# Wheat Glutenin Subunits in Relation to Baking Quality Parameters

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A Thesis
Submitted in Fulfilment
of the Requirements for the Degree of
Doctor of Philosophy
at
Lincoln University

by

Cuiyun Luo

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Lincoln University
New Zealand

1999

This is to certify that the work described in this thesis was planned, implemented and described by Cuiyun Luo under our direct supervision at Lincoln University, Crop & Food Crown Research Institute, New Zealand and Institute National de la Rechereche Agronomique (INRA), France.

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# Abstract

of a thesis submitted in fulfilment for the requirements for
the degree of Doctor of Philosophy at Lincoln University, New Zealand

## Wheat Glutenin Subunits in Relation to Baking Quality Parameters

**by**

**Cuiyun Luo**

Wheat gluten has unique properties that make it suitable for bread-making. As a result bread-making quality is closely associated with gluten quantity and quality. This research was conducted to look at the relationship between varieties, environment, wheat glutens and potential bread-making quality. Specifically the objectives were: 1) to assess the environmental effect on the quantities of gluten and its subunits across a range of genotypes (both high and low molecular weight glutenins); 2) to quantify the relationships among glutenin subunits (both high molecular weight and low molecular weight) and baking quality parameters, and the relationships among those quality parameters themselves; 3) to quantify specific allelic (Glu-1 & Glu-3) effects on bread-making quality and interpret the effects using genetic expression models.

Two sets of materials, a GXE trial and a set of recombinant lines were created. Fourteen New Zealand cultivars or lines with various baking quality were chosen for GXE trial and these were given six treatments that varied in fertiliser type (nitrogen or sulphate) and application time (early or late application). Based on the allele information, five recombinant lines were chosen for later analyses and quality tests. The quality tests were: wholemeal flour protein, white flour protein, hardness, SDS sedimentation volume, Pelshenke time and 10-gram mixograph.

The data obtained indicated that bread-making quality could be improved by late nitrogen application. Cultivars' responses to the environmental changes varied, they could be either stable or more responsive. Higher SDS sedimentation and mid-line peak values of the mixograph were mainly related to higher protein content. Pelshenke time values were mainly related to high molecular weight glutenin subunit score, which represents their qualitative or allelic differences.

Allelic differences were shown to significantly affect quality parameters. Possession of the null allele of Glu-A1 resulted in inferior values for most of the flour quality tests when compared to alleles 2* and 1. Possession of allele 5+10 for Glu-D3 was significantly related to longer Pelshenke times and greater SDS sedimentation volumes. Possession of different Glu-A3 alleles significantly affected wholemeal flour protein content, sedimentation volumes and mid-line peak values of the mixograph. Possession of allele d for Glu-A3 could be a valuable high quality predictor in breeding programmes.

Bread-making quality is a complex matter influenced by many factors apart from glutenins. This thesis has demonstrated a combined genetic and agronomic approach to wheat quality improvement. However, there remains substantial scope for further research in this area.

# Acknowledgments

This PhD research was a cooperative enterprise among three research organisations: Lincoln University, Christchurch, New Zealand; Crop and Food Crown Research Institute, Christchurch, New Zealand and Unite Amelioration Des Plantes (INRA), Clermont-Ferrand, France.

I am most indebted to Dr. Gerard Branlard (INRA) and Dr. W.B. Griffin (Crop and Food Crown Research Institute), the joint supervisors of this research. Without their consistent encouragement and assistance this project could not have reached completion. They contributed greatly through the entire research procedure, from networking with colleagues and institutes across continents to final revisions of the draft. Their abundant knowledge and experience in wheat quality research has been an invaluable resource for experimental design and data analysis and I am grateful for their guidance. I am particularly indebted to Dr. Branlard for his stoic patience with my limitations in spoken French, a challenge for us both during my sojourn at INRA.

I would like to thank my supervisor at Lincoln University, Dr. D. L. McNeil, for his indispensable contribution to this project. His expertise in experimental design and data analysis helped to shape the research from its inception. His supervisory experience and constructive advice guaranteed steady progress.

I would like to thank the following staff for their wide-ranging advice and technical assistance: Mrs Helen Searle in the Lincoln University laboratory; Mr D. Heffer and D. Jack for their assistance in the field trials; Professor G. Kuan, Dr. S. Warwick and Mr. R. Martin for their advice on fertiliser design; Mme. Mireille Dardevet, who advised on spectrophotometer and densitometer techniques and Isabelle Gateau for her assistance with electrophoresis; M. Rene Saccomano and Jean-Pierre Martinant for mixograph and technology testing; Mme Josiane Gourdon for SDS sedimentation and M. Fabrice Lagoute for the Pelshenke test. The support of INRA director Dr. Dumas de Vaulx is recognised and deeply appreciated.

Many thanks are due to Dr. A.G. Fautier for his contribution at the initial stages of the project. I wish to thank both George Hill, former head of the Plant Science Department at Lincoln University for his support and encouragement, and his wife Dr Roberta Hill. Her warmth and positive attitude toward life eased the burdens along the way.

The completion of this research was facilitated by the PhD research fellowship from The International Union of Biochemistry and Molecular Biology (IUBMB), the Freemasons Scholarship of New Zealand and a Lincoln University postgraduate scholarship. I wish to express my gratitude to all these organisations for their support.

Finally, I thank my daughter and all members of my family, both far and near, for their love, understanding and belief in me, which sustained me through challenging times.

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# Abbreviations

| | |
|---|---|
| AA | amino acid |
| AACC | American Association of Cereal Chemists |
| BCA | bicinchoninic acid |
| C | cross linker of SDS-PAGE |
| E | early in fertiliser supplying |
| ER | endoplasmic reticulum |
| FP | flour protein |
| GQ | glutenin quantity (mg/100mg flour) |
| GS | glutenin subunit(s) |
| GXE | genotype and environment interaction |
| HAR | hardness of grain (%) |
| HMW-GS | high molecular weight glutenin subunit(s) |
| HMWS | HMW-GS score |
| HPLC | high-performance liquid chromatography |
| HQ | HMW-GS quantity (mg/100mg flour) |
| kda | 1,000 dalton (unified mass unit) |
| L | late in fertiliser supplying |
| LMW-GS | low molecular weight glutenin subunit(s) |
| LQ | LMW-GS quantity (mg/100mg flour) |
| Max | maximum |
| mg | milligram(s) |
| Min | minimum |
| min | minute(s) |
| ml | millilitre(s) |
| MPT | mid-line peak time of mixograph |
| MPV | mid-line peak value of mixograph |
| N | nitrogen fertiliser (Nitrolime) |
| NIR | near infrared reflectance |

| | |
|---|---|
| PEL | Pelshenke test (minute) |
| R-sq | R square (%) |
| RER | rough endoplasmic reticulum |
| RILs | recombinant inbred lines |
| S | sulphur fertiliser (Gypsum) |
| SDS | sodium dodecyl sulphate |
| SDS-PAGE | sodium dodecyl sulphate-polyacrylamide gel electrophoresis |
| SED | SDS sedimentation test (ml) |
| T | gel concentration of SDS-PAGE |
| WFP | wholemeal flour protein |
| µg | microgram |
| µl | microlitre(s) |

# Chapter 1
# Introduction

## *1.1 Wheat industry*

Wheat is the leading cereal grain produced, consumed and traded in the world today. Bread, leavened and unleavened has been a staple food for humans throughout recorded history. Wheat has the widest adaptation among all cereal crops and is grown in some 100 countries around the world. World wheat production has exhibited steady growth from 1960 to 1990, and has almost tripled from the 1960 level, to around 600 million tons. Today, wheat provides more food for people than any other food. Cereal grains provide 68% of world food supplies, and wheat contributes almost one-third of all cereal production, meaning around 23% of the food in the world comes directly from wheat or wheat products (Oleson, 1994).

## *1.2 Bread wheat and bread*

### *1.2.1 Wheat types*

Based on the suitability for baking bread, wheat is normally divided into hard, semi-hard and soft wheat. Hard wheat has a physically hard kernel that yields flour with high gluten and consequently high protein content and this is suitable for producing a western style loaf of bread or pasta. Soft wheat is characterised by a lower protein level and is most suitable for producing cakes and biscuits. Semi-hard wheat have some combination of the hard and soft wheat quality characteristics and is utilised in unleavened breads such as chapattis as well as Asian steamed bread and certain noodles.

#### *I Bread wheat*

Bread baking wheat is semi-hard or hard with a medium to high protein content. Bread flours must absorb a relatively large amount of water, and good bread baking dough must also have the capacity to stretch into a large volume. Bread baking dough is expanded by gas from

the fermenting yeast and then must retain that volume when cooked.

All these wheat types are commonly grouped as bread wheat (or common wheat) which provides about 95% of world wheat production; the remaining 5% are provided by cultivated durum wheat, which is mainly processed into pasta.

### *II Pasta and noodles*

Pasta are made from dried dough that forms the basis of much Italian cuisine, as well as Chinese, Japanese, Korean, and Southeast Asian cuisines. It is believed that Marco Polo introduced pasta to Europe from China. Scholars now believe that the Chinese were eating noodles as early as the first century. Italian pastas, such as spaghetti and macaroni, are traditionally made from semolina flour derived from tetraploid durum wheat; Asian style noodles are normally made from common wheat.

### *III Biscuit wheat and Others*

Biscuit, cake and pastry are made from soft wheat and form weak gluten. Wheat is also used to make beer, whisky, and industrial alcohol. Its by-products of the flour milling, brewing, and distilling, are used as feed of livestock. A minor amount of wheat is used as a coffee substitute in Europe. The unique properties of gluten have been explored and exploited in edible packaging, coating, inks, cosmetics and hair care products.

## *1.2.2 Bread*

Bread covers a wide range of products. Loaves may appear in different shape and size, vary in volume, density, crust and crumb characteristics. The appearance and texture of bread are affected by the flour type (white or wholemeal), flour quality and baking method. The basic steps for making bread are the same: mixing of the ingredients, rising or fermentation, kneading, moulding into loaf shapes, further rising, baking, cooling and slicing if desired. Bread supplies a significant portion of the nutrients required for growth and maintenance of health.

Leavened products have become an important part of the daily diet, and wheat is the only grain suitable for leavened bread. This is due to the presence of a unique elastic gluten complex that provides a matrix for the gases to form the characteristic crumb texture (light, airy and porous, yet chewy) of bread. This unique texture offers the wider popularity and appeal of

leavened breads and defines the most essential functional characteristic of wheat gluten. Another feature of leavened breads is a well-defined crust. The most common type of leavened bread is white pan bread, which has a number of variants, such as yeasted doughnuts, cinnamon rolls, coffee cakes, Danish and puff pastries, French croissants and brioche, steamed bread in China, Philippines and Japan, however, they may require different amounts of gluten for optimum quality.

One of the primary determinants for bread texture is the quantity and quality of gluten protein. The gluten must form a continuous matrix that can stretch to hold the carbon dioxide and other gases which result from the yeast fermentation, which allows increased loaf volumes.

## *1.3 Baking quality and its relationship to flour protein*

### *1.3.1 Wheat and flour quality*

Wheat quality is defined by an almost infinite number of different food products which contain flour, starch, gluten, bran, whole or cracked grains. In general terms, wheat needs to be sound, clean, fully mature, and free from contamination. The cereal or food technologist often needs to predict variation in end-product quality based on variation in wheat grain or flour. Quality of flour is defined by its inherent physical-chemical qualities, and the suitability of those for the preferences of the consumer. The quality of a particular flour is not necessarily low or high until it is judged in the context of a particular end-use. Quality assessment of wheat is divided into tests based on physical and chemical criteria (Morris and Rose, 1996).

### *1.3.2 Baking quality*

For the consumer, quality relates to the senses: sight, sound, feel (touch and mouth feel), smell, and taste. Determining the bread making ability of wheat by test baking has been of major importance to wheat research. Generally, individual bread quality is determined and described by the following criteria:

- Form, overall appearance,
- Crust, surface,
- Leavening/crumb grain,

- Elasticity,
- Structure,
- Smell and taste/flavour

Commercial bread bakers often add gluten to dough if the proteins in the flour do not provide enough strength and elasticity. Some food companies add gluten to bread to increase the protein content of the bread above specified minimum standards.

Baking quality can be partially predicted by a combination of flour chemical criteria and tests for individual components of the overall baking procedure.

### *1.3.3 The role of protein on baking quality*

The protein quantity of the flour can strongly influence the bread making quality, often the higher the protein content, the better the bread making quality. However, qualitative differences among the proteins of different cultivars also affect bread making quality, even though their protein quantity is the same.

## *1.4 Flour protein and gluten*

Wheat flour proteins are the crucial component in relation to bread making quality, both quantity and quality being important. Protein quantity is primarily determined by the environment and by the rate of nitrogen fertiliser application. In contrast, protein quality is much less affected by the environment and is mainly genetically controlled. The genetic constitution of wheat is important because all quality traits are the result of the expression of genes and their interaction with the environment.

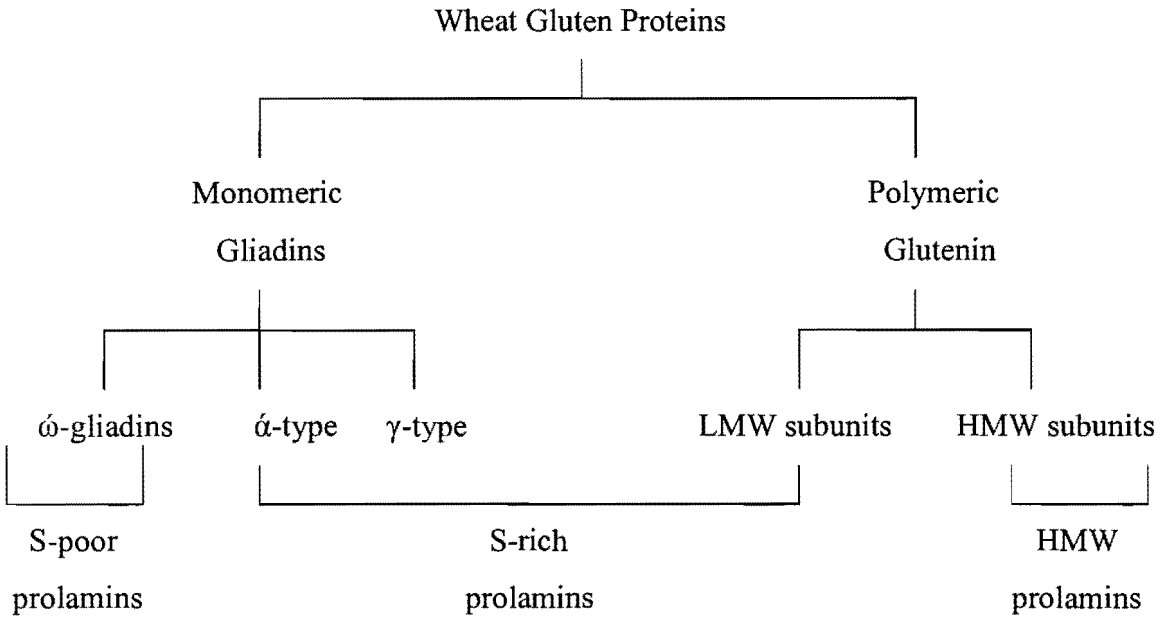
During the 1996 6th International Gluten Workshop, a common gluten language was established, to avoid poor interaction and confusion between groups (Wrigley *et al.*, 1996). This thesis uses their nomenclature system.

