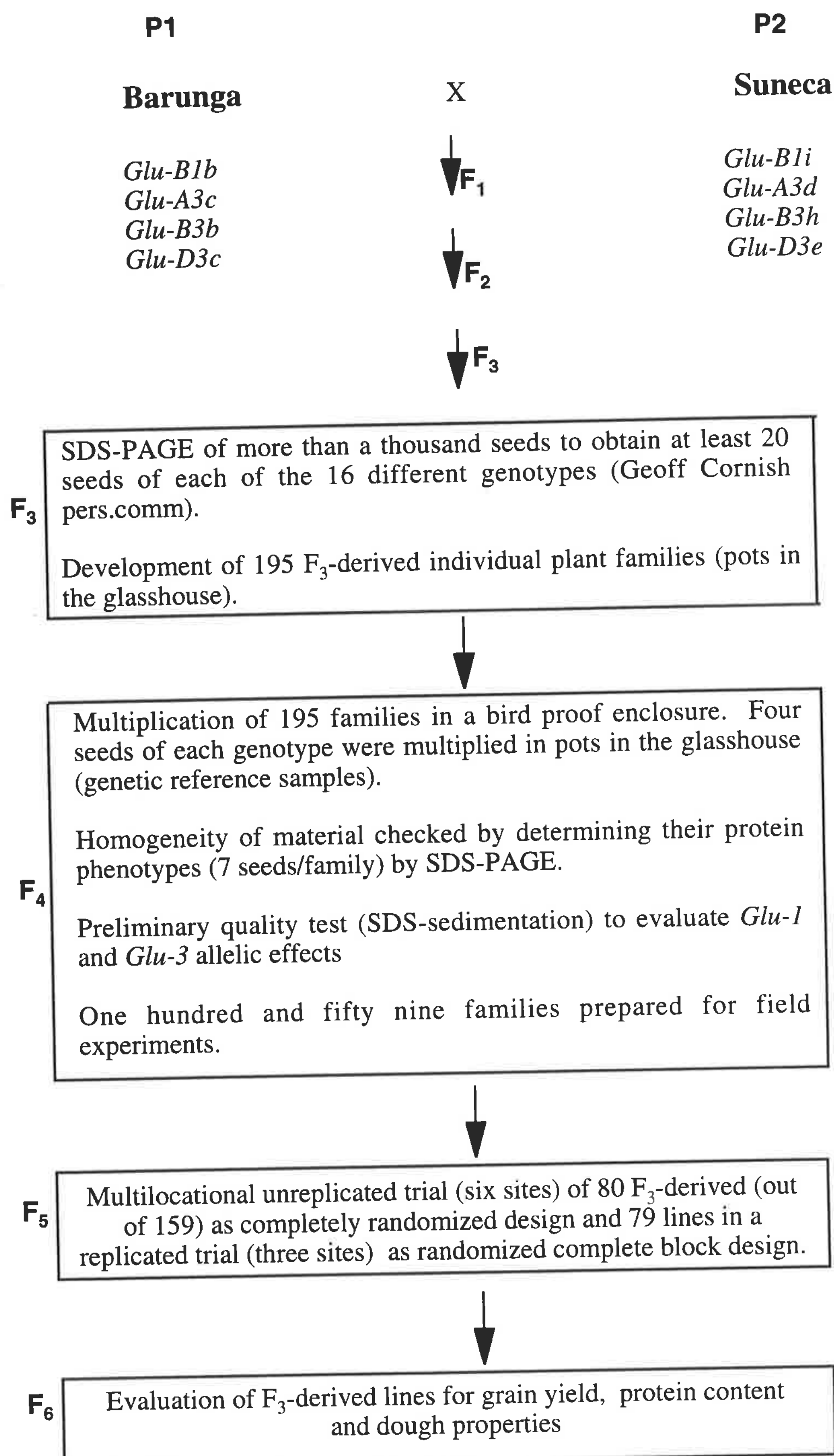


Figure 7.1 Procedure used to develop 159 F_3- derived lines from a cross between parents Suneca and Barunga and the quality and yield evaluations in the F_4-F_6

7.2.2 Germination

Twenty half-seeds containing the embryo portion (the other half had been used for electrophoresis) from each of the RILs were surface sterilised for about 1-2 min with 70% ethanol in Eppendorf centrifuge tubes and rinsed three times with distilled water. The petri dishes and filter papers were also sterilised in boiling distilled water and left for a few minutes to cool.

The seeds were placed in an incubator at 25°C for about 12 hours and in a refrigerator at 4°C for 1-2 days until the seeds produced a radicle and then the seedlings were placed back in an incubator at 25°C for 1-2 days until the coleoptile emerged. The seedlings were transplanted into 25 cm plastic pots containing sterilised recycled potting medium and then grown under glasshouse conditions. No artificial lighting or heating was supplied. Some seeds did not establish, mainly due to their having insufficient endosperm.

7.2.3 Multiplication

One hundred and ninety-five F_3-derived F_4 lines obtained from the separate single plants were sown as single rows for further multiplication in a bird proof enclosure in September 1993. At the same time four seeds of each line were separately multiplied in the glasshouse as genetic reference samples for further study and retesting of their band patterns. To minimise the chance of mislabelling and contamination, all lines within each genotype were sown together without any randomisation. Since the amount of seeds for each line was limited, 5m rows were sown for each line in the order as presented in Fig 7.2.

7.2.4 Electrophoresis

The RILs harvested from the bird-proof enclosure were checked electrophoretically as a test of their correct identification and homogeneity. For this purpose seven seeds from each line were randomly selected and checked using SDS-PAGE (Fig 7.3) as described in Chapter 3 (Materials and methods).

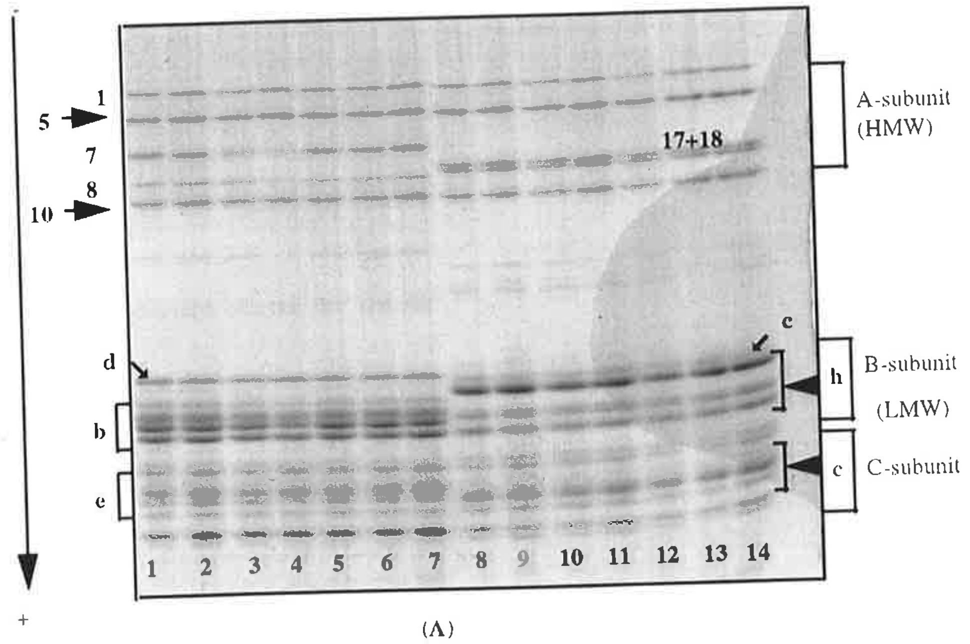
Figure 7.2 The layout for multiplication of 195 F4-derived F5 progeny from (Barunga x Suneca) grown in the bird-proof enclosure in September of 1993. The lines indicated by the shaded square were not included in the field experiments. There were two field experiments. The first consisting of five lines from each genotypes grown as a randomised complete block (unreplicated) and the remaining lines were included in a two-replicated randomised block experiment (see section 7.2.6 for more details).

Genotype no.	Band Patterns						Lines within genotypes	no of lines		
	Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*		Total	Exp 1	Exp 2
1	1	7+8	5+10	c	b	c	B E G J L M Q S V R P Y T U X Z	16	5	7
2	1	7+8	5+10	c	b	e	A B C D E F G H O P Q R U	13	5	6
3	1	7+8	5+10	c	h	c	A B C E F G H J K L N O	12	5	6
4	1	7+8	5+10	c	h	e	A B C D E F G H I J M P Q R	14	5	7
5	1	7+8	5+10	d	b	c	A B D E G M R S T V W X Y Z C	15	5	6
6	1	7+8	5+10	d	b	e	A B C D E G H I J N O P Q	13	5	5
7	1	7+8	5+10	d	h	c	A C D E F G I J K M L N P Q	14	5	7
8	1	7+8	5+10	d	h	e	A B D E F G H I J L M N	12	5	5
9	1	17+18	5+10	c	b	c	A B C D E F G H I J K L M N O P Q R S	19	5	7
10	1	17+18	5+10	c	b	e	B C D G H I J	7	5	0
11	1	17+18	5+10	c	h	c	A B C D E H J K L M N O P Q R	15	5	6
12	1	17+18	5+10	c	h	e	A C D E F G H I J K L M N	13	5	7
13	1	17+18	5+10	d	b	c	A B D E F	5	5	0
14	1	17+18	5+10	d	b	e	A C D F G H I J L M	10	5	4
15	1	17+18	5+10	d	h	c	A C D E F J K L	8	5	2
16	1	17+18	5+10	d	h	e	A B C D E F G H I	9	5	4
Total	-	-	-	-	-	-	-	**195**	**80**	**79**

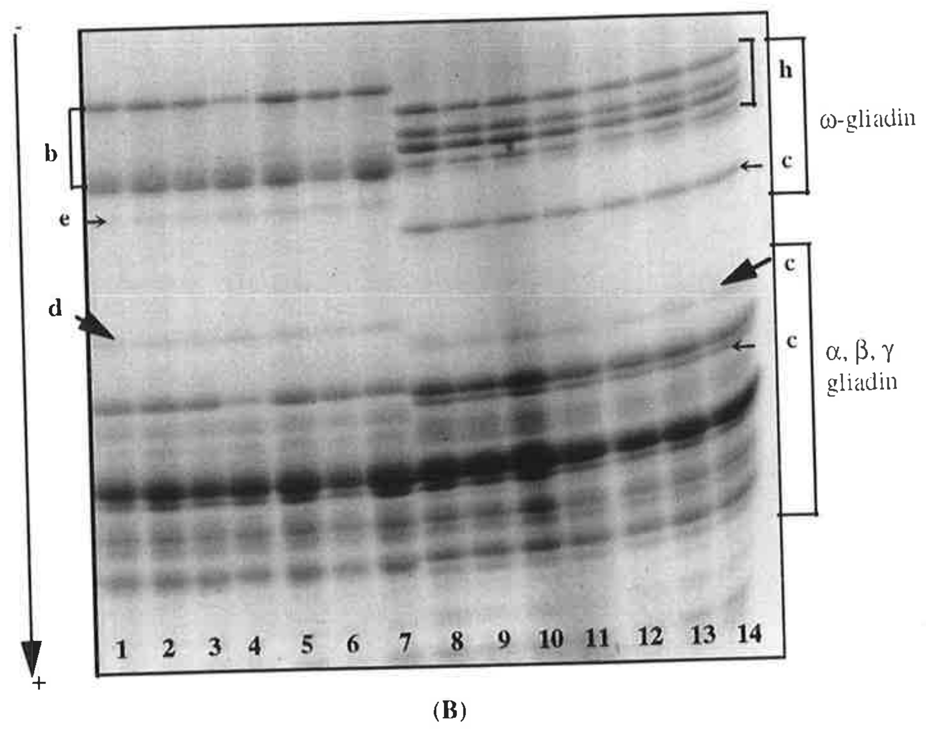
Figure 7.3 Test of homogeneity for F3-derived progeny from Barunga and Suneca using SDS-PAGE method developed by Singh *et al.* (1991b). Seven seeds of each of two lines were checked on each gel , numbered 1 to 7 and 8 to 14 respectively.

(A) SDS-PAGE pattern of glutenin subunits.

(B) SDS-PAGE pattern of gliadin bands



(A)



(B)

7.2.5 Quality testing

Grinding

Ten grams of untempered grain (11-12% moisture content) from each line was ground on the falling number model 3100 laboratory mill fitted with a 0.8 mm screen to produce wholemeal samples.

NIR

Total protein concentration in the flour was determined by Near-Infrared Reflectance spectroscopy-NIR (Technicon Infra-Alyser 450) at the Cereal Laboratory at the South Australian Research and Development Institute (The NIR instrument had been calibrated for a range of protein concentrations by a Kjeldahl analysis).

SDS-sedimentation test

Since the number of lines within each genotype ranged between 5 and 19 (mostly around 10 (see Fig 7.2), the SDS-sedimentation test was performed on all the lines available or maximum of ten randomly selected lines. The procedure used for the SDS-sedimentation test was similar to that reported in Chapter 4 (Materials and Methods).

7.2.6 Yield trials

Only one hundred and fifty-nine lines (out of 195) multiplied in the birdcage were incorporated into the field experiments mainly because some lines did not produce sufficient seed. On the basis of seed availability, the lines were divided into two sets and sown in two different experimental designs. In the first experiment, five lines from each genotype were selected and grown as an unreplicated design (completely randomised) at six locations. Therefore 80 lines out of 159 representing all 16 different genotypes were sown at Minnipa, Kimba, Winulta, Roseworthy Campus, Kapunda and Lowbanks in 1994. In the wheat breeding program conducting at the Waite Institute, it is the usual procedure to include a check variety and some standards varieties to allow for statistical correction of soil variation and varietal comparison within experiments. In this trial, Angas was used as a check variety every third plot and Aroona, Warigal, BT-Schomburgk, Barunga and Yarralinka as standards with five replications as shown in Fig 7.4. The arrangement and dimensions of plots used for the field experiments was the same to those employed in the previous experiment (Chapter 4, Material and methods)

Figure 7.4 Field arrangement for F3-derived F6 lines from (Barunga x Suneca) counducted in two sets of experimental designs as completely randomised design (unreplicated) and completely randomised block design (replicated).

The symbols used in the Figure:

(A)= Angas-the check variety, systematic layout

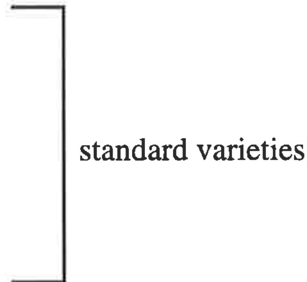
AR=Aroona

BA=Barunga

BT=BT-Schomburgk

WA=Warigal

YA=Yarralinka



The F3-derived lines were allocated at random to the unlabelled plots (eg plots 3, 4, 6 etc in the unreplicated experiment and plots 3, 4, 5, 7 etc in the replicated experiments.

Unreplicated experiment at:
Minnipa
Kimba
Winulta
RAC
Kapunda
Lowbanks

Plot number									
1 BT	2 (A)	3	4	5 (A)	6	7	8 (A)	9	10 BA
11	12	13 (A)	14	15	16 (A)	17	18	19 (A)	20 WA
21	22 (A)	23	24	25 (A)	26 BT	27 AR	28 (A)	29 YA	30
.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.
141	142 (A)	143	144 YA	145 (A)	146	147	148 (A)	149	150

Plot number

replicated experiments at:
Winulta
Minnipa
RAC

Plot number																
rep 1	1	2 (A)	3	4	5	6 (A)	7	8	9	10 (A)	11	12	13	14 (A)	15	16 AR
	17	18	19	20 (A)	21	22	23	24 (A)	25 WA	26 BT	27	28 (A)	29	30	31 BT	32 (A)
	33	34 (A)	35	36	37	38 (A)	39	40	41	42 (A)	43	44	45	46 (A)	47	48
	49 AR	50	51	52 (A)	53	54	55 WA	56 (A)	57	58	59	60 (A)	61	62 BA	63 BA	64 (A)
	65	66 (A)	67	68	69 YR	70 (A)	71	72	73 YR	74 (A)	75	76	77	78 (A)	79	80
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	113.	.	.	.	.	.	.	120.	121.	.	.	.	.	.	.	128.
rep 2	129	130 (A)	131	132	133 YR	134 (A)	135	136 AR	137	138 (A)	139	140	141	142 (A)	143 WA	144
	145	146	147	148 (A)	149	150	151 BT	152 (A)	153	154	155	156 (A)	157	158	159	160 (A)
	161	162 (A)	163	164	165 AR	166 (A)	167	168	169 BT	170 (A)	171	172	173	174 (A)	175	176
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Plot number	225 BA	226 (A)	227	228	229	230 (A)	231	232	233	234 (A)	235	236	237	238 (A)	239	240 BA

The remaining lines were sown in a two-replicate randomised block trial design at Winulta, Minnipa and Roseworthy Campus in 1994. Angas was again used as the check variety every fourth plot and six replicates of Aroona, Warigal, BT-Schomburgk, Barunga and Yarralinka were also included.

The decimal code for the growth, the same as that reported in Chapters 4 and 5, was used to record the time of anthesis at Roseworthy Campus in 1994.

7.2.7 Growing conditions

Good rains in the cereal belt of South Australia (early June) allowed preparation and sowing to proceed. Seeding commenced on 10th of June and was completed on 8th of July. The South Australian cereal zone had less than average rainfall during the winter months and September except at Winulta which had useful rains in early September (Table 4.1). Early November rains and the absence of drying northerly winds improved the prospects for some of the later maturing areas such as Winulta, but generally the experiments reported here did not recover from the drought. Due to the late start to the season and the dry winter and spring conditions very little leaf disease developed on wheat in 1994. Boron toxicity was a major yield limiting factor in 1994 as roots were required to extract moisture from the subsoil.

7.2.8 Statistical analysis

The results were analysed statistically with the Genstat 5 computer package. As the plant material grown in the bird proof enclosure was not randomised, a fully acceptable analysis of variance was not possible. Therefore a preliminary factorial analysis was calculated using the lines within genotypes as replicates for the HMW and LMW loci as factors. Since the combinations of alleles at the *Glu-1* and *Glu-3* loci were in unequal frequencies, analysis of variance models (modified ANOVA) based on regression analysis were used to determine the relative contributions of the *Glu-B1* and *Glu-A3*, *Glu-B3*, *Glu-D3* loci. That is, all four loci were fitted into the regression model, allowing the detection and measurements of main effects and interloci interactions. If there was interaction, only the interaction between pairs of loci, rather than that of greater complexity was examined.

The yield data obtained from the field plots at different sites were analysed using Genstat 5 to detect genetic variation among the F_3-derived F_6 progeny and to detect genotype x site interactions resulting from the different environmental conditions. The proportion of the variation accounted for by genotypes and lines within genotypes was also calculated by using the variance components.

7.3 Results

7.3.1 Bird-proof enclosure experiment

Grain yield and quality characters of F_3-derived progeny at the small scale level

The results of analysis of variance for grain yield, protein concentration and SDSS volume is given in Table 7.3.

Table 7.3 Mean square and statistical significance of yield and quality characteristics measured on 16 genotypes, each represented by different numbers of F_3-derived progeny from (Suneca x Barunga) grown in a bird-proof enclosure in 1993.

Source of variance	d.f	Mean Square		
		GY(a)	PC	SDSS
Genotypes	15	11668***	7.14***	134.22**
Residual	144	4848	0.94	22.87
Total	159 (b)			

(a)= see abbreviation list.
(b) for those genotypes with less than ten lines (see Table 7.2) the missing data was estimated by the Genstat program.

Analysis of variance showed that the parameters were all highly significant different between the progenies. There was a wide range of variation for yield of grain, SDSS volume and protein concentration among the F_3-progenies (Table 7.4). The mean grain yield between families varied from 140 to 284 g/row. The SDSS volume ranged from 57 to 70 with the low range of SDSS volume reflecting the comparatively small differences between the dough quality of the parents. The overall average grain protein concentration for the progenies varied from 12 to 14.8%.

The significant genotypic effect, particularly on protein concentration and to a lesser extent yield, was somewhat unexpected, but probably related to the lack of randomisation of the genotypes. The systematic effect of non-randomisation can be easily seen on protein concentration (see Table 7.4). The mean value of protein concentration of the most southerly four genotypes was less than the others. As it has been reported that environmental factors are unimportant for the quality characteristics such as sedimentation volume (Preston *et al.*, 1982), it does not seem that this systematic effect on protein concentration is likely to affect the SDS-sedimentation test.

Table 7.4 Mean of the grain yield (g/row), protein concentration and SDS-sedimentation values of 16 genotypes in F_3 derived F_4 progeny of cross between Barunga and Suneca grown in a bird-proof enclosure.

Genotypes	Grain yield (gr/row)		Protein concentration (%)		SDSS volume (ml)	
	Mean	SE†	Mean	SE	Mean	SE
1 7+8 5+10, c b c	210.0	21.12	14.0	0.14	64.5	1.81
1 7+8 5+10, c b e	226.6	22.50	14.4	0.43	57.3	1.57
1 7+8 5+10, d b c	200.3	22.69	14.5	0.21	57.4	1.01
1 7+8 5+10, d b e	214.9	11.40	14.4	0.44	59.3	1.08
1 7+8 5+10, c h c	140.9	11.12	14.1	0.46	64.6	1.08
1 7+8 5+10, c h e	171.8	14.25	14.8	0.23	64.5	1.85
1 7+8 5+10, d h c	198.9	19.58	14.6	0.33	65.0	1.62
1 7+8 5+10, d h e	211.8	19.98	14.6	0.33	61.0	1.41
1 17+18 5+10, c b c	226.7	33.82	14.5	0.26	61.9	1.37
1 17+18 5+10, c b e	237.3	23.91	14.1	0.23	62.4	0.53
1 17+18 5+10, d b c	182.8	17.24	14.8	0.33	62.1	1.75
1 17+18 5+10, d b e	250.8	15.37	14.0	0.21	65.9	1.70
1 17+18 5+10, c h c	284.3	50.20	13.0	0.14	70.6	1.47
1 17+18 5+10, c h e	239.5	22.11	12.8	0.33	68.4	1.19
1 17+18 5+10, d h c	214.7	29.71	12.1	0.42	62.2	2.20
1 17+18 5+10, d h e	250.0	30.39	12.7	0.26	66.6	1.26

† standard error

7.3.2 Effect of allelic variation in protein bands on dough properties and grain yield

Effect of glutenin subunits on SDS-sedimentation volume

The results of analysis of variance for main and interaction effects of alleles at the *Glu-B1*, *Glu-A3*, *Glu-B3* and *Glu-D3* loci is given in Table 7.5. The main effects showed that the progeny with HMW glutenin subunit alleles 17+18 had a highly significant (P<0.001) greater SDSS volume than the 7+8 alleles at *Glu-B1* (65 ml vs. 62 ml) (Fig 7.5). These results are consistent with those of Payne *et al.* (1984a) and Branlard and Dardevet (1985b). Similarly the LMW glutenin band *Glu-A3d* was associated with a highly significant (P<0.001) greater SDSS volume than its counterpart allele (*Glu-A3c*) (65 ml vs. 61 ml). Likewise, at the *Glu-B3* locus, allele *b* gave a significantly greater SDSS volume than the *h* allele (P<0.05) (64 ml vs. 62 ml). A greater maximum resistance in direct measurements of dough strength for alleles *d* compared to *c* at the *Glu-A3* and *b* compared to *h* at the *Glu-B3* loci has been reported by Gupta *et al.* (1991). The *Glu-D3* alleles (i.e. *c*, *e*) did not show significantly different mean SDSS volumes.

Table 7.5 Mean square and level of significance for SDS-sedimentation volume, protein concentration and grain yield of F3-derived F5 progeny from (Suneca x Barunga) grown as unreplicated rows in a bird-proof enclosure in 1993.

Source of variation	Mean Square		
	SDSS (a)	PC	GY
Glu-B1	344.5 ***	29.8 ***	47839 ***
Glu-A3	486.5 ***	21.3 ***	2411 ns
Glu-B3	114.3 *	0.0 ns	182 ns
Glu-D3	0.9 ns	0.0 ns	19338 *
Glu-B1 x Glu-A3	4.0 ns	36.1 ***	22115 *
Glu-B1 x Glu-B3	7.1 ns	0.9 ns	12275 ns
Glu-A3 x Glu-B3	53.5 ns	0.9 ns	8930 ns
Glu-B1 x Glu-D3	184.7 ***	1.7 ns	390 ns
Glu-A3 x Glu-D3	0.1 ns	2.5 ns	1947 ns
Glu-B3 x Glu-D3	106.3 *	0.5 ns	4747 ns
Residual	24.7	0.9	4922

a=see abbreviation list

As shown in Table 7.5, two interloci interactions (*Glu-B1 x Glu-D3*, *Glu-B3 x Glu-D3*) were significant for SDSS volume. In both of these interactions, the *Glu-D3* locus was present. With respect to the interaction between *Glu-B1* and *Glu-D3*, the largest SDSS volume was observed in the combination of 17+18 and *e* (Table 7.6). Likewise for the

interaction of *Glu-B3* and *Glu-D3* the largest SDSS was observed in the combination of *b* and *c*.

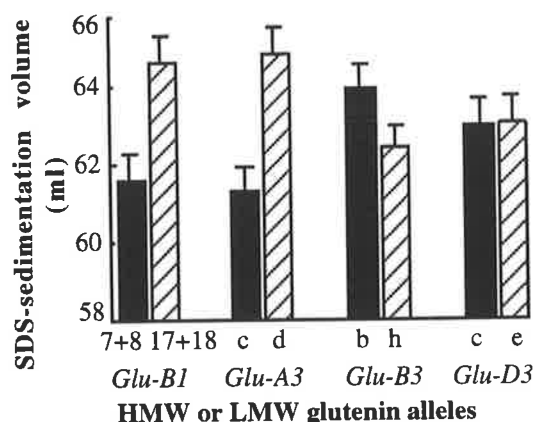


Figure 7.5 Mean SDS-sedimentation volume for F3-derived F5 progeny from (Barunga x Suneca), grown as unreplicated rows in a bird-proof enclosure in 1993 for specific alleles of HMW (*Glu-B1*) and LMW (*Glu-A3*, *Glu-B3* and *Glu-D3*) glutenin loci.

Table 7.6 The mean SDS-sedimentation volume for F3-derived F5 progeny from (Barunga x Suneca), grown in a bird-proof enclosure in 1993 with specific combinations of *Glu-B1*(17+18, 7+8), *Glu-B3* (*b*, *h*) with *Glu-D3* (*c*, *e*) alleles.

Glutenin subunit interaction		*Glu-D3*	
		c	e
Glu-B1	7+8	62.8	60.7
	17+18	63.4	66.1
Glu-B3	*b*	64.8	63.3
	h	61.6	63.3

Effect of glutenin subunits on protein concentration

The allelic effect of the *Glu-B1* and *Glu-A3* loci on protein concentration were, apparently, highly significant. Lines possessing glutenin subunit band 7+8 had a higher protein concentration than those having 17+18. Similarly at the *Glu-A3,* the *c* allele gave a higher protein level than its counterpart. However, examination of Fig 7.2 and Table 7.4 which outlines the spatial relationship of the lines in the bird-proof enclosure suggests that the differences in protein concentration were probably due to the non-randomisation.