Gluten proteins are those proteins that give the unique viscoelastic properties to dough made from wheat flour, and gluten is the mass remaining when dough is thoroughly washed under running water. Although generally gluten refers to the relevant proteins from wheat grain, from the nutritional view, gluten may also be used to cover prolamines and glutelins from the grain of rye, triticale, barley and possibly oats. Wheat gluten is composed of glutenin

and gliadin.

The glutenin proteins are polymeric, with disulfide bonds joining the individual glutenin polypeptides. The single chain polypeptides obtained after reduction of the disulfide bonds are referred to as 'subunits'. Techniques such as SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) or SE-HPLC (Size Exclusion High Performance Liquid Chromatography) divide glutenin subunits into two distinct groups: high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunit (LMW-GS). The LMW-GS overlap with some of the gliadins after SDS-PAGE.

**Figure 1-1** Classification of wheat Gluten proteins based on structural homologies and genetical relationships (Shewry and Miflin, 1955)




The composition of wheat gluten determines the 'strength' of the flour and whether or not it is suitable for biscuit or bread making. LMW-GS are present in much larger proportions than HMW-GS, 3-4 times as much by weight. They are also significantly different from HMW-GS in terms of their amino acid composition. Variation in gluten composition, in type or amount of subunits among classes and cultivars greatly influences gluten quality and baking quality.

## *1.5 The role of gluten components on baking quality*

The importance of HMW-GS in controlling the bread making quality has been determined in many studies, but only a proportion of the variation in bread making quality among wheat cultivars is explained. Part of the variation on bread making quality caused by HMW-GS could be the result of quantitative difference among them, and the presence of certain HMW-GS is positively correlated with improved bread making quality (Branlard and Dardevet, 1985b; Payne *et al.*, 1987b).

Gliadin polypeptides are associated with both direct and indirect measures of bread making quality. Some studies have suggested that the variation in bread making quality is not the result of the variation in gliadin polypeptides themselves, but rather the variation in LMW-GS, because LMW-GS and gliadin genes are tightly linked. Gliadins have the greatest influence on dough extensibility (Branlard and Dardevet, 1985a).

The functional role of LMW-GS has received relatively little attention until recently. Allelic variation in LMW-GS is also important for explaining dough quality variation in bread wheats. Together with HMW-GS, they explain 90% variation in dough resistance and 25% variation in dough extensibility. Genetic variation in LMW-GS has been shown to be the primary factor in differences in gluten viscoelastic properties in both bread and durum wheat (Gupta and Shepherd, 1987 & 1988). Allelic variation in LMW-GS and HMW-GS have an cumulative effect on dough properties. Some LMW glutenin alleles increase dough extensibility and reduce the dough development time (Gupta *et al.*, 1989c).

## *1.6 The objectives of the project and the layout of the thesis*

### *1.6.1 Objectives*

A major objective of research on wheat proteins has been to define the molecular basis of variation in bread making quality and to identify the polypeptides of greatest importance. During the last 10-15 years, in defining the molecular basis of bread making performance, much of the interest has centred around the effects of specific polypeptides of the gluten complex, especially the HMW-GS.

From a breeding point of view, dough quality can be improved more effectively by

selecting for both Glu-1 (where HMW-GS are coded) and Glu-3 (where LMW-GS are coded) alleles together, than for either of these alone. Favourable effects on dough properties can be combined to maximise genetic potential. Understanding the roles of specific Glu-3 alleles is still limited in comparison to Glu-1 allele, and Glu-3 alleles need to be tested in a wider range of genotypes to determine their relative ranking in predicting dough properties. Only very few Glu-3 alleles have been tested for their significance on dough properties. A thorough knowledge of the different alleles in relation to baking quality is needed for effective progress by traditional or molecular breeding.

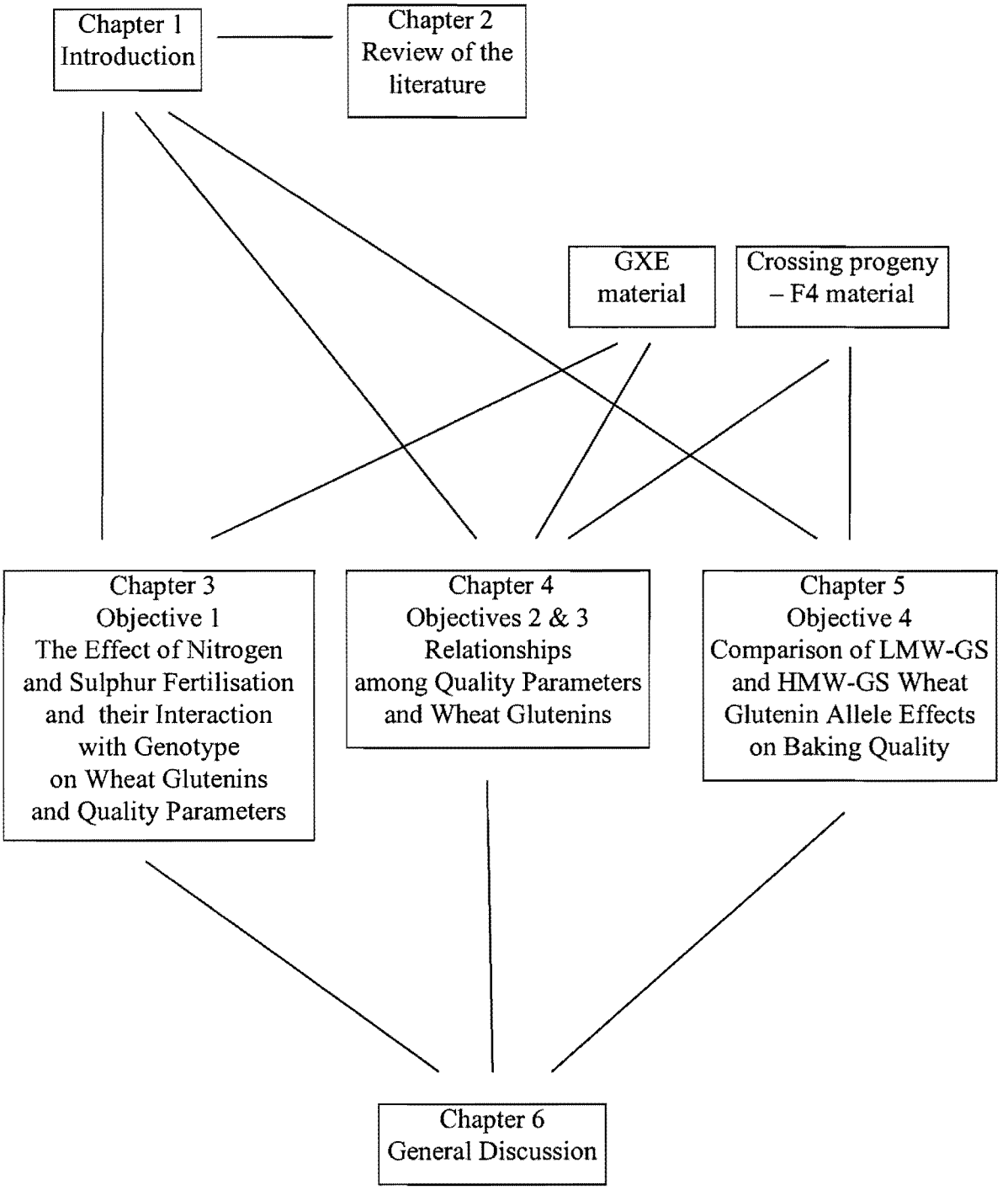
The objectives of this project are:

- to study the environment effects on glutenin (HMW-GS & LMW-GS) quantities, baking quality and their interaction
- to study the relationship between LMW-GS & HMW-GS and quality parameters
- to investigate the possibility of predicting baking quality
- to compare some LMW-GS alleles in relation to baking quality

The information provided by this research should assist wheat quality breeders in selecting their breeding materials and predicting baking quality.

### *1.6.2 Layout of the thesis*

**Figure 1-2** Diagram of the relationship between the objectives and the chapters



# Chapter 2

# Review of the Literature

## *2.1 Background*

Wheat is a member of the grass family. It belongs to the group of grasses called cereals or cereal grains. Other important cereals include rice, corn, barley, sorghum, oats, millet, triticale, and rye. Wheat is the world's most important food crop. Hundreds of millions of people throughout the world depend on food made from the kernels (seeds or grains) of the wheat plant. The kernels are ground into flour to make breads, cakes, cookies, crackers, macaroni, spaghetti, and other foods. The leading wheat-producing countries include Australia, Canada, China, France, India, Russia, Ukraine, and the United States.

Wheat is the only grain suitable for leavened bread. This is due to the presence of the unique elastic protein complex (the gluten complex) that provides a matrix for gases to form the characteristic open texture of bread.

The economic and technological importance of cereal storage proteins has provided an important stimulus for their study. In addition, they have been an attractive model system for molecular biologists interested in the mechanisms of gene regulation. The results of these studies have provided a valuable insight into the molecular basis for various quality traits and have indicated strategies for improvement through the application of molecular genetics (Oleson, 1994; The World Book Multimedia Encyclopedia, 1995).

### *2.1.1 History of Bread Making*

Scholars believe that about 11,000 years ago people in the Middle East took the first steps toward agriculture. It is believed that the Egyptians discovered bread baking (Seibel and Brummer,1991). Mural paintings show that sourdough was already used in Egypt as early as the 13th century BC. Findings from eastern Mediterranean regions suggest that bread baking was known around 1800 B.C. Historians believe the Egyptians learned to make yeast bread about 2600 B.C. The ancient Greeks learned bread making from the Egyptians and later taught the method to the Romans. From Egypt, the art of bread making came to Israel and then to

Greece. Around 776 B.C. the conclusion of the Olympic Games was celebrated with a feast that included bread made from wheat and barley. In Greece, a special bread cult existed and the Demeter, the goddess of bread, was worshipped (Seibel, 1994; Encarta 98, 1997).

Romans learned to make bread from the Greeks after they conquered Greece. Around 100 BC, there were hundreds of small bakeries and even some baking companies in Rome. By 100 AD, the Romans had taught the technique to people in many parts of Europe. By the Middle Ages, most European cities had bakeries. At this time, white bread was preferred by the rich, the common people consumed dark bread and whole grain meal bread. Brewer's yeast was developed in the 15th century and was also used for the production of bread. The production of baker's yeast began in Europe around 1900, and then, white bread was a common food. The baking industry, including bread processing techniques and equipment, significantly improved bread quality by developing new products for special purposes (Drews, 1976).

### *2.1.2 Classification and genetics*

Wheat is a diverse family of related grasses. Over two dozen individual species have been characterised as members of the genus *Triticum*. Of these, only common wheat (*T. aestivum* L.) is studied in this PhD research project.

### *2.1.3 Morphology*

Wheat is an annual plant with various height, attaining an average of 1.2 metre. Wheat has primary and secondary roots, the secondary roots are thicker and stronger than the primary roots and anchor the plant securely in the soil. The root system mostly lies in the upper 38-50cm of soil. Apart from the main stem, wheat plants have several tillers. Each leaf a wheat plant has a sheath and blade. The wheat head, or spike forms at the top of each main stem and tiller. The head is composed of a many-jointed stem, carries clusters of flowers that called spikelets. Many varieties of wheat have bristly hairs, called awns of beards, which are extended from the spikelets. A typical wheat spike bears 30-50 grains (The World Book Multimedia Encyclopedia, 1995).

## *2.2 Grain Properties*

### *2.2.1 Introduction*

Wheat grain is a biological entity - a living, breathing, complex collection of tissues and organs. Wheat grain is the seed of the wheat plant, normally 4-8 mm long, depending on the variety and condition of growth. The colour of the grain results from the pigments present in the seed coat or pericarp, mostly are brown with the various shades, some of the varieties can be red or purple (Cornell and Hoveling, 1998).

### *2.2.2 Grain structure and composition*

The wheat grain is divided into three main parts: bran, endosperm and embryo. The bran is the seed coat and aleurone layer, rich in B vitamins and minerals. The endosperm is about 80% of the grain weight, and is the part milled into flour, is rich in starch and proteins, consisting of albumins, globulins, gliadins and glutenins, the starch granules also contain protein and lipids as minor constituents, the amounts are related to the size of the granules. The embryo (or germ) is a rich source of B & E vitamins and oils, and consists of several parts necessary for a new wheat seedling. Wheat germ is separated from flour during the milling process because the oils can cause the rancidity during flour storage. Wheat germ is valuable and used in many other processed products, such as: a dietary supplement of vitamin E, soap or cosmetic products (Cornell and Hoveling, 1998).

### *2.2.3 Grain quality*

Grain quality is generally assessed by texture (hardness), colour, degree of contamination, percentage of broken kernels, moisture content, and baking quality. In order to test the processing quality, some form of test milling is usually required and the flour produced must be evaluated for its dough properties, as well as the final processed products such as bread, biscuits and pasta.

It is the inherent characteristics associated with each wheat type that makes them suitable for particular end user, as well as unsuitable for others. Grain hardness is a key determinant of wheat utilisation and affects the grain's performance during the milling procedure. Particle Size Index (PSI) has long been used to classified the soft or hard wheat (AACC, 1985), it can also be assessed by Near Infrared Reflectance (NIR) method (AACC,

1985) and a single kernel crunching device (Martin *et al.*, 1993).

Apart from hardness, kernel mass and morphology, as well as protein quantity and quality are other intrinsic traits that affect end-use quality. Generally, millers prefer uniformly large, well-filled, plump kernels. Kernel morphology has also been used to classify grain for market categories. Kernel mass is typically expressed as Thousand Kernel Weight. The single kernel crunching device provides measures of kernel weight and outer dimension, as well as hardness and moisture.

Moisture content determines the storability of grain, the relative concentration of other kernel constituents (eg. proteins), and the amount of additional water needed during modifying before milling. For safe storage, wheat moisture must be lower than 14.5%, to prevent the growth of various moulds.

A final intrinsic quality trait of grain is soundness, or sprouting - germination of grains in the field prior to harvest. The inferior quality of sprouted grain relates to the presence of carbohydrases, proteases and other hydrolytic enzymes normally associated with germination. When sprouting occurred, the proteins and starch had undergone a lot of chemical changes and had lost its desirable properties for the procedure of grain storage, milling, making of the flour and end product.

To promote the orderly marketing of wheat, most developed countries have a system of classes and grades to give both the buyer and seller an estimation of the potential quality (Morris and Rose, 1996).

## *2.3 Baking quality*

### *2.3.1 Introduction*

Overall breadmaking quality of wheat depends on several factors which correspond to the ability to produce quality bread. The most important factors are water absorption, loaf volume, internal and external loaf characteristics, and tolerance to mixing and fermentation. All these quality factors are correlated to physical and chemical properties of the flour or dough. End-product quality and processing quality are closely linked. Wheat flour is about 75-80% starch on a dry weight basis, also containing protein, oils, crude fibre, ash, pentosans and water (Fennema, 1996).

### *2.3.2 Dough formation*

Three main stages are involved in bread making:

- Preparation of the dough
- Fermentation
- Baking

#### *I Gluten network*

Preparation of the dough is of prime importance to bread making. A dough is formed when gluten proteins hydrate, together with the damaged starch granules. The glutenin fraction of the gluten forms an extensive three-dimensional network due to the following:

- Hydrogen bonding between the amide groups on side chains of glutamine (by far the most abundant amino-acid moiety present) and other groups
- Hydrophobic interactions between aromatic rings; also alkyl groups
- Ionic bonds between acidic and basic side chains
- Disulphide bonds formed from cysteine side chains

The viscoelastic properties of the dough depend heavily on these reactions and are modified by the presence of a roughly equal amount of gliadin protein, which also contains a large amount of glutamine moiety, together with some sulfhydryl groups and disulphide bonding. Gliadin has a much lower molecular weight than glutenin, allowing it to fit between subunits of glutenin, and thus exerting a significant effect on dough properties, particularly extensibility. Both glutenin and gliadin contain about 15% proline (mole %), probably related to the β-spiral conformation proposed for both types of proteins. Intramolecular disulfide bonds also probably help to keep the molecules of glutenin more tightly coiled (Cornell & Hovelling, 1998).

#### *II Physical and chemical transformation of flour*

Several physical, chemical and biochemical transformations occur during mixing and kneading of a mixture of wheat flour and water. Under the applied shear and tensile forces, gluten proteins absorb water and partially unfold. The partial unfolding of protein molecules facilitates hydrophobic interactions and sulfhydryl disulphide interchange reactions, which result in formation of thread like polymers. These linear polymers in turn are believed to interact with each other, presumably via hydrogen bonding, hydrophobic associations and

disulphide cross linking, to form a sheet like film capable of entrapping gas. These transformations cause an increasing dough resistance during mixing until a maximum is reached, which is followed by a decrease in resistance, as this network structure breaks down. The breakdown involves alignment of polymers in the direction of shear and some scission of disulphide cross links, which reduces the polymer size. The time taken to reach maximum dough strength during kneading is used as a measure of wheat quality for bread making, a longer time (within limits) indicating better quality (Fennema, 1996).

Available information indicates that a specific pattern of disulphide cross-linked associations among LMW-GS and HMW-GS in the gluten structure may be far more important to bread quality than the absolute amount of this protein. It is possible that in good quality wheat varieties, more of the LMW-GS may polymerise with HMW-GS, whereas in poor-quality wheat varieties, most of the LMW-GS may polymerise among themselves.

### *2.3.3 Quality parameters*

The assessment of flour quality may be grouped into two categories: laboratory end-product tests and component tests. End-product tests tend to produce a summation of quality - the sum of all the components of quality as well as their interaction. They are generally considered the best predictors of commercial end-product quality, however, they require more time, flour and equipment, and therefore are more expensive compared to component tests.

Component tests tend to assess one or more fundamental properties, or components, of flour, to predict the end-product quality. The tests are quick, require relatively small amounts of sample, and are therefore relatively cheap to run. They are better at identifying particular components which affect quality, but sometimes have limited ability to predict commercial end-product quality. They are often used primarily in cultivar development (Morris and Rose, 1996).

Small-scale tests of breadmaking quality are essential in wheat breeding programs in order to select suitable lines at an early stage. A large number of tests are available for quality evaluation, classification and screening of the early generations in breeding programs. In an efficient and effective wheat breeding program for high quality cultivars, critical quality differences must be identified early. Several tests for breadmaking quality have been developed specifically for the generally small samples available in the early generations.

Several tests such as farinograph, extensograph, mixograph and alveograph can

estimate the dough mixing or viscoelastic properties. Other bread making quality tests like the Pelshenke dough ball test, the Zeleny sedimentation test and the SDS (sodium dodecyl sulphate) sedimentation test give valuable information on protein quality associated with baking. The quality tests used for this PhD research project are explained in the following sections:

### *I Technology test*

Near Infrared Reflectance (NIR) is used to estimate wholemeal and white flour protein content, hardness and moisture level. It involves scanning a sample in the infrared part of the light spectrum, determining absorbency at certain wave lengths and computing a factor value by comparison with a pre-tested calibration set of samples. An international collaborative study established the integrity of the NIR method for measuring wheat hardness. An NIR index of hardness was developed, in which the theoretical range of wheat hardness extends from 0 to 100, with the harder wheat having the higher values. Results for a series of wheats with a very wide range of hardness stretched from 3.8 to 92.4. The NIR index is proposed as a rapid method for testing wheat for hardness, using simple commercial bench-type NIR instruments (Williams and Sobering, 1986).

Grain kernel hardness is a characteristic often used in wheat classification (Meppelink, 1974; Symes, 1961), as it affects the manner of grain breakdown in a mill and the behaviour of flours during their subsequent use. Hardness is often the first prediction test applied. The test is simple, rapid and can be done on small amount of seeds. Hard wheat produces flour with a higher percentage of damaged starch. In bread making, higher starch damage causes higher water absorption in the dough and, subsequently, higher bread yield (Stenvert, 1974) and better keeping properties. Hardness is closely related to important flour properties such as protein and starch content (Moss *et al.*, 1973; Newton *et al.*, 1927).

When evaluating bread baking potential, once the hardness of the wheat had been identified, protein quantity would be the next major concern (Finney and Barmore, 1948; Bushuk *et al.*, 1969; Pomeranz *et al.*, 1970).

### *II SDS sedimentation test*

The SDS Sedimentation Test (Axford *et al.*, 1979), employs water and then sodium dodecyl sulfate (SDS) and lactic acid for dispersion of the flour proteins. Good correlation (r =

0.76-0.82) has been obtained between sediment volumes and loaf volumes using this test. It gives a simple, overall assessment of protein quality and is used extensively as an early-generation screen for breeding quality bread wheat. Normally, the SDS sedimentation data were consistent with the volumes of baked loaves.

### *III Pelshenke test*

There are several variants of this test. Pelshenke (1933) first introduced the test to determine the baking and gluten quality of wheat varieties and strains. Bread volume is determined by the quantity of gas which the dough can retain during fermentation. The gas-holding power of gluten is affected by its quality as well as by its quantity, and can be determined by making a dough ball, mixed with yeast and water, from wholemeal flour, which is allowed to ferment in water at a constant temperature. The dough ball first sinks to the bottom, then rises to the surface after 10-15 minutes as a result of the development of carbon dioxide gas. The increasing pressure in the interior of dough, which can expand freely on all sides, causes the ball to finally burst and the dough particles to sink to the bottom. The time taken from the dough being put initially into the water until the dissolved dough particles sink to the bottom is recorded as the Pelshenke time, normally as minutes.

Pelshenke test has made a valuable contribution to baking research as it characterises baking quality by determining diastatic power as well as the quantity and quality of gluten. It is generally consistent with the baking results.

This PhD research used a modified version by Dr. Gerard Branlard developed at INRA, Clermont-Ferrand, France. All samples were tested at INRA, Clermont-Ferrand.

### *IV Mixograph*

In 1939 Dr. E. B. Working of the National Manufacturing Company of Lincoln, Nebraska, designed and built a device for determining the physical dough properties, that required only 35g of flour, known as the mixograph (Swanson, 1943). Finney and Shogren (1972) downsized the 35g mixograph to 10g of flour for measurement of physical dough properties and prediction of breadmaking quality in early generation plant breeders' lines. Later, the mixograph was further modified to require only 5g or 2g of flour (Finney, 1989; Rath *et al.*, 1990). The standardised mixograph is regarded as one of the most useful methods to determine and predict bread making properties. Dough consistency, as measured by the

height of the Mixogram, was correlated with grain hardness (Martinant *et al.*, 1998).

In un-mixed doughs, diffusion is the only driving force to slowly move water to the centre of the flour particles. Mixing provides an additional mechanism, since as the hydrated particles are rubbed against each other, their surface is removed and a new layer of particles is exposed to the excess water in the system. As this is repeated, the flour particles slowly become completely worn away or hydrated, more and more water is absorbed to hydrate the protein and starch, and the resistance of the system to extension gradually increases to an optimum. Mixing beyond this optimum causes excess water absorption and the dough strength collapses. Thus, the height of the mixing curve gradually increases to a peak, indicating an optimally mixed dough for bread making. The height of the peak often is recorded as peak value, and the time for the mixing to reach the peak value is recorded as peak time. In this PhD research, Mid-line Peak Value (MPV) and Mid-line Peak Time (MPT) were selected as the critical indicators from the 11 parameters recorded by the 10-gram mixograph.

### *2.3.4 Relationship between the quality parameters*

A better understanding of the relationships between the quality parameters will assist traders, growers and breeders. It will provide an early indication for growers so that they can adapt their management practices, and a guideline for selection during the breeding procedure.

#### *I Protein quantity and loaf volume*

Protein content of wheat is accepted as the most important criterion for most aspects of processing capability and nutritional value. Bushuk *et al.* (1969) observed that loaf volume is positively correlated with protein content and suggested a linear regression, whereas protein content on development time has a curvilinear regression. Within a cultivar, most variation in loaf volume results from variation in protein quantity.

#### *II Protein quantity and hardness*

As early as 1927, Newton *et al.* (1927) reported that no relationship exists between protein content and wheat hardness. On the other hand, Greenaway (1969) obtained a high positive correlation between hardness and protein content. Stenvert and Kingswood (1977) found that for wheat grown under the same environmental conditions, hardness increased with increasing protein content; however, the rates were cultivar dependent. The hardness of de-

branned wheat was strongly affected by moisture content. Measurements of hardness on de-branned wheat did not show any significant correlation with protein content. This observation confirms that bran has a definite influence on results of grain hardness evaluation (Obuchowski *et al.* 1980b).

### *III Protein quantity and SDS sedimentation*

SDS sedimentation is one of the most suitable screening tests in a breeding program. Kitterman and Barmore (1969) noted that sedimentation value was positively related to protein content. A high correlation exists between SDS sedimentation values and some quality parameters (Mazzoni, 1988). Very high sedimentation value could be undesirable if it required extra mixing time in the baking process. A statistical analysis revealed that mixograph development time and SDS sedimentation volume were both able to account satisfactorily for variations in gluten strength (Dexter *et al.*, 1980). Baker *et al.* (1971) claimed that Zeleny Sedimentation test and mixograph peak development time (MPT) were among the most repeatable tests. Although the SDS sedimentation test has been widely adopted, it does not always differentiate strong and 'extra strong' cultivars (Pritchard, 1993; Pritchard *et al.* 1994).

### *IV Glutenin and sedimentation, MPT*

Zhu *et al.* (1996) observed that the amount of HMW-GS and its relative proportion were positively correlated with sedimentation value. Payne *et al.* (1987) and Gupta *et al.* (1989) suggested that the positive effects of LMW-GS have been entirely due to increased total glutenin rather than qualitative superiority of specific subunits, similar possibilities could occur for HMW-GS as well. Singh *et al.* (1990) found that the glutenin quantity was highly positively correlated with loaf volume and mixograph MPT, and they suggested the glutenin has a direct effect on functionality.

### *V MPV and MPT of mixograph*

Khatkar *et al.* (1996) found that mixing properties were significantly influenced by protein content. The height of mixograph (MPV) is most frequently reported, and is correlated with grain hardness (Martinant *et al.*, 1998; Finney and Shogren, 1972), which is less significantly correlated with the other mixograph parameters. Strong relationships exist between loaf volume and MPV (Dachkevitch *et al.*, 1989). Later investigation found that MPV

has a very highly significant correlation with Glu-1 quality score and loaf volume (Branlard *et al.*, 1992; Payne, 1987a). Glu-1 quality score is the value given to each HMW-GS allele according to its effect on the baking quality by Payne or Branlard. Generally, alleles that have better influence on quality will have a higher score. MPT showed no significant correlation with loaf volume and most other quality factors (Dong *et al.*, 1992; Preston *et al.*, 1992).

### *2.3.5 Summary and conclusion*

Fowler *et al.* (1975) suggested that only hardness, dough development and protein content are necessary to describe the baking quality of wheat. Orth *et al.* (1972) used simple correlation and regression to predict loaf volume. They suggested that protein content, sedimentation value and dough development time provides the most useful information for predicting baking quality. They also found that curvilinear regression best describes some of the relationships for these parameters. Branlard *et al.* (1990) compared 46 technological parameters and recommended that Pelshenke and mixograph result, because of their high heritability should be included when selecting for wheat quality.

Improved understanding of the relationships between quality parameters can make a selection program more effective. Baker *et al.* (1971b) presented a model to provide some understanding for the complex nature of breadmaking quality, and claimed that prediction of loaf volumes, from flour measurements, were generally successful.

Early generation selection is a potentially cost effective breeding strategy; however, its usefulness depends on the reliability, complexity and speed of the tests done on small seed samples. A predicted quality trait could present the genotypic value of a line for any particular character. Through a simple, accurate and widely accepted definition of wheat quality, identification and manipulation of genetic and environmental factors will fulfil the baking quality requirements of the consumer.

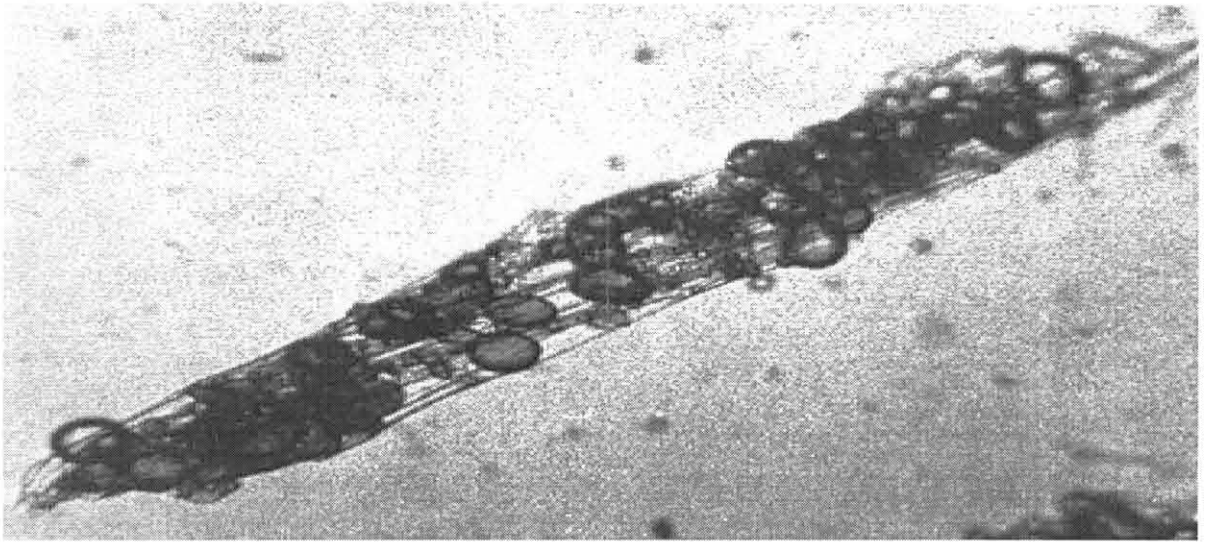
## *2.4 Gluten*

The word gluten comes from a Latin word meaning glue. All wheat gluten proteins are synthesised on the rough endoplasmic reticulum (RER), with a signal peptide that is cleaved as it directs the polypeptide into the ER lumen. Then proteins fold and disulphide bonds are formed within the ER (Shewry, 1996). The precise mechanisms and the role of other proteins are still not understood in detail (Shewry, 1995b; Richard *et al.*, 1996). Their chemical and physical differences contribute to their functional characteristics. Gliadin is monomer and soluble in aqueous alcohol solutions with molecular weight around 40-60kDa. Glutenin is the native (unreduced) oligomeric molecules; they are grouped as HMW-GS and LMW-GS by SDS-PAGE, the molecular weight of HMW-GS is between 80-160kDa, for LMW-GS, between 23-68kDa. Over 50 individual gluten proteins can be separated by two-dimensional electrophoresis under reducing and denaturing conditions (Shewry, 1986, 1994). All the individual proteins contribute in some way to the functional properties of whole gluten. The gluten proteins have been studied intensively because they determine the viscoelasticity of dough, and are considered essential for bread making quality (Shewry, 1995a).