Effect of glutenin subunits on yield of grain

The data showed that alleles at *Glu-B1* had significantly different grain yield values (P<0.001). As shown in Fig 7.6, progeny with high molecular subunits bands 17+18 had a higher yield of grain than the 7+8 but this, as with protein concentration was probably due to the absence of randomisation. There was also a significant difference between alleles at *Glu-D3* for grain yield. Progeny carrying allele *e* had higher grain yields than those having *c*. In this case, the contrasting lines were grown in pairs and in seven of the eight pairs, the *e* allele lines were higher yielding than the *c* lines, so spatial relationships were unlikely to be involved.

The significant interaction between alleles at the *Glu-B1* and *Glu-A3* loci on grain yield was also likely to reflect their spatial relationships in the sowing plants (Fig 7.2).

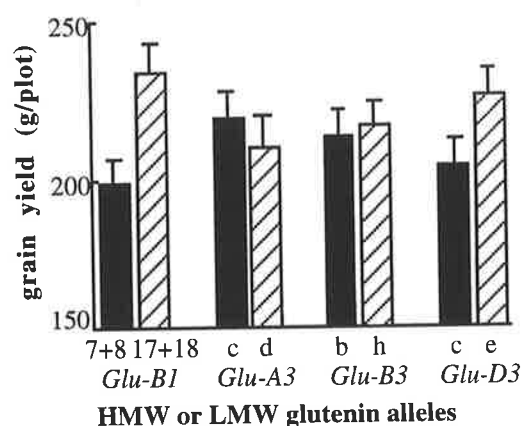


Figure 7.6 Mean grain yield for F3-derived F5 progeny from (Barunga x Suneca), grown in a bird-proof enclosure in 1993 and carrying specific alleles of HMW (*Glu-B1*) and LMW (*Glu-A3*, *Glu-B3* and *Glu-D3*) glutenin loci.

The correlation matrix between grain yield and each of sodium dodecyl sulphate (SDS) sedimentation volume and protein concentration was computed. A significant negative correlation was observed between grain yield and protein concentration (r=-0.20; P<0.05). Flour protein concentration was also negatively correlated with SDSS volume (r=-0.28 P<0.001). This negative correlation suggested that differences in dough strength (as measured by SDSS test) of these progeny were mainly due to protein quality rather than quantity. The trend of decrease in dough strength with increased flour protein concentration has also been reported by Gupta *et al.* (1992) and Bariana *et al.* (1993).

There was no relationship between yield of grain and SDSS volume amongst F_3 progenies suggesting that a simultaneous improvement of yield and quality can be achieved.

7.3.3 Yield analysis of F_3-derived F_6 lines

Five lines of 16 genotypes at six sites, unreplicated trial

To investigate the efficiency of selection for breadmaking quality in an early generation and its effects on grain yield in later generations, the study was continued by growing the F_3-derived F_6 lines at six different country sites. The results of analyses for grain yield are presented here and the results of quality analyses will be presented later (see Chapters 8 and 9). The results of the analysis of the experiment conducted as a completely randomised design over six sites (pooled data for the unreplicated trial) showed that there were no significant differences between genotypes for grain yield. By contrast there was a highly significant difference between sites for grain yield (Table 7.7) but no significant interaction between genotypes and sites.

Table 7.7 Mean squares and probabilities from analysis of variance for grain yield of F_3-derived F_6 lines from (Barunga x Suneca), five lines each of 16 different genotypes grown at six sites in 1994.

Source of variation	d.f	Grain yield	
		Mean square	Probability
Site	5	2553288	<0.001
Genotype	15	6290	0.72
Site x Genotype	75	7423	0.71
Residual	384	8249	-
Total	479		-

Analysis of variance for separate sites showed that the differences between genotypes was significant or close to significant at Minnipa and Kapunda (Table 7.8), therefore in the second step of analysis these sites were examined separately.

Table 7.8 Variance ratio of grain yield for 80 F3-derived F6 lines from (Barunga and Suneca), of 16 different genotypes grown at six sites in 1994.

Sites	Grain yield	
	F value	P
Lowbanks	1.5	0.15
Kapunda	1.8	0.06
Kimba	1.3	0.25
Minnipa	1.8	0.05
Roseworthy Campus	0.6	0.86
Winulta	0.9	0.60

As shown in Table 7.9 the difference between alleles was only significant for the *Glu-B3* locus at Minnipa. Progeny carrying the *b* allele had a higher grain yield than those having *h* (Fig 7.7).

Table 7.9 F-value and probabilities from analysis of variance of grain yield for different glutenin loci of 80 F3-derived F6 lines from (Barunga x Suneca) grown as a completely randomised design at Minnipa and Kapunda in 1994

Source of variation	Minnipa		Kapunda	
	F value	P	F value	P
Glu-B1	0.2	ns	2.1	ns
Glu-A3	0.2	ns	3.5	ns
Glu-B3	5.9	<0.05	1.1	ns
Glu-D3	1.9	ns	0.0	ns
Glu-B1 x Glu-A3	1.7	ns	2.4	ns
Glu-B1 x Glu-B3	6.8	<0.05	0.6	ns
Glu-A3 x Glu-B3	0.1	ns	3.6	ns
Glu-B1 x Glu-D3	1.6	ns	3.1	ns
Glu-A3 x Glu-D3	1.7	ns	0.8	ns
Glu-B3 x Glu-D3	0.4	ns	0.0	ns

The only significant interaction was observed between two non-homoelogous loci *Glu-B1* and *Glu-B3* at Minnipa. The highest grain yield (185 g/plot) was obtained with the 17+18 and *b* combination compared to 17+18, *h* combination which had lowest grain yield (138 g/plot). This difference was not so pronounced in the comparison of the other two allelic combinations 7+8, *h* and 7+8, *b* (159 g/plot vs 157 g/plot).

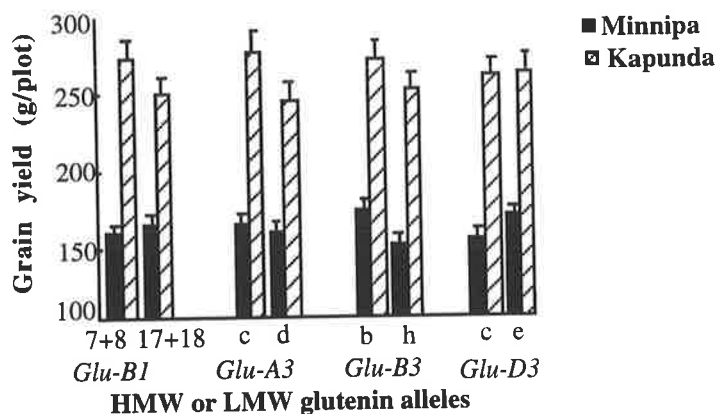


Figure 7.7 Mean grain yield of F3-derived F6 lines from (Barunga x Suneca) carrying specific HMW (*Glu-B1*) or LMW (*Glu-A3*,*Glu-B3* and *Glu-D3*) glutenin loci (data from Minnipa and Kapunda in 1994).

Replicated lines at three sites

The other F3-derived F6 progeny were grown in a completely randomised block design with two-replicates at three sites (Winulta, Minnipa and Roseworthy Campus). The analysis of variance over the sites (Pooled data) showed that there were highly significant differences between sites and genotypes for grain yield (Table 7.10). However their interaction was not significant.

Table 7.10 Mean square and probabilities from analysis of variance for grain yield of 79 F3-derived F6 lines from (Barunga x Suneca) grouped into 14 different genotypes and grown at three sites (Winulta, Minnipa and Roseworthy Campus) in 1994.

Source of variation	d.f	Grain yield	
		Mean square	Probability
Site	2	7543108	<0.001
Residuals (1)	3	584	-
Genotype	13	12255	<0.001
Site x Genotype	26	5388	0.07
Residual (2)	429	3695	-
Total	473		-

Amongst the 14 different genotypes, the highest and lowest grain yield for each site was determined (Fig 7.8, Table 7.11). In general the highest and lowest grain yields were accompanied by the presence of alleles derived from Suneca, particularly those coding

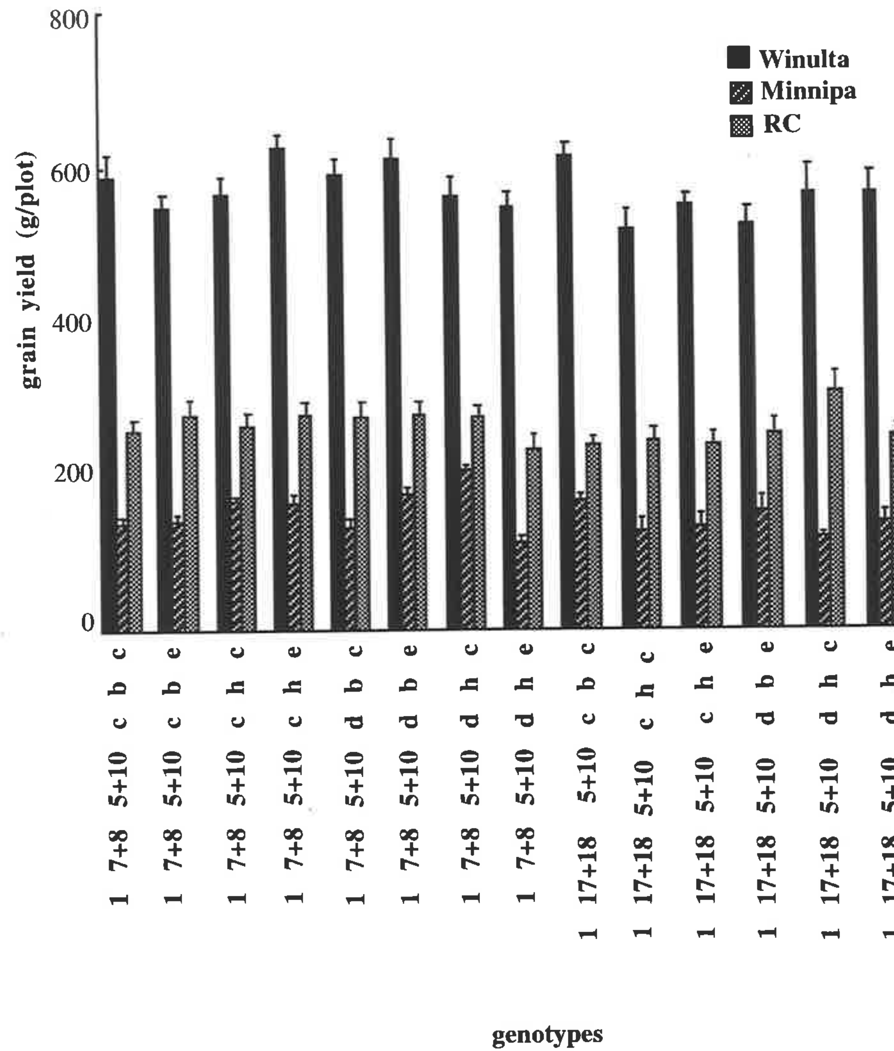


Figure 7.8 The mean yield of F_3-derived F_6 lines from(Barunga x Suneca), grouped into 14 different genotypes and grown at three sites (Winulta, Minnipa and Roseworthy Campus) in a completely randomised block design (replicated) in 1994.

LMW glutenin subunits. This suggested that those alleles controlling grain yield in Suneca (a non-South Australian variety) were most likely to be associated with the variation for grain yield under South Australian conditions and that these may be linked with the glutenin genes. It is notable that the allele *b* at *Glu-B3* locus was not involved with the variation for grain yield.

Table 7.11. The genotypes of F3-derived F6 lines from (Barunga x Suneca), showing high and low grain yield at three sites (Winulta, Minnipa and Roseworthy Campus) in the replicated trial in 1994.

Site	Glutenin genotype specification										
	Lowest grain yield						Highest grain yield				
Winulta	1	17+18	5+10	c	h	c	1	7+8	5+10	c	h	e
Minnipa	1	7+8	5+10	d	h	e	1	7+8	5+10	d	h	c
Roseworthy	1	7+8	5+10	d	h	e	1	17+18	5+10	d	h	c

When the analyses of variance were performed for separate sites, the results showed that differences between genotypes for grain yield were significant at Winulta and Minnipa (Table 7.12).

Table 7.12 Variance ratio of grain yield for 79 F3 derived F6 lines from (Barunga x Suneca) grouped to 14 different genotypes and grown in two replications at three sites (Winulta, Minnipa and Roseworthy Campus) in 1994.

Sites	Grain yield	
	F value	P
Winulta	1.97	0.03
Minnipa	4.28	<0.001
Roseworthy Campus	1.10	0.36

These two sites were examined in a second analysis. Results showed that the main effects of alleles at the *Glu-B1* and *Glu-3* loci were not significant for grain yield (Table 7.13). The highly significant difference between genotypes at Winulta and Minnipa for grain yield and the non-significance of allelic variation at the *Glu-B1* and *Glu-3* loci, suggested that there was no linkage between genes controlling the glutenin subunits with thoseresponsible for grain yield. This was also confirmed by computing the percentage of variance accounted for by genotypes. The results showed that for grain yield, only 5% of variation was accounted for by genotypes (glutenin subunits). These results are in contrast

to those of Carrillo *et al.* (1990) and McLendon *et al.* (1993) who reported genetic linkage between glutenin loci and grain yield.

Table 7.13 F-value and probabilities from analysis of variance of grain yield for different glutenin loci of 79 F_3-derived F_6 lines from (Barunga x Suneca) conducted as a two-replicate randomised block design at Winulta and Minnipa in 1994.

Source of variance	Winulta		Minnipa	
	F value	P	F value	P
Glu-B1	2.70	ns	3.24	ns
Glu-A3	0.44	ns	0.51	ns
Glu-B3	1.62	ns	0.15	ns
Glu-D3	0.00	ns	2.09	ns
Glu-B1 x Glu-A3	0.13	ns	0.29	ns
Glu-B1 x Glu-B3	1.40	ns	9.93	<0.001
Glu-A3 x Glu-B3	0.07	ns	0.11	ns
Glu-B1 x Glu-D3	0.53	ns	1.41	ns
Glu-A3 x Glu-D3	0.37	ns	1.18	ns
Glu-B3 x Glu-D3	7.35	<0.001	8.00	<0.001

The highly significant interaction between two non-homoelogous loci of *Glu-B1* and *Glu-B3* at Minnipa showed that subunit bands 7+8 in the presence of allele *h* gave the highest grain yield (167 g/plot) and 17+18 in the presence of allele *h* gave the lowest (133 g/plot) (Fig 7.9).

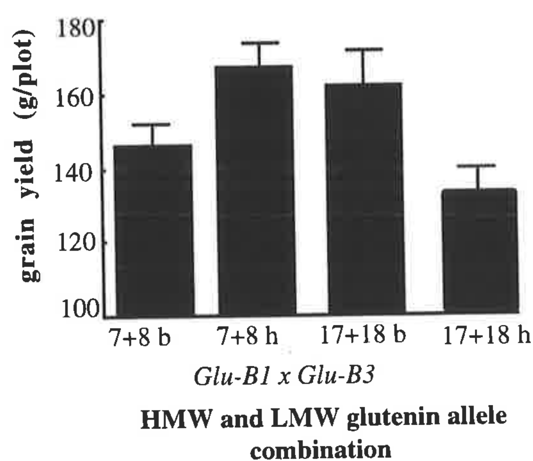


Figure 7.9 Mean grain yield of 79 F_3-derived F_6 lines from (Barunga x Suneca) showing specific interaction between HMW (*Glu-B1*) and LMW (*Glu-B3*) glutenin alleles (data from Minnipa in 1994).

The interaction between *Glu-B3* and *Glu-D3* observed at both Winulta and Minnipa was also quite substantial. The highest grain yield at Winulta was observed with the combination of alleles *b* and *c* (598 g/plot) (Fig 7.10a), whereas at Minnipa the combination of *h* and *c* was highest yielding (166 g/plot) (Fig 7.10b).

To provide an explanation for these contrasting results at Winulta and Minnipa, the correlations between maturity and grain yield at these two sites were computed. At Minnipa there was a significantly positive correlation between grain yield and maturity (r=0.49), while at Winulta the correlation was negative and non-significant (r=-0.13).

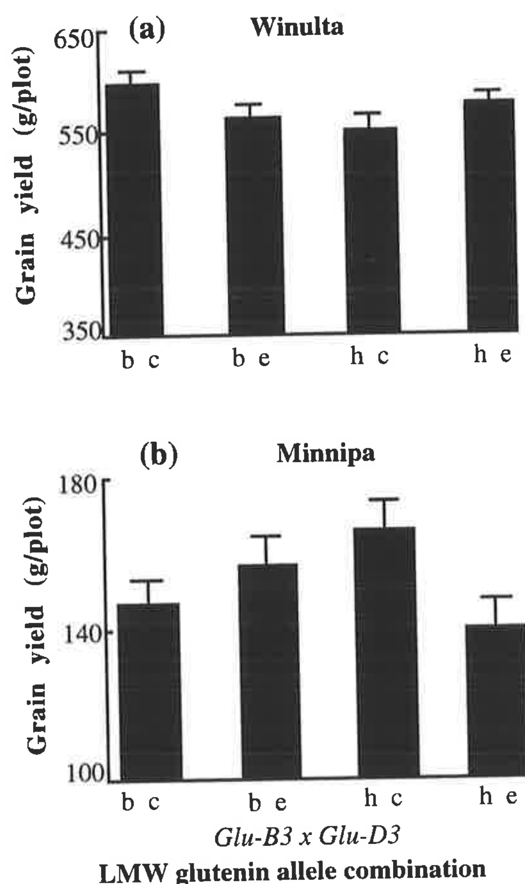


Figure 7.10 Mean grain yield of 79 F3-derived F6 lines from (Barunga x Suneca) showing the interaction effects of specific combination alleles of LMW glutenin loci (*Glu-B3* x *Glu-D3*). Data from Winulta **(a)** and Minnipa **(b)** in 1994.

7.3 Discussion

The positive effects of the LMW glutenin subunits on quality as measured by SDSS can be most likely attributed to their greater amounts of total glutenin (Payne *et al.*, 1984c), or number of bands as reported by Gupta and Shepherd (1990a). With respect to number of bands at the *Glu-A3* locus, it has been reported that the *d* allele codes three bands, whereas allele *c* codes only a single band (Cornish, 1994). However visual observation on gels (Chapter 3, section 3.3.2) showed that the number of bands coded by these two alleles are the same. Therefore it seems that the greater SDSS volume of the *d* allele could be due to its higher molecular weight product (see Fig 7.3).

In respect to the differences between *b* and *h* at *Glu-B3*, the greater SDSS volume associated with *b* (Fig 7.5) could be attributed to its larger number of bands as suggested by Gupta *et al.* (1990b). The apparent number of bands and related LMW glutenin subunits, particularly at *Glu-B3* locus which commonly has the largest number of bands, depends upon the method of fractionation. In the inheritance study (Chapter 3, Fig 3.2) visual observation showed that the allele *b* at *Glu-B3* has three extra bands compared to *h*, which conflicts with the identification by Cornish (1994) (Chapter 5, Table 5.3).

It seems that the positive effect of the glutenin alleles cannot be fully predicted by the main effects of each allele alone, as interactions occur between different loci. In the present study the effects of the alleles at *Glu-B1* and *Glu-B3* varied when different *Glu-D3* alleles were present (Table 7.6). The role of *Glu-D3* in the two loci interaction on SDS-sedimentation was similar to that reported by Lafiandra *et al.* (1993) who found that the presence of one allele at the *Glu-D3* locus had a negative effect on gluten properties as measured by SDS-sedimentation. This negative effect was mainly due to its interaction with other subunits, resulting in a reduction in the size of the glutenin polymers.

Although there was an apparent significant effect of the alleles at *Glu-B1* in early generation on grain yield (Table 7.5), however this was probably due to the lack of randomisation. The results of the yield analysis of F_3-derived F_6 lines at the later generation showed that alleles at *Glu-B3* were significantly associated with grain yield at Minnipa (unreplicated experiment) (Table 7.9) , but this effect on grain yield was not observed at Kapunda and in the replicated experiment (Table 7.13). The only consistent effect was in the interaction between the *Glu-B3* and *Glu-D3* at Winulta and Minnipa (replicated trial) (Table 7.13). These occasional effects probably reflect the large number of genes controlling grain yield, such as those affecting maturity, being influential at some locations. The presence of *Glu-D3* in an interaction with *Glu-B3* at two sites (Winulta and Minnipa) in the replicated trial (Table 7.13) suggests that the*Glu-D3* effect on grain yield observed in an early generation might ~~to~~ be real (Table 7.5).

Although subunit bands 17+18 coded by the gene at *Glu-B1*, and allele *d* at *Glu-A3* were associated with greater SDS sedimentation (Fig 7.5), the selection of the lines on the basis of these positive effects had no effect on grain yield. This is in agreement with O'Brien *et al.* (1989) who reported that positive effects of glutenin alleles does not limit selection for grain yield. As quality testing in early generations, prior to yield evaluation, is cost-effective this would allow only those lines with acceptable quality to be tested for yield in later generations. This has a potential psychological advantage for the breeder, in that it will no longer always be the highest yielding lines that are subsequently discarded because of quality defects.

Improvement in dough properties of bread wheats could be achieved by replacing of a superior allele in place of those associated with weak dough properties. This may take a considerable length of time and a great deal of effort. For example, at the Waite Institute, it has taken about 10 years to substantially reduce the proportion of the population with the subunit bands 2+12 and increase 5+10 in the wheat breeding program (A.J. Rathjen, pers. comm.). Therefore before any elimination or substitution of glutenin subunit alleles is undertaken, the value of the glutenin allele must be clearly demonstrated to the breeder.

CHAPTER 8

CONTRIBUTION OF ALLELES CONTROLLING HMW AND LMW GLUTENIN SUBUNITS TO DOUGH PROPERTIES IN F_6 RECOMBINANT INBRED LINES (RILS) FROM A BREAD WHEAT CROSS

8.1 Introduction

It is widely accepted that the rheological characteristics of wheat flour, when mixed with water to form a viscoelastic dough, are due to its gluten proteins (Dimler, 1965; Wall, 1979). This protein complex consists of two major fractions: gliadin and glutenin. Gliadin molecules consist of a complex mixture of polypeptides which, when fractionated by gel electrophoresis at low pH, separate into four groups, alpha-, beta-, gamma-and omega-gliadins (Woychik *et al.,* 1961; Wall, 1979). Glutenins consist of polypeptide chains (subunits) similar to those of gliadin, but cross-linked by disulphide bonds into higher level polymers. When treated with a reducing agent such as sodium dodecyl sulphate (SDS), the glutenin molecules dissociate into high molecular weight (HMW) (Payne and Corfield, 1979) and low molecular weight (LMW) (Jackson *et al.*, 1983) glutenin subunits.

The storage proteins of wheat flour typically consist of 50% gliadins, 10% HMW and 40% LMW glutenin subunits (Payne and Corfield, 1979; Payne *et al.,* 1984a). The bread-making quality of wheat flour is dependent not only on the concentration of protein but also the nature of the gluten proteins (Finney and Barmor, 1948). Protein quality differences between varieties are considered to be due mainly to different combinations of endosperm storage protein variants (Payne *et al.,* 1984a).

The study of the diversity of wheat storage proteins and their effects on technological quality has progressed substantially in the last decade. The alleles for HMW glutenin subunits have been determined for both bread (Payne and Lawrence, 1983) and durum wheats (Branlard *et al.,* 1989). An association between HMW glutenin subunits and different flour quality criteria has been established by several workers (Payne *et al.,* 1981a; Moonen *et al.,* 1982; Branlard and Dardevet, 1985b). There is also wide variation among varieties in the electrophoretic patterns of LMW glutenin subunits and the association between some LMW glutenin subunits and quality characteristics has been studied (Payne, 1987; Gupta and Shepherd, 1987; 1988; Gupta *et al.,* 1991). LMW glutenin genes are located on the short arms of group 1 chromosome and are closely linked with the genes controlling the gliadin subunits. Genetic and biochemical evidence has revealed that the LMW glutenin subunits alone are likely to be the cause for the

relationship between *Glu-3/Gli-1* alleles and flour quality parameters (Payne *et al.*, 1987c; Pogna *et al.*, 1988)

It is now accepted that both HMW and LMW glutenin subunits contribute to the rheological properties of dough and their effects on dough quality appear to be mostly additive (Gupta and Shepherd, 1988; Gupta *et al.*, 1989; Pogna *et al.*, 1990). A study of several varieties of Australian bread wheats has shown that the LMW subunits of glutenin have a much stronger correlation with the dough quality (as determined by extensograph) than the HMW subunits (Metakovsky *et al.*, 1990).

Correlation between individual protein bands and dough quality can be studied in different ways. F_2 derived progeny is one of the most reliable methods. This procedure has the advantage of analysing the association between two traits in a range of genetic backgrounds, and thus any correlations detected are likely to be robust. Causal relationships may be more easily defined in F_2 derived progeny than with cultivar sets, due to a more random variation in background differences of the former.

In an earlier study (see Chapter 4) the relative contribution of HMW and LMW glutenin subunits was evaluated by using random recombinant inbred lines similar to those reported by Gupta *et al.* (1994a). However in these studies only a limited number of lines were available for each genotype and, therefore, mean effects claculated over the restricted number of lines could not provide reliable estimates of associations or direct effects of the major loci coding glutenin subunits. Moreover, those lines represented only 33 of the 64 homozygous allelic combinations expected based on six segregating loci so a considerable number of genotypes were missing. The study described here was designed to overcome some of these problems. In particular, it involves variation in four alleles at the *Glu-B1*, *Glu-A3*, *Glu-B3* and *Glu-D3* loci and several independent isolates (lines) of all 16 genotypic combinations of these alleles. This permitted partitioning the genetic variance associated with the four glutenin loci into main and interaction effects.

8.2 Material and methods

8.2.1 Plant material

The plant material originated from a cross between Barunga and Suneca as reported in Chapter 7. This cross was made mainly to find which glutenin loci need to be changed to upgrade South Australian Hard wheat (Barunga) to the Prime Hard (Suneca) standard, and whether the background genotype can appreciably affect the physical dough quality. Barunga was released at the University of Adelaide (AWCC, 1994) and has similar quality characteristics to other good quality Hard wheats, but is superior to Machete which is the

most widely grown Hard wheat in South Australia. Suneca, used as the other parent, was released at the University of Sydney (AWCC, 1983) and has not been widely grown in South Australian trials as it has low grain yields under South Australian conditions. This parent is classified as a Prime Hard wheat and has a higher milling yield than most other wheats in cultivation in Australia. A comparison of the quality parameters of these two parents has been given elsewhere (Table 7.1). The procedure for the development of the RILs was presented in Chapter 7 (Materials and methods).