### *2.4.1 Gluten structure*

Amend and Belitz (1990, 1989) studied extended particle from wheat flour mixed with water by various microscopic techniques. On the basis of these results, they have developed a clear model of gluten's formation and elasticity. According to the model, when flour is mixed with water, the native gluten proteins (present as irregular globular structures), build a three-dimensional network. The individual strands of this network are extended during kneading and simultaneously stretched because of their elasticity. As a result of the tensile stress, small plate-like films are formed at the junctions of the protein strands, which build up in layers to form a type of membrane. Strong stretching tears the films or membranes, but the layers remain intact. This model explains the process of gluten agglomeration and starch extraction out of dispersion.

**Figure 2-1** Light microscopic photograph of an extended particle from wheat flour; gluten is visible in the form of strand, extended between the starch granules in the direction of the applied stress (Meuser, 1994).



### *2.4.2 Genetics of gluten composition*

Wheat belongs to the grass family, Poaceae (Gramineae), and makes up the genus *Triticum* L. Bread wheat is a hexaploid with 42 chromosomes, originated from two major evolutionary events in which three diploid species are presumed to have participated. Each of the 21 chromosomes of wheat has been classified into 3 genomes A, B and D, representing the 3 ancestral diploid species. The chromosomes of hexaploid wheat fall into 7 homoeologous groups, each composed of 3 chromosomes and each exhibiting a high level of functional identity. The chromosomes of bread wheat are all either metacentric or submetacentric.

The gluten proteins are controlled by four loci on each genome: one for HMW-GS, named Glu-1; one for LMW-GS, named Glu-3; and two for gliadin, named Gli-1 and Gli-2.

HMW-GS genes are located on the long arm of chromosomes 1A, 1B, and 1D, respectively named Glu-A1, Glu-B1 and Glu-D1. HMW-GS genes have extensive allelic variation, and segregate independently from the Gli-1 loci. Polypeptide bands on the SDS-PAGE gels are identified as specific allele using a lower-case letter, eg. Glu-A1a, (Payne and Lawrence, 1983).

LMW-GS are controlled by genes at Glu-A3, Glu-B3 and Glu-D3 loci on the chromosome short arms 1AS, 1BS and 1DS, respectively. Among a collection of 222 hexaploid wheat from 32 countries, 20 LMW-GS band patterns were detected. Although six

alleles are coded by Glu-A3, many cultivars do not exhibit any LMW-GS controlled by this locus. The greatest polymorphism exists for subunits encoded by Glu-B3, where nine alleles have been identified. Five alleles are coded by Glu-D3 (Gupta and Shepherd, 1990a). LMW-GS have been linked to genes coding for ω- and γ-gliadins. Sreeramula and Singh (1997) found two new LMW-GS, Glu-4 and Glu-5, and suggested that Glu-5 is coded by a gene on Chromosome 7. The location of the Glu-4 gene is not yet known. Both Glu-4 and Glu-5 are faster moving than Glu-3 in SDS-PAGE, and have different amino acid (AA) composition from Glu-1, Gli-1 and Glu-3. Due to the complexity of the patterns and the fact that some of the bands overlap, it is difficult to score these protein subunits on SDS-PAGE unless carried out regularly on a routine base. The nomenclature system of Gupta and Shepherd (1990a) is adopted for naming the LMW-GS alleles in this thesis.

Most genes coding for ω- and γ-gliadin are tightly clustered at three homologous loci at the distal end of the short arm of chromosomes 1A, 1B and 1D, named Gli-A1, Gli-B1 and Gli-D1. They are close to the LMW-GS coding genes Glu-3. Genes coding for α- and β-gliadin are tightly clustered at a single locus on each of the short arms of the chromosomes of group 6, named as Gli-A2, Gli-B2 and Gli-D2 respectively. Other genes coding for ω-gliadins were also found proximal to Gli-1 loci and named Gli-A4, Gli-A5 and Gli-B3 (Metakovsky *et al.*, 1997).

### *2.4.3 Gluten functionality*

On the basis of their amino acid sequences, gluten proteins can be divided into sulphur-rich and sulphur-poor groups due to the distribution of cysteine residues. This in turn leads to three groups based on molecular weight (Shewry *et al.*, 1955; Tatham *et al.*, 1990)

#### *I Disulphide bonds*

The disulphide bond is one of the most important factors affecting and stabilising the structure of proteins. It is formed by the interaction and oxidation of sulphydryl groups of two cysteine residues. If the two cysteine residues comprising the bond are in the same protein chain, the disulphide bridge is intramolecular. Intermolecular disulphide bonds are formed between cysteine residues in two different protein chains. Disulphide crosslinks do not hold the protein molecule in the form of a rigid structure, and they can enter into interchange reactions with other molecules, either large or small, containing free sulphydryl groups. Such molecules may be present naturally in flour, or they may be added intentionally to affect the physical

properties of the dough.

The importance of disulphide bonds in maintaining the structural/functional properties of dough was clearly demonstrated in early experiments by adding reducing agents to the flour mixes. The reducing agents undergo interchange reactions with the disulphide bonds of the gluten proteins, resulting in weaker dough and increased solubility of the glutenin proteins (Sullivan *et al.*, 1940; de Deken *et al.*, 1955; Kauffman *et al.*, 1986). Studies of sulphur deficient grain have shown that the sulphur status differentially affects protein synthesis. Sulphur deficiency increases the synthesis of low sulphur-containing prolamins (most notable the ω-gliadins and HMW-GS), which result in weaker and less extensible dough (Randall *et al.*, 1986).

These studies indicated the importance of intermolecular disulphide bonds in dough/gluten rheology. An understanding of which proteins are disulphide bonded and whether these disulphide bonded polymers are genetically determined, or of a more random nature, should help to explain the differences in glutenin polymers between "good" and "poor" quality wheat (Shewry and Tatham, 1997). Cereal chemists have long been interested in disulphide bonds in gluten because of their role in determining functionality.

### *II Structural models of glutenin*

The polymeric glutenins, in addition to the non-covalent interactions, interact via inter- and intra- molecular disulphide bonds and contribute to elasticity. There is a general correlation between the content of insoluble glutenin polymers and dough strength (Field *et al.*, 1983; MacRitchie, 1987; Gupta *et al.*, 1993a).

The proteins that comprise the polymeric glutenin complex markedly affect the rheological properties of dough. These properties are influenced by a number of factors: the molecular weight distribution of the polymers; the density of covalent (and non-covalent) bonds; the number of disulphide bonds between the polymers; whether they are linear or branched; and the strength of the bonds between individual proteins of the polymer complex.

Disulphide interchange has been investigated extensively as one possible mechanism for explaining the rheological properties of wheat flour dough (Hird 1966; Hird and Yates 1961a/b; Hird *et al.* 1968; Frater and Hird 1963; Frater *et al.* 1960, 1961; Mauritzen and Stewart 1963; Jones and Carnegie 1969a/b, 1971; Jones *et al.* 1974; Redman and Ewart 1967a/b). Their results suggest that a comparatively small number (less than 4%) of the

disulphide bonds present in gluten proteins determines the development time of a dough, and 11-13% determine the resistance to mixing. 25-35% of the total thiol groups are concerned in both dough development and tolerance to mixing. The numbers of rheologically important thiol and disulphide groups were found to differ between wheat varieties, the differences being significantly related to the rheological properties of doughs derived from them. MacRitchie (1987a) found that better quality gluten has higher levels of glutamine and asparagine, and lower levels of salt soluble proteins. Defining the polymeric structure of glutenin, and how it is affected by different compositions of glutenin subunits, will be one of the major challenges in the coming years.

The final structure and properties of gluten depend on amounts and types of specific proteins. The structures of the individual subunits comprising glutenin polymers are now known in some detail, but the polymeric structure of glutenin is little understood. Several models have been proposed.

### *a Head to tail model*

Ewart (1968) proposed his "linear glutenin hypothesis", which explains many of the technologically important characteristics of dough. In this model, glutenin molecules are considered as linear chains of polypeptide subunits called 'concatenations'. It suggested that HMW-GS join head-to-tail through disulphide bonds to provide a backbone to the gluten complex. LMW-GS are crosslinked into the backbone through disulphide bonds, and gliadins are combined into the network by hydrogen and hydrophobic bonds. The important feature of Ewart's model was that the individual subunits of glutenin have a conformation that could be stretched when a shearing force was applied, which conversely recoils to its original conformation (ie. its lowest-energy state) when the force was removed. His model is very similar to that of Field *et al.* (1987), for which there is now considerable experimental evidence.

### *b Tail to tail model*

Kasarda (1989) proposed another model, in which the glutenin subunits are linked by inter-chain disulphide bonds. LMW-GS are linked to each other by disulphide bonds in the unrepetitive C-terminal domains (ie. tail-to-tail) rather than head-to-tail as suggested by Ewart. In this model, the HMW-GS are not linked directly to each other but are linked into the gluten

polymers via LMW-GS. They are linked to N- and C-termini of different LMW-GS, resulting in an anti-parallel packing of the repetitive domains of the LMW-GS.

### *2.4.4 Comparison of glutenin alleles to baking quality*

It is generally accepted that glutenin is the most important variable for breakmaking quality. HMW-GS play a major role, but since LMW-GS are present in much greater amounts, considerable effort has also been made to establish their role in breadmaking quality (Gupta and Shepherd, 1987, 1988; Payne *et al.*, 1987b; Gupta *et al.*, 1989a/c, 1990c/d, 1994b/c; Metakovsky *et al.*, 1990).

Dough resistance correlated best with Glu-B3, which is also the most variable locus (Gupta and Shepherd, 1988). Glu-A3 and Glu-D3 are related strongly to dough extensibility. Allele b of Glu-D3 accounted for nearly all the more extensible wheat, although allele b of Glu-A3 was also present in these extensible wheats (Gupta and Shepherd, 1988; Metakovsky *et al.*, 1990). Some alleles for LMW-GS and HMW-GS have shown cumulative effects on bread making quality (Gupta and Shepherd, 1987; Gupta *et al.*, 1989c; Payne *et al.* 1987b). Thus, a cumulative quality score based on all the three classes of proteins may be a better predictor of quality than one based only on a single class of protein (Metakovsky, 1990).

By using recombinant inbred lines and biotypes, Gupta *et al* (1990c/e, 1994b/c) demonstrated that for Rmax (maximum dough resistance), Glu-A3, allele c is better than e; and for Glu-B3, allele b is better than c. They concluded that the effects of individual Glu-3 or Glu-1 alleles on Rmax were largely additive, thus together accounting for about 80% of the variability in Rmax among these progeny. Interaction between Glu-3 and/or Glu-1 loci also affected Rmax significantly, accounting for 10% of its variation. Glu-D3 showed significant effects on extensibility. They confirmed that dough strength can be improved without increasing grain protein levels, and therefore, without compromising grain yield. They concluded that HMW-GS alone are insufficient to account for differences in quality, and that breeding lines should not be selected or discarded based only on their HMW-GS composition. LMW-GS must also be taken into consideration (Gupta *et al.*, 1989c).

A limited number of biochemical (Autran *et al.*, 1987) and genetic (Pogna *et al.*, 1990) studies have separated the effects of LMW-GS alleles from the genetically linked gliadin (Gli-1) alleles. Their results indicated that the positive effects associated with the Gli-1/Glu-3 alleles were mainly due to the Glu-3 alleles. The alleles at the Glu-A3 locus affected both quantity

and the size of the polymers. The positive effects of the glutenin subunits could be attributed primarily to their capacity to form inter-molecular disulphide linkages. LMW-GS affected the quantity and/or size distribution of the polymers, due to differences in the amounts and type of subunits produced.

However, influence of gliadin alleles on dough strength was evidenced from Italian and French wheat collections (Metakovsky *et al.*, 1997). Some gliadins would be linked, through disulphide bridges to glutenin network. In a large collection of European cultivars, approximately 90%, 60% and 85% of dough strength, tenacity and extensibility were respectively explained, when HMW-GS, LMW-GS, ω-gliadins and grain hardness were included in the explanatory model (Branlard *et al.*, 1997).

In Australian wheat cultivars, LMW-GS provided better predictions than HMW-GS for Rmax (Gupta *et al.*, 1990d). Cornish *et al.* (1993) catalogued information about the gluten alleles of nearly 600 wheats in GENEJAR and summarised that: null alleles are detrimental to extensibility; the Glu-3 pattern of b b b gives the best extensibility, particularly in combination with the Glu-1 alleles b b a; Glu-3 b b c gives excellent extensibility; Glu-B3 c, d and g alleles have medium to weak dough properties, and should be avoided in breeding strategies; and for Glu-3, the best combinations are b b b, b b c, and c b c.

### *2.4.5 Summary and conclusion*

The effects of Glu-1 and Glu-3 alleles in a wider range of genotypes are needed before the ranking can be regarded as fixed for use in predicting dough properties. A better understanding of the effects of individual alleles on quality parameters will provide clearer information for bread making quality breeders.

## *2.5 Nitrogen and Sulfur Effects on Bread Making Quality parameters*

### *2.5.1 Introduction*

Wheat quality is determined by genotypic, agronomic, and environmental factors, and the interactions among them. Environmental factors such as soil fertility, irrigation, location, diseases and pest can all affect wheat quality. Environment can vary the protein content between 7-17% within one cultivar.

In contrast to the considerable research effort directed towards understanding the genetic basis of gluten quality, the effects of environmental factors (soil, climate, agronomic practices, diseases, etc.) have received only minor attention (Autran, 1997).

Various levels of available nitrogen and sulphur have been systematically studied. Wrigley *et al.* (1984a) found that nitrogen variability affects mainly flour protein content, whereas sulphur variability affects protein composition. Flour of low sulphur content gave excessively tough dough having high values for Rmax and long mixograph development times (Moss *et al.*, 1981; Macritchie, 1992).

For quality evaluation of cultivars, it is necessary to distinguish between variance influenced by genotypic effects (G), by environmental effects (E) or environment and genotype interaction (GXE). Fowler and de la Roche (1975) reported a large environmental effect for protein content and a relatively small effect for MPT. The GXE was small compared to the cultivar and environment effects on physical, chemical, or rheological properties. Baker and Kosmolak (1977) found that both cultivar and environment had a large effect on all measured quality parameters and that GXE was relatively unimportant for flour protein content. Baezinger *et al.* (1985) determined that for total protein percentage, the environment effect was greater than genotype effect. Lukow and McVetty (1991) reported that both cultivar and environment had a significant effect on baking quality parameters.

### *2.5.2 Effect of nitrogen*

N fertilisation level influences the accumulation of the different classes of storage and metabolic proteins by modifying the relative composition of the protein pool; it intervenes not only as a quantitative parameter but also as a qualitative one (glutenin to gliadin ratio). The glutenins, and more especially the different types of aggregates, considered to be key quality predictors, are also exposed to several quantitative variations directly related to N fertilisation (Jia *et al*, 1996a).

Scheromm *et al.* (1992) have demonstrated the relationship between N fertilisation and the amount of polymeric fractions and type of aggregation of the proteins. The amount of polymeric fractions has proved to be important in explaining the variation in flour quality (Huebner and Wall, 1976; Bietz, 1984; Dachkevitch and Autran, 1989).

All the key physiological processes that occur during grain filling depend not only on nitrogen fertilisation, but also on the maturation conditions (temperature and water

availability). During this sensitive period, especially after the milky stage, variation in these maturation conditions can disturb both protein accumulation and protein aggregation kinetics (Kasarda, 1989; Randall and Moss, 1990). All these effects can potentially modify the technological quality of wheat flour by increasing or masking the influence of nitrogen supplies.

Lelley *et al.* (1997) demonstrated that N fertiliser substantially increased the total protein content of the endosperm, which improved all the quality parameters (except water absorption). Increased protein content led to an increased ratio of HMW-GS to LMW-GS, due to a relatively higher increase of HMW-GS compared to LMW-GS, and to a changed glutenin / gliadin ratio.

N fertilisation applied at the early stages of wheat plant developments tends to increase yield, whereas N supplied at late stages increases the amounts of all grain protein fractions, which improves the baking quality properties. The responses to increasing N fertilisation of diverse types of glutenin polymers can vary according to growing conditions. Environmental factors appear to be the main source of differences in polymerisation modes and polymer distribution (Jia *et al.*, 1996a&b).

High yield and good bread making quality are important features in today's wheat market. Both can be improved through nitrogen (N) fertilisation strategies, such as the rates and timings of N fertilisation (Martin *et al.*, 1992) and the source of N fertilisation (Peltonen and Virtanen, 1994). Many studies have shown that the increase in flour protein content resulting from N application can lead to changes in protein composition (Branlard *et al.*, 1983; Triboi, 1983; Fullington *et al.*, 1983; Gupta *et al.*, 1992). The repartition between soluble and insoluble polymers causes differences in protein expression and therefore glutenin functionality and dough strength (Jia *et al.*, 1996a).

Schinkel and his colleagues (1990, 1991) found GXE variances and increasing heritability with varying N input. Oettler (1996), Feil and Fossati (1995) found an inverse relationship between grain yield and protein content, and concluded it will be difficult to improve both grain yield and protein level in Triticale. Genotypes that have a high stability show low interaction with the environment, which is desirable for selecting for performance in diverse environments.

### *2.5.3 Effect of sulphur*

Sulphur, unlike carbon and nitrogen, can be utilised in its most highly oxidised naturally occurring form, sulphate. Sulphate reduction is then necessary for the formation of sulphur-containing amino acids and proteins (Schiff and Hodson, 1973; Roy and Trudinger 1970; Shiff, 1980).

Sulphur supply has relatively little effect on protein concentration, but considerable influence on protein quality. The ω-gliadins decrease in proportion as sulphur supply increases. Bread loaf volume increases with sulphur supply because of its association with greater dough extensibility (Randall and Wrigley, 1986). S deficiency limits yields of wheat grain and the grain has low S content, a higher N: S ratio and greater hardness. S deficient flours produce tougher dough, which is more resistant and has lower extensibility (Randall *et al.*, 1981; Moss *et al.*, 1981).

Baking quality improves with increased protein content and sulphur containing amino acids within the protein (Fajersson, 1961; MacRitchie, 1984). Background soil S levels had more influence on the grain S levels than applied superphosphate (Ramins *et al.*, 1975). The importance of adequate S levels in wheat grains for high baking quality has been well established from experiments which have manipulated N & S levels (Wrigley *et al.*, 1980; Moss *et al.*, 1981; Timms *et al.*, 1981). S levels showed no relationship to bake score even though they ranged from 0.10 to 0.17%, therefore the absolute requirement of S for acceptable baking wheat requires further resolution.

### *2.5.4 Interaction of N & S*

The responses of quality to sulphur supply are closely related to responses to nitrogen, since it is the ratio of sulphur to nitrogen in the grain that determines protein quality. Hence, it is possible to induce the effects of sulphur deficiency by excessive application of nitrogen fertiliser. The lack of response of baking quality to very high protein concentrations is related to an increase in N / S ratio in the grain (Schnug *et al.*, 1993). Nitrogen enhances the uptake of sulphur in the flour, and MPT increased with increases in nitrogen and sulphur (Wooding *et al.*, 1994). Late foliar N treatment can induce S-deficiency in the developing grain, presumably due to the S from the roots being unable to keep pace with the acutely increased availability of N (Kosmolark, 1980; Wrigley *et al.*, 1980)

### *2.5.5 Summary and conclusion*

Quality stability is a key objective of wheat breeders, producers and processors, so an understanding of the environmental effects is critical. Ideally, the breeder needs to know the relative size of the genetic and environmental components of the quality variation observed. Even though there has been a lot of research investigating N and S effects on yield and protein content, very little has looked at their combined effect on different quality parameters. Our knowledge of genetic aspects of gluten quality needs to be complemented by a better understanding of gluten quality and environmental factors.

## *2.6 Analysis of the glutenin subunits (GS)*

The unique ability of wheat flour to produce leavened bread depends primarily on the correspondingly unique physical-chemical properties of gliadin and glutenin. It has been proposed that only a fraction of glutenin above a critical or threshold molecular size forms effective molecular entanglements that would contribute to dough strength (MacRitchie, 1994; Gupta and MacRitchie, 1994c). It is difficult to measure the molecular size distribution of polymeric glutenin. Measuring the amount of "functional glutenin", composed of particularly large molecules, is relatively easier from the amount of insoluble residue protein remaining after extraction of flour with suitable solvents. The relationship between dough mixing characteristics and the amount of unextractable residue or polymeric protein in flour is well established, and it supports the view that glutenin molecular size, solubility and functionality are inter-related.

### *2.6.1 SDS-PAGE of GS separation*

Because of its simplicity, speed and the microgram amounts of sample proteins required, SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) has become the most widely used method for determining the complexity and molecular masses of constituent polypeptides in a protein sample. It has proven eminently suitable for characterisation of all glutenins. The SDS denatures the proteins into random coils that carry a negative charge, which allows separation on the basis of size and an estimation of molecular weight (Weber and Osborn, 1969).

The LMW-GS comprise about one-third of the total seed proteins in wheat and are the second most abundant class of storage proteins after gliadins. However, their characterisation in terms of their allelic variation, chromosomal location of genes and influence on functional properties has not received the attention the HMW-GS have. The first determination of the chromosomal location of genes encoding LMW-GS was made using 2-dimensional (2-D) electrophoresis techniques. But these techniques are complicated and slow. The first linkage mapping studies used 1-D SDS-PAGE or a modified 2-D SDS-PAGE procedure that allowed the analysis of up to 20 samples per gel (Singh and Shepherd, 1990a). A two-step 1-D SDS-PAGE procedure for the first time allowed the details of allelic variation, inheritance and functional properties of LMW-GS in a large number of wheat cultivars and segregating progeny to be studied (Gupta *et al.*, 1988, 1989c&1990b). The original two-step procedure was later modified to reduce interference from albumin / globulins and to give improved resolution of LMW-GS (Gupta and Shepherd, 1990a; Singh *et al.*, 1990). Graybosch and Morris (1990) developed an alternative one step 1-D procedure following the sequential extraction procedure of Burnouf and Bietz (1989). Singh (1991) and his colleagues described a much simplified procedure for 1-D separation of glutenin subunits, suitable for rapid screening of a large number of samples. It gives much improved resolution for both HMW and LMW-GS. The procedure is based on a sequential extraction method described earlier by Marchylo *et al.* (1989) and provides a glutenin preparation with very little contamination from other classes of seed proteins. The original method has been modified and adapted to allow rapid glutenin preparation from single kernels. Singh's method takes about 3h to prepare highly purified glutenins from 18 samples and to have them ready for loading on to SDS gels. It is easy to use and highly reproducible. The glutenin banding patterns obtained by this procedure are similar to those obtained using the two step procedure (Singh *et al.*, 1990a). Felix and Branlard (1996, personal communication) further improved Singh's method by modifying the concentration and cross-linkage of acrylamide gel; using a linear gel instead of a gradient gel; and running the gels at a lower constant current and temperature. This technique is simple, rapid, efficient and reproducible. It improves the resolution of both LMW-GS and gliadins, compare to other 1-D methods. Using this technique, genetic analysis of different bread wheats has revealed new subunits and alleles, which could be related to quality parameters of dough.

### *2.6.2 Spectrophotometry for glutenin quantification*

The Pierce BCA (bicinchoninic acid) Protein Assay is a highly sensitive method for the spectrophotometric determination of protein concentration (Smith *et al.*, 1985). This reagent system combines the reaction of protein with Cu2+ in an alkaline medium (yielding Cu1+) with a highly sensitive and selective detection reagent bicinchoninic acid for Cu1+. The BCA Protein Assay offers the researcher a flexible and easy assay procedure, which eliminates the precisely timed reagent additions and vortexing necessary with the Lowry method (Lowry *et al.*, 1951). Additional advantages include compatibility with ionic and non-ionic detergents, a stable working reagent, less protein-to -protein variation than with other methods, and broad linear working ranges with excellent sensitivity (Pierce, 1991).

The Pierce BCA Protein Assay is also flexible. It allows the researcher to choose the best assay protocol for any given situation. Changing the incubation time and/or temperature can change the sensitivity of the assay. Although the final colour does not reach a true endpoint, the colour stability after cooling to room temperature is sufficient to allow absorbency readings of multiple samples.

Fu and Sapirstein (1996a&b) developed a relatively simple spectrophotometric procedure for measuring the concentration of insoluble glutenin in flour. Protein concentration as low as 4ug/ml can be accurately determined in the sample. They found the relationship between insoluble glutenin content determined by this spectrophotometric procedure and dough mixing requirements was very strong. The advantages of the spectrophotometric approach include its simplicity, low cost, small sample size, minimal use of reagents, speed and convenience in handling many samples in a short time.

### *2.6.3 Densitometer for quantification of LMW & HMW-GS*

Densitometers measure the density of contrasting areas on gels. They use procedures that include moving across a matrix and finding areas that are significantly different from the background (ie. the spots or bands), allocating each a numerical location, then measuring and displaying the zone location and dimensions. Densitometers also measure maximum intensity of the zone and more importantly, integrate the areas under the peaks. Peak area values are proportional to signal strength and therefore reflect target concentration (Hawcroft, 1997).

Scanning densitometers use a laser light source and in practice the major limitation to resolution is often the electrophoresis separation and not the scanning procedure. Most

densitometers move the gel at a constant speed perpendicular to a narrow, fixed, parallel light beam, the transmission of which is detected by a photomultiplier. The peak absorption for Coomassie Blue R-250-protein complexes varies between 560-575nm depending on both the protein and the solvent (Hames, 1990).

Some densitometers incorporate automatic integration of the densitometric record, which allows automatic integration of the areas under each peak. By this means the amounts of a single component in different gels may be estimated. Densitometer output is increasingly being computerised by integrated or stand-alone microcomputer hardware. Thus allows the data to be analysed at will, including direct comparison of two or more scans from different gels. Different proteins bind Coomassie Blue to different extents, so quantifying a particular protein by staining requires a standard curve for that particular protein. This applies whether quantification is by scanning or elusion. However, if another protein is used as a standard, this allows the relative amounts of the specific protein to be determined in multiple samples. In this situation the linearity of dye absorbency with mass still needs to be determined for the proteins under study. The overall extent of staining for any protein varies depending on the time period used for staining and de-staining, upon the gel thickness and concentration, both of which affect the diffusion rate of dye molecules. For optimal reproducibility of data, these variables should be kept constant.

## *2.7 Summary*

One dimensional SDS-PAGE has been combined with a protein extraction procedure to give a simpler and more reliable protocol for obtaining well resolved patterns of gliadin, HMW-GS and LMW B and C subunits from the endosperm of a single wheat grain (Singh *et al.*, 1991; Gupta and MacRitchie, 1991a). It has greatly facilitated genetic and other studies on these seed storage proteins.

It is possible to determine the SDS-PAGE band patterns of segregating lines in a wheat breeding program, and if the information about band combinations that are important for quality parameters is available, selection for quality potential can be made at a very early stage in the breeding program. Much is known about the influence of the different HMW glutenin alleles on quality parameters (Payne, 1987a), but less information is available on the effects of

LMW subunits on quality and particularly the interaction between the different alleles at these loci.

To approach the problem, this PhD research created a set of recombinant inbred lines (RILs) from wheat populations involving New Zealand cultivars or lines, and measured their quality parameters and gluten protein characteristics to find out:

- whether there are significant differences between the different alleles at Glu-3 (LMW-GS) loci for different quality parameters,
- how these alleles affect the quality parameters,
- whether there is an additive effect with HMW-GS and which combinations offer the best potential quality
- and the predicability of the quality parameters in RILs by gluten protein analysis

As the stability of quality is a key objective for wheat breeders, producers and processors, it is essential to understand the effects of environment. Even though there has been a lot of research on the N and S effect on yield and protein content, very little has looked at their effect on other quality parameters.

To investigate the N, S effect on baking quality, this project designed a field trial involving 14 cultivars/lines and measured their quality responses to different N and S treatments in order to clarify:

- whether N, S affect quality
- the extent of the interaction between genotype and N, S fertilisation on the quality parameters,
- and the predicability of the quality parameters for GXE material

Both sets of results, for the GXE and RILs, also provide a better understanding of the relationship among the quality parameters measured.

# Chapter 3

# The Effect of Nitrogen and Sulphur Fertilisation and their Interaction with Genotype on Wheat Glutenins and Quality Parameters

## *3.1 Abstract*

The effects and interactions of Nitrogen, Sulphur and genotype on baking quality parameters have been investigated on 14 New Zealand wheat cultivars or lines. N and S treatments were applied separately early and late during the growing season, and late N and S were also supplied together. For each of 168 samples generated by the experiment, we analysed the amount of HMW-GS and LMW-GS, and measured quality parameters such as: grain hardness, protein content, Pelshenke, SDS sedimentation and mixograph rheology properties. The results show that: (a) Genotype has a strong influence on all the tested quality parameters and is the greatest source of quality variation. Genotype is also the only significant source for the quantity variation of HMW-GS and LMW-GS. (b) N application increases all the tested quality parameters. N application timing is not generally significant, but late N application produces the greatest effect. (c) Late S application is not necessary for optimising most of the tested quality parameters. However, late N & S together maximise the Pelshenke values and mid-line peak value of the mixograph. (d) Of the 14 tested NZ cultivars, the genotype Kotare has the highest quantity of glutenin, HMW-GS & LMW-GS, flour protein percentage, SDS sedimentation and mid-line peak value. It also has above average hardness and Pelshenke results, and shorter mid-line peak time values. (e) Good quality lines are recommended for specific and diverse environments.

**Keywords**: baking quality; HMW-GS; LMW-GS; quality parameters; hardness; Pelshenke test; SDS sedimentation; mixograph; nitrogen; sulphur; genotype by environment (GxE) interaction

## *3.2 Introduction*

In contrast to the considerable research effort directed towards understanding the genetical bases of gluten quality, environmental factors have received relatively little attention (Autran 1996). Genotype has generally been found more important than environment for gluten quality parameters in bread and durum wheats, except for protein content. GXE interaction is small compared to cultivar and environment effects (Ames *et al.*, 1999; Baker and Kosmolak, 1977; Baezinger *et al.*, 1985; Fowler and de la Roche, 1975). Baking quality was significantly affected by both cultivar and environment (Lukow and McVetty, 1991). Oettler (1996), Feil and Fossati (1995) found genotypes that had a high stability showed low interaction with the environment. This is desirable if selecting for use in diverse environments.