Five Australian hard wheat varieties Aroona, Yarralinka, Warigal, BT-Schomburgk and Angas along with the Barunga parent were also included in this study. The overall means of the quality parameters of these varieties give a better estimation of quality than Barunga alone, and therefore can be used for a comparative analysis of the results obtained with the RILs.

8.2.2 Field experiments

Two field experiments were conducted in 1994 as reported in Chapter 7 (Materials and methods). In brief, the first experiment comprised five lines from each genotype grown as a randomised complete block (unreplicated trial) at six sites (Minnipa, Kimba, Winulta, Roseworthy Campus, Kapunda and Lowbanks) and the second, contained the remaining lines sown as a two-replicate randomised block (replicated trial), at three sites (Winulta, Minnipa and Roseworthy Campus). The location and brief description of these experimental sites have been given elsewhere (Chapter 7, Materials and methods). The arrangement and dimensions of plots used for the field experiments were similar to those in previous experiments (Chapter 4, Material and Methods). In this Chapter the terms unreplicated trial and replicated trial have been used as the description of the two experiments.

Maturity

Maturity score was recorded at the time of anthesis similar to the procedure described in Chapters 4, 5 and 7 (Material and methods).

8.2.3 Quality tests

This experimental material was grown in a drought season in 1994 (Chapter 7, section 7.2.7) and therefore there was not enough seed available for complete quality analysis from most of the sites except Winulta. Therefore this site was chosen for quality evaluation. Quality tests, including tempering, milling, NIR measurements, extensograph

and SDS sedimentation was conducted according to procedures described in Chapter 4 (Material and Methods).

8.2.4 Statistical analyses

The statistical procedures for analysing the individual and combination of alleles for both sets of experiments were similar to those reported in Chapter 7. The analyses were carried out using the Genstat 5 statistical package. Correlation coefficients between different measurements were obtained using simple correlation calculations. The proportion of variation accounted for by genotypes and lines for the replicated experiment was also calculated from the variance components of two separate analysis of variance to account for the variable number of lines within genotypes. The first partitioned variance into genotypes and the second, lines within genotypes.

8.3 Results

8.3.1 Testing of the distribution of genotypes

Although equal number of lines within each genotypes (five for each genotype) were grown in the unreplicated trial, in the replicated trial, the number of lines within each genotypes differed and two genotypes out of the 16 were missing. To test whether these RILs lines were randomly distributed between the genotypes, the differences between the observed and theoretical frequencies was tested with a chi-square test. Table 8.1 presents the occurrence of the allelic combinations at *Glu-B1*, *Glu-A3*, *Glu-B3* and *Glu-D3* loci among the 79 F_6 RILs. The chi-square value of 17.2 obtained from this analysis was not significant (15 degrees of freedom at 5 percent level of significant), indicating a random representation of the genotypes.

8.3.2 Distribution of quality parameters between parents and F_3-derived families

The mean quality and agronomic measurements for Barunga, the other six Hard reference varieties and the RILs for replicated and unreplicated trials are presented in Tables 8.2 and 8.3. The full details of the quality analysis for the six Hard reference varieties is given in the next chapter. With the F_3-derived genotypes, the mean values for the quality parameters were higher for most of the genotypes in both trials when compared to Barunga and the reference hard varieties. In the previous study, a comparison between Barunga and Suneca (Table 7.1) showed that Barunga has a greater Rmax than Suneca. The mean effects of their glutenin alleles can be seen by comparing the genotypes corresponding to the patterns of the two parents (Tables 8.2 and 8.3). Comparison between progeny with a Suneca pattern (17+18, *d h e*) with those having a Barunga pattern (7+8, *c b c)* for both

trails showed that the former had higher extensibility and extensibility-protein ratio particularly for the replicated trial.

Table 8.1 Frequency of 16 genotypes for combinations of the glutenin alleles in the F_3-derived F_6 families from (Barunga x Suneca) and goodness of fit to the expected frequencies.

Genotypes	Glutenin loci				observed frequency	expected frequency	χ^2
	Glu-B1	*Glu-A3*	*Glu-B3*	*Glu-D3*			
1	7+8	c	b	c	7	4.94	0.86
2	7+8	c	b	e	6	4.94	0.22
3	7+8	c	h	c	6	4.94	0.22
4	7+8	c	h	e	7	4.94	0.86
5	7+8	d	b	c	6	4.94	0.22
6	7+8	d	b	e	5	4.94	0.00
7	7+8	d	h	c	7	4.94	0.86
8	7+8	d	h	e	5	4.94	0.00
9	17+18	c	b	c	7	4.94	0.86
10	17+18	c	b	e	0	4.94	4.94
11	17+18	c	h	c	6	4.94	0.22
12	17+18	c	h	e	7	4.94	0.86
13	17+18	d	b	c	0	4.94	4.94
14	17+18	d	b	e	4	4.94	0.18
15	17+18	d	h	c	2	4.94	1.75
16	17+18	d	h	e	4	4.94	0.18
Total					**79**		**χ^2=17.2**

The analysis of variance for the replicated trial conducted as a two-replicate randomised block showed that the differences between genotypes were highly significant for most of the measurements (Table 8.4). The differences between 79 lines within each genotype were also highly significant for most of the measurements.

As shown in Table 8.4, the absolute values of variance (F value) attributed to lines within genotypes for different measurement (quality and agronomic characters) were mostly higher than genotypes, indicating that quality differences observed are due to differences between lines as well as the glutenin genotypes. To further investigate this finding the percentage of variance accounted for by genotypes, lines and block was calculated for all of the quality and agronomic characters (Table 8.5).

Table 8.2 Mean quality parameters of Barunga, six South Australian hard wheats and 79 F_3-derived F_6 families (14 different glutenin genotypes) from Barunga x Suneca) grown as a two-replicate randomised block (replicated trial) at Winulta in 1994[a].

Phenotypic classes	No lines	Characters									
		Quality							Agronomic		
		PC	Rmax	Ext	E/P	WA	FY	SDSS	M	GY	PY
Barunga 7+8 c b c	4	9.3	265.7	14.5	1.58	61.3	63.8	57.2	54.2	695.7	64.7
Hard wheats	6	9.6	229.0	14.3	1.49	60.8	65.6	58.5	48.7	669.0	64.2
Genotypes											
7+8 c b c	7	10.3	285.1	16.1	1.56	58.8	67.4	63.3	44.6	587.6	60.5
7+8 c b e	6	10.3	276.9	16.3	1.59	58.6	67.4	62.7	46.0	548.7	56.5
7+8 c h c	6	9.9	257.5	14.9	1.50	60.4	67.5	62.2	47.4	656.5	65.0
7+8 c h e	7	10.1	271.3	15.9	1.57	60.8	66.7	60.1	47.1	625.6	63.2
7+8 d b c	6	10.7	290.4	17.4	1.63	60.2	68.3	68.4	45.8	591.3	63.3
7+8 d b e	5	10.8	291.2	18.0	1.66	59.2	67.6	68.2	47.8	611.4	66.0
7+8 d h c	7	11.4	294.0	18.6	1.63	60.4	66.3	75.3	51.6	562.6	64.1
7+8 d h e	5	11.0	310.4	16.7	1.51	58.4	67.4	69.3	45.7	550.2	60.5
17+18 c b c	7	10.6	282.0	15.3	1.45	59.4	66.3	61.8	46.4	613.6	65.0
17+18 c b e	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
17+18 c h c	6	11.2	312.8	17.0	1.53	59.4	69.1	63.4	46.3	520.7	58.3
17+18 c h e	7	11.2	291.5	17.5	1.55	58.8	69.0	68.5	47.1	552.1	61.8
17+18 d b c	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
17+18 d b e	4	11.0	299.9	17.4	1.59	58.4	67.0	75.2	46.4	527.1	58.0
17+18 d h c	2	10.7	286.2	17.2	1.61	60.0	68.3	64.0	46.2	565.7	60.5
17+18 d h e	4	10.9	279.7	18.1	1.67	59.6	67.5	70.0	45.9	566.2	61.7
Mean over lines	-	**10.7**	**287.2**	**16.8**	**1.57**	**59.6**	**67.5**	**67.5**	**46.8**	**572.9**	**61.3**
LSD of Hard wheats (%5)	-	**0.7**	**25.3**	**2.1**	**0.25**	**2.0**	**2.9**	**1.7**	**2.8**	**224.2**	-

a=see abbreviation list
NI=no information

Table 8.3 Mean quality parameters of Barunga, six South Australian hard wheats and 80 F_3-derived F_6 families (16 different glutenin genotypes) from (Barunga x Suneca), grown in one completely randomized block (unreplicated trial) at Winulta in 1994[a].

Phenotypic classes	No lines	Characters									
		Quality							Agronomic		
		PC	Rmax	Ext	E/P	WA	FY	SDSS	M	GY	PY
Barunga 7+8 c b c	5	9.7	262.8	14.3	1.50	59.8	65.1	55.0	55.2	637.2	61.8
Hard wheats	6	9.8	240.1	14.4	1.47	60.3	66.4	55.7	53.0	697.4	68.3
Genotypes											
7+8 c b c	5	10.1	281.4	15.6	1.54	59.0	65.1	64.0	47.4	616.6	62.3
7+8 c b e	5	10.3	281.4	16.4	1.61	58.6	68.4	58.2	47.8	649.0	66.8
7+8 c h c	5	10.1	286.8	15.1	1.50	59.2	68.8	56.0	46.0	582.6	58.8
7+8 c h e	5	10.2	289.8	15.6	1.54	58.6	68.5	57.4	50.0	625.0	63.7
7+8 d b c	5	10.6	292.4	15.8	1.50	60.0	67.5	64.6	51.2	588.2	62.3
7+8 d b e	5	10.9	311.8	17.1	1.57	58.4	66.9	62.6	50.6	601.6	65.6
7+8 d h c	5	10.4	324.8	16.2	1.56	57.6	69.3	67.2	52.2	556.2	57.8
7+8 d h e	5	10.6	310.8	16.7	1.57	57.6	69.3	60.0	49.6	536.6	56.9
17+18 c b c	5	10.0	262.2	15.1	1.52	58.8	68.9	58.0	49.2	583.4	58.3
17+18 c b e	5	10.0	266.6	16.3	1.63	57.0	67.5	57.4	49.8	613.2	61.3
17+18 c h c	5	11.6	295.6	16.8	1.45	59.6	68.8	60.6	45.6	515.4	59.8
17+18 c h e	5	10.9	298.6	18.0	1.86	59.2	68.7	61.0	50.0	543.6	59.2
17+18 d b c	5	10.9	316.6	17.5	1.60	56.8	69.1	65.6	52.6	555.0	60.5
17+18 d b e	5	11.5	306.2	17.4	1.50	57.8	69.6	64.0	52.4	555.6	63.9
17+18 d h c	5	10.6	318.8	16.9	1.59	58.0	67.9	62.8	48.0	520.2	55.1
17+18 d h e	5	10.9	302.0	17.0	1.57	58.2	70.3	62.2	49.6	561.2	61.2
Mean over lines	-	**10.6**	**296.6**	**16.5**	**1.57**	**58.4**	**68.6**	**60.8**	**49.5**	**575.2**	**61.0**
LSD of Hard wheats (%5)	-	**0.7**	**18.3**	**1.9**	**0.13**	**1.5**	**1.8**	**4.0**	**3.6**	**126.9**	-

a=see abbreviation list

Table 8.4 Variance ratio of the quality and agronomy measurements for genotypes and lines within genotypes derived from (Barunga x Suneca) sown as a two-replicate randomised block at Winulta in 1994

Measurement	Variance ratio			
	Genotypes		Lines	
	Fvalue	P	Fvalue	P
Quality				
protein concentration (%)	3.9	<0.001	6.7	<0.001
maximum resistance (BU)	3.0	<0.001	6.2	<0.001
extensibility (cm)	5.3	<0.001	3.2	<0.001
extensibility/protein ratio	3.1	<0.001	1.9	0.002
water absorption (ml)	3.2	<0.001	16.3	<0.001
flour yield (%)	2.6	0.003	4.1	<0.001
SDS-sedimentation volume (ml)	10.3	<0.001	10.1	<0.001
Agronomic characters				
maturity (anthesis)	4.0	<0.001	5.5	<0.001
grain yield (g/plot)	2.0	0.027	3.7	<0.001

Table 8.5 Percentage of variance component attributed to genotypes (glutenin subunits), lines within genotypes (same glutenin bands) and blocks for quality and agronomic characters of 79 F_3-derived F_6 lines from (Barunga x Suneca), grown as a two-replicate randomised block at Winulta in 1994.

Measurement	Percentage of variance component		
	genotypes	lines	block
Quality data			
Flour yield	8	92	-†
Protein concentration	16	84	-
Water absorption	9	91	-
Extensibility	22	76	2
E/P ratio	12	85	3
Maximum resistance	9	90	1
SDS-sedimentation volume	40	60	-
Agronomic characters			
Maturity (anthesis)	18	79	3
Grain yield (g/plot)	5	95	-

† negative variance components, so the value has been excluded when calculating the percentage of variance component.

The high percent of variation for most of the measurements accounted for by lines within genotypes indicates that genes other than the segregating glutenin loci have large effects on the measurements recorded.

With respect to an unreplicated trial, the analysis of variance showed that there are only significant differences between genotypes for maximum resistance (Rmax) and water absorption (Table 8.6).

Table 8.6 Mean squares in analysis of variance for quality and agronomic characters of genotypes derived from (Barunga x Suneca), grown in a completely randomized block (unreplicated trial) at Winulta in 1994.

Measurement	Variance ratio	
	F value	P
Quality		
protein concentration (%)	1.3	0.21
maximum resistance (BU)	2.0 * (a)	0.03
extensibility (cm)	0.8	0.69
extensibility/protein ratio	0.7	0.75
water absorption (ml)	1.8 *	0.05
flour yield (%)	0.7	0.81
SDS-sedimentation volume (ml)	1.7	0.07
Agronomic characters		
maturity (anthesis)	1.3	0.26
grain yield (g/plot)	0.9	0.60

a=see abbreviation list for significant level

The major difference between the replicated and unreplicated trials is the greater precision obtained with the replicated trial compared to the one completely randomised block. In the unreplicated trial, the background genetic variation plus the residual variation together made up "residual". Thus the lack of significance in the unreplicated trial for most quality parameters is due to the large background genetic variation compared to that attributable to the genotypes (glutenin band patterns). For these reasons in the analysis of the results the main emphasis is given to the replicated trial.

8.3.3 Effect of allelic variation in protein bands on quality parameters

Individual allelic effects on dough properties of 79 F3-derived F6 lines (replicated trial)

The data summed over all genotypes and lines showed that allelic variation at the *Glu-A3* locus had a highly significant effect (P<0.001) on Rmax (Table 8.7). This trend was also observed for SDS-sedimentation, another indication of dough strength. However the relative size of the variance (F value) for this criterion was around twelve times that observed for Rmax (Table 8.7), indicating that SDS-sedimentation is less affected by environmental effects within the experiment than the direct measurement of dough strength, in agreement with the report by Preston *et al.* (1982). Although SDS-sedimentation volume is not a direct measure of strength like Rmax, the progeny with LMW glutenin subunit allele *d* had a higher Rmax and SDS-sedimentation volume than *c* (Fig 8.1). Allelic variation at the three remaining loci, *Glu-B1* (17+18, 7+8), *Glu-B3* (*b*, *h*) and *Glu-D3* (*c*, *e*) did not significantly alter Rmax or SDS-sedimentation volume.

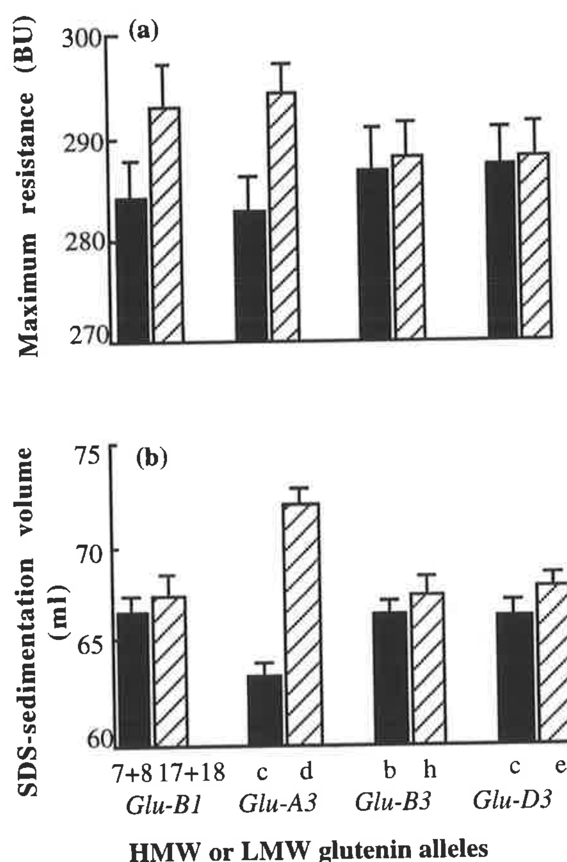


Figure 8.1 Mean **(a)** maximum dough resistance and **(b)** SDS-sedimentation volume for progeny carrying specific alleles of HMW (*Glu-B1*) and LMW (*Glu-A3*, *Glu-B3* and *Glu-D3*) glutenin loci. Data from 79 F3-derived F6 lines, obtained from (Barunga x Suneca) and grown as a two-replicate randomised block at Winulta in 1994.

Table 8.7 Mean squares from analysis of variance for quality and agronomic characters of 79 F_3-derived F_6 families from (Barunga x Suneca), grown as a two-replicate randomised block at Winulta in 1994[a].

Source of variation	d.f	quality characters							agronomic characters	
		PC	Rmax	Ext	E/P	WA	FY	SDSS	M	GY
Glu-B1	1	5.7 **	2929.6 ns	1.4 ns	0.04 ns	1.71 ns	107.5 ns	14.5 ns	13.6 ns	17088.0 ns
Glu-A3	1	10.1 **	6248.8 **	99.9 **	0.22 **	0.01 ns	6.8 ns	2281.5 **	35.1 ns	2773.0 ns
Glu-B3	1	1.7 ns	2.3 ns	3.9 ns	0.00 ns	4.70 **	28.7 ns	27.0 ns	81.1 **	10241.0 ns
Glu-D3	1	0.0 ns	49.0 ns	2.0 ns	0.03 ns	3.70 *	1.9 ns	3.1 ns	17.1 ns	4.0 ns
Glu-B1 x Glu-A3	1	7.8 **	7082.8 **	9.1 ns	0.02 ns	0.26 ns	41.6 ns	53.3 ns	28.9 ns	791.0 ns
Glu-B1 x Glu-B3	1	0.5 ns	891.3 ns	26.2 **	0.15 **	0.51 ns	631.6 **	6.1 ns	36.6 *	8883.0 ns
Glu-A3 x Glu-B3	1	0.5 ns	285.1 ns	0.1 ns	0.00 ns	2.14 ns	122.3 ns	2.7 ns	0.0 ns	433.0 ns
Glu-B1 x Glu-D3	1	0.8 ns	552.6 ns	6.8 ns	0.00 ns	0.41 ns	0.6 ns	735.2 **	11.9 ns	3349.0 ns
Glu-A3 x Glu-D3	1	0.1 ns	763.4 ns	6.2 ns	0.03 ns	3.25 *	9.7 ns	6.5 ns	40.0 ns	2345.0 ns
Glu-B3 x Glu-D3	1	1.3 ns	130.4 ns	8.3 ns	0.01 ns	0.58 ns	0.1 ns	268.6 **	108.6 **	46512.0 **
Residual	147	0.6	879.9	2.8	0.01	0.59	33.5	27.1	9.1	6327.0
Total	158	0.8	952.3	3.7	0.01	0.66	37.3	47.1	11.2	6480.0

a=see abbreviation list

A highly significant difference between alleles at the *Glu-A3* locus was also observed for protein concentration. Lines carrying *Glu-A3d* had a higher protein concentration and protein yield than those possessing the *Glu-A3c* allele (11% vs 10.5%) (Fig 8.2). Although bands 17+18 were associated with a highly significant higher protein concentration than 7+8 (11% vs 10.6%), their effect on Rmax and SDS-sedimentation was not significant, suggesting that variation in protein concentration associated with allelic differences did not account for their effect on protein quality. It seems that the higher protein concentration associated with lines carrying 17+18 glutenin bands was not sufficient to make a significant difference to dough strength. It is notable that the difference between alleles at the *Glu-B1* locus was not pronounced when protein yield per plot (grain yield x protein concentration) was considered (Fig 8.2b).

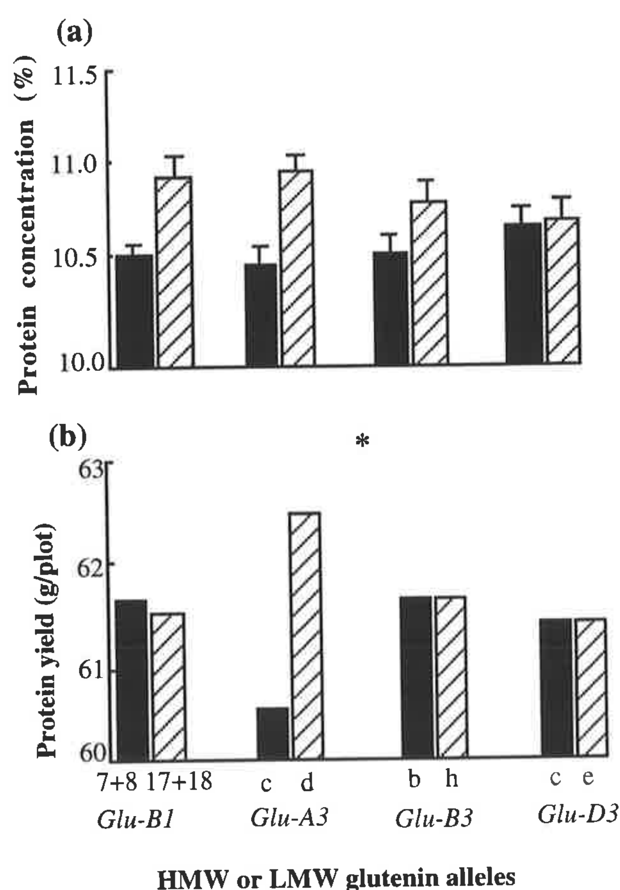


Figure 8.2 Mean **(a)** protein concentration and **(b)** protein yield for progeny carrying specific alleles of HMW (*Glu-B1*) and LMW (*Glu-A3*, *Glu-B3* and *Glu-D3*) glutenin loci. Data from 79 F3-derived F6 lines, obtained from (Barunga x Suneca) and grown as a two-replicate randomised block at Winulta in 1994.
* The protein yield was not analysed.

Since extensibility is more highly correlated to flour protein concentration than maximum resistance, as reported by Gupta *et al.* (1992), the extensibility/protein concentration ratio (E/P) was calculated. The *Glu-A3* locus showed significant differences in both

extensibility and extensibility/protein ratio (Table 8.7) with the main effects of these alleles on extensibility, protein concentration and extensibility/protein ratio given in Table 8.8. The progeny carrying the *d* allele were more extensible than those possessing the *c* allele. Variation in extensibility due to the *Glu-A3* locus was not attributable to protein concentration, as the difference in E/P ratio was highly significant between the *d* and *c* alleles. Lines possessing the *Glu-A3d* allele had a higher E/P ratio than those possessing the *Glu-A3c* allele, in agreement with the results of Gupta *et al.* (1991).

Table 8.8 Mean effect of alleles at the *Glu-A3* locus on extensibility, protein concentration and extensibility/protein ratio in 79 F3-derived F6 lines from (Barunga x Suneca), grown as a two-replicate randomised block at Winulta in 1994.

Allele	Number samples	Ext		PC		E/P ratio	
		Mean	SE	Mean	SE	Mean	SE
d	66	17.7	0.20	11.0	0.10	1.61	0.02
c	92	16.1	0.19	10.5	0.10	1.54	0.01

Interaction between allelic effects on quality parameters of 79 F3-derived F6 lines (replicated trial)

The analysis of variance showed that the relative effect of an allele from a given locus depended upon the alleles present at the other glutenin loci (Table 8.7). This was most evident with the combination of the effects of alleles at the *Glu-B1* and *Glu-A3* loci on Rmax. The absolute values of the sum of square for interaction between alleles at *Glu-B1* (17+18, 7+8) with alleles at *Glu-A3* (*c*, *d*) was higher than with *Glu-A3* alone, indicating that the *Glu-B1* locus interacts with *Glu-A3* in dough strength. The highest Rmax was obtained with the combination of 7+8 and *d* from *Glu-B1* and *Glu-A3*, respectively (296 BU) (Fig 8.3a)

There was also a significant interaction between the alleles at the *Glu-B1* and *Glu-A3* loci on protein concentration. Lines carrying allele combinations 7+8 and *d* had the highest protein concentration (11.0%) (Fig 8.3b), similar to the result obtained with Rmax. These results indicate that these alleles seem to act in a complementary fashion to increase protein concentration and develop high gluten strength.

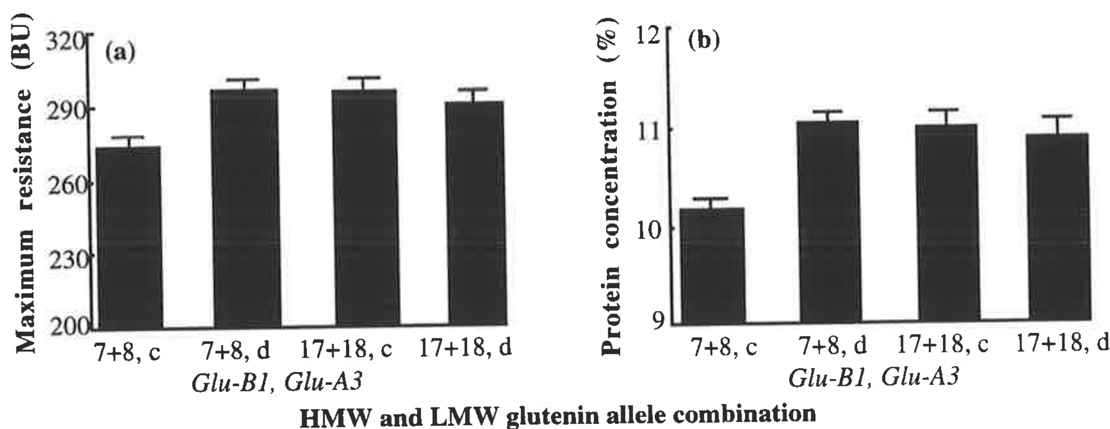


Figure 8.3 Mean **(a)** maximum dough resistance and **(b)** protein concentration values for progeny carrying specific combinations of HMW (*Glu-B1*) and LMW (*Glu-A3*) glutenin alleles. Data from 79 F3-derived F6 lines, obtained from (Barunga x Suneca) and grown as a two-replicate randomised block at Winulta in 1994.

Two significant interactions were also noted for SDS-sedimentation. Both of these involved alleles at the *Glu-D3* locus as there was a highly significant interaction between *Glu-B1* x *Glu-D3* and *Glu-B3* x *Glu-D3*. Lines with subunit bands 17+18 had a greater SDSS volume than those possessing the 7+8 bands only when the *Glu-D3e* allele was present (70.7 ml) (Fig 8.4a). This same trend was observed earlier in the bird-proof enclosure experiment (Table 7.6). With respect to the *Glu-B3* x *Glu-D3* interaction, the highest SDSS volume was obtained when the alleles *Glu-B3b* and *Glu-D3e* were both present (67.9 ml) (Fig 8.4b).

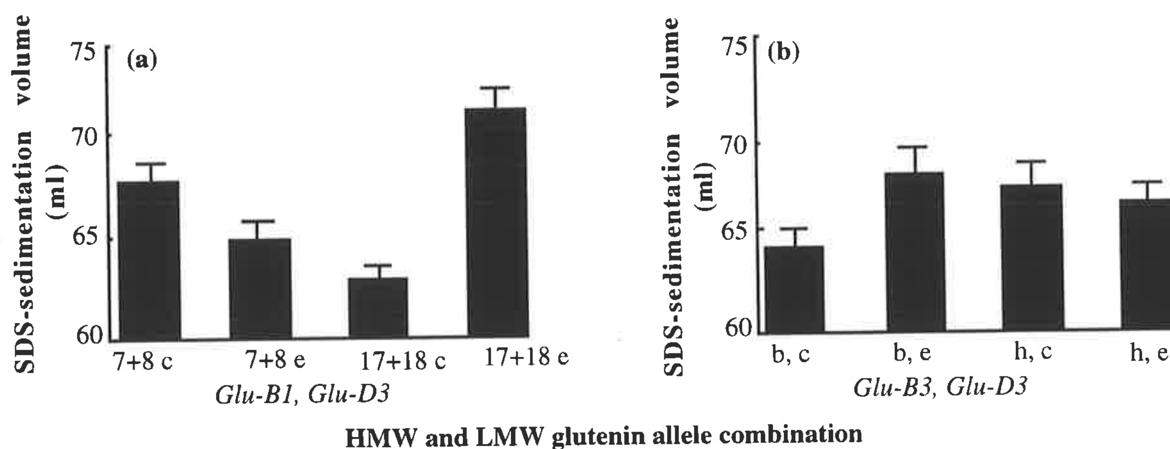


Figure 8.4 Mean SDS-sedimentation volume for progeny carrying specific combinations of **(a)** *Glu-B1 x Glu-D3* or **(b)** *Glu-B3 x Glu-D3* glutenin alleles. Data from 79 F3-derived F6 lines, obtained from (Barunga x Suneca) and grown as a two-replicate randomised block at Winulta in 1994.

With respect to extensibility and extensibility/protein ratio, although the main effects of *Glu-B1* and *Glu-B3* alleles were not significant, there was a highly significant interaction between these alleles (Table 8.7). Subunit bands 17+18 present with *Glu-B3h* bands gave the greatest extensibility (17.4 cm), whereas the least extensibility was observed in the presence of *Glu-B3b* (16.0 cm) (Fig 8.5a). This trend was quite different with extensibility/protein ratio. The highest E/P ratio was observed with the 7+8 and *b* combination (1.6) (Fig 8.5b)

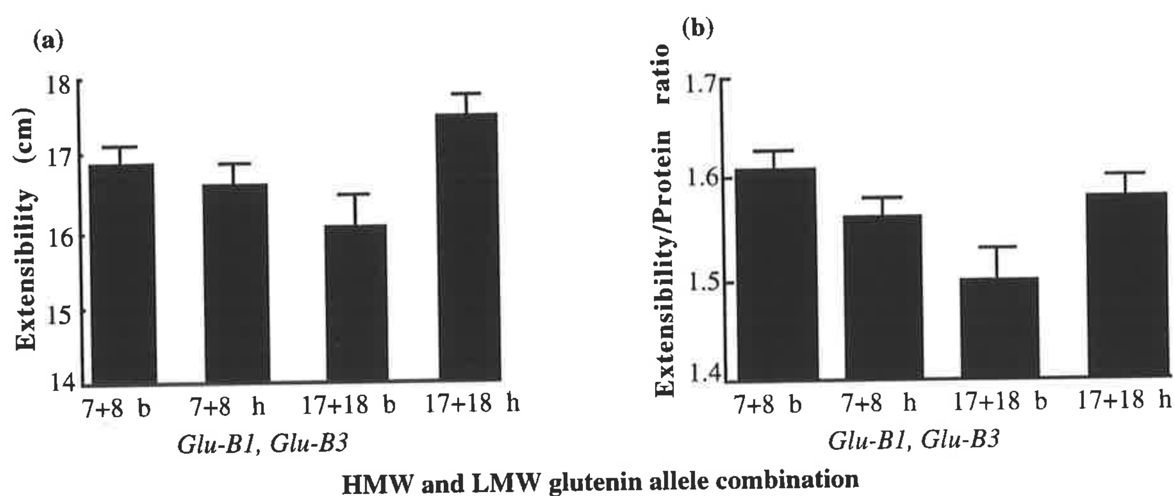


Figure 8.5 Mean **(a)** dough extensibility and **(b)** extensibility/protein ratio for progeny carrying specific combinations of HMW and LMW glutenin alleles. Data from 79 F3-derived F6 lines, obtained from (Barunga x Suneca) and grown as a two-replicate randomised block at Winulta in 1994.

Flour yield and water absorption

With flour yield, the main effects of *Glu-B1* and *Glu-3* alleles were not significant, however the interaction between *Glu-B1* and *Glu-B3* was highly significant ($P<0.001$) (Table 8.7). Comparison of means showed that progeny carrying the subunit bands 17+18 have a significantly higher flour yield than those with bands 7+8 only when the *Glu-B3h* allele was present (1.7% higher than the 7+8, *h* combination) (Fig 8.6), similar to the results obtained for extensibility. These two alleles are present in the Suneca parent which has been found to give more extensible dough and higher flour yield than Barunga (Table 7.1).

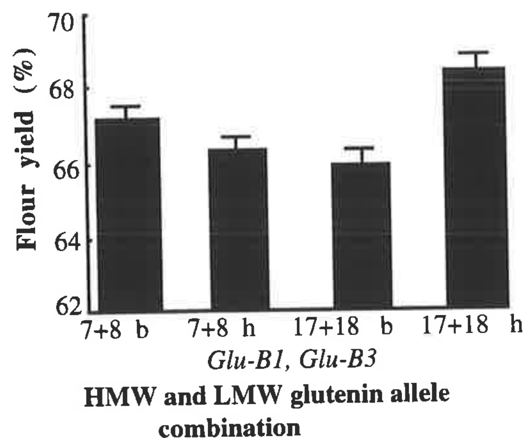


Figure 8.6 Mean flour yield (%) for progeny carrying specific combinations of HMW (*Glu-B1*) and LMW (*Glu-B3*) glutenin alleles. Data from 79 F3-derived F6 lines, obtained from (Barunga x Suneca) and grown as a two-replicate randomised block at Winulta in 1994.

The main effects for water absorption were highly significant for two of the LMW glutenin loci (Table 8.7). As shown in Fig 8.7, progeny carrying the *h* allele at *Glu-B3* had a higher water absorption than those having the *b* allele. Similarly at the *Glu-D3* locus, lines possessing allele *c* gave a higher water absorption than those carrying allele *e*.

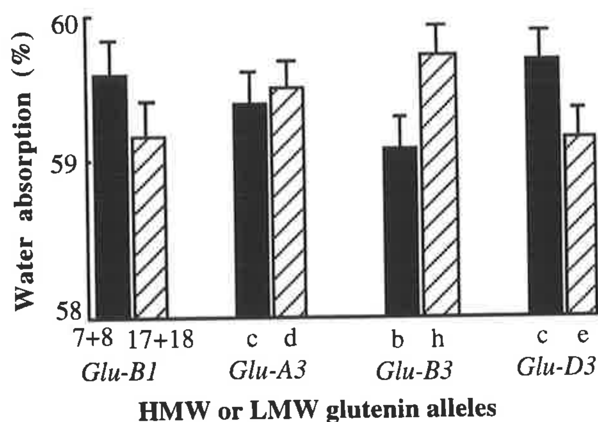


Figure 8.7 Mean water absorption for progeny carrying specific alleles of HMW (*Glu-B1*) and LMW (*Glu-A3*, *Glu-B3* and *Glu-D3*) glutenin loci. Data from 79 F3-derived F6 lines, obtained from (Barunga x Suneca) and grown as a two-replicate randomised block at Winulta in 1994.

Interaction effects between alleles on water absorption were also observed between *Glu-A3* and *Glu-D3* (Fig 8.8). The highest water absorption (60.3%) was observed in the presence of the *d* and *c* alleles at the *Glu-A3* and *Glu-D3* loci respectively.

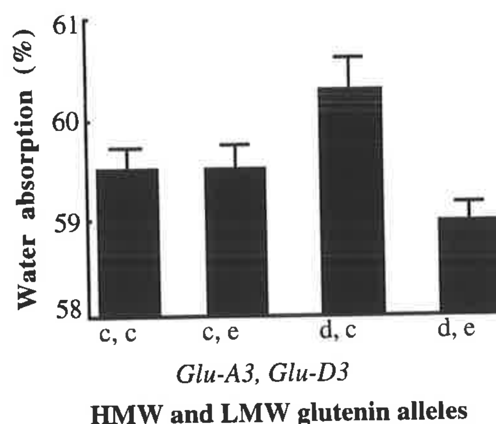


Figure 8.8 Mean water absorption (%) for progeny carrying specific combinations of *Glu-A3* and *Glu-D3* glutenin alleles. Data from 79 F3-derived F6 lines, obtained from (Barunga x Suneca) and grown as a two-replicate randomised block at Winulta in 1994.

Effects of individual alleles on dough properties of 16 genotypes (5 lines each), grown in an unreplicated trial

In this experiment, only Rmax and water absorption showed significant differences between genotypes (Table 8.6). Although there were no significant differences between genotypes for the other quality characteristics through the overestimation of the residual variance, the overall mean of the main effects of each locus of 20 samples (mean of 4 loci over 5 lines) could provide some information about their allelic relationship on these quality parameters (Table 8.9). Moreover this allows a comparison of the results from this experiment with those from the replicated trial and provides an opportunity to look for confirmatory evidence of the effect detected in the unreplicated trial. The same trend even though non-significant would tend to confirm the statistical significance in the other trial. Conversely, where the trend in the non-replicated experiment was in the opposite direction, the significant result would more likely be a result of statistical aberration.

To evaluate the effects of the different alleles on Rmax and water absorption, the lines were grouped for the presence of individual alleles at each locus, and these values were subjected to analysis of variance (Table 8.10). The data showed a highly significant difference between the Rmax values for the alleles at *Glu-A3*. The difference was also significant for alleles at *Glu-B3*. As shown in Fig 8.9, progeny with the *Glu-A3* allele *d* gave a higher Rmax value than progeny with allele *c* (310 BU vs 282 BU). This trend was similar to that reported for the replicated trial at the same site (Fig 8.1a), indicating that the difference between alleles at *Glu-A3* for Rmax was consistent. Likewise progeny possessing the *h* allele at *Glu-B3* gave a higher Rmax than those having the *b* allele (303 BU vs 289 BU).

Table 8.9 Comparison of main effects of glutenin subunit alleles in F_3-derived F_6 lines from (Barunga x Suneca) in two experiments where statistically significant effects on quality and agronomic characters had been established by analyses of variance in one or other on both experiments. The data obtained from experimental material grown as a completely randomised block (unreplicated trial) and a two replicate randomised block (replicated trial) at Winulta in 1994 [(a)].

comparison	characters								
	PC	Rmax	Ext	E/P	WA	FY	SDSS	M	GY
Unreplicated trial									
Glu-B1									
17+18	10.8	-	-	-	-	-	-	-	-
7+8	10.4	-	-	-	-	-	-	-	-
significant level	ns	-	-						
Glu-A3									
d	10.8	310.4	16.8	1.56	58.0	-	63.6	50.8	-
c	10.4	282.8	16.1	1.56	58.9	-	59.1	48.2	-
significant level	ns	**	ns	ns	*	-	**	**	-
Glu-B3									
b	-	289.8	-	-	58.3	-	-	50.1	-
h	-	303.4	-	-	58.7	-	-	48.9	-
significant level		*			ns			ns	
Glu-D3									
c	-	-	-	-	58.6	-	-	-	-
e	-	-	-	-	58.3	-	-	-	-
significant level	-	-	-	-	ns				
Replicated trial									
Glu-B1									
17+18	11.0	-	-	-	-	-	-	-	-
7+8	10.6	-	-	-	-	-	-	-	-
significant level	**	-	-	-	-	-	-	-	-
Glu-A3									
d	11.0	293.9	17.7	1.61	59.5	-	70.7	47.4	-
c	10.5	282.5	16.1	1.54	59.5	-	63.2	46.4	-
significant level	**	**	**	**	ns	-	**	ns	-
Glu-B3									
b	-	286.6	-	-	59.2	-	-	46.1	-
h	-	287.8	-	-	59.8	-	-	47.4	-
significant level	-	ns	-	-	**	-	-	**	
Glu-D3									
c	-	-	-	-	59.8	-	-	-	-
e	-	-	-	-	59.2	-	-	-	-
significant level	-	-	-	-	*	-	-	-	-

a=see abbreviation list

Table 8.10 Mean squares from an analysis of variance for quality and agronomic characters of 80 F_3-derived F_6 families from (Barunga x Suneca) grown in a completely randomised block (unreplicated trial) at Winulta in 1994 [a].

Source of variation	d.f	quality characters							agronomic characters	
		PC	Rmax	Ext	E/P	WA	FY	SDSS	M	GY
Glu-B1	1	3.3 ns	49.6 ns	12.2 ns	0.01 ns	1.8 ns	19.9 ns	0.8 ns	1.8	29684.0 ns
Glu-A3	1	3.2 ns	15262.8 **	9.1 ns	0.00 ns	4.1*	33.4 ns	414.0 **	130.0 **	20193.0 ns
Glu-B3	1	0.2 ns	3685.6 *	0.1 ns	0.00 ns	0.7 ns	33.9 ns	16.2 ns	31.2 ns	32361.0 ns
Glu-D3	1	0.3 ns	40.6 ns	10.4 ns	0.04 ns	0.4 ns	1.0 ns	80.0 ns	18.0 ns	8841.0 ns
Glu-B1 x Glu-A3	1	0.0 ns	127.5 ns	0.0 ns	0.00 ns	0.1 ns	2.8 ns	0.4 ns	6.0 ns	5040.0 ns
Glu-B1 x Glu-B3	1	1.6 ns	103.5 ns	4.2 ns	0.00 ns	3.2*	8.4 ns	33.8 ns	42.0 ns	44.0 ns
Glu-A3 x Glu-B3	1	4.6 **	775.0 ns	2.7 ns	0.02 ns	2.7*	20.1 ns	1.2 ns	7.2 ns	1505.0 ns
Glu-B1 x Glu-D3	1	0.1 ns	248.5 ns	0.3 ns	0.00 ns	0.0 ns	35.8 ns	39.2 ns	8.4 ns	300.0 ns
Glu-A3 x Glu-D3	1	1.2 ns	324.0 ns	1.4 ns	0.10 ns	0.3 ns	22.2 ns	14.4 ns	39.2 ns	2965.0 ns
Glu-B3 x Glu-D3	1	0.6 ns	456.0 ns	0.4 ns	0.00 ns	0.8 ns	80.2 ns	5.0 ns	16.2 ns	78.0 ns
Residual	69	1.0	819.0	4.5	0.02	0.7	50.3	33.0	17.0	8204.0 ns
Total	79	1.0	982.6	4.5	0.02	0.7	47.2	36.5	16.7	8444.0 ns

a= see abbreviation list

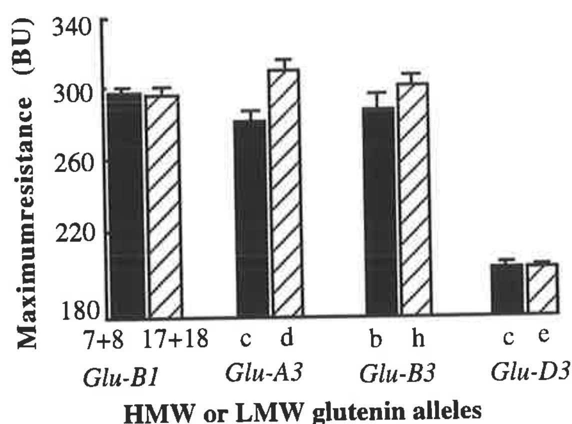


Figure 8.9 Mean maximum dough resistance for progeny carrying specific alleles of HMW (*Glu-B1*) and LMW (*Glu-A3*, *Glu-B3* and *Glu-D3*) glutenin loci. Data from 80 F_3-derived F_6 lines (five lines within each genotype) obtained from (Barunga x Suneca) and grown in a completely randomised block at Winulta in 1994.

The *c* allele at *Glu-A3* gave a significantly higher water absorption than the *d* allele (Fig 8.10), whereas in the replicated trial, the trend was in the opposite direction. By contrast, although there were no significant differences between alleles at *Glu-B3* and *Glu-D3* for water absorption in the unreplicated trial, these loci had the same trends and significantly different water absorption values in the replicated trial (Fig 8.7), confirming the statistical conclusions of a significant effect.

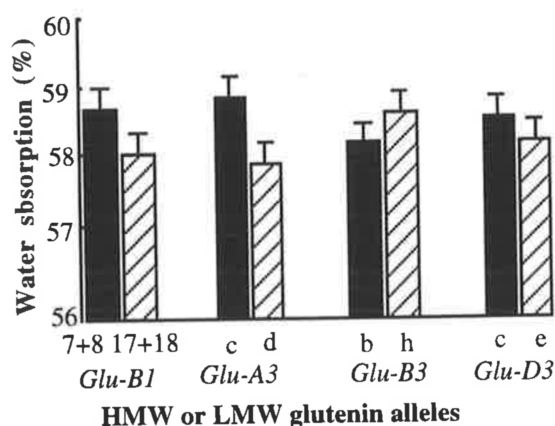


Figure 8.10 Mean water absorption (%) for progeny carrying specific alleles of HMW (*Glu-B1*) and LMW (*Glu-A3*, *Glu-B3* and *Glu-D3*) glutenin loci. Data from 80 F_3-derived F_6 lines (five lines within each genotype) obtained from (Barunga x Suneca) and grown in a completely randomised block at Winulta in 1994.

With the other quality parameters, the main effect of the allelic differences at *Glu-A3* on SDS-sedimentation were quite pronounced, but the overall effect of the genotypes was not significant in the analysis of variance. The trend of the allelic effects for this quality parameter was similar to those reported for Rmax. The progeny possessing allele *d* gave a higher SDSS volume than those carrying allele *c* (63 ml vs 59 ml) (Fig 8.11), in agreement with the results reported in the replicated trial.

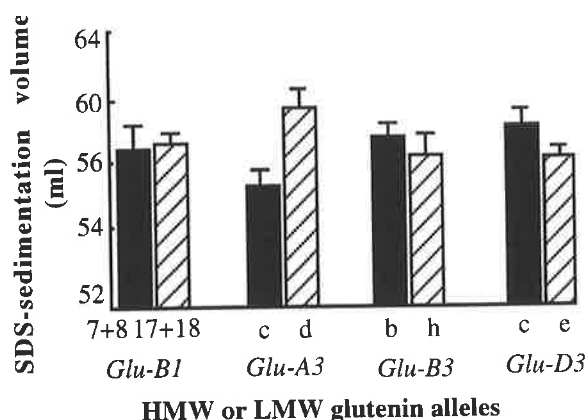


Figure 8.11 Mean SDS sedimentation volume for progeny carrying specific alleles of HMW (*Glu-B1*) and LMW (*Glu-A3*, *Glu-B3* and *Glu-D3*) glutenin loci. Data from 80 F3-derived F6 lines (five lines within each genotype) obtained from (Barunga x Suneca) and grown as a completely randomised block at Winulta in 1994.

Interactions between allelic effects on dough properties of the 16 genotypes (unreplicated trial)

The effects of the alleles at the different glutenin loci on Rmax were additive (Table 8.10). As shown in Table 8.3 the highest Rmax was observed with genotypes carrying the 7+8 *d h c* alleles (324 BU). This genotype also showed the highest SDSS volume (67 ml). This was consistent with the individual effect of each allele, because genotypes carrying the alleles *d* and *h* at *Glu-B3* and *Glu-D3* had a greater Rmax than their counterpart alleles.

The effects of individual alleles on water absorption were mostly additive. However two interacting loci were noted, involving *Glu-B1 x Glu-B3* and *Glu-A3 x Glu-B3* (Table 8.10). The *Glu-B1* allele 7+8 in the presence of *Glu-B3* allele *b*, gave the highest value for water absorption (59%) (Fig 8.12a). The *Glu-A3c* allele in the presence of allele *Glu-B3* allele *h* gave a higher water absorption than the *d* and *b* combination (59.5%) (Fig 8.12b).

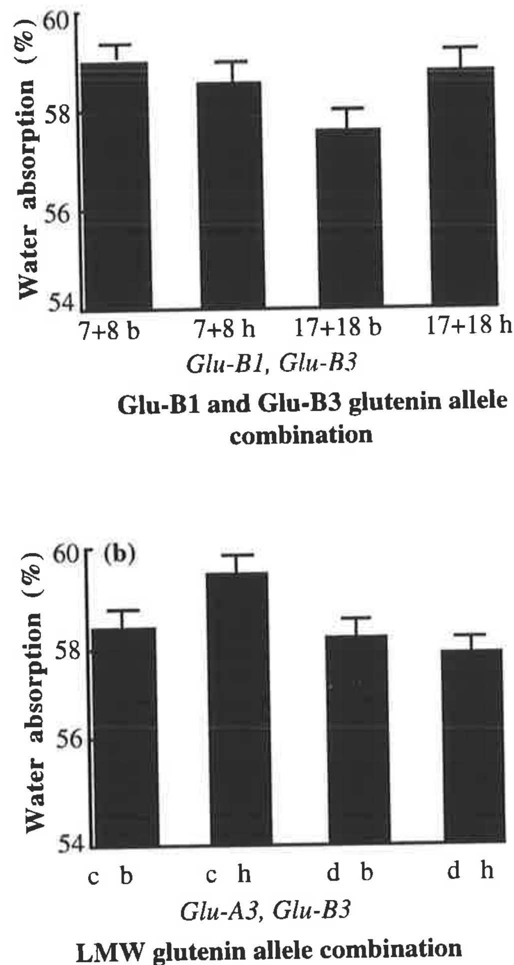


Figure 8.12 Mean water absorption for progeny carrying specific alleles **(a)** of *Glu-B1* and *Glu-B3* or **(b)** *Glu-A3* and *Glu-B3* glutenin loci. Data from 80 F3-derived F6 lines (five lines within each genotype), obtained from (Barunga x Suneca) and grown as a completely randomised block at Winulta in 1994.

Maturity

The effect of glutenin subunits on maturity were only significant for the *Glu-B3* locus in the replicated trial (Table 8.7) and *Glu-A3* for the unreplicated trial (Table 8.10), indicating possible linkage between these LMW glutenin loci and genes controlling maturity. This was opposite with the results from the previous study (Chapter 4, section 4.3.5) which showed that maturity and glutenin subunits are independent attributes. Lines with the *Glu-A3d* allele had earlier maturity than those with *Glu-A3c* (Fig 8.13). Difference in maturity were also observed with the alleles at the *Glu-B3* locus in the replicated trial, where lines with *Glu-B3h* tended to have earlier maturity. The opposite trend was observed in the unreplicated trial, suggesting that the effect in the replicated trial was due to experimental variation

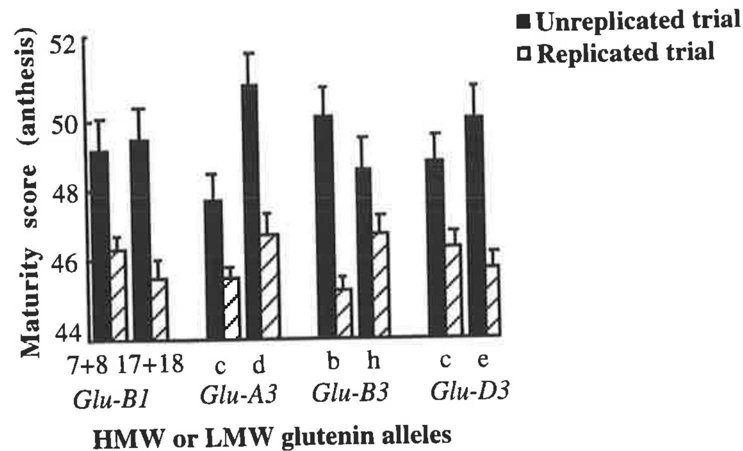
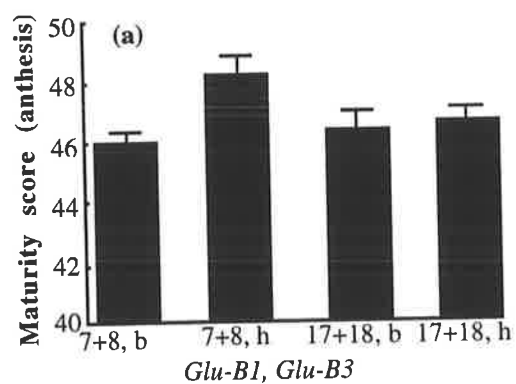
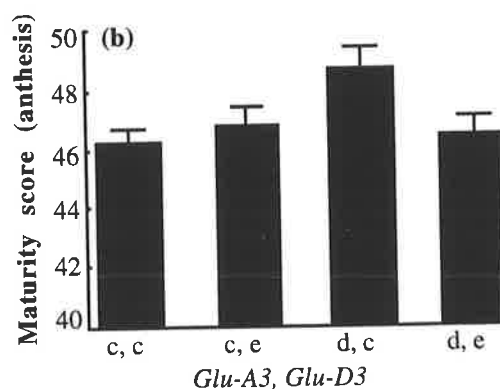


Figure 8.13 Mean maturity score for progeny carrying specific alleles for HMW (*Glu-B1*) and LMW (*Glu-A3*, *Glu-B3* and *Glu-D3*) glutenin loci. Data obtained from unreplicated and replicated trials conducted at Winulta in 1994.

Three significant interactions were also noted for the maturity of the lines in the replicated trial. An interaction was observed between the non-homeologous loci *Glu-B1* and *Glu-B3*. Lines possessing the *Glu-B1* allele 7+8 in the presence of *Glu-B3h* showed the earliest maturity (score=48.2) (Fig 8.14a). A second interaction was observed between alleles at *Glu-A3* and *Glu-D3*. The earliest maturity occurred with progeny carrying the allelic combination of *d* and *c* at these loci (score=48.6) (Fig 8.14b). A third interaction was observed between the *Glu-B3* and *Glu-D3* loci. The combination of *h* and *c* allele showed the earliest maturity (score=57.6) in the RILs (Fig 8.14 c). It is notable that none of these interactions were significant for the unreplicated trial. The trend of these interactions with those of unreplicated trial showed that combination of *d* and *c* at *Glu-A3* and *Glu-D3* was only the same and for the two other interaction the trends was in the opposite direction.