High yield and good bread making quality are important features in today's wheat market. Both of these features can be improved through nitrogen (N) fertilisation strategies, such as the rates and timings of N fertilisation (Jia *et al.*, 1996; Martin *et al.* 1992) and the source of N fertilisation (Lelly *et al.*, 1996; Peltonen and Virtanen, 1994). Wrigley *et al.* (1984) found that N variability mainly affects flour protein content, whereas sulphur variability affects protein composition. However, some studies have shown that the increase in flour protein content resulting from N application can also lead to changes in protein composition (Fullington *et al.*, 1983, Gupta *et al.*, 1992). Increased total protein content improved all the quality parameters (except water absorption). It was also associated with an increased ratio of HMW-GS to LMW-GS, due to a relatively greater increase of HMW-GS amount compared to LMW-GS amount.

Both grain yield and quality responses to S fertiliser have been found to be associated with unfertilised grain S concentations of $< 0.12\%$ and N:S ratios greater than 17:1 in the grain. These critical values are now generally accepted for bread making wheat (Haneklaus *et al.*, 1992 a&b; Moss *et al.*,1981; Randall *et al.*, 1981; Randall and Wrigley, 1986; Wrigley *et al.*, 1984b). N enhances the uptake of S in the flour, and mixograph optimum mixing time increased with increases in N and S (Wooding *et al.*, 1994).

Quality stability is a key objective of wheat breeders, producers and processors, so an understanding of the environmental effects is critical. Ideally, the breeder needs to know the relative size of the genetic and environmental components of the quality variation observed. Even though there has been a lot of research investigating the N, S effect on yield and protein

content, very little attention has been addressed at their combined effect on different quality parameters.

Our aim for this work was fourfold. (1) To define the variation of quality parameters induced by N, and S treatments, genotypes and the interaction between the genotypes and treatments. (2) To quantify the effects of N and S fertilisation, alone and in combination on HMW-GS & LMW-GS amounts in the grain, and other baking quality parameters. (3) To differentiate the effects of N, S supply and timing in relation to wheat quality parameters. (4) To test the quality performance of the 14 NZ cultivars and their response to the environment.

## *3.3 Materials and Methods*

### *3.3.1 Field Experiment*

Fourteen New Zealand wheat cultivars or lines were grown in a field trial at the Henley Block of Lincoln University (43° 39′ S, 172° 28′ E). The soil type was a Templeton Silt Loam. The previous crop of barley was not fertilised in order to reduce soil N availability. The sample for soil testing was collected from 40 spots randomly around 0.57 hectare for the trial. The S concentration from the soil test result was 8ppm.

The soil test results had shown relatively low P (phosphate) (16ppm), while preparing the soil, triple super-phosphate (100kg/ha, 1-2% S and 23% P) was applied.

The trial had 6 treatments (T1-T6) differing in the amount and timing of N and S application. The 6 treatments are listed in Table 3-1. Each treatment had two replications. Eighty grams of seeds were sown for each individual 6 $m^2$ plot, the sowing date was 20/9/95.

**Table 3-1.** Treatments applied to the GXE trial

| Treatment | T1 | T2 | T3 | T4 | T5 | T6 | Note |
|---|---|---|---|---|---|---|---|
| Time | | early | late | early | late | late | early=booting; late=flowering |
| N (kg/ha) | 0 | 0 | 0 | 200 | 200 | 200 | Nitrolime: 27% N (466g/plot) |
| S (kg/ha) | 0 | 50 | 50 | 0 | 0 | 50 | Gypsum: 18% S (175g/plot) |

Prior to machine harvesting of the entire plot, random heads were hand harvested from

each plot. There were a total of 168 samples available for each quality test. The hand harvested grain was used for quantifying LMW-GS and HMW-GS through the combined methods of spectrophotometry, SDS-PAGE and densitometer scanning; the machine harvested grain was used for all the other quality tests.

### *3.3.2 Analytical methods*

Analysis of the material was carried out at INRA, Station D'Amelioration des Plantes, Clermont-Ferrand, France.

#### *I Protein separation by SDS-PAGE*

All 168 samples were tested by SDS-PAGE. The protocol is based on Singh *et al.* (1991). In order to have better resolution for both HMW-GS and LMW-GS, the concentration of the gel (T) and the cross linker (C) were modified as follows: T = 12.8%, C = 0.99%. This is standard practice for analysis in the lab of Dr. G. Branlard, INRA, Clermont-Fd, France.

#### *II Glutenin concentration by pectrophotometry*

The Pierce BCA Protein Assay protocol (Pierce, 1991) was used for the spectrophotometer measurements.

Samples were prepared for SDS-PAGE and spectrophotometry following the same initial steps. Gliadin was removed by 50% propanol, the supernatant containing the gliadin was discarded. Then 500µl of extracting solution was added to the residue. This solution consisted of 0.05M sodium phosphate and 2% SDS. After vortexing, the solution was left to extract for 1h, followed by sonication for 30s at 10W, then centrifuged for 5 minutes at 10,000g. The supernatant was then divided into two samples of 100µl and 200µl. The 200µl sample was prepared for SDS-PAGE using the method of Singh *et al.* (1991). The 100µl sample was added to 400µl of water to make a final amount of 500µl. Then 100µl was taken and mixed with 2ml working reagent (Pierce, 1991) in a labelled test tube. The tube containing the sample was incubated at 37°C for 30min, cooled to room temperature and 1ml pipetted into measuring wells, and the absorbancy measured at 562nm against a standard. The standard was prepared and measured simultaneously with each load of samples. A standard curve was prepared with a sequence of protein (BSA) concentrations: 0, 100, 200, 300, 400 µg/ml. The samples were read on Kontron Instruments-Uvikon 930. The concentration of each sample was

calculated using the CALC. MODE procedure.

### *III Densitometry*

The densitometer system used for analysing the GXE glutenin subunits gel was a Hoefer Scientific Instruments, GS 365 W Version 3.02.

After electrophoresis, the gel was placed between glass plates, fixed by Sellotape, the HMW & LMW-GS zones were marked, the clearest area on the gel used to set as background zero, and the darkest band set at 80% for Gain Control. After scanning, data smoothing and quick integration, the areas and percentages of HMW-GS and LMW-GS were determined. The absolute amount of HMW-GS and LMW-GS were calculated from the combined results of the Densitometer and Spectrophotometer measurement. Preliminary measurements had proved linear relationship between glutenin subunits quantity and Coomassie stained subunits.

### *IV Quality tests*

A Near Infrared Reflectance (NIR) instruments (Inframatic 8620 Perten Instruments, Hamburg, Germany) was used to estimate the wholemeal flour protein content and hardness, white flour protein content and moisture level. This result was also used for calculating the amount of water to add to the flour for the mixograph test (Martinant *et al.*, 1998).

For the SDS sedimentation test, 5g of wholemeal flour was used, according to the procedure described by Axford *et al.* (1979).

The Pelshenke test was carried out by using 10g of wholemeal flour. The original procedure (Pelshenke, 1933) was employed for determining the dough swelling time. A temperature controlled cabinet was used to standardise the dough swelling conditions.

Mixographic measurements were carried out on a 10g flour sample (AACC Method 54-40A, 1992). Dough hydration was obtained by taking into account flour protein content, flour moisture and grain hardness (Martinant *et al.*, 1998). The mixograph curves were computed by Mixsmart® software. Mid-line Peak Value (MPV) and Mid-line Peak Time (MPT) were the major parameters used in our study.

### *V Statistical analysis*

Statistical analysis was by the computer statistics software Statgraphics Plus, Minitab and SAS for Windows.

## *3.4 Results and Discussion*

An understanding of the results of the trial requires an understanding of the significance as well as the absolute and relative magnitudes and the individual optima of the main effects and of the interactions. In addition the stability and individual magnitudes of the interactions for each wheat cultivar are important in determining their utility and limitations in a wheat quality breeding program. Therefore, the discussion will follow this sequence in the presentation of the results.

### *3.4.1 The significance of the main effects*

**Table 3-2.** Overall trial quality parameter mean values, ranges and significance. values (P values) for the main effects of; N/S treatment (T) and genotype (G) and their interactions

| Source | Glutenin Quantity (mg/100mg flour) | HMW-GS Quantity (mg/100mg flour) | LMW-GS Quantity (mg/100mg flour) | Whole-meal Flour Protein (%) | Flour Protein (%) | Hard-ness | Pelsh-enke (min) | SDS Sedimen-tation (ml) | Mid-line Peak Value (%) | Mid-line Peak Time (min) |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameter Abbreviation | GQ | HQ | LQ | WFP | FP | HAR | PEL | SED | MPV | MPT |
| Mean | 4.3 | 1.2 | 3.2 | 11.8 | 10.9 | 70 | 145 | 52 | 47 | 3.7 |
| Upper Limit | 6.9 | 2.2 | 5.6 | 17.2 | 17.0 | 100 | 254 | 79 | 72 | 7.9 |
| Lower Limit | 2.6 | 0.6 | 2.0 | 9.5 | 7.9 | 42 | 32 | 26 | 37 | 2.0 |
| Treatment P | 0.053 | 0.118 | 0.151 | **0.000** | **0.000** | **0.000** | 0.076 | **0.000** | **0.000** | 0.273 |
| Genotype P | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** |
| Interaction P | 0.993 | 0.953 | 0.999 | **0.000** | **0.000** | **0.014** | 0.816 | 0.369 | **0.028** | 0.721 |

(Values in **bold** are significant at $p < 0.05$)

# These abbreviations will be used throughout for the indicated quality parameters.

As shown in table 3-2, the fertiliser treatments had significant effects on half of the measured quality parameters. These significant effects were for "wholemeal flour protein percentage", "flour protein percentage", "grain hardness", "SDS Sedimentation value", and "Mid-line Peak Value". The genotypic effect was significant on all measured parameters. The interaction between treatment and genotype was significant for WFP, FP, hardness and Mid-

line Peak Value. Generally (four out of five times) if the treatment effect was significant so was the interaction effect. Nitrogen & Sulphur fertiliser addition treatments and their interaction with genotype did not significantly affect the quantity of HMW-GS and LMW-GS. Glutenin subunits quantity was strongly under genetic control, though the treatment effect was close to ($p=0.053$) significant for total glutenin quantity.

Like Fowler and de la Roche (1975), our results show a significant environmental effect on flour protein content, and mixograph peak value, but not on mixograph peak time. Our results also support Baker and Kosmolak's (1977) observation, that cultivar had a large effect on all quality parameters. However, our results differ from theirs by showing that environment only had significant effects on some of the quality parameters, and that the GxE interaction in our trial was significant for flour protein content.

### *3.4.2 Relative magnitudes of the trial effects on the measured quality parameters*

**Table 3-3.** The relative magnitude of the range of variation induced in the measured quality parameters by Treatments (T), Genotypes (G), and their interaction (TxG)

| Source | Glutenin Quantity | HMW-GS Quantity | LMW-GS Quantity | Whole-meal Flour Protein | Flour Protein | Hard-ness | Pelsh-enke | SDS Sedimen-tation | Mid-line Peak Value | Mid-line Peak Time |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | 11.8* | 15.4 | 12.2 | 8.3 | 10.3 | 10.7 | 11.8 | 11.0 | 7.6 | 9.7 |
| Genotype | 25.7 | 40.2 | 29.6 | 17.3 | 22.5 | 32.7 | 69.2 | 35.1 | 23.5 | 48.6 |
| Interaction | 45.2 | 65.6 | 54.2 | 32.3 | 41.4 | 48.3 | 96.3 | 53.3 | 37.5 | 69.5 |

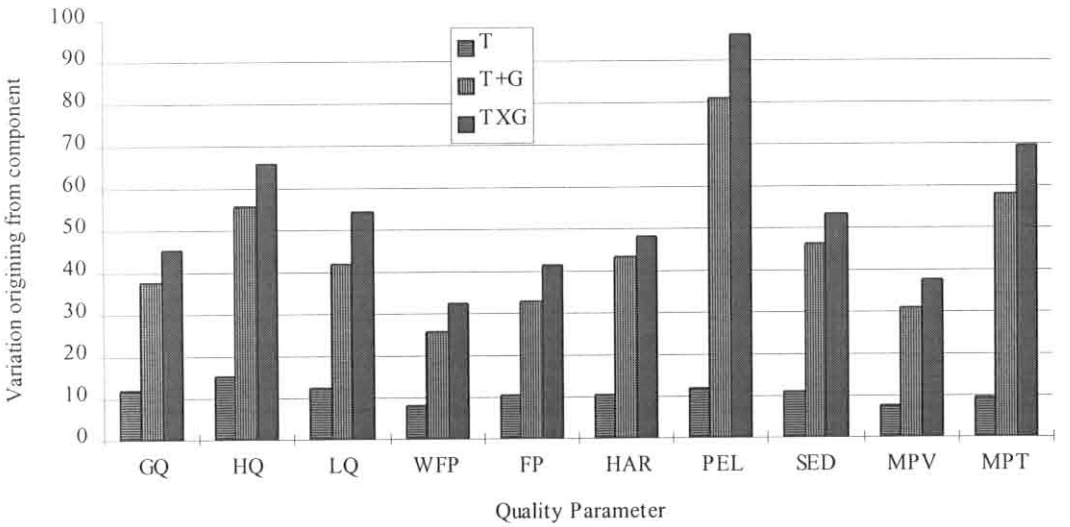
***Values in the table are calculated as**

$$[(A-B)/(A+B)]/2*100$$

Where A = (highest mean value), and B=. (lowest mean value)

**For each of the Treatment, and Genotype main effects and their Interaction.**

**Figure 3-1.** The relative magnitude of the range of variation induced in the measured quality parameters by Treatments (T), the potential additive range of Treatments plus Genotypes (T+G), and the actual range of the interaction (TxG) showing the additional increase in range due to the interaction




**Values in the vertical axis are calculated as referred in Table 3-3**

The abbreviations used for the quality parameters on the horizontal axis are the same as used in Table 3-1.

The three bars for each parameter represent the variation induced by treatment (left hand bar), treatment + genotype (middle bar), treatment x genotype (right hand bar).

Though frequently significant, as indicated in Table 3-2, the variation in mean values resulting from the treatment effects was less than 16% (and mostly less than 12%) for all parameters irrespective of their significance (Table 3-2 & Fig 3-1). Variation due to genotype was greater in all cases (averaging 34% across the 10 quality parameters) particularly for HMW-GS, LMW-GS, hardness, Pelshenke, SDS sedimentation and mid-line peak time. The maximum variation for all parameters was when the GXE interaction was also taken into account. Breeding strategies must take this interaction effect into account, although this additional variation never exceeded 10%. The additional effects of the interactions were generally small, approximally equal to the environmental effect. This suggests that generally when N & S fertiliser level was the cause of the range of environments, the genotype controlled

the wheat quality, which simplifies breeding for enhanced quality.

In contrast to our results Baezinger *et al.*, (1985) found the environmental effect was greater than the genotypic effect. From our results, although protein was significantly affected by both the main effects and their interaction, the genotypic variation for protein content was greater.