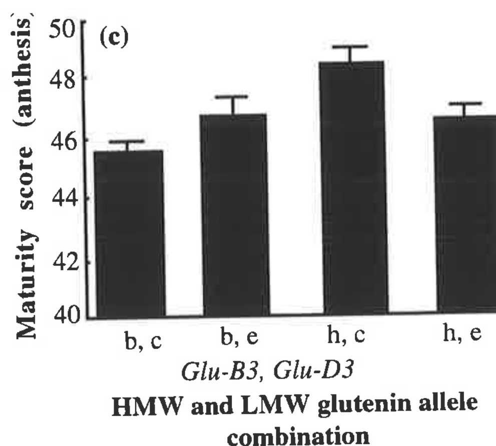


Figure 8.14 Mean maturity score for progeny carrying specific combinations of **(a)** *Glu-B1 x Glu-B3* **(b)** *Glu-A3 x Glu-D3* and **(c)** *Glu-B3 x Glu-D3*. alleles. Data from 79 F3-derived F6 lines, obtained from (Barunga x Suneca) and grown as a two-replicate randomised block at Winulta in 1994.

8.3.4 Relationship between quality parameters and agronomic traits

The associations between the six quality characteristic and two agronomic traits were calculated using simple correlation for both experiments (Table 8.11).

Table 8.11 Correlation matrix between quality parameters and agronomic traits for F3-derived F6 lines obtained from (Suneca x Barunga) and grown as unreplicated and replicated trials at Winulta in 1994.

Quality parameters	agronomic traits	
	maturity	grain yield
Unreplicated trial		
extensibility (cm)	0.40 *(a)	-0.18
protein concentration (%)	0.36 *	-0.41*
extensibility/protein ratio	0.16	0.15
maximum resistance (BU)	0.04	-0.05
water absorption (%)	-0.01	-0.13
flour yield (%)	0.21	-0.04
SDS-sedimentation volume (ml)	0.47 *	-0.18
Replicated trial		
extensibility (cm)	0.20 *	-0.16 *
protein concentration (%)	0.30 *	-0.43 *
extensibility/protein ratio	-0.03	0.20 *
maximum resistance (BU)	0.15	-0.18 *
water absorption (%)	0.24 *	0.18 *
flour yield (%)	-0.14	-0.13
SDS-sedimentation volume (ml)	0.23 *	-0.14

a=see abbreviation list

There were significant correlations between maturity and three major quality parameters ie. extensibility, protein concentration and SDS-sedimentation for both experiments. Since maturity and protein concentration were positively correlated, the positive correlation between maturity and extensibility was not unexpected. In the drought conditions prevailing in 1994, it was not surprising that the later maturity lines had lower grain yields but it was not expected that the early maturity lines would have a higher protein concentration and hence greater dough extensibility.

The positive correlation between maturity and SDS-sedimentation volume indicates that the earlier lines produce higher sedimentation values than those with late maturity, as

reported by Schlehuber *et al.* (1967). The only significant correlation between maturity and water absorption was observed in the unreplicated trial, and it seems that this relationship is mainly due to the linear relationship between protein concentration and water absorption as reported by Finney (1979).

8.4 Discussion

In general, the relationship between glutenin subunits and breadmaking quality is very dependent on the type of genetic material used for such studies. For example, by using different cultivars or breeding lines it is quite possible that alleles at each of the six loci controlling glutenin subunits (*Glu-1* and *Glu-3* loci) may not show the same effects as with RILs because pleiotropy or genetic linkage or common ancestory can affect the association between glutenin subunits and breadmaking quality. The RILs provide an opportunity to look at the causal relationship more closely than is possible with a cultivar set, due to a more random background variation in RILs compared to likelyhood of common ancestry among cultivars with the same genotype and the higher level of replication of lines within genotypes which can be achieved with the RILs. This permits the estimation of genetic effects of particular alleles in several lines and, if linkage and epistatic effects are of limited importance, a measurement of the direct effects of major loci of glutenin subunits of bread wheat. At the same time, examination of many different lines (isolates) with the same allelic composition for glutenin subunits can indicate whether loci other than the glutenins influence the quality characteristics. In the present study it was found that the significant within genotype between line differences occurred for all quality parameters investigated (Table 8.4).

It is generally recognised that breadmaking quality is determined not only by the properties of the gluten proteins but also other protein fractions (gliadins-albumins-globulins) and non-protein factors (lipids, starch). Aggregating HMW albumins (Gupta and Shepherd, 1987) and the globulins or triticins (Singh and Shepherd, 1988) present in the glutenin complex may have some influence on the functionality of glutenin and thus on flour quality (MacRitchie, 1987). Also it has been reported that changes in lipids (Chung *et al.*, 1982) and carbohydrates (McMaster and Bushuk, 1983) may affect the bread making quality. This occurred here as seen in the high percentage of the total variance which was accounted for by lines within genotypes. The low percentage of variance in Rmax associated with genotypes was somewhat surprising (Table 8.5). In a previous study using random selected lines (Chapter 6, Table 6.3), it was observed a high percentage of the variance for Rmax was accounted for by genotypes (glutenin subunits). Therefore it appears that the background genotype can appreciably affect the Rmax and this is particularly notable here as the glutenin alleles under investigation did not have particularly large effects on Rmax (e.g. 310 and 282 BU for the allele *d* and *c* at *Glu-A3* respectively).

Data from both experiments (Tables 8.7 and 8.9) generally showed that the *Glu-A3* alleles had a major influence on dough properties. The subunit coded by the *d* allele was associated with increased dough strength (as measured by Rmax and estimated by SDSS)

(Table 8.9), and higher grain protein, which in turn affected the extensibility of dough and consequently higher extensibility/protein ratio as significantly observed in the replicated trial (Table 8.8) and reported by several other researchers (Gupta *et al.*, 1991; Singh *et al.*, 1991a). Further, it appears that the *d* allele was associated with a higher E/P ratio, suggesting that the inherant extensibility of the glutenin subunit coded by this allele are greater than those from the *c* allele.

Although the *Glu-B3* locus is known to be responsible for the largest number of LMW subunits amongst Glu-3 loci (MacRitchie, 1992; Cornish, 1994) and it has been advised to select for LMW glutenin alleles with the largest number of bands (Cornish, 1994). However the *Glu-B3* locus had no significant effect on maximum resistance and extensibility (Tables 8.7 and 8.9). The visual observation showed that *Glu-B3b* coded an extra band compared with *Glu-B3c* in the B subunit region (Chapter 3, Fig 3.3). In contrast, Cornish (1994) reported that the *h* allele at the *Glu-B3* locus had one extra band than *b*. It seems that the actual number of bands visualised for the LMW glutenin subunits, particularly at the *Glu-B3* locus, depends upon the method of fractionation and that this may invalidate any extrapolation from band number to relative extensibility.

Although several researchers have shown that the HMW glutenin subunits loci generally make a substantial contribution to dough strength (as measured by Rmax and SDSS), in the present study no significant difference was observed between the alleles at *Glu-B1* (17+18, 7+8) (Table 8.7). Lagudah *et al.* (1988) also found no differences in maximum resistance between F_3-derived lines possessing bands 7+8 or 17+18, whereas Payne *et al.* (1984a) and Branlard and Dardevet (1985b) found a significant positive effect of bands 17+18 on SDSS volume. The 17+18 allele occurs frequently in strong Australian wheats (Wrigley *et al.*, 1982; Lawrence, 1986) and has been shown to be related to high SDSS volume. Metakovsky *et al.* (1990) also reported that HMW glutenin bands 17+18 of the B genome was strongly related to high Rmax and the 7+8 bands to a low Rmax. However Gupta *et al.* (1991) reported that the effect of these two alleles 17+18 and 7+8 on Rmax were equal in the Australian wheat set. In the present study all the RILs had the strong 5+10 subunits in their background and this may have prevented differences between alleles 17+18 and 7+8 at *Glu-B1* being expressed. Alternatively, the differences between 17+18 and 7+8 are influenced by unidentified environmental factors which were not present at Winulta where the grain analysed here was grown.

Although allelic variation at the *Glu-B1* locus had a smaller main effect on gluten quality than variation at *Glu-A3*, its interaction effect on Rmax was quite large. Genotypes containing 7+8 at *Glu-B1* and the *d* allele at *Glu-A3* gave greater strength than the other combinations indicating that HMW glutenin and LMW glutenin subunits together explain portion of the variation for quality and furthermore this indicated that the effects of

glutenin subunits on dough properties were not totally additive. As reported by Lafiandra (1993), the LMW subunits encoded at the *Glu-D3* can interact with other subunits and reduce the size of glutenin polymers which, in turn, result in a reduction in the amount of insoluble aggregated protein and hence lower SDS-sedimentation. This interaction effect was clearly seen between *Glu-B1 x Glu-D3* and *Glu-B3 x Glu-D3* in the analysis of SDS-sedimentation volume (Fig 8.4). Therefore the relative importance of interaction effects found here and reported by other researchers (Gupta *et al.*, 1994a; Carrillo *et al.*, 1990) suggest that these should be included in predictive models (Chapter 5, section 5.3.2) when breeding for breadmaking quality. When establishing the contribution of an allele for quality, one should take into account interactions with alleles at other loci which affect the phenotypic expression of the allele being considered.

The significant effects of allelic differences at the *Glu-A3* and *Glu-B3* on maturity could also affect quality. *Glu-A3d* was associated with early maturity (high score) and showed higher dough strength and extensibility than the counterpart allele particularly in the replicated trial. There was also a large difference in grain protein concentration (Fig 8.2) with the progeny with allele *d* having earlier maturity and higher grain protein concentration which resulted in improved breadmaking quality. However, the association between early maturity and high protein was highly unexpected under drought conditions.

From the viewpoint of wheat improvement, it would be an advantage if the correlation between yield and quality characteristics were low and statistically non-significant rather than negative. In the study here it was observed that correlations between grain yield and quality characteristics were mostly negative and low (Table 8.11). The negatively significant correlation between grain yield and quality characteristic for this group of RILs suggested that simultaneous improvement would be difficult. Amongst these quality parameters, the highest correlations were between grain yield and protein concentration for both sets of data. Although the negative correlations could have been due to the drought season, it is not uncommon for high grain yields to be associated with low protein through dilution of the protein through greater starch production.

This study showed that the qualitative composition of protein subunits within HMW or LMW group can determine rheological properties of dough. However, it does not show how they interact with each other in the manifestation of dough strength. The study described in the next chapter was designed to find some relationship between biochemical aspects of quality and the glutenin subunits.

As the main point of this study, it was observed that lines within genotypes were a source of variation for most of the quality parameters. The comparison of two parents used in this study showed that both of them have relatively good dough properties (Tables 7.1, 8.2 and

8.3), indicating that they had been efficiently selected for glutenin band patterns conferring high strength in the plant breeding programs. Moreover comparison between those lines having similar band patterns with their parents showed that progeny having Suneca pattern had higher extensibility and E/P ratio than those possessing Barunga pattern (Tables 8.2 and 8.3), suggesting that it would be advantageous to change from the *Glu-A3c* to *Glu-A3d* to improve Barunga. Variation between lines within genotypes for quality parameters was most likely due to the genes at other than the glutenin loci, and that selection within segregating populations for suitable enduse qualities using the conventional large scale tests needs to be undertaken as well.

CHAPTER 9

BIOCHEMICAL EFFECTS OF ALLELIC VARIATION OF HMW AND LMW GLUTENIN ALLELES IN THE RILs FROM (BARUNGA X SUNECA)

9.1 Introduction

Glutenin and gliadins are the major storage proteins of the wheat endosperm. The glutenin polymers consist of HMW and LMW glutenin subunits linked via cysteine residues (Moonen *et al.*, 1985; Kasarda, 1989). The wheat endosperm also contains some albumins and globulin subunits (Gupta and Shepherd, 1990b; Singh *et al.*, 1990). The ability of the HMW glutenin subunits to polymerise differs from that of the LMW glutenin subunits. It has been reported that all HMW glutenin subunits have cysteine residues towards both ends of the molecules, whereas not all the LMW glutenin subunits sequenced have been shown to have a cysteine residue in the N-terminal region (Shewry *et al.*, 1992; Lew *et al.*, 1992). This may affect the conformation (e.g shape or linear size) of the polymers being formed (Kasarda, 1989).

It is well established that the molecular size distribution of polymeric glutenin fraction is an important factor in determining dough characteristics. Orth and Bushuk (1972) were first to find that polymers with the largest molecular weight have the greatest effect on dough strength. Conversely the monomeric fraction, including gliadins, do not form inter-molecular disulphide linkages, have a limited size range (30-80K) and have little effect on the size of proteins. It has been reported that the large aggregates apparently determine resistance to extension, whereas the smaller gliadins contribute plasticity (Wrigley, 1994).

The polymeric structures that are formed by HMW and LMW glutenin subunits, have been shown to have a high correlation with different quality parameters in some Australian wheat sets (MacRitchie *et al.*, 1991; Gupta *et al.*, 1993). Variation in extensibility and maximum resistance can be attributed to changes in the total protein composition and polymeric protein composition. In a study using genetic lines differing in amounts and number of HMW or LMW subunits of glutenin, it has been shown that the HMW subunits have more pronounced effects on Rmax than do the LMW subunits when compared on an equal weight basis (Gupta *et al.*, 1990b). It has been postulated that the size distribution of the polymers can be modified by different glutenin subunits (Popineau *et al.*, 1994). Payne and Corfield (1979) and Bietz and Wall (1980) found that the HMW and LMW subunits of glutenin combine at random to give a continuous array

of different-sized aggregates which make up glutenin. Despite these advances in our understanding of the relationship between dough properties and polymeric protein (glutenin) at the subunit (allelic) or polymer (functional) level, the role of individual glutenin subunits in the formation of polymeric proteins and the resulting effects on functional properties have not been investigated in detail.

In the previous study (Chapter 8, Fig 8.1) it was found that the effect of allele *d* at the *Glu-A3* locus on dough properties was greater than its counterpart allele. Biochemical study of this could provide information which can be used to explain the variation in flour quality. The main aim of this study was to evaluate the relationship between quality parameters and quantities of protein fractions (as measured by SE-HPLC), the glutenins, gliadins and albumins/globulins in relation to different alleles at the *Glu-1* and *Glu-3* loci.

9.2 Materials and methods

9.2.1 Plant materials

F_3-derived F_6 progeny

A set of F_3-derived F_6 progeny selected from cross between Suneca and Barunga segregating for one locus coding for HMW (*Glu-B1*) and all loci for LMW glutenin subunits (*Glu-A3, Glu-B3* and *Glu-D3*), grown at Winulta in 1994 (see Chapters 6 and 8 for more details), was analysed. Six Australian hard wheat varieties Aroona, Yarralinka, Warigal, BT-Schomburgk, Barunga and Angas were used here for a comparative analysis with the results of RILs in a manner similar to those reported by Gupta *et al.* (1993). The glutenin subunits and dough properties of the derived progeny and varieties have been described earlier (Tables 8.2 and 8.3).

9.2.2 Field experiment

The two experimental designs in previous study (Chapter 8, Material and methods) were also used in this study. In brief, 16 different genotypes (based on the band patterns) with five lines within each genotypes (a total of 80 lines) were grown as a randomised complete block (unreplicated trial) and the remaining lines (79 out of 159) in a two-replicate randomised block (replicated trial). These latter lines representative only 14 of the 16 different genotypes, mainly because there was insufficient seed for other two genotypes to be sown in this experiment (Chapter 7, Fig 7.4). Both these experiments was grown at several sites, however only one site (Winulta) was chosen for protein

composition analysis, mainly because this site had previously been considered for quality evaluation.

9.2.3 Protein extraction and SE-HPLC analysis

Protein composition in terms of proportions of the three main groups of proteins (polymeric, gliadins, and albumins-globulins) was analysed using size-exclusion high-performance liquid chromatography (SE-HPLC) (Batey *et al.*, 1991).

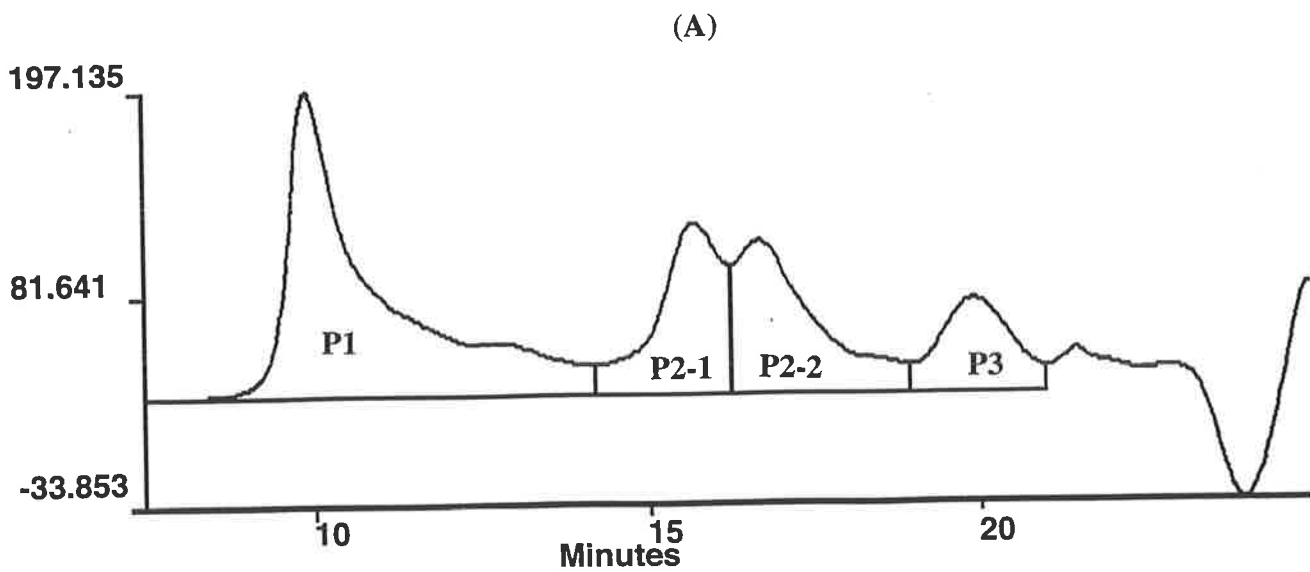
Ten milligrams of white flour, produced on a Brabender Quadrumat Junior mill (Chapter 8, Materials and Methods), was extracted to determine the total endosperm proteins. The extraction was performed using 1 ml SDS phosphorous buffer (50 mM Na_2HPO_4 and 50 mM NaH_2PO_4 in 2% SDS solution, pH 6.8) by 30 sec sonication (Branson Sonifier model B-12 cell disrupter, Branson Sonic Power Company, Danburg, CT) at power setting 5 (output 10 W). Samples were then centrifuged at 12000 RPM for 15 minutes. The supernatant was filtered through a 0.45 μm (Millex-HV13) millipore and placed in a WISP 712 automatic sampler. Acetonitrile water (1:1) mixture with 0.1% (v/v) trifluoroacetic acid was used as the eluent. This gave much improved resolution of peaks and also a much longer column life (Batey *et al.*, 1991) than the previous method described by Singh *et al.* (1990).

The sequence of the SE-HPLC program was set using the Waters Software Package in a Digital Professional 350 Computer. The Waters HPLC system (Waters WISP 710B liquid chromatography) comprises two 510 model pumps, a WISP 712 automatic sampler and a Lambda-Max Model 481 UV-visible detector. Pump control and data acquisition were achieved with a Digital Professional 350 Computer.

The 20μl of each sample, containing total unreduced proteins, was injected by the automatic sampler and separated on a protein Pack 300 Column (Waters Protein Pak^{TM} 200 SW Size-exclusion analytical column 7.5 x 300mm). The columns were eluted isocratically by elution buffer at the flow rate of 0.5 ml/min at 26°C (for a total 40 minutes for each sample).

The elution profile resulting from the SE-HPLC was divided into three main peaks corresponding to polymeric protein, gliadins and albumins/globulins, respectively. These major peaks were eluted between 9 and 21 min as detected by the spectrophotometer at a wavelength of 214 nm (Fig 9.1). Peak 1 (P1) containing mainly glutenin and only a small proportion of aggregated albumins and globulins (Batey *et al.*, 1991), was considered as glutenin and in this study referred to as polymeric protein.

(A)



(B)

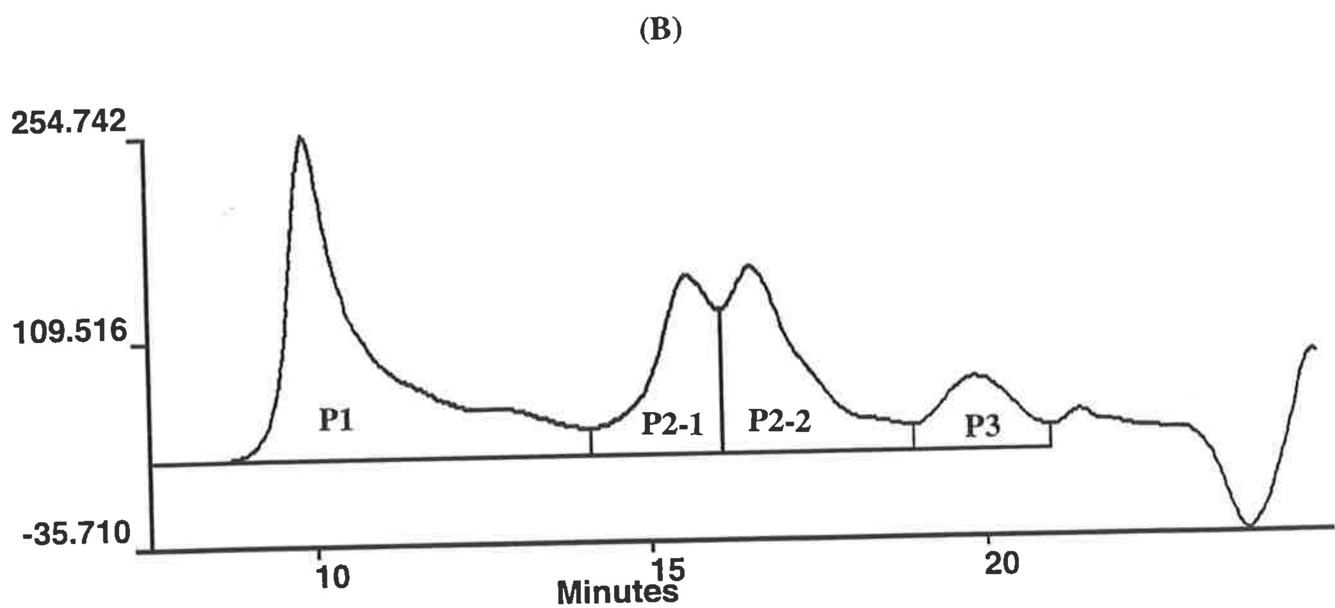


Figure 9.1 The size exclusion high performance liquid chromatography (SE-HPLC) traces of **(A)** Barunga and **(B)** Yarralinka with their respective expanded traces.

The symbols used in this Figure are peak area 1 (P1), peak area 2 (P2) and Peak area 3 (P3). It is notable that the gliadin fraction can be separated into two subfractions (P2-1 and P2-2).

Peak 2 (P2), consisting of gliadin, could be divided into two adjacent peaks, corresponding to the high chromatogram, using the Waters Software Package. The lowest points on the SE-HPLC curves (valleys) were used as cut-off points for calculating areas under the individual peaks (Fig 9.1). In the study here, the percentage of each protein fraction of the total protein was calculated and therefore the terms %P1, %P2-1, %P2-2 and %P3 were used to represent these.

The percentage of each protein fractions in flour can be related to dough properties as reported by Gupta *et al.* (1992), assuming a close to complete extraction of protein, as has been reported by a few studies (Singh *et al.*, 1990; Batey *et al.*, 1991). The percentage of each fractions in the flour was also calculated as follows:
percentage of glutenin in flour (Glu%Fl)=(percentage of protein in flour x %P1)/100
percentage of gliadin in flour (Gli%Fl)=(percentage of protein in flour x %P2)/100
percentage of albumin-globulins in flour (Albg%Fl) =(percentage of protein in flour x %P3)/100

Studies have shown that the ratio of polymeric (mainly %P1) to monomeric (Mo=%P2+%P3) (Gupta *et al.*, 1992) and also glutenin (%P1) to gliadin (%P2) (Blumenthal *et al.*, 1991) can be used as an indication of dough strength, therefore these ratios were calculated. In study here these ratios were shown as P/M and P1/P2, respectively.

9.2.4 Statistical analysis

The statistical analysis were similar to those reported previously (Chapter 8, Materials and Methods). In both experiments individual and combinations of alleles were subjected to an analysis of variance. In brief, for the unreplicated trial, lines within genotypes were treated as the residual mean square, whereas for the replicated trial, blocks x lines within genotypes were used as the residual mean square. The amount of variation accounted for by genotypes and lines was also calculated for different protein fraction measurements using the variance components. Simple and multiple linear regressions were also calculated in efforts to determine the basic measurements necessary to identify wheat quality. With respect to the six varieties grown with the RILs, the data for both experiments were pooled and analysed as a randomised complete block design with nine replications.

9.3 Results

9.3.1 Variation of protein composition and protein content with rheological quality

In the first step of the analysis, the different genotypes were subjected to an analysis of variance (Table 9.1). The protein concentration, extensibility and maximum resistance in the replicated trial were all highly significantly different between the genotypes, whereas for the unreplicated trial the only significant difference was observed for Rmax. With the SE-HPLC data, differences between genotypes were significant for most of the parameters for the replicated trial. However for the unreplicated trial, it was observed that the sub-fractions of gliadin (%P2-1 and %P2-2) were significantly different between genotypes. It is notable that flour glutenin (Glu%Fl) was significantly affected by the genotypes for both experiments. Since the number of lines within each genotype were different for the replicated trial, it was impossible to analyse lines within genotypes together with genotypes, therefore an analysis of variance was performed separately on the total of 79 lines attributed to 14 different genotypes (Table 9.1). The analysis of variance showed that there were highly significant differences between lines for all measurements.

The significant differences for most of the quality and quantity parameters for the replicated trial shows that the differences between the parameters were more evident in a two-replicate randomised block rather than in the unreplicated trial. This is mainly due to genetic variation in the genetic backgrounds of the glutenin genotypes which could not be partitioned through the lack of replication of the individual lines within genotypes, affecting the characteristics protein concentration and dough properties.

The nature of the replicated trial provided an opportunity to evaluate the amount of variation for protein composition accounted for by genotypes and lines, similar to that reported for quality parameters (Chapter 8, section 8.3.2). The results showed that for all of the protein composition measurements, the percent of variation accounted for by lines was very high, compared to the genotypes (Table 9.2), suggesting that the genes controlling protein composition were often other unidentified genes in the genetic background rather than the glutenin genes. Specific carbohydrates (Huebner and Wall, 1979; Bushuk *et al.*, 1980; 1984; McMaster and Bushuk, 1983) and lipids (Hoseney *et al.*, 1970; MacRitchie, 1977; Pomeranz, 1980; Bekes *et al.*, 1983; Bushuk *et al.*, 1984; Zawistowska *et al.*, 1984) interact with specific proteins of the gluten complex to form large aggregates contributing to gluten rheology.

Table 9.1 Mean squares for the quality parameters and SE-HPLC data of F_3-derived F_6 lines from (Barunga x Suneca), grown as a completely randomised block (unreplicated trial) and a two-replicate randomised block (replicated trial) at Winulta in 1994 [a]

Source of variation	d.f	quality parameters				SE-HPLC data								
		PC	Ext	E/P ratio	Rmax	%P1	%P2-1	%P2-2	%P2	%P3	P1/P2	Mo	P/M	Glu%Fl
Unreplicated trial														
Genotypes	15	1.3 ns	3.6 ns	0.01 ns	1650.8 *	3.1 ns	5.3 ***	4.7 **	4.5 ns	0.6 ns	0.01 ns	3.15 ns	0.004 ns	0.4 *
Residual	64	1.0	4.6	0.02	826.0	2.3	0.7	2.0	2.7	0.5	0.007	2.31	0.003	0.2
Total	79													
Replicated trial														
Genotypes	13	2.4 ***	14.3 ***	0.04 ***	2463.7 ***	4.2 **	15.2 ***	10.0 ***	7.5 ***	1.4 ***	0.015 ***	4.2 **	0.005 **	0.5 ***
Residual (1)	143	0.6	2.7	0.01	806.8	2.2	0.8	1.5	2.2	0.5	0.005	2.2	0.002	0.1
Total	157													
Lines within genotypes	78	1.3 ***	5.6 ***	0.02 ***	1627.4 ***	3.4 ***	3.6 ***	4.1 ***	4.1 ***	0.8 ***	0.009 ***	3.4 ***	0.004 ***	0.27 ***
Residual (2)	78	0.2	1.7	0.01	262.3	1.6	0.5	0.7	1.3	0.3	0.003	1.6	0.002	0.06
Total	157													

a= See abbreviation list
(1) This include the sum of square related to lines within genotypes.
(2) This include the sum of square related to genotypes.

Table 9.2 Percentage of the variance of the SE-HPLC measurements and protein proportions attributable to genotypes and the lines within genotypes from (Barunga x Suneca), grown as a two-replicate randomised block at Winulta in 1994.

Measurements (a)	percentage of variance component		
	genotypes	lines	block †
SE-HPLC measurements			
%P1	6	94	-
%P2-1	40	60	-
%P2-2	27	73	-
%P2	16	84	-
%P3	11	89	-
Protein proportion			
P1/P2	13	87	-
P/M	6	94	-
Glu%Fl	18	82	-
Gli%Fl	13	87	-
Albg%Fl	21	79	-

a=see abbreviation list
† negative variance component, so the value has been excluded when calculating the percentage of variance.

9.3.2 Interrelationship between protein composition, protein content and dough quality

Protein concentration is an important criterion for most aspects of dough properties. However, it has been reported that an increase in protein concentration is accompanied by a disproportionate increase in the gliadin component relative to the other components (Bariana *et al.*, 1993). To evaluate these relationships, the correlation matricies of protein composition, protein concentration and dough properties for both experiments were computed (Table 9.3).

Examination of the correlation matrix of protein composition data and protein concentration (Table 9.3) for both experiments showed that there was a significant negative relationship between flour protein concentration and percentage of glutenin in the protein (%P1) (Fig 9.2a). By contrast there was a positive correlation between flour protein concentration and percentage of gliadin in the protein (%P2) (Fig 9.2b). However their percentage in the flour (i.e Glu%Fl and Gli%Fl) showed a highly positive

Table 9.3 Correlation matrix between quality parameters, SE-HPLC measurements and protein proportions for F_3 derived RILs from (Barunga x Suneca), grown as a completely randomised block (unreplicated trial) and a two-replicate randomised block (replicated trial) at Winulta in 1994 [(a)].

Measurement	Correlation matrix															
	Ext	PC	E/P	Rmax	SDSS	%P1	%P2-1	%P2-2	%P2	%P3	P1/P2	Mo	P/M	Glu%Fl	Gli%Fl	Albg%Fl
Unreplicated trial																
Ext	1.00															
PC	0.66 *	1.00														
E/P	0.69 *	-0.06	1.00													
Rmax	0.17	0.17	0.05	1.00												
SDSS	0.40 *	0.47 *	0.08	0.22	1.00											
%P1	-0.22	-0.27 *	-0.06	0.19	0.01	1.00										
%P2-1	0.18	0.28 *	0.00	0.14	0.01	-0.42 *	1.00									
%P2-2	0.21	0.26 *	0.02	-0.27 *	-0.04	0.67 *	-0.26 *	1.00								
%P2	0.33 *	0.44 *	0.02	-0.15	-0.03	-0.91 *	0.49 *	0.72 *	1.00							
%P3	-0.30 *	-0.48 *	0.07	-0.07	0.03	0.03	0.26 *	-0.28 *	-0.44 *	1.00						
P/P2	-0.28 *	-0.34 *	-0.05	0.17	0.04	0.96 *	-0.45 *	-0.73 *	-0.98 *	0.27 *	1.00					
Mo	0.22	0.27 *	0.06	-0.19	-0.01	-1.00	0.42 *	0.67 *	0.91 *	-0.03	-0.96 *	1.00				
P/M	-0.22	-0.25 *	-0.07	0.20	0.02	1.00	-0.40 *	-0.68 *	-0.91 *	0.04	0.97 *	-1.00	1.00			
Glu%Fl	0.61 *	0.94 *	-0.10	0.25*	0.50 *	0.08	0.13	0.03	0.13	-0.48 *	-0.01	-0.08	0.01	1.00		
Gli%Fl	0.63 *	0.95 *	-0.07	-0.09	0.36 *	-0.53 *	0.40 *	0.45 *	0.70 *	-0.53 *	-0.61 *	0.53 *	-0.51 *	0.79 *	1.00	
Albg%Fl	0.50 *	0.72 *	-0.02	0.14	0.57 *	-0.25 *	0.09	0.05	0.12	0.27 *	-0.14	0.25 *	-0.23 *	0.65 *	0.61 *	1.00
Replicated trial																
Ext	1.00															
PC	0.67 *	1.00														
E/P	0.70 *	-0.07	1.00													
Rmax	0.47 *	0.56 *	0.11	1.00												
SDSS	0.58 *	0.51 *	0.27 *	0.42 *	1.00											
%P1	-0.01	-0.29 *	0.24 *	-0.19 *	-0.11	1.00										
%P2-1	-0.03	0.14	-0.18	0.04	-0.03	-0.42 *	1.00									
%P2-2	0.12	0.33 *	-0.15	0.28 *	0.13	0.67 *	-0.26 *	1.00								
%P2	0.08	0.43 *	-0.29 *	0.29 *	0.09	-0.91 *	0.49 *	0.72 *	1.00							
%P3	-0.15	-0.33 *	0.13	-0.25 *	0.04	0.03	0.26 *	-0.28 *	-0.44 *	1.00						
P/P2	-0.05	-0.37 *	0.27 *	-0.24 *	-0.10	0.96 *	-0.45 *	-0.73 *	-0.98 *	0.27 *	1.00					
Mo	0.01	0.29 *	-0.24 *	0.19 *	0.11	-1.00	0.42 *	0.67 *	0.91 *	-0.03	-0.96 *	1.00				
P/M	-0.02	-0.29 *	0.24 *	-0.19 *	-0.12	1.00	-0.40 *	-0.68 *	-0.91 *	0.04	0.97 *	-1.00	1.00			
Glu%Fl	0.70 *	0.92 *	0.04	0.50 *	0.48 *	0.08	0.13	0.03	0.13	-0.48 *	-0.01	-0.08	0.01	1.00		
Gli%Fl	0.55 *	0.94 *	-0.16	0.55 *	0.43 *	-0.53 *	0.40 *	0.45 *	0.70 *	-0.53 *	-0.61 *	0.53 *	-0.51 *	0.79 *	1.00	
Albg%Fl	0.51 *	0.68 *	0.33 *	0.34 *	0.51 *	-0.25 *	0.09	0.05	0.12	0.27 *	-0.14	0.25 *	-0.23 *	0.65 *	0.61 *	1.00

a=see abbreviation list

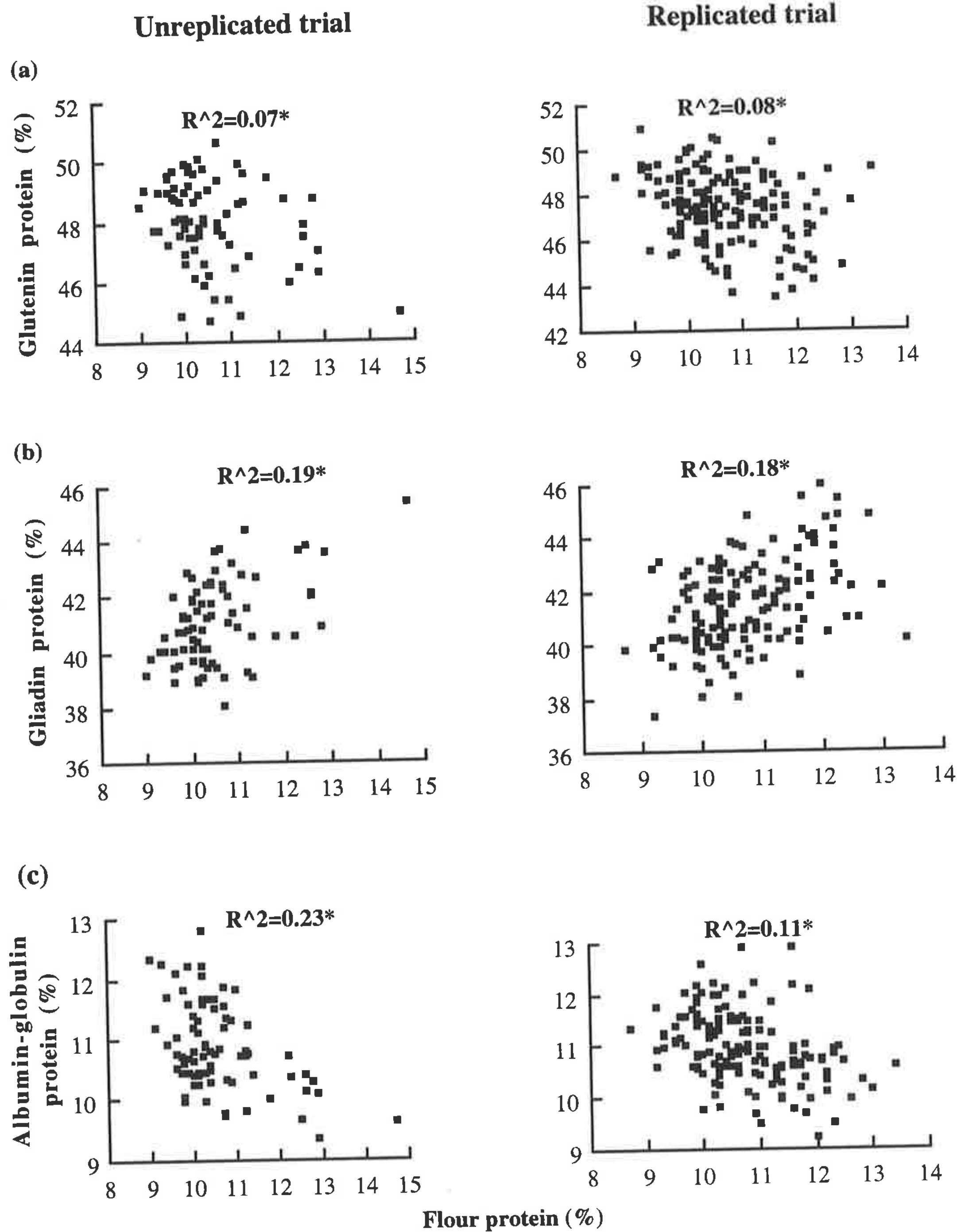


Figure 9.2 Percent of different components of protein in relation to flour protein (**a**); percent glutenin in protein (%P1) (**b**); percent gliadin in protein (%P2) (**c**); percent albumin-globulin in protein (%P3) for F3-derived F6 lines grown as a completely randomised block (unreplicated trial) and a two-replicate randomised block (replicated trial) at Winulta in 1994.

* significant correlation

relationship with flour protein concentration indicating that their total amount increased generally with increasing flour protein concentration. The trend of %P1 and %P2 against flour protein in this study was similar to that described by others (Doekes and Wennekes, 1982; Gupta *et al.,* 1992).

It has been reported that the aggregating high molecular weight albumins (Gupta and Shepherd, 1987) and the globulins or triticins (Singh and Shepherd, 1988) present in the glutenin complex have some influence on the functionality of glutenin and thus on quality (MacRitchie, 1987). In the case of albumin-globulin groups of proteins, the percentage in the protein (%P3) significantly decreased with increasing flour protein concentration (Fig 9.3c), whereas the percentage of this group in the flour (Albg%Fl) showed a highly positively correlation with flour protein (Table 9.3).

9.3.3 Physical dough parameters

Extensograph statistics plotted against percentage of glutenin in protein (%P1) and Glu%Fl for flours from the F_3-derived wheat lines (Fig 9.3), indicated that there was no correlation between extensibility and %P1 for either experiment (Fig 9.3a) but as expected, extensibility was highly correlated with Glu%Fl (Table 9.3). There was a significant correlation between extensibility/protein ratio with %P1 for the replicated trial (r=0.24), however for the unreplicated trial there was no relationship at all (r=-0.06) (Fig 9.3b).

It was also observed that the percentage of gliadin in protein (%P2) was correlated to extensibility in the unreplicated trial. By contrast there was a negative correlation between extensibility-protein ratio and %P2 (r=-0.29) in the replicated trial. However this relationship were not significant for the unreplicated trial (r=0.02). This negative correlation probably indicated that an increase in protein content is accompanied with the disproportionate increase in the gliadin component, resulting in lower extensibility. It is notable that none of the gliadin subfractions (%P2-1 and %P2-2) showed any relationship with extensibility for either trial (Table 9.3).

The percentage of albumin-globulin in protein (%P3) showed a significant negative correlation with extensibility in the unreplicated trial. This was not observed with the E/P ratio for either experiment, mainly because of the negative correlation between protein concentration and %P3 (Fig 9.2c).

The only relationship between extensibility and protein composition was seen in the unreplicated trial, suggesting that protein composition particularly %P1 and %P2 did not described the variation of in extensibility and therefore other loci must have been involved in controlling dough extensibility. The high percent of variation for this quality parameter accounted for by genetic background (Table 8.5) and the high correlation between extensibility and Glu%Fl both indicate that extensibility was not controlled by glutenin subunits within this genetic material.

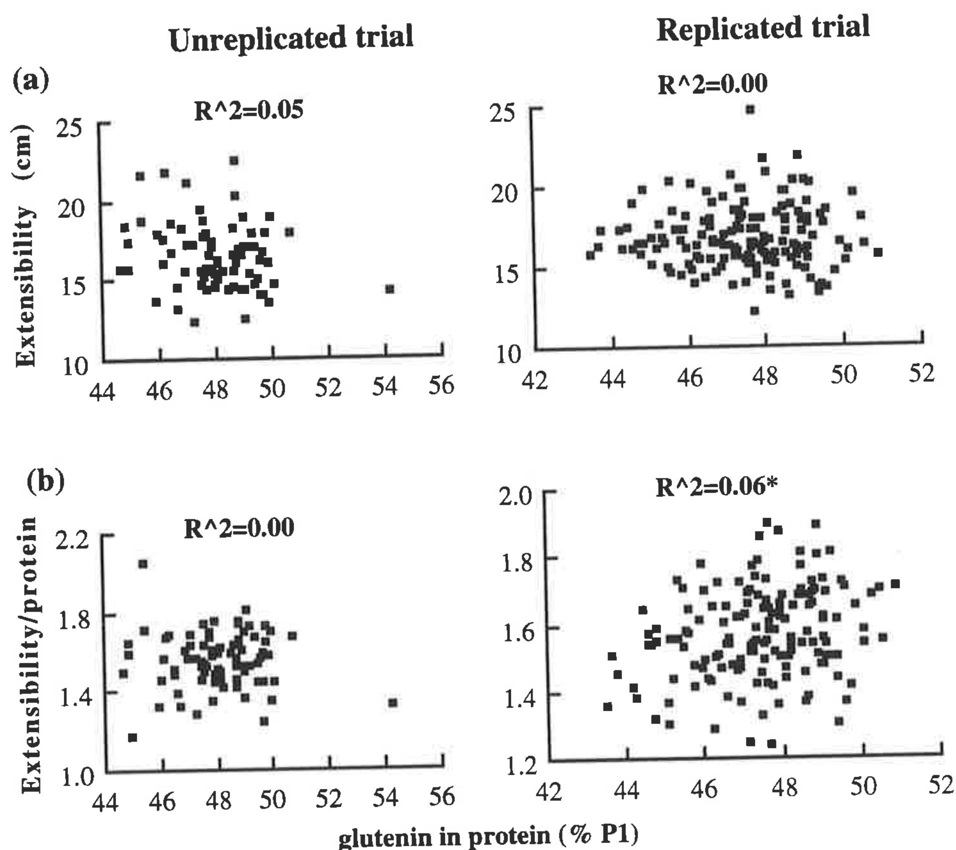


Figure 9.3 Extensograph extensibility **(a)** and extensibility/protein ratio **(b)** as a function of percent glutenin in protein (%P1) for F3-derived F6 lines grown as a completely randomised block (unreplicated trial) and a two-replicate randomised block (replicated trial) at Winulta in 1994.
* significant correlation

With respect to Rmax, there was no significant correlation between Rmax and proportion of protein glutenin (%P1) for the unreplicated trial, and for the replicated trial this correlation was not strong (Table 9.3, Fig 9.4a). Rmax showed a correlation with Glu%Fl, but the r value was lower than that for the dough extensibility-flour glutenin relationship (Table 9.3, Fig 9.4b). This indicated that protein composition or other components such as polysaccharides and lipids rather than component concentrations have a role in dough strength. This can also be seen in the lower relationship between

Rmax and protein concentration (Table 9.3) compared to the extensibility-protein relationship.

The relationships between Rmax and %P2 (including its subfractions) and %P3 were significant. The first subfraction of gliadin (%P2-1) was not significant for either trial, however the second subfraction (%P2-2) showed a significant negative correlation with Rmax. In relation to the total percent of gliadin in protein (%P2), a significant correlation was only observed in the replicated trial. In the case of albumin-globulin percentage in protein (%P3), a negative significant correlation was observed only in the replicated trial.

In the study here there was no relationship between SDSS and the proportions of the different protein fractions (Table 9.3). This quality parameter, however showed highly significant correlation with the Glu%Fl, Gli%Fl and Albg%Fl. This indicates that, in addition to the SDS-insoluble glutenin, other proteins present in the glutenin complex may influence the SDSS volume.

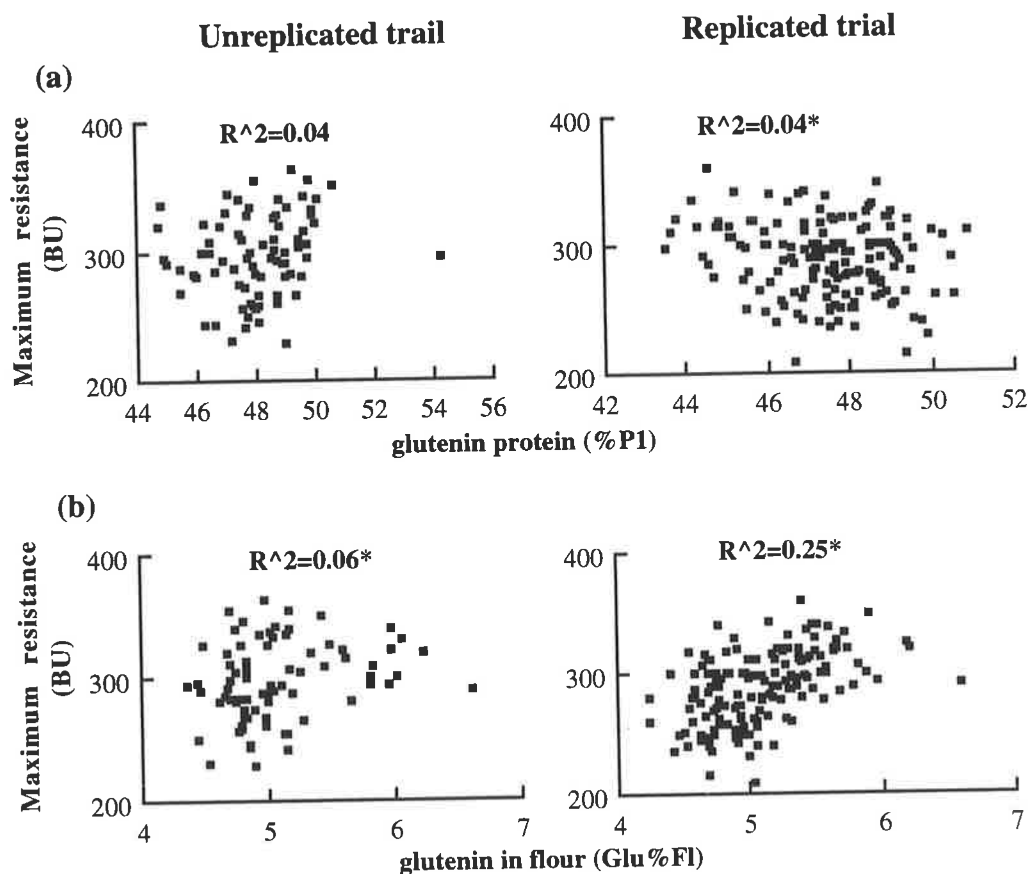


Figure 9.4 Extensograph maximum resistance (Rmax) as a function of **(a)** glutenin in protein (%P1) and **(b)** percent of glutenin in flour for F3 derived F6 lines grown as a completely randomised block (unreplicated trial) and a two-replicate randomised block (replicated trial) at Winulta in 1994.

* significant correlation

9.3.4 Effect of glutenin alleles on relative quantities of protein fractions

The relationship between dough strength and particular components of large protein aggregates have been reported by several researches (Huebner, 1970; Huebner and Wall, 1976; Field *et al.*, 1983; Miflin *et al.*, 1983; Bushuk, 1985; 1987). Therefore the allelic effects of the glutenin subunits were analysed in this section (Table 9.4). For convenience the main effects of glutenin subunits with a significant effect on protein composition have been summarised in Table 9.5.

Individual effects of the glutenin loci on SE-HPLC measurements

Glu-A3 locus

In the previous study it was observed that the alleles at the *Glu-A3* locus were significantly involved with dough properties and that the *d* allele gave a higher Rmax and extensibility than its counterpart allele (Fig 8.1a and Table 8.8). As shown in Table 9.4 the alleles at *Glu-A3* were significantly different for most of the protein parameters analysed for both trials except for peak 3 and protein concentration. The greater effect of the *d* allele at *Glu-A3* on dough properties was accompanied by a greater proportion of higher polymeric protein (%P1) than its counterpart allele for the unreplicated trial (Fig 9.5) showing that the positive effect of this allele was mainly due to its capacity to form large aggregates of proteins. The trend of allelic effect at *Glu-A3* was similar in the replicated trial (Fig 9.5), although the difference between the alleles on polymeric protein (%P1) was not significant.

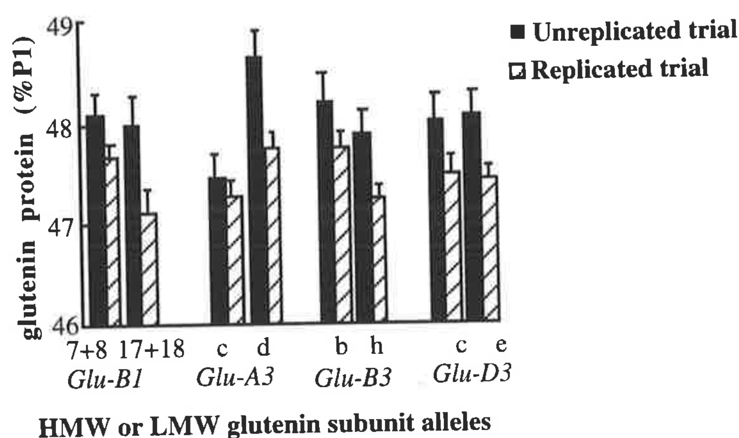


Figure 9.5 The proportion of glutenin protein (%P1) of the F3 derived F6 progeny from (Barunga x Suneca), carrying specific alleles of the HMW (*Glu-B1*) and LMW (*Glu-A3*, *Glu-B3* and *Glu-D3*) glutenin loci. Data from experimental material grown as a completely randomised block (unreplicated trial) and a two-replicate randomised block (replicated trial) at Winulta in 1994.

Table 9.4 Significant main and interaction effects of the glutenin loci on relative quantities of the protein fractions (as measured by SE-HPLC) for F3-derived F6 lines grown as a completely randomised block (unreplicated trial) and a two-replicate randomised block (replicated trial) at Winulta in 1994.