### *3.4.3 Treatment effect on measured quality parameters*

**Table 3-4.** Mean values for the individual treatments for the measured quality parameters

| Treatment | | | Glutenin Quantity (mg/100mg flour) | HMW-GS Quantity (mg/100mg flour) | LMW-GS Quantity (mg/100mg flour) | Whole-meal Flour Protein (%) | Flour Protein (%) | Hard-ness | Pelsh-enke (min) | SDS Sedimen-tation (ml) | Mid-line Peak Value (%) | Mid-line Peak Time (min) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *No* | *Time of Applica-tion* | *Applied Ferlizer* | | | | | | | | | | |
| T1 | | | 4.32 | 1.17 | 3.15 | 11.24 | 10.19 | 65.04 | 140.14 | 49.64 | 45.53 | 3.85 |
| T2 | E* | S | 4.14 | 1.15 | 2.99 | 11.33 | 10.34 | 66.72 | 149.43 | 51.93 | 46.26 | 3.76 |
| T3 | L | S | 4.09 | 1.09 | 3.00 | 11.02 | 9.95 | 64.81 | 142.29 | 48.21 | 44.44 | 3.90 |
| T4 | E | N | 4.52 | 1.23 | 3.29 | **12.46** | **11.59** | **74.28** | 135.29 | **56.11** | **49.12** | 3.59 |
| T5 | L | N | 4.59 | 1.26 | 3.32 | **12.53** | **11.64** | **75.12** | 148.64 | 52.32 | 48.58 | 3.67 |
| T6 | L | S&N | 4.38 | 1.21 | 3.17 | **12.44** | **11.59** | **72.08** | 151.61 | **55.57** | **49.35** | 3.61 |
| LSD (at p<0.05) | | | 0.38 | 0.16 | 0.30 | 0.61 | 0.71 | 6.69 | 29.14 | 5.57 | 3.15 | 0.54 |

(Values in **bold** are significant different from T1 at $p < 0.05$)

*E = early, L = late as in Table 3-1.

In this trial the application of any N fertiliser significantly increased protein quantity and grain hardness (Table 3-4). Application of early N and late N+S significantly increased SDS sedimentation and mid-line peak value. Application of late N alone also increased these values, though not significantly, suggesting the effect was also a general effect of increased N availability. The addition of Early or Late N alone or in combination with late S caused increases, although non-significant, in HMW-GS, LMW-GS, glutenin quantity, SDS sedimentation and mid-line peak value. The statistical contrast of [Early or Late N] against [Early or Late S] was, however, significant ($P<0.05$) for HMW-GS, LMW-GS, and glutenin quantity. In all instances the S fertilised treatments mean was lower than the N treatments and the unfertilised control lay closer to the S treatments mean than the mid point of the two fertilised treatments values. No treatment had a significant effect on Pelshenke or mid-line peak time. The use of S fertilisation alone had no significant effect on any parameter relative

to the control.

As concluded by Fajersson (1961) and MacRitchie (1984), S doesn't seem to play a crucial role in controlling the quality parameters measured. Application of S did however, maximise the results for Pelshenke and mid-line peak value when applied late together with N. Applied alone it may possibly have reduced the glutenin quantity by a relatively small amount (4% on average in this trial). This result supports the conclusions of Wooding *et al.*, (1994).

Our observations on the effects of N agree with the results of Martin *et al.*, (1992), Wrigley *et al.*, (1984), Jia *et al.*, (1996) and Lelly *et al.*, (1996). Our results for S have not shown the longer mixing times and greater hardness with lower S, found by Moss *et al.*, (1981) and Randall *et al.*, (1981). According to Martin (1997), when the soil S concentration is >4ppm, normally S application will have not give a yield response. As our soil S concentration was 8ppm, the lack of effect on quality was not unexpected.

### *3.4.4 Quality comparison among the genotypes*

**Table 3-5.** Genotype mean values for the measured quality parameters

| Genotype | | Name* | Glutenin Quantity (mg/100mg flour) | HMW-GS Quantity (mg/100mg flour) | LMW-GS Quantity (mg/100mg flour) | Whole-meal Flour Protein (%) | Flour Protein (%) | Hard-ness | Pelsh-enke (min) | SDS Sedimen-tation (ml) | Mid-line Peak Value (%) | Mid-line Peak Time (min) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Identity Number | Quality Class# | | | | | | | | | | | |
| 1 | B | Kokako-s | 4.19 | 1.07 | 3.12 | 11.20 | 10.23 | 63.48 | 216.75 | 60.67 | 50.25 | 4.20 |
| 2 | B | Oroua-f | 3.78 | 1.06 | 2.73 | 12.00 | 10.98 | 80.83 | 146.75 | 46.00 | 43.62 | 3.66 |
| 3 | A | Domino-f | 4.23 | 1.16 | 3.07 | 11.42 | 10.34 | 70.49 | 159.50 | 60.33 | 44.93 | 5.16 |
| 4 | A | PB13058-w | 4.39 | 1.23 | 3.16 | 11.88 | 10.81 | 85.99 | 121.17 | 64.92 | 45.94 | 4.01 |
| 5 | D | Impact-f | 4.24 | 1.11 | 3.13 | 10.68 | 9.41 | 67.16 | 62.33 | 45.42 | 43.23 | 3.00 |
| 6 | D | Larnoch-w | 4.03 | 0.92 | 3.11 | 12.30 | 10.74 | 63.24 | 55.25 | 39.92 | 41.22 | 4.83 |
| 7 | A | Kotare-w | 5.23 | 1.15 | 4.09 | 14.37 | 13.82 | 76.39 | 174.42 | 70.50 | 62.08 | 2.70 |
| 8 | C | Karamu-s | 3.74 | 0.86 | 2.88 | 10.85 | 9.86 | 70.54 | 57.25 | 38.00 | 45.84 | 2.23 |
| 9 | A | Otane-s | 4.20 | 1.27 | 2.93 | 10.85 | 10.08 | 57.53 | 159.83 | 52.17 | 46.88 | 4.39 |
| 10 | D | Pernel-w | 4.26 | 1.00 | 3.26 | 12.33 | 11.11 | 73.16 | 175.17 | 58.25 | 48.04 | 3.90 |
| 11 | B | Rongotea-f | 4.63 | 1.38 | 3.26 | 12.25 | 11.55 | 78.13 | 156.17 | 48.83 | 51.45 | 3.03 |
| 12 | D | Tui-w | 4.42 | 1.38 | 3.04 | 11.83 | 11.08 | 83.37 | 156.75 | 43.08 | 44.22 | 2.75 |
| 13 | A | Morahi-s | 4.69 | 1.55 | 3.14 | 12.19 | 11.71 | 48.98 | 193.08 | 50.75 | 49.27 | 3.72 |
| 14 | B | 27.330-f | 4.73 | 1.46 | 3.27 | 11.56 | 10.62 | 56.18 | 189.50 | 53.33 | 44.02 | 4.64 |
| Average | | | 4.34 | 1.19 | 3.16 | 11.84 | 10.88 | 69.68 | 144.57 | 52.30 | 47.21 | 3.73 |

#Rankings of the Genotypes into quality classes (A = best, D = worst) were based on trial

results, commercial baking data and experience, and were subjectively ranked by Dr. W. B. Griffin (wheat breeder with Crop and Food Crown Research Institute Lincoln, New Zealand)
*For the cultivars, -s = spring wheat; -f = spring/winter wheat; -w = winter wheat.

There was a large range of values for all quality parameters, across the genotypes as indicated in Table 3-5. Genotype 7 (Kotare), a very good baking quality cultivar, had the highest protein content, SDS sedimentation value and mid-line peak value, but amongst the lowest mid-line peak times and HMW-GS to LMW-GS ratios. Genotype 8 (Karamu), a very poor baking quality cultivar, had low values for all parameters except hardness.

Branlard *et al.* (1992) indicated that wheat quality cannot be evaluated by a single indirect quality test, because numerous influential factors are involved and our data agree with that conclusion. Therefore, multivariate analyses and comparison of these parameters and overall quality are needed for these data, which are presented in chapter 4 of this thesis. Evaluation of the relationships between the individual quality parameters, and determination of the most influential individual quality parameter in relation to the overall baking quality will be carried out in Chapter 4.

### *3.4.5 The individual line responses to the environment*

In order to understand the individual reactions of each genotype to the changing N and S fertilisation environments, we analysed each genotype's regression for a parameter relative to the average performance of all the material from this trial for that parameter using the model of Finlay and Wilkinson (1963). Thus a genotype with exactly average response to the environment would have an R-square of 100% and slope of 1. Only the quality parameters with significant GxE interactions (as shown in Table 1) were analysed. The results of this analysis are shown in Table 3-6 and Figure 3-2.

**Table 3-6.** Finlay & Wilkinson (1963) regression analysis results showing significance for individual lines relative to the average response of 14 wheat cultivars for three quality parameters that showed a significant Genotype X Treatment (GXT) interaction

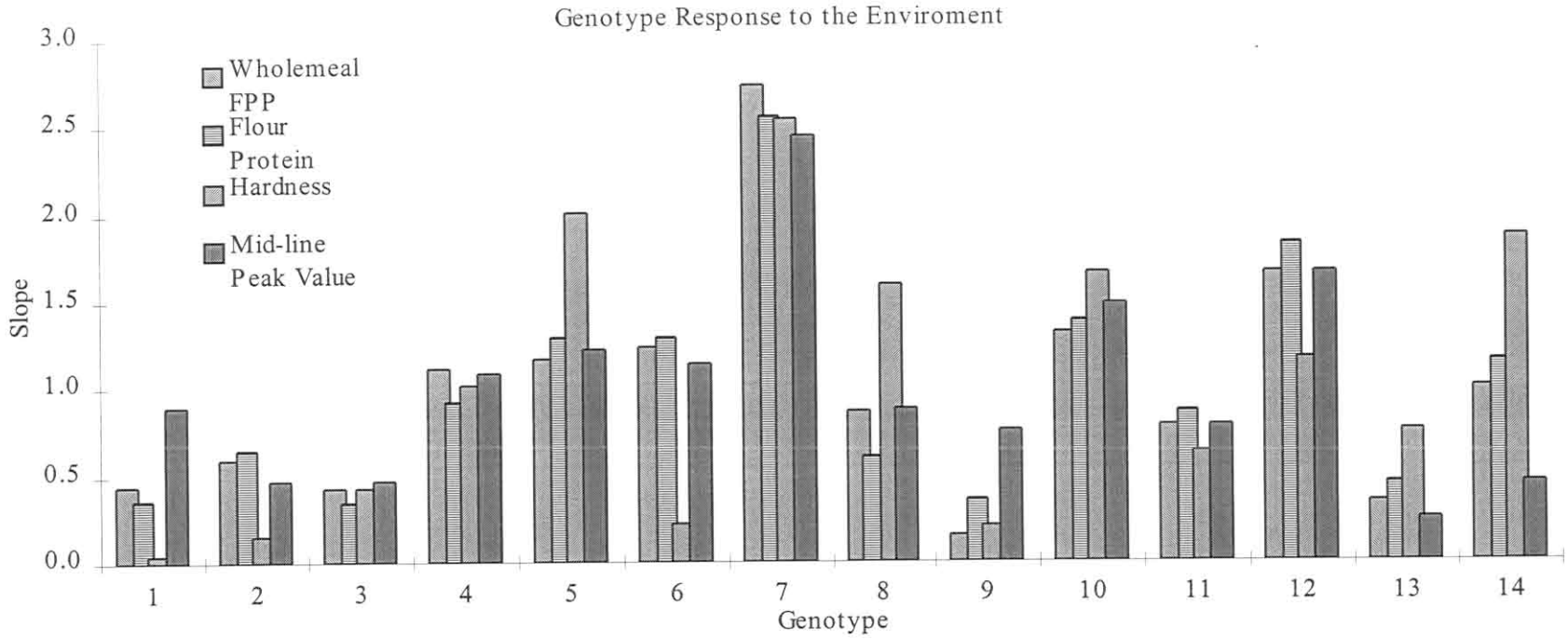
| Genotype | Name of | Wholemeal FP | | | | Hardness | | | | Mid-line Peak Value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| quality | the Cultivars | R-sq% | P value | Min | Max | R-sq% | P value | Min | Max | R-sq% | P value | Min | Max |
| B | Kokako-s | 7.2 | 0.204 | 9.8 | 12.3 | 0.0 | 0.930 | 47.9 | 71.5 | 10.9 | 0.157 | 43.6 | 59.9 |
| B | Oroua-f | 12.2 | 0.143 | 10.8 | 13.3 | 0.0 | 0.676 | 70.0 | 86.3 | 0.0 | 0.377 | 38.4 | 49.5 |
| A | Domino-f | 48.1 | **0.007** | 10.7 | 12.1 | 6.2 | 0.218 | 64.2 | 80.1 | 32.1 | **0.032** | 42.1 | 47.9 |
| A | PBI3058-w | 66.7 | **0.000** | 10.6 | 13.0 | 27.9 | **0.045** | 74.4 | 99.6 | 47.5 | **0.008** | 40.6 | 51.0 |
| D | Impact-f | 83.1 | **0.000** | 9.5 | 11.8 | 66.8 | **0.000** | 51.7 | 85.2 | 42.0 | **0.013** | 37.7 | 53.0 |
| D | Larnoch-w | 87.7 | **0.000** | 10.8 | 13.5 | 0.0 | 0.409 | 58.1 | 71.1 | 59.2 | **0.002** | 37.0 | 46.7 |
| A | Kotare-w | 86.7 | **0.000** | 11.9 | 17.2 | 61.4 | **0.002** | 45.7 | 96.8 | 58.5 | **0.002** | 54.2 | 71.9 |
| C | Karamu-s | 60.5 | **0.002** | 9.6 | 12.2 | 45.0 | **0.010** | 52.8 | 86.8 | 44.2 | **0.011** | 42.6 | 51.8 |
| A | Otane-s | 0.0 | 0.466 | 10.4 | 11.8 | 0.0 | 0.689 | 43.7 | 66.6 | 19.8 | 0.082 | 42.8 | 53.6 |
| D | Pernel-w | 82.7 | **0.000** | 11.0 | 13.6 | 76.7 | **0.000** | 59.7 | 84.6 | 74.5 | **0.000** | 43.0 | 53.3 |
| B | Rongotea-f | 54.2 | **0.004** | 10.8 | 13.3 | 7.0 | 0.206 | 64.7 | 90.7 | 35.5 | 0.240 | 46.4 | 55.9 |
| D | Tui-w | 85.6 | **0.000** | 10.1 | 13.5 | 63.5 | **0.000** | 72.8 | 97.6 | 66.9 | **0.000** | 38.4 | 50.9 |
| A | Morahi-s | 5.0 | 0.238 | 11.2 | 13.7 | 41.9 | **0.014** | 42.3 | 60.2 | 0.0 | 0.506 | 45.5 | 55.0 |
| B | 27.330-f | 72.6 | **0.000** | 10.2 | 12.5 | 84.8 | **0.000** | 43.6 | 72.9 | 5.1 | 0.236 | 40.5 | 49.2 |

(p values<0.05 are in **bold**, and represent a significant correlation with the group average performance for the indicated quality parameter)

From Table 3-6, genotypes 4 (PBI3058), 5 (Impact), 7 (Kotare), 8 (Karamu), 10 (Pernel) and 12 (Tui) significantly correlated with the group reaction to the environment for all of the four tested parameters. This suggests these cultivars respond to the environment in a way representative of many wheat cultivars for all the measured quality parameters. They could therefore be used as controls for measuring generalised environmental effects on quality. Genotype 1 (Kokako), 2 (Oroua) and 9 (Otane) did not correlate with the group reaction to the environmental effect for any of the quality parameters which showed a significant GXT interaction. This implies the form of their response is different from the average response in any particular environment, at least for those modified by different N and S fertilisation regimes. This response may be positive, so they may be beneficial in environments which are

usually deleterious to quality. However, careful scrutiny of their response in each environment would be needed. Their lack of average response means they would make poor controls for assessing environmental effects on quality.

**Figure 3-2.** Slopes of the responses of individual genotypes to the environment in comparison to the average group performance across all lines for a Finlay & Wilkinson (1963) regression analysis




*Number of the genotype are presented as in Table 5&6.