Variables	Variance ratio of SE-HPLC analysis (a)								
	PC	%P1	%P2-1	%P2-2	%P2	%P3	P1/P2	P/M	Glu%Fl
Unreplicated trial									
Glu-B1	3.4 ns	0.1 ns	1.3 ns	0.1 ns	0.7 ns	1.7 ns	0.3 ns	0.0 ns	3.0 ns
Glu-A3	3.3 ns	12.2***	8.0 ***	9.2 ***	16.4 ***	3.6 ns	14.8 ***	12.6 ***	9.7 ***
Glu-B3	0.3 ns	1.0 ns	81.1 ***	13.5 ***	2.1 ns	1.3 ns	1.6 ns	1.0 ns	0.1 ns
Glu-D3	0.3 ns	0.0 ns	10.4 ***	1.6 ns	0.3 ns	2.2 ns	0.0 ns	0.0 ns	0.1 ns
Glu-B1 x Glu-A3	0.0 ns	0.6 ns	0.2 ns	2.1 ns	1.0 ns	0.4 ns	0.8 ns	0.7 ns	0.0 ns
Glu-B1 x Glu-B3	1.6 ns	0.8 ns	4.3 **	0.0 ns	1.3 ns	0.6 ns	1.2 ns	0.8 ns	1.7 ns
Glu-A3 x Glu-B3	4.8 **	0.0 ns	2.7 ns	1.4 ns	0.0 ns	0.0 ns	0.1 ns	0.0 ns	5.1 **
Glu-B1 x Glu-D3	0.1 ns	0.0 ns	2.1 ns	1.5 ns	0.1 ns	0.2 ns	0.1 ns	0.0 ns	0.1 ns
Glu-A3 x Glu-D3	1.2 ns	0.0 ns	0.1 ns	0.2 ns	0.3 ns	1.6 ns	0.1 ns	0.0 ns	1.0 ns
Glu-B3 x Glu-D3	0.6 ns	3.1 ns	2.2 ns	2.8 ns	0.5 ns	4.5 **	1.7 ns	3.1 ns	2.0 ns
Replicated trial									
Glu-B1	9.0 ***	4.9 **	13.9 ***	4.7 **	17.3 ***	16.2 ***	11.3 ***	5.7 **	3.8 ns
Glu-A3	15.9 ***	2.4 ns	23.5 ***	0.6 ns	5.4 **	2.6 ns	4.2 **	2.4 ns	23.7 ***
Glu-B3	2.6 ns	3.0 ns	154.3 ***	44.4 ***	4.0 **	0.3 ns	4.2 **	2.9 ns	1.1 ns
Glu-D3	0.1 ns	0.1 ns	7.2 ***	1.6 ns	0.3 ns	0.3 ns	0.2 ns	0.1 ns	0.1 ns
Glu-B1 x Glu-A3	12.3 ***	0.2 ns	7.9 ***	9.8 ***	0.9 ns	0.9 ns	0.4 ns	0.3 ns	10.6 ***
Glu-B1 x Glu-B3	0.8 ns	1.2 ns	0.3 ns	1.7 ns	0.6 ns	0.5 ns	1.0 ns	1.1 ns	2.1 ns
Glu-A3 x Glu-B3	0.8 ns	5.6 **	10.7 ***	2.5 ns	11.5 ***	4.4 **	9.3 ***	5.8 **	0.0 ns
Glu-B1 x Glu-D3	1.3 ns	0.0 ns	1.4 ns	0.3 ns	0.1 ns	0.0 ns	0.1 ns	0.0 ns	1.3 ns
Glu-A3 x Glu-D3	0.2 ns	0.4 ns	6.5 **	4.3 **	0.0 ns	1.0 ns	0.1 ns	0.3 ns	0.0 ns
Glu-B3 x Glu-D3	2.1 ns	2.2 ns	1.4 ns	1.6 ns	0.1 ns	6.1 **	0.8 ns	1.8 ns	0.5 ns

(a)=see abbreviation list

Table 9.5 Comparison of main effects of glutenin subunit alleles related to F_3-derived F_6 lines from (Barunga x Suneca) showing significant effects on protein parameters measured by SE-HPLC. The data obtained from experimental material grown as a completely randomised block (unreplicated trial) and a two-replicate randomised block (replicated trial) at Winulta in 1994 [a].

comparison	protein parameters								
	PC	%P1	%P2-1	%P2-2	%P2	%P3	P1/P2	P/M	Glu%FI
Unreplicated trial									
Glu-B1									
17+18	10.8	48.0	20.6	20.6	41.2	10.8	1.17	0.93	-
7+8	10.4	48.1	20.4	20.5	40.9	11.0	1.18	0.93	-
significant level	ns	ns	ns	ns	ns	ns	ns	ns	-
Glu-A3									
d	10.8	48.6	20.3	20.1	40.3	-	1.21	0.95	5.2
c	10.4	47.5	20.8	21.0	41.8	-	1.14	0.90	4.9
significant level	ns	***	***	***	***	-	***	***	***
	-								
Glu-B3	-	-							
b	-	-	19.7	21.1	40.8	-	1.19	-	-
h	-	-	21.4	20.0	41.3	-	1.16	-	-
significant level	-	-	***	***	ns	-	ns	-	-
Glu-D3									
c	-	-	20.2	-	-	-	-	-	-
e	-	-	20.8	-	-	-	-	-	-
significant level	-	-	***	-	-	-	-	-	-
Replicated trial									
Glu-B1									
17+18	11.0	47.1	21.0	21.2	42.2	10.7	1.12	0.89	-
7+8	10.6	47.7	20.5	20.7	41.2	11.1	1.16	0.91	-
significant level	***	**	***	**	***	***	***	**	-
Glu-A3									
d	11.0	47.7	20.2	20.9	41.2	-	1.16	0.91	5.2
c	10.5	47.3	21.0	20.9	41.9	-	1.13	0.90	5.0
significant level	***	ns	***	ns	**	-	**	ns	***
Glu-B3									
b	-	-	19.6	21.6	41.2	-	1.16	-	-
h	-	-	21.5	20.3	41.9	-	1.13	-	-
significant level	-	-	***	***	**	-	**	-	-
Glu-D3									
c	-	-	20.5	-	-	-	-	-	-
e	-	-	20.9	-	-	-	-	-	-
significant level	-	-	**	-	-	-	-	-	-

a=see abbreviation list

Consistent effects for the alleles at the *Glu-A3* locus was observed on %P2-1, %P2, P1/P2, and Glu%Fl across the two trials (Tables 9.4 and 9.5). The *d* allele gave lower percentages of first gliadin subfraction (%P2-1) and total gliadin fraction (%P2) for both trials. This effect was accompanied by a higher glutenin/gliadin ratio (Fig 9.6), suggesting that the stronger dough property attributed to the *d* allele was associated with a lower gliadin proportion.

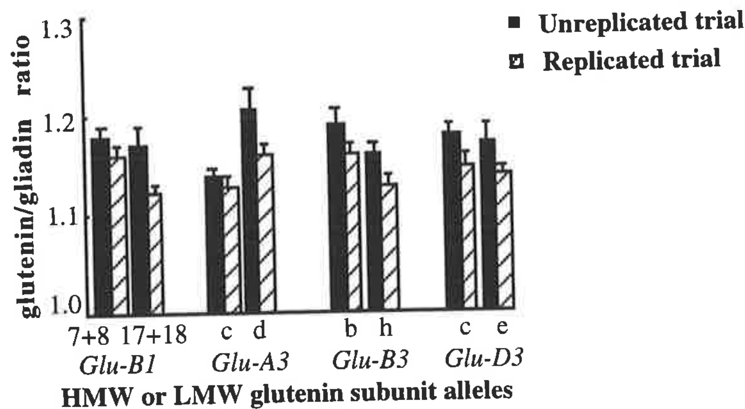


Figure 9.6 The glutenin/gliadin ratio (P1/P2) of F3 derived F6 progeny from (Barunga x Suneca), carrying specific alleles of HMW (*Glu-B1*) and LMW (*Glu-A3*, *Glu-B3* and *Glu-D3*) glutenin loci. Data from experimental material grown as a completely randomised block (unreplicated trial) and a two-replicate randomised block (replicated trial) at Winulta in 1994.

Another result obtained from these experiments showed that the *d* allele was associated with a higher Glu%Fl in both experiments (Fig 9.7), indicating that the greater Rmax and extensibility attributed to the *d* allele (Table 8.7) could be explained by the higher Glu%Fl.

Glu-B3 locus

The difference between the *b* allele and *h* at the *Glu-B3* locus was significant for both gliadin subfractions (%P2-1 and %P2-2) for both trials (Table 9.4), with the difference being more pronounced for %P2-1 (Table 9.5). The *h* allele gave a higher %P2-1 than *b*. By contrast, the second peak of the gliadin fraction was higher for progeny carrying the *b* allele. It seems that the second subfraction of gliadin has a less important role in forming the gliadin fraction, because the ratio of glutenin to gliadin was higher for *b* than for *h* for both trials (Fig 9.6). This indicates a genetic ability of *Glu-B3b* to form more high molecular weight polymers.

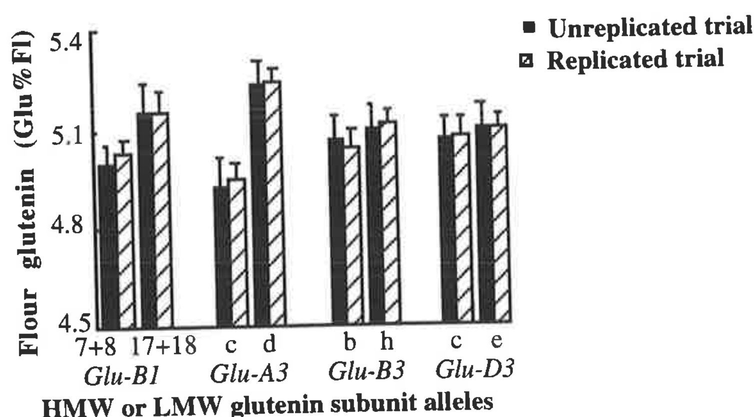


Figure 9.7 The proportion of glutenin in the flour (Glu%Fl) in F3 derived F6 progeny from (Barunga x Suneca), carrying specific alleles of HMW (*Glu-B1*) and LMW (*Glu-A3*, *Glu-B3* and *Glu-D3*) glutenin loci. Data from experimental material grown as a completely randomised block (unreplicated trial) and a two-replicate randomised block (replicated trial) at Winulta in 1994.

Glu-B1 locus

The differences between glutenin bands 17+18 and 7+8 coded for by the *Glu-B1* locus were significant for most of the protein parameters in the replicated trial (Table 9.4), however their effects on dough properties were not significant (Tables 8.7 and 8.9). A higher proportion of glutenin (%P1) (Fig 9.5), glutenin:gliadin (Fig 9.6), albumin-globulin (%P3), and polymeric:monomeric ratios (Table 9.5) associated with the 7+8 bands were accompanied by a lower maximum resistance (284 BU vs. 293 BU). Bands 17+18 were above 7+8 for concentration of the gliadin fraction. The glutenin subunit bands 17+18 also gave a significantly higher protein concentration than 7+8 in the replicated trial (Fig 8.3). It was notable that the 17+18 bands were associated with a higher protein concentration (Table 9.5), whereas its counterpart allele i.e 7+8 was associated with higher percentage of glutenin protein (%), indicating that these two alleles could have compensatory effects on dough quality parameters. This may be a reason for the non-significant differences between the alleles at the *Glu-B1* locus on Rmax, extensibility and extensibility/protein ratio in both trials.

Glu-D3 locus

The effects of the alleles at the *Glu-D3* were not significant on protein composition for either trial. However it was observed that the alleles at this locus differed significantly in their ability to form the components of the first subfraction of the gliadin peak (%P2-1) (Fig 9.8). In general the *e* allele formed an higher proportion of first peak area of gliadin than its counterpart allele.

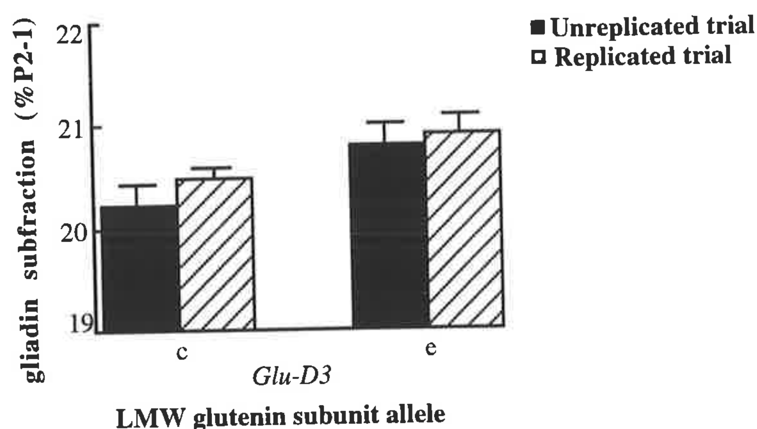


Figure 9.8 The gliadin subfraction (P2-1) of the F_3 derived F_6 progeny from (Barunga x Suneca), carrying alternative alleles of the LMW (*Glu-D3)* glutenin locus. Data from experimental material grown as a completely randomised block (unreplicated trial) and a two-replicate randomised block (replicated trial) at Winulta in 1994.

Interaction effects on glutenin proportions

Glu-B1-Glu-A3

As described in the previous study (Tables 8.7 and 8.9), both LMW and HMW glutenin alleles had large effects on dough resistance and the effects of individual glutenin loci were largely additive, with some minor interloci interactions (non-additive) effects. Similar additive and non-additive effects of these loci were also observed for protein composition in the replicated trial (Table 9.4). While the difference between the *Glu-A3* alleles in relation to Glu%Fl was highly significant with the effect of the *d* allele higher than its counterpart (Fig 9.7), its interaction with the *Glu-B1* locus indicated that the *d* allele in the presence of the 7+8 allele at *Glu-B1* gave the highest proportion of Glu%Fl (Fig 9.9). This interaction was accompanied by a high protein concentration as reported in Chapter 8 (Fig 8.3b). It is notable that this interaction was not pronounced for the two combinations 17+18, *c* and 17+18, *d,* suggesting that the HMW and LMW subunits do not combine at random to give different-sized aggregates.

A combination of alleles 7+8 and *d* was accompanied by a lower %P2-1 compared to the 7+8, *c* combination, whereas, for second subfraction of gliadin the trend was the opposite (Fig 9.9b). This could provide an explanation for the greater effect of the 7+8 and *d* combination on maximum resistance observed in the previous study (Fig 8.3).

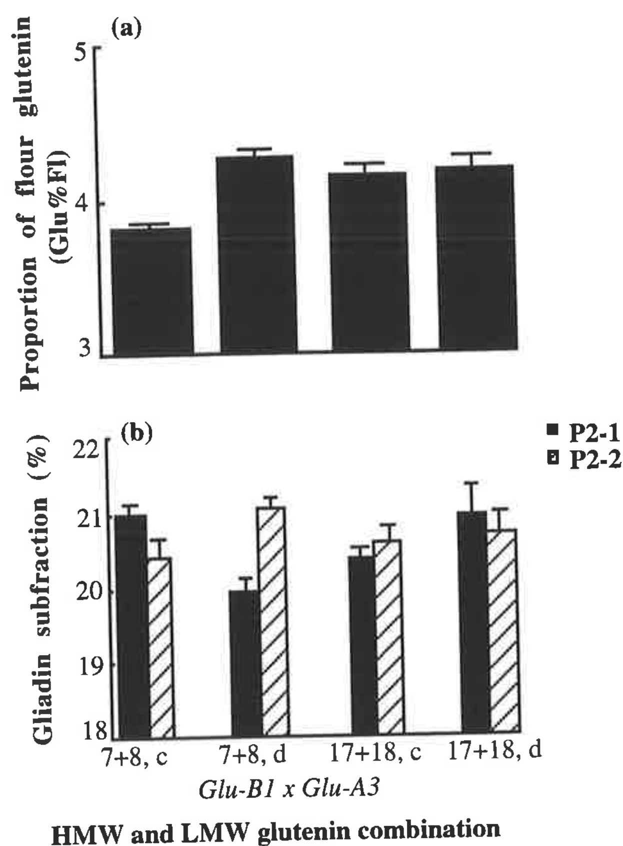


Figure 9.9 Flour glutenin (Glu%Fl) (**a**), first subfraction (%P2-1) and second subfraction (%P2-2) (**b**) of the F3 derived F6 progeny from (Barunga x Suneca), carrying alternative combination of alleles at the *Glu-B1* and *Glu-A3* loci. Data from experimental material grown as a two-replicate randomised block (replicated trial) at Winulta in 1994.

Glu-A3-Glu-B3

The interloci interactions between *Glu-A3* and *Glu-B3* were significant for %P1, %P2, P1/P2 and P/M ratios (Table 9.4). The *d* allele at *Glu-A3* formed less gliadin when *b* was present at *Glu-B3* (Fig 9.10a). This can be easily seen when the ratios of glutenin/gliadin and polymeric to monomeric proteins were taken into consideration (Fig 9.10b). This interaction was not significant on dough strength (Table 8.7). This is in contrast to the conclusion of Batey *et al.* (1991) who reported that higher P1/P2 and P/M ratios can lead to higher dough strength.

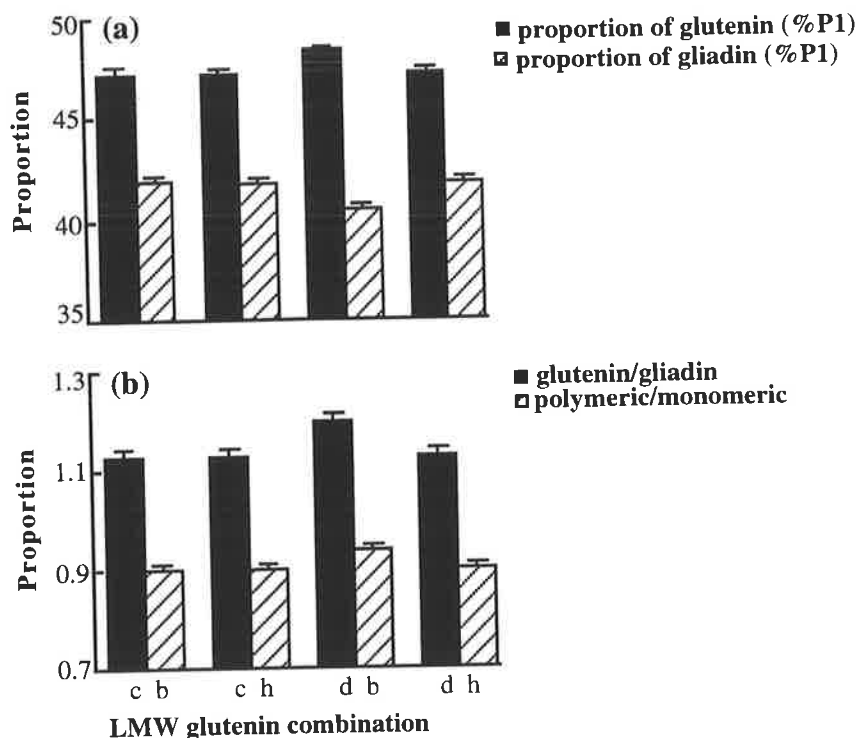


Figure 9.10 The proportion of **(a)** glutenin (%P1) and gliadin (%P2) **(b)** glutenin/gliadin (P1/P2) and polymeric/monomeric(P/M) ratios of the F3-derived F6 progeny from (Barunga x Suneca), carrying alternative combination of alleles at the *Glu-A3* (*c*, *d*) and *Glu-B3* (*b*, *h*) loci. Data from experimental material grown as a two-replicate randomised block (replicated trial) at Winulta in 1994.

9.3.5 Variety survey

The six varieties included in the two experiments were also examined by SE-HPLC analyses. The allelic constitution at the *Glu-1* and *Glu-3* loci of each of the six cultivars along with the local score for HMW and LMW devised by Cornish (1994), similar to those reported in Chapter 5 (Tables 5.2 and 5.3), is given in Table 9.6. As shown, these cultivars were very similar for their LMW glutenin subunits, allowing an evaluation of different HMW glutenin scores with protein composition.

Table 9.6 HMW and LMW glutenins and quality score for six South Australian wheat varieties.

Variety	HMW loci (*Glu-1*)				LMW loci (*Glu-3*)			
	1A	1B	1D	score	A3	B3	D3	score
Angas	1	7+8	2+12	6	c	j	c	14
Aroona	1	7+8 7+9	2+12	6	c	b	c	13
Barunga	1	7+8	5+10	8	c	b	c	13
BT-Schomburgk	1	7+8 7+9	2+12 5+10	7	c	b	c	13
Warigal	-	7+8 7+9	2+12 5+10	6	c	b	c	13
Yarralinka	2*	17+18	5+10	10	c	b	b	13

There was a wide range of variation in quantitative composition and rheological quality amongst the cultivars studied (Table 9.7), although these cultivars were genetically related.

Analysis of variance showed that the differences between varieties were significant for dough strength (as measured by Rmax) whereas their differences for the extensibility-protein ratio were not significant. There were also significant differences between varieties for most of the glutenin composition components.

No trend was apparent for %P1 as flour protein increased, whereas Glu%Fl showed a strong linear relationship with flour protein (r= 0.92). By contrast, the percentage of gliadin in protein increased with increasing protein content (r=0.57) as found by others (Doekes and Wennekes, 1982; Gupta *et al.*, 1992; also see section 9.3.2). Similarly to glutenin the percentage of gliadin in the flour (Gli%Fl) was highly correlated with flour protein (r=0.94). In the case of the albumin-globulin group of proteins, the percentage in the protein decreased with increasing flour protein (r=-0.54). However, its total amount (i.e. Albg%Fl) showed no correlation with flour protein concentration.

Table 9.7 Mean quality parameters and SE-HPLC data for six South Australian varieties, grown in the completely randomised block (unreplicated trial) and in the two-replicate randomised block (replicated trial) at Winulta in 1994 and analysed as a randomised complete block design with nine replications.[a]

Cultivar	quality parameters				SE-HPLC data							
	PC	Ext	E/P	Rmax	%P1	%P2-1	%P2-2	%P2	%P3	P1/P2	P/M	Glu%Fl
Angas	9.5	13.9	1.44	204.3	47.1	20.0	21.6	41.5	11.3	1.13	0.89	4.5
Aroona	10.1	15.3	1.48	203.2	46.4	20.3	22.1	42.5	11.1	1.09	0.87	4.7
Barunga	9.6	14.4	1.52	264.1	46.8	19.4	21.8	41.2	12.0	1.14	0.88	4.5
BT-Schomburgk	9.9	14.3	1.51	243.3	47.3	19.9	21.6	41.4	11.3	1.14	0.90	4.7
Warigal	9.3	13.7	1.45	207.6	46.7	20.7	20.9	41.6	11.7	1.12	0.87	4.4
Yarralinka	9.8	14.6	1.47	289.4	46.4	19.7	23.9	43.6	10.0	1.06	0.87	4.5
LSD (0.05)	0.5	-	-	14.9	0.8	0.6	0.8	0.8	0.5	0.03	-	0.2

a=see abbreviation list

The differences between varieties were only significant for dough strength (as measured by Rmax) (Table 9.8). Amongst the varieties, Yarralinka had the highest Rmax (289 BU) (Table 9.7). With respect to its SE-HPLC measurements it was observed that Yaralinka showed significantly the highest %P2 and lowest %P3. Although the P1/P2 ratios were highly significant between varieties, the lowest ratio was measured for Yarralinka. This is in contrast to the findings of Batey *et al.* (1991) who reported that a higher P1/P2 ratio corresponds to higher dough strength. It seems that the higher Rmax related to this variety was most likely due to its 17+18 or 2* glutenin bands which were absent in the other varieties (Table 9.6). The former glutenin subunit bands gave lower glutenin/gliadin ratios previously (Fig 9.6).

Table 9.8 Variance ratio and probabilities from the analysis of variance for quality parameters and SE-HPLC data of six South Australian varieties grown in the completely randomised block (unreplicated trial) and the two-replicate randomised block (replicated trial) at Winulta in 1994 and analysed as randomised complete block design with nine replications.

measurements	variance ratio	
	value	probability
Quality parameters		
protein concentration	3.1	0.20
extensibility	2.0	0.10
maximum resistance	60.8	<.001
extensibility-protein ratio	0.5	0.76
Protein parameters (SE-HPLC)		
glutenin fraction (%P1)	2.1	0.08
first gliadin fraction (%P2-1)	5.1	0.001
second gliadin fraction (%P2-2)	16.5	<.001
total gliadin fractions (%P2)	11.3	<.001
albumin-globulin fraction (%P3)	15.1	<.001
glutenin/gliadin ratio (P1/P2)	6.1	<.001
polymeric/monomeric ratio (P/M)	2.0	0.10
percentage of glutenin in flour (Glu%Fl)	3.1	0.02

To test whether the quality score can reflected the quantitative aspect of the various allele as reported by Singh *et al.* (1990), the correlation between HMW quality score with Rmax were calculated. The HMW quality score showed a high correlation with Rmax (r=0.88). A multiple linear regression showed that approximately 77% of variation in Rmax was accounted for by the HMW score, whereas when the LMW score was also included, the percentage of variation was slightly increased (79%).

Since no significant differences were observed in extensibility and extensibility/protein ratios for these varieties, no variation in these could be accounted for by the HMW or LMW scores.

Correlations between quality parameters and quantitative protein composition were also studied by multiple regression techniques. In the first step, the relationship between Rmax and SE-HPLC parameters was considered. Approximately 51% of the variation in Rmax was accounted for by %P1, %P2-2, monomeric fractions (%P2+%P3) and polymeric:monomeric (P/M) ratio together. Regarding the extensibility, 18% of the variation could be accounted for by the albumin-globulin fraction. These trends changed when Glu%Fl, Gli%Fl and Albg%Fl were considered. More than 56% of variation in extensibility could be accounted for by Glu%Fl and Gli%Fl. Likewise 12% of the variation in Rmax was accounted for by flour albumin-globulin.

9.4 Discussion

The correlation of certain proteins with quality is of potential value in a plant breeding program. In the past, to develop improved varieties, a breeder had to make crosses between varieties, and multiply many progeny to test their quality before undertaking selection. Small scale tests such as the polyacrylamide gel electrophoresis (PAGE) patterns of seed storage proteins are capable of analyzing single grains in early generations saving both much time and expense. With the advent of High-Performance Liquid Chromatography (HPLC) further improvements in protein analytical methodology become possible. This technique has been widely used in attempts to determine quantitative variation of protein composition, potentially providing a basis for manipulating the functionality of polymeric protein and hence of wheat flour in a plant breeding program.

In the present study, the relationship between protein composition and dough properties is discussed first mainly because the information gained from this type of study can provide an explanation for the differences in the effects of various glutenin alleles on dough properties. The correlation matrix on the properties of the RILs showed that as flour protein increased, the proportion of glutenin (%P1) fell, however the correlation was not strong (Table 9.3). By contrast the proportion of gliadin increased. As total protein increased, the total amount of each group increased, as both flour glutenin and flour gliadin contents were highly correlated with flour protein (Table 9.3).

The protein balance in terms of %P1, %P2 and %P3 showed a fundamental relationship with Rmax. This can be easily seen in the high correlations between Rmax and these components, particularly in the replicated trial (Table 9.3), with the relationship being negative with %P1 and %P3. This result did not support the general conclusion of Orth and Bushuk (1972) who reported that polymers with the largest molecular weights have the greatest effect on dough strength. The correlation coefficient between extensibility and flour glutenin was somewhat higher than that with flour protein, particularly for the replicated trial (r=0.70 vs r=0.67), indicating that the flour glutenin content has a more important role in controlling extensibility than the total protein.

Analysis of variance for the replicated trial using two replication of individual lines showed that the differences between parameters were more evident than in the unreplicated trial (Table 9.1). These results, together with high amount of variation accounted for by lines, suggesting that the genetic background affects either the level of expression of the glutenin subunit genes or the dough properties directly.

In the study here, although there was a difference between subunit bands 7+8 and 17+18 for the glutenin proteins (Table 9.5), these subunits did not have large effects on Rmax (Tables 8.7 and 8.9) compared to the alleles at *Glu-D1* (5+10 vs 2+12) which gives about 100 BU difference (A.J. Rathjen, pers.comm.). The controversial reports about the influence of alleles at *Glu-B1* on dough characteristic (Chapter 8, Discussion) may reflect the genetic background or, more likely, environmental effects. With respect to the genetic background it has been reported that subunit bands 17+18 or 7+8 in the presence of genetic background of 5+10 did not significantly affect the percent of glutenin (Galili *et al.*, 1986). In contrast, the results here showed that, in the presence of a genetic background of 5+10, there was a significant difference between these two groups of glutenin bands for the percent of glutenin (Table 9.5). This is similar to the results of Kolster and Krechting (1993) who compared the amount of subunits produced by two varieties containing an identical set of HMW glutenin alleles [(*Glu-A1* (null); *Glu-B1* (6+8) and *Glu-D1* (2+12)] and found that within a single site, the relative amount of HMW subunits produced varied between these two varieties. Another reason for the non-significant difference between alleles at *Glu-B1* for Rmax may be due to their compensatory effects on dough quality. The glutenin subunit bands 17+18 had a higher protein concentration, whereas its counterpart allele gave a higher percentage of glutenin (Table 9.5). Consequently the generally greater strength of 17+18 than 7+8 on Rmax amongst Australian varieties (e.g Yarralinka and Excalibur) cannot be attributable by the results here to any particular effect on protein composition.

The results here showed that the higher Rmax attributed to Yarralinka was accompanied by a lower glutenin/gliadin ratio (Table 9.7). This trend was also observed with RILs, particularly for the replicated trial (Fig 9.6), both in contrast to the conclusion of Blumenthal *et al.* (1991). This evidence suggests that the presence of subunit bands 17+18 qualitatively affects dough strength by changing the size distribution of the polymers. This may result from differences in the structure of the allelic subunits with consequent effects on their ability to form high molecular polymers. This ability of the HMW glutenin subunits is related to their cysteine residues towards both ends of the molecules (Shewry *et al.*, 1992; Lew *et al.*, 1992). This is thought to allow the HMW subunits to form long polymers linked by head-to-tail disulphide bonds. Thus differences in the location and or number of these residues would result in differences in the reactivity of these subunits and hence in the visco-elastic properties of a dough (Graveland *et al.*, 1985).

This survey showed that the alleles at the *Glu-A3* locus had a significant effect on most of the protein composition parameters, particularly for the unreplicated trial (Table 9.4). The consistent effect of alleles at *Glu-A3* across the two trials was seen in the results for the gliadin fraction (%P2), glutenin:gliadin ratio (P1/P2) and flour glutenin (Glu%Fl)

(Table 9.5). This indicated that the higher Rmax of the *d* allele at *Glu-A3* (Figs 8.1 and 8.9) had its origin in the protein balance in terms of glutenin:gliadin (P1/P2) ratio (Fig 9.6) similar to that shown by Blumenthal *et al.* (1991). The opposite effect of this allele in relation to the gliadin fraction (%P2) indicated that the higher amounts of glutenin were associated with less gliadin (Table 9.5). This was consistent with the results of Gupta *et al.* (1994b) who reported that the relative quantities of gliadin coded by alleles at the *Glu-A3* locus were inversely associated with Rmax values. In a study relating quantitative aspects of glutenin alleles to flour quality, Gupta *et al.* (1990b) found that the positive effects of LMW subunits on Rmax were due to an increased total glutenin content and that the LMW alleles associated with high dough strength generally had a greater number and or/intensity of bands. In studies involving LMW subunits (Payne *et al.*, 1987c; Gupta *et al.*, 1989), comparisons between null alleles (absence of bands) and alleles coding for several bands revealed that the positive effects of LMW glutenin subunits were due to increased total glutenin content rather than a qualitative superiority of specific subunits.

The allelic effect at *Glu-B3* on protein composition was only pronounced on the gliadin fraction and the P1/P2 ratio (Table 9.4). Although allele *b* had a greater glutenin/gliadin ratio (Table 9.5) and was expected to give higher dough strength (Blumenthal *et al.*, 1991), its effect on dough strength was not significant (Figs 8.1 and 8.9). This indicated that the balance between the two subfractions of gliadin may also have a role in controlling dough strength.

Another interesting result in the present study was found with the alleles at the *Glu-D3* locus. In the previous study it was observed that the alleles at this locus (*c* and *e*) had no significant effect on dough quality (Tables 8.7, 8.9) and a similar trend was observed in the study here (Table 9.5). The effect of the alleles at *Glu-D3* on protein composition was not significant except on the first gliadin subtraction. Comparison of the allelic effects at other glutenin loci (i.e *Glu-B1*, *Glu-A3* and *Glu-B3*) on this gliadin subfraction showed that the *d* allele at *Glu-A3* with a significantly greater effect on Rmax than *c* also had the lower %P2-1 (Table 9.5). Hence there did not seem to be any causal relationship between %P2-1 and dough properties. Nor is there any supporting evidence here for the alleles at the *Glu-D3* locus having a negative effect as reported by Lafiandra *et al.* (1993).

The interaction between *Glu-B1* and the *Glu-3* loci, particularly with *Glu-A3* could provide a biochemical explanation for the interaction effect of these loci on Rmax. The greatest protein concentration (Fig 8.3) and flour glutenin concentration (Fig 9.9a) was found with the 7+8 and *d* combination and this was accompanied by the highest Rmax, indicating that the interaction between these glutenin loci operated through the quantity of glutenin. This is in contrast to the report of Gupta *at al.* (1994b) who noted that the interaction between

Glu-B1 and *Glu-D1* on Rmax mainly operated at the level of polymer formation (size distribution).

It has been reported that the proportion of glutenin in total protein, as measured by percent area of the first SE-HPLC peak had a highly significant positive correlation with the dough properties of a set of diverse wheat cultivars (Singh *et al.*, 1990). However in this study no trend between Rmax and %P1 or Glu%Fl was observed. Examination of the data in Table 9.7 demonstrates the absence of this relationship. The most striking example is the comparison between the Aroona and Yarralinka. Although the proportion of glutenin in their protein was similar (46.4%), Yarralinka produced a much stronger dough (Rmax=289 BU vs 203). This emphasised that the proportion of the total glutenin (%P1) cannot fully explain dough strength, agreeing with the results of Gupta *et al.* (1992).

CHAPTER 10

GENERAL DISCUSSION

Proteins are recognised as the most important components governing bread-making quality. Although a high protein content is often related to good bread making quality, protein quantity alone cannot explain all the variation. Other factors such as protein 'quality' are also important. This has motivated much of the cereal chemistry research over the last century, from the pioneering work of Osborne (1907).

Protein quality has been shown to be largely governed by the structure of the gluten proteins which are mainly made up of glutenin and gliadin. It is widely accepted that variation in protein quality is mainly due to the differences in the glutenin proteins. The subunit composition of glutenin, the molecular weight distribution, and the aggregative tendency with other proteins and non-protein constituents are likely to be particularly important in this regard. In the present study, electrophoresis and high performance liquid chromatography were used as the basic techniques to evaluate the importance of particular protein components in breadmaking quality.

10.1 Linkage between *Gli-1* and *Glu-3* loci

An inheritance study of LMW glutenin subunits was facilitated using a triple translocation stock (Gupta and Shepherd, 1993) and the 1-dimensional electrophoresis technique developed by Singh *et al.* (1991b). It was observed that the individual LMW glutenin subunits in the cross between the breeding line (W1 x MMC)/Wl/10 and Halberd, were always inherited as a block with their parental gliadin alleles. These results confirmed earlier findings of Gupta *et al.* (1994a) who also studied the segregation behaviour of these LMW glutenin subunits and gliadins in progeny of the same cross. It is obvious that the level of recombination between the LMW glutenin loci and the gliadin loci is extremely low (Singh and Shepherd, 1984; 1988). Therefore the gliadin pattern can served as a reliable marker for the identification of LMW glutenin bands and the analysis of the gliadins can aid in the identification of LMW glutenin loci in segregating populations. From a breeding point of view, both *Glu-3* and *Gli-1* provide useful genetic markers for quality, and in certain cases only the *Gli-1* provides the positive markers, viz, the null *Glu-3* alleles (Gupta and Shepherd, 1990a).

10.2 Individual glutenin loci

Although the relationship between glutenin subunits and breadmaking quality has been established by many authors using several different approaches, a major limitation of these studies has been their failure to record the relative magnitude of the effect of the genetic background. In this thesis, evaluation of several selections of each glutenin genotype in RILs from a hybrid between two bread wheat parents has permitted the effects of the genetic background on the glutenin properties to be measured. As both parents used in this study had good breadmaking quality, this comparison was made in a good quality background. This study also was one of the first to examine both classes of glutenin subunits subunits (HMW and LMW) simultaneously, since LMW fractionation techniques, developed by Singh *et al.* (1991b) recently allowed reliable identification of the large number of glutenin genotypes present in a segregating population.

The effects of allelic variation at the *Glu-1* loci (Chapter 4) on dough strength (as measured by SDSS volume) of unselected recombinant inbred lines showed that the individual HMW glutenin loci can be ranked *Glu-B1*>*Glu-A1*>*Glu-D1* with respect to their effects on SDSS values (Table 10.1).

Table 10.1 Comparison of the effects of different alleles at *Glu-1* loci on SDS-sedimentation volume, over two locations in 1992 and three in 1993 using RILs from [Halberd x (Wl x MMC)/Wl/10].

		SDS-sedimentation value				
		1992		1993		
locus	allele	Winulta	Minnipa	Palmer	RC	Yeelanna
Glu-A1	1	45.9	48.9	75.3	72.5	74.5
	2*	47.8	51.3	79.0	73.8	75.7
difference		**-1.9**	**-2.4**	**-3.7**	**-1.3**	**-1.2**
Glu-B1	17+18	48.8	51.6	81.8	76.1	79.6
	20	45.9	49.1	74.0	71.2	72.2
difference		**+2.9**	**+2.5**	**+7.8**	**+4.9**	**+7.4**
Glu-D1	5+10	47.5	50.8	75.4	72.2	74.4
	2+12	46.2	49.3	78.0	73.6	75.4
difference		**+1.3**	**+1.5**	**-2.6**	**-1.4**	**-1.0**

This ranking differed from that of Gupta *et al.* (1994a) on the basis of effects of different alleles on maximum resistance with the greatest effect attributed to the *Glu-D1* locus.

However, the same rankings were observed when both studies were compared on the basis of extensograph measurements (Table 10.2).

Table 10.2 Comparison of the effects of different alleles at the *Glu-1* loci on maximum resistance, using RILs from [Halberd x (Wl x MMC)] in two studies.

locus	allele	Rmax values in two studies			
		Gupta *et al.* (1994a)	difference	current study	difference
Glu-A1	1	205	**+5**	219	**0**
	2*	210		219	
Glu-B1	17+18	250	**+75**	247	**+90**
	20	175		157	
Glu-D1	5+10	265	**+95**	255	**+100**
	2+12	170		155	

Unlike with SDS-sedimentation, the direct measurement of dough strength with extensograph gave large differences between the different alleles at the *Glu-D1* locus in the present study and that of Gupta *et al.* (1994a). This change in ranking was most likely due to the difference in the method of quality evaluation. SDS-sedimentation is based on the solubility of glutenin in the sodium dodecyl sulfate solvent and strong wheats normally have less SDS soluble glutenin and consequently more insoluble glutenin in their flours (Dachkevitch and Autran, 1989; Bietz, 1984). The dough strength measured by maximum resistance reflects the capacity of each glutenin subunit to form large-size polymers as reported by Gupta *et al.* (1994b). Since subunit 5 has been found to have one more cysteine residue than subunit 2 (Greene *et al.*, 1988), it is likely to form polymers with a greater molecular weight than subunit 2 (Shewry *et al.*, 1992), resulting in higher dough strength.

Comparisons of the *Glu-3* loci on SDSS over two seasons and several locations (Table 10.3) showed that the alleles at the *Glu-A3* locus had a greater effect on SDSS than those at the other two loci. Comparisons between the two loci *Glu-A3* and *Glu-B1* in the normal wheat growing season of in 1993 (Tables 10.1 and 10.3), showed that allelic variation at the *Glu-A3* locus had a greater effect on SDSS than at the *Glu-B1* locus.

Table 10.3 Comparison of the allelic effects of *Glu-3* loci on the SDS-sedimentation volume at five location over two years, using RILs from [Halberd x (Wl x MMC)].

locus	allele	SDS-sedimentation value				
		1992		1993		
		Winulta	Minnipa	Palmer	RC	Yeelanna
Glu-A3	*c*	48.4	51.7	80.1	75.7	78.2
	e	43.5	46.5	70.1	67.2	67.8
difference		**+5.0**	**+5.2**	**+10.0**	**+8.5**	**+10.4**
Glu-B3	*b*	49.0	51.7	83.8	77.4	80.5
	c	45.5	49.0	72.3	70.1	71.3
difference		**+3.5**	**+2.7**	**+11.5**	**+7.3**	**+9.2**
Glu-D3	*b*	46.6	49.5	76.4	74.1	74.2
	c	46.8	50.3	77.5	72.3	75.6
difference		**-0.2**	**-0.8**	**-1.1**	**+1.8**	**-1.4**

The generally greater dough strength of genotypes with subunit bands 17+18 compared to subunit 20 at *Glu-B1* is likely to be due to the different number and or positions of the cysteine residues as reported by Gupta *et al.* (1994b). However, there has been some inconsistency in reports of the effects of these subunit bands (17+18) on dough characteristics compared to 7+8 (Branlard and Dardavet, 1985b; Lawrence *et al.*, 1987; Payne, 1987; Lagudah *et al.*, 1988). This may indicate that both of these pairs of glutenin subunit bands have an ability to form large polymers and hence to increase the visco-elastic properties of dough, but that their actual effects are environmentally influenced. In the present study using RILs from (Barunga x Suneca) (Chapter 8) it was found that the subunit pairs 17+18 and 7+8 did not show significant differences for dough strength (as measured by Rmax and estimated by SDSS). This could be attributed to their compensatory effects on dough strength (Chapter 9) or their ability to interact with other components such as lipids (MacRitchie, 1984) through differences in protein composition and polymer formation.

The effects of allelic variation at the *Glu-3* loci on Rmax of RILs from [Halberd x (Wl x MMC)] could not be compared with those of Gupta *et al.* (1994a), mainly due to the limited number of RILs which were used here in the extensograph evaluation. But the allelic effects at *Glu-3* could be compared on the basis of SDS-sedimentation tests over two years (1992 and 1993) and several locations (Table 10.3). This study showed that allelic variation at the *Glu-A3* locus had a significant influence on dough characteristics and that allele *c* at this locus gave a significantly greater SDSS volume than the *e* allele. It seems that the low dough strength of the null type *Glu-A3e* is likely to be due to its failure to produce any protein product (Chapter 3). Payne *et al.* (1987c) also found a significant

negative effect of the null allele at the *Glu-A3* locus on dough quality and Gupta *et al.* (1989) found significantly low dough strength associated with *Glu-A3e* from the bread wheat variety Kite.

Allele *b* at the *Glu-B3* locus with an extra subunit band (Chapter 3) was associated with a consistently higher SDS-sedimentation volume than its counterpart allele *c* over seasons and locations.

In the study with (Barunga x Suneca) RILs the allelic effects at *Glu-A3* (*d* vs *c*), *Glu-B3* (*b* vs *h*) and *Glu-D3* (*c* vs *e*) were evaluated (Table 10.4) on the basis of extensograph data. This study showed that only allelic variation at the *Glu-A3* locus had a significant influence on dough characteristics (Table 8.9). Although the *c* allele at this locus had a significantly higher dough strength than the *e* allele in the [Halberd x (Wl x MMC)/Wl/10] cross (Chapter 4), its effect on dough strength was lower than the *d* allele in the (Barunga x Suneca) cross. The effects of alleles at the *Glu-B3* locus on maximum resistance were not significant and were less than those reported by Gupta *et al.* (1994a) (Table 10.4). Several studies have shown that allelic variation at the *Glu-D3* locus has no significant effect on dough strength (Gupta *et al.*, 1994a; Nieto *et al.*, 1994). This feature was also observed in the present study, indicating that the effects of alleles at the *Glu-D3* locus on dough strength are relatively low compared to the other *Glu-3* loci, using SDS-sedimentation or extensograph tests.

Table 10.4 Comparison of the allelic effect of the *Glu-3* loci on extensograph parameters of RILs from (Barunga x Suneca) in two experiments.

locus	allele	quality parameters			
		Rmax		E/P ratio	
		unreplicated trial	replicated trial	unreplicated trial	replicated trial
Glu-A3	*c*	282.8	282.5	1.56	1.54
	d	310.4	293.9	1.56	1.61
difference		**-27.6**	**-11.4**	**0.0**	**-0.7**
Glu-B3	*b*	289.8	286.6	1.56	1.57
	h	303.4	287.8	1.55	1.57
difference		**-13.6**	**-1.2**	**+0.1**	**0.0**
Glu-D3	*c*	297.3	287.0	1.53	1.55
	e	295.9	287.5	1.58	1.59
difference		**+1.4**	**-0.5**	**-0.5**	**-0.4**

The *Glu-B3* locus is known to code for the largest number of LMW subunits amongst *Glu-3* loci (MacRitchie, 1992; Cornish, 1994) and it has been suggested that number of bands

may be an important criterion in assessing the value of these alleles in promoting dough strength (Cornish, 1994). In the present study, the *Glu-B3* locus had no significant effect on extensibility (Tables 8.7 and 8.9), although *Glu-B3b* coded for an extra B subunit band in gels compared with *Glu-B3c* (Chapter 3). In contrast, Cornish (1994) reported that the *h* allele of *Glu-B3* produces an extra band over allele *b*. The actual number of LMW subunit bands detected in gels, particularly at the *Glu-B3* locus, depends upon the method of fractionation and thus it may be unreliable to extrapolate from band number to relative extensibility.

10.3 Interaction between loci

The effect of allelic variation at two or more loci on rheological properties (Chapters 4 and 8) was not solely additive. Epistatic effects have also been reported in the analysis of the effects of HMW glutenin alleles (Carrillo *et al.*, 1990; Rousset *et al.*, 1992; Kolster *et al.*, 1991; Payne *et al.*, 1987b). The results obtained in the present study showed that significant epistasis can occur between alleles at some of the loci investigated with respect to their effects on dough characteristics, particularly SDS-sedimentation volume (Table 10.5).

Table 10.5 Interactions between different glutenin loci on SDS-sedimentation volume, observed in RILs from two parent combinations.

parent crosses and experiment			
[Halberd x (Wl x MMC)/Wl/10]		(Barunga x Suneca)	
1992 †	1993 ‡	unreplicated	replicated
Glu-B1 x Glu-D1 ***[a]	-	-	*Glu-B1 x Glu-D3* **
Glu-A1 x Glu-B3 ***	-	-	*Glu-B3 x Glu-D3* **
Glu-D1 x Glu-B3 ***	*Glu-D1 x Glu-B3* ***	-	-
Glu-A3 x Glu-B3 ***	-	-	-
Glu-A1 x Glu-D3 *	-	-	-
Glu-B1 x Glu-D3 ***	-	-	-
-	-	-	-
-	-	-	-

a=see abbreviation list for significant level
†, ‡ analysis of variance at Winulta and Yeelanna site.

In the study of the RILs, the interaction between different loci on dough strength (as measured by SDSS) was more common in the [Halberd x (Wl x MMC)/Wl/10] than the (Barunga x Suneca) cross. Moreover these interactions occurred most frequently with the *Glu-1* loci, perhaps not surprisingly. It was notable that the largest number of significant interactions were observed at Winulta site which was affected by rain damage. With

respect to Rmax the only significant interaction was observed between *Glu-B1 x Glu-A3* in the replicated trial and for extensibility between *Glu-B1 x Glu-B3* (Table 8.7). Since in the study of (Barunga x Suneca) RILs the effect of the HMW and LMW glutenin subunits on dough quality were mostly additive, it was expected that the genotype with highest dough strength would possess the alleles with highest individual effects (viz 17+18, *d h c*). This prediction was correct in the unreplicated trial (319 BU, Table 8.3), however with the replicated trial, the highest maximum resistance was observed for the combination 17+18, *c h c* (313 BU, Table 8.2), but the only difference between these two genotypes was for the alleles *d* and *c* at the *Glu-A3* locus. Comparisons of those lines having the same band patterns as their parents showed that progeny having the Suneca pattern (17+18 *d h e*) had a higher extensibility and E/P ratio than those possessing the Barunga pattern (7+8 *c b c*) (Table 8.2 and 8.3). These results all suggested that the dough quality of Barunga can be improved solely by altering *Glu-A3c* to *Glu-A3d*, because the alleles at the other three loci (i.e *Glu-B1*, *Glu-B3* and *Glu-D3*) did not show significant differences over the genetic backgrounds.

10.4 Linkage, pleiotropy and environmental effects

The allelic composition of the *Glu-1* and *Glu-3* genes alone did not provide a completely reliable prediction of dough strength, therefore there must be other genes involved (as yet not characterised), in addition to the environmental effects that modify the genotypic influence of the glutenin genes. In the study involving the RILs of (Barunga x Suneca) (Chapter 8), it was observed that only 9% of variance in maximum resistance could be accounted for by the glutenin genotypes (band patterns) (Table 8.5), whereas in the study using the random lines of [(Turkey x Warigal) x Warigal], where the background differences were probably minimised by the selection regime, 84% of variance for Rmax was accounted for by glutenin band pattern (Table 7.3). Although in the former study highly significant differences on Rmax were observed between alleles at the *Glu-A3* locus (Tables 8.9, 10.4), their effects were not as large as those in the latter study (Table 10.5) and those reported for biotypes having the subunit bands 5+10 compared to 2+12 at *Glu-D1* (90 BU) (Lawrence *et al.*, 1987). This indicates that the genetic background had a large influence in determining dough quality and that it is necessary to select for an appropriate genetic background in conjunction with the alleles with consistent effects. It appears that selection within segregating populations for suitable enduse qualities still requires the use of conventional large scale tests, and not just seed protein composition.

Inconsistent effects of allelic difference at the *Glu-D1* locus were observed in the RILs from [Halberd x (Wl x MMC)/Wl/10] in the different seasons (1992 and 1993) (Table 10.2). These differences in ranking of the alleles at *Glu-D1* were most likely due to environmental effects. Another indication of the importance of the environment effects on dough properties was observed in the experiment reported in Chapter 6, while [(Turkey x

Warigal) x Warigal] genotypes having either 5+10 or 2+12 at the *Glu-D1* locus gave different effects over sites (Table 10.6). In this particular study the greater maximum resistance normally occurring with group having bands 5+10 was not found to occur at Palmer. Likewise the greater extensibility/protein ratio usually found with groups possessing subunit bands 2+12 did not occur at Yeelanna. These results indicating the occurrence of considerable genotype x environment interaction effects, point to the need for more detailed studies on this aspect of quality expression.

Table 10.6 The mean maximum resistance and extensibility/protein ratio of two groups of random lines (labelled as group 5 and group 2, see Chapter 6) from the cross [(Turkey x Warigal) x Warigal] grown at four locations in 1992.

Site	Rmax		E/P ratio	
	group /5/	group /2/	group /5/	group /2/
Yeelanna	277.6	158.7	1.45	1.45
Walpeup	347.0	241.7	1.53	1.71
Mudamuckla	273.6	230.7	1.35	1.44
Palmer	244.7	238.0	1.48	1.69

In the study of RILs from [Halberd x (Wl x MMC)/Wl/10] it was found that sprouting resistance was significantly associated with alleles controlling HMW glutenin subunits but not with the LMW subunits (Table 4.4). A similar association was also reported by Lukow *et al.* (1989) but in this case between gliadin proteins and Falling Number. Therefore it is worth investigating these associations further because it would be valuable in selecting for sprouting resistance if the alleles with a higher Falling Number were accompanied with higher dough strength. It was also observed that genes controlling grain hardness (measured by particle size index) were linked to the LMW glutenin subunits (Table 4.7). Another indication of linkage between glutenin subunits and interesting agronomic traits was found in the study of RILs from (Barunga x Suneca) for grain yield, with the *Glu-D3* locus showing a consistent interaction with *Glu-B3* on grain yield at two experimental sites in the replicated trial (Table 7.13). These results all suggest that selection for glutenin subunits can lead to changes in agronomic or other quality characteristics.

10.5 Breeding strategy

In the study with (Barunga x Suneca) RILs, the alleles at *Glu-A3* showed a highly consistent effect on dough quality over the two experiments (Table 10.4), although their effect was not as large as those reported for *Glu-D1* and *Glu-B1* (Table 10.2). It was also

found that a high percentage of the variance components were attributed to line within genotypes effects reflecting differences in the genetic background. This can be clearly shown by examining an example of the different lines within a genotype from the replicated trial (Table 10.7) where there was a wide range for quality parameters (means of 265-340 for Rmax, 15.4-19.0 for extensibility and 1.41-1.71 for E/P ratio). To investigate this further, diverse lines within a single glutenin genotype should be selected and evaluated for other major components such as gliadins (on the group 6 chromosomes), lipids and starch in relation to physical flour dough properties.

Comparison of Barunga and Suneca with standard hard wheat variety (Table 7.1) showed that Suneca had a higher flour yield than Barunga (1.3% and -0.4% respectively, compared to the standard variety). This indicates that not only altering the *Glu-A3c* to *Glu-A3d* is necessary to upgrade Barunga; attention must be concentrated on milling quality. The mean flour yields for the RILs were higher than that of Barunga (Tables 8.2 and 8.3), indicating that the incorporation of Suneca genetic material into Barunga can lead to a higher flour yield. Breeders using backcross procedures, together with electrophoresis techniques and milling tests, could expect to rapidly recover an appropriate background with the desired protein patterns.

Table 10.7 Variation between lines within a single genotype having band patterns 17+18 *c h c* at the *Glu-B1*, *Glu-A3*, *Glu-B3* and *Glu-D3* loci, from (Barunga x Suneca), grown as a two-replicate randomised block at Winulta in 1994.

Lines No. (within genotype)	Quality parameters (a)					
	Rmax		Ext		E/P	
	rep 1	rep 2	rep 2	rep 1	rep 1	rep 2
1	306	287	15.2	17.8	1.30	1.51
2	317	333	15.7	19.7	1.29	1.60
3	312	321	16.5	15.3	1.54	1.50
4	320	360	16.0	19.0	1.36	1.57
5	270	260	15.7	15.0	1.69	1.72
6	340	328	20.2	18.0	1.70	1.55

a=see abbreviation list

It was noted that the interaction found in the study of [Halberd x (W1 x MMC)] RILs was not necessarily similar to that of the (Barunga x Suneca) cross (Table 10.5), indicating that the degree of interaction can be affected by genetic backgrounds. For this reason, fixation

of particular glutenin alleles in a breeding population probably may not be a wise strategy, since fixation of the six glutenin loci on the basis of additive effects might eliminate the chance of selecting genotypes that have superior performance in end-use quality.

The results of this study provide information for devising improved strategies for wheat breeders in selecting for improved quality characteristics. While incorporation of *Glu-A3d* is expected to improve dough properties, the breeder must:

a) test many lines within genotypes to maximize favourable background effects

b) conduct trials over a number of environments to measure genotype x environment effects

c) examine the data closely to avoid undesirable linkage effects

Further investigations aimed at identifying the genes involved in controlling dough extensibility are required.

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