In Figure 3-2, cultivars whose slope is >1 are more responsive to changes in the environment. Their improvement in response is greater than the average genotype in response to a favourable environment. Equally their deterioration in response is greater in poorer environments. Thus, they show a greater than average environmental effect. Kotare due to its very high mean values in the "good" environment, was still quite good even in the "poor" environment (see maximum & minimum in Table 3-6). Those with a slope<1 respond to the environment less than the average response, ie. they do not change much with differing environments. The smaller the slope, the more stable the genotype performance in different environments. Thus, genotypes such as Otane & Domino showed better stability than average across all environments.

This information is of benefit to both wheat breeders and wheat farmers. Both need to understand how their cultivars might respond to differing environments. Highly responsive types might provide the best quality in a particular area, on a particular soil type, or in a particular season, but less responsive types might be more suitable for general recommendation over a wider area or across seasons. For these cultivars however, this relationship need not hold for environmental changes induced by treatments other than the N & S fertilisation regimes used here.

## *3.5 Conclusions*

In these trials we found that the amount of HMW-GS and LMW-GS are genetically determined and their quantity is increased only slightly by (late) N application, although not to a significant level. N application does, however, significantly increase the wholemeal and white flour protein percentage, hardness, SDS sedimentation and mid-line peak value of the mixograph. It also increases Pelshenke time, but not significantly.

The variation in quality parameters is mostly a result of the genotypic difference. The interaction of fertiliser regime and genotype also significantly affects wholemeal and white flour protein percentage, hardness and mid-line peak value.

The good quality cultivars: PBI 3058 and Kotare are recommended when grown in specific environments; other high quality lines: Domino, Otane and Morahi are recommended for their stable quality performance over more diverse environments.

Further investigations will be carried out in attempt to reveal: (a) the relationship of the individual quality parameters with and without the environmental effect, and (b) the relationships between the quantity of glutenin, HMW-GS & LMW-GS and the measured quality parameters.

# Chapter 4

# Relationships among Quality Parameters and Wheat Glutenins

## *4.1 Abstract*

Quality parameters were measured on two sets of New Zealand wheat samples collected from: 1) a field trial designed to investigate genetic by environmental variation (GXE), and 2) F4 progeny from 5 different populations involving 6 parents. The measured quality parameters included wholemeal flour protein content (WFP), white flour protein content (FP), grain hardness (HAR), SDS sedimentation volume (SED), Pelshenke time (PEL), and mixograph mid-line peak value (MPV) and mid-line peak time (MPT). For the GXE material the HMW-GS and LMW-GS were also quantified. Relationships investigated included those: (1) among quality parameters and quantity of HMW-GS/LMW-GS/glutenin, (2) among all the measured quality parameters for the GXE material, (3) among quality parameters for the F4 progeny. The first set of relationships provided an indication of whether allelic difference or quantity of glutenin was likely to affect the quality parameters measured. The latter two sets of data were used to create regression predictions of SED, PEL, MPV and MPT for the GXE and F4 materials respectively. Predictions of these more difficult to measure quality parameters were made using the more basic parameters, such as WFP, FP, HAR, and SED, plus the information of the genotype HMW score. If successful the prediction could provide a quick method for selection in early breeding stages. However the equations derived for one set of material had little if any applicability to the other set of material suggesting they were genotype and environment specific and thus likely to be of little use in general breeding programmes.

**Keywords**: baking quality; glutenin, HMW-GS; LMW-GS; SDS-PAGE; quality parameters; wholemeal flour protein (WFP); flour protein (FP); hardness (HAR); Pelshenke test (PEL); SDS sedimentation (SED); mid-line peak value (MPV) and mid-line peak time (MPT) of mixograph; correlation and regression, HMW score (HMWS)

## *4.2 Introduction*

Identification of wheat quality is important for plant breeding, cereal chemistry, and the commercial wheat industry. Prediction is needed for both traders and growers, to allow the traders to sell and to provide growers with suitable management options. Small-scale tests of bread making quality are essential in wheat breeding programs in order to select suitable materials at an early stage so effective progress can be made. A number of tests are available for quality evaluation, classification and screening of the early generations in breeding programs.

Protein content of wheat is generally accepted as the most important criterion for most aspects of processing capability and nutritional value. Within a cultivar, most variation in loaf volume results from variation in protein quantity. Bushuk *et al.* (1969) observed that loaf volume is positively correlated with protein content. The very high positive correlation between flour and grain protein indicates that milling had no differential effect on protein content in the cultivars studied. They attempted to predict loaf volumes from a combination of flour quality measurements, and suggested a linear regression on protein content and a curvilinear regression on development time.

Saxena *et al.* (1997) observed a positive correlation between protein content/total glutenin and overall sensory score for traditional bakery products, such as Tandoori roti, in India. Zhu *et al.* (1996) also observed that the content of HMW-GS and its proportion were positively correlated with sedimentation value. Payne *et al.* (1987) and Gupta *et al.* (1989) suggested that the positive effect of LMW-GS have been entirely due to increased total glutenin rather than qualitative superiority of specific subunits, and a similar scenario could occur for HMW-GS as well. Singh *et al.* (1990) suggested that glutenin quantity was highly and positively correlated with loaf volume and mixograph peak development time, indicating glutenin has a direct effect on functionality.

Grain kernel hardness is a characteristic often used in wheat classification (Meppelink 1974, Symes 1961). Hardness is often the first prediction test applied. In bread-making, the higher starch damage associated with hard wheat causes higher water absorption in the dough and, subsequently, higher bread yield (Stenvert, 1974). Hardness is closely related to several important flour properties (Moss *et al.* 1973, Newton *et al.* 1927). When evaluating bread baking potential, once the hardness of the wheat had been identified, protein quantity would be

the next measurement (Finney and Barmore, 1948; Bushuk *et al.*, 1969; Pomeranz *et al.*, 1970).

As early as 1927, Newton *et al.* (1927) reported that no relationship exists between protein content and wheat hardness. On the other hand, both positive correlations (Greenaway, 1969; Stenvert & Kingswood, 1977a&b) and negative correlations (Moss *et al.*, 1973) between hardness and protein content have been found. Obuchowski *et al.* (1980b) confirmed that bran had a definite influence on results of grain hardness evaluation.

The SDS sedimentation test is very useful in breeding for quality. Mazzoni *et al.* (1988) observed high correlations between SDS sedimentation values and some quality parameters in wheat. Baker *et al.* (1971a) claimed that Zeleny sedimentation value and mixograph development time were among the most repeatable tests. Kitterman and Barmore (1969) noted that Zeleny sedimentation value was positively related to protein content. However, very high sedimentation values could be undesirable if it required extra mixing time in the baking process. Fowler and de la Roche (1975) concluded that the results of kernel hardness, protein quantity and rate of dough development could provide sufficient information for the minimum requirements of baking quality. Dexter (1980) found that mixograph development time (MPT) and SDS sedimentation volume together satisfactorily accounted for most variation in gluten strength. The SDS sedimentation test has been widely adopted, but it does not always differentiate effectively between wheats of different quality, especially strong and 'extra strong' cultivars (Pritchard, 1993; Pritchard *et al.*, 1994).

Baking quality is not characterised by one single property, but a number of properties affecting the baking quality, such as ash content, fat content and method of milling, as well as the quantity and quality of gluten. The Pelshenke test (Pelshenke, 1933) has contributed significantly to baking research as it characterises the baking values of wheat and flour by determining the diastatic power, together with the quantity and quality of gluten.

Khatkar *et al.* (1996) found that mixing properties were significantly influenced by protein content. The height of the mixogram (MPV) may be correlated with grain hardness (Martinant *et al.*, 1998), and is strongly correlated to loaf volume (Dachkevitch *et al.*, 1989). Mixogram peak time (MPT) shows no significant correlation with loaf volume or other quality factors (Dong *et al.*, 1992; Preston *et al.*, 1992).

Jardine (1963) claimed that strength is mainly determined by environmental conditions and also remarked that a single test is less satisfactory for strength. Therefore, several tests are probably necessary to satisfactorily understand and predict wheat or flour quality. Fowler *et al.*

(1975) suggested that only hardness, dough development and protein content are necessary to describe the baking quality of wheat. Orth *et al.* (1972) used simple correlation as a guide for prediction of loaf volume by regression analysis. They suggested that the protein content, sedimentation value, and dough development time would provide the most useful information, and that curvilinear regression would better describe some of the relationships for the parameters. Branlard *et al.* (1991) compared 46 technological parameters and recommended that Pelshenke and mixograph results be included for selection of wheat quality, because of their high heritabilities. Baker *et al.* (1971b) presented a model which attempted to describe the complex nature of bread making quality, and claimed that the prediction of loaf volumes on the basis of flour measurements were successful.

Early generation selection is a potentially cost effective breeding strategy; but its usefulness depends on the reliability, complexity, and speed of the tests done on small seed quantities. It would therefore be of great advantage if simpler or very small sample size tests (e.g. protein content, hardness, high molecular weight glutenin scores) could be used effectively to predict values of the more complex tests (MPT, MPV, Pelshenke, sedimentation value) consistently across environments and genotypes. The predicted quality traits should present the genotypic value of a line for any particular character. Once quality is defined by these methods, genetic factors can be identified and manipulated, and the environmental effects can be assessed to fulfil the baking quality requirements of the consumer.

In this study quality tests that may be used in a wheat breeding programme were investigated by measuring the relationships among: 1) The quality parameters of protein content, hardness, SDS sedimentation, Pelshenke, MPV and MPT of the mixograph. 2) The quality parameters and Glutenin/HMW-GS/LMW-GS. Two sets of data were used: one from a GXE trial and the other from a set of F4 lines grown in a single environment. The aim was to determine whether the relationships found were generally applicable across genotypes and environments, or restricted to the individual sets of materials tested.

## *4.3 Materials and Methods*

### *4.3.1 Materials*

#### *I Field Experiment*

Fourteen New Zealand wheat cultivars or lines were grown in a field trial at the Henley Block of Lincoln University (43° 39′ S, 172° 28′ E). The soil type was a Templeton Silt Loam, the previous crop was barley. The trial had 6 treatments (T1-T6) differing in the amount and timing of N and S application. The 6 treatments are listed in Table 1. Each treatment had two replications. Eighty grams of seeds were sown for each individual 6 $m^2$ plot, the sowing date was 20/9/95. The names and quality characteristics of the 14 cultivars used in the trial are given in table 5 of chapter 3.

**Table 4-1.** Treatments applied to the GXE trial

| Treatment | T1 | T2 | T3 | T4 | T5 | T6 | Note |
|---|---|---|---|---|---|---|---|
| Time | | early | late | early | late | late | early-booting; late-flowering |
| N (kg/ha) | 0 | 0 | 0 | 200 | 200 | 200 | Nitrolime: 27% N (466g/plot) |
| S (kg/ha) | 0 | 50 | 50 | 0 | 0 | 50 | Gypsum: 18% S (175g/plot) |

Random heads were hand harvested for each plot before machine harvesting the entire plot. There were a total of 168 samples available for each quality test. The hand-harvested grain was used for quantifying LMW-GS and HMW-GS through the combined methods of spectrophotometry, SDS-PAGE and densitometer scanning; and the machine harvested grain was used for all the other quality tests.

#### *II Crossing Progeny*

From 14 New Zealand lines, ten crosses were made in 1995 and their progenies grown at Crop & Food Research Institute under the supervision of Dr. W. B. Griffin. From the ten crosses, five F3 populations were selected and analysed for individual plant variation by SDS-PAGE. The F3 SDS-PAGE results were used to group the progenies by common band patterns for F4 bulk quality tests. A total of 52 F4 bulks were tested for wholemeal flour protein (WFP),

hardness, SDS sedimentation and Pelshenke, and 50 F4 bulks were tested by mixograph analysis. SDS-PAGE of F3 progeny and the quality tests for the F4 materials were carried out in 1997&1998, at INRA, Clermont-Ferrand, France.

### *4.3.2 Analytical methods*

Analysis of the material were carried out at INRA, Station D'Amelioration des Plantes, Clermont-Ferrand, France.

#### *I Protein separation by SDS-PAGE*

All 168 samples were tested by SDS-PAGE. The protocol is based on Singh *et al.* (1991). In order to have better resolution for both HMW-GS and LMW-GS, the concentration of acrylamide/bisacrylamide concentration was constant, the gel parameters T and C were modified as follows: T = 12.8%, C = 0.99%. This is a standard practice for analysis by the lab of Dr. G. Branlard, INRA, Clermont-Fd, France.

#### *II Glutenin concentration by pectrophotometry*

The Pierce BCA Protein Assay protocol (Pierce, 1991) was used for the spectrophotometer.

Samples were prepared for SDS-PAGE and spectrophotometry following the same initial steps. Gliadin was removed by 50% propanol, the supernatant containing the gliadin was discarded. Then 500µl of extracting solution was added to the residue. This solution consisted of 0.05M sodium phosphate and 2% SDS. After vortexing, the solution was left to extract for 1h, followed by sonication for 30s at 10W, then centrifuged for 5 minutes at 10,000g. The supernatant was then divided into two samples of 100µl and 200µl. The 200µl sample was prepared for SDS-PAGE using the method of Singh *et al.* (1991). The 100µl sample was added to 400µl of water to make a final amount of 500µl. Then 100µl was taken and mixed with 2ml working reagent (Pierce, 1991) in a labelled test tube. The tube containing the sample was incubated at 37°C for 30min, cooled to room temperature and 1ml pipetted into measuring wells, and the absorbancy measured at 562nm against a standard. The standard was prepared and measured simultaneously with each load of samples. A standard curve was prepared with a sequence of protein (BSA) concentrations: 0, 100, 200, 300, 400 µg/ml. The

samples were read on Kontron Instruments-Uvikon 930. The concentration of each sample was calculated using the CALC. MODE procedure.

### *III Densitometry*

The densitometer system used for analysing the GXE glutenin gel was a Hoefer Scientific Instruments, GS 365 W Version 3.02.

After electrophoresis, the gel was placed between glass plates, fixed by Sellotape, the HMW&LMW-GS zones were marked, the clearest area on the gel used to set as background zero, and the darkest band set at 80% for Gain Control. After scanning, data smoothing and quick integration, the areas and percentages of HMW-GS and LMW-GS were determined. The absolute amount of HMW-GS and LMW-GS were calculated from the combined results of the Densitometer and Spectrophotometer measurement. Preliminary measurements had proved linear relationship between glutenin quantity and Coomassie stained subunits.

### *IV Quality tests*

A Near Infrared Reflectance (NIR) instruments (Inframatic 8620 Perten Instruments, Hamburg, Germany) was used to estimate the wholemeal flour protein content and hardness, white flour protein content and moisture level. This result was also used for calculating the amount of water to add to the flour for the mixograph test (Martinant *et al.*, 1998).

For the SDS sedimentation test, 5g of wholemeal flour was used, according to the procedure described by Axford *et al.* (1979).

The Pelshenke test was carried out by using 10g of wholemeal flour. The original procedure (Pelshenke, 1933) was employed for determining the dough swelling time. A temperature controlled cabinet was designed to better control the condition of dough swelling.

Mixographic measurements were carried out on a 10g flour sample (AACC Method 54-40A, 1992). Dough hydration was obtained by taking into account flour protein content, flour moisture and grain hardness (Martinant *et al.*, 1998). The mixograph curves were computed by Mixsmart® software. Mid-line Peak Value (MPV) and Mid-line Peak Time (MPT) were the major parameters used in our study.

### *V Calculation of HMW-GS score*

Branlard's HMW-GS score (Branlard *et al.*, 1992) was used for calculating the HMW-

GS score for both F4 and GXE material. The scores were given according to their functionality on bread making quality, e.g. for Glu-A1, allele 2* is scored 30, whereas the null allele scored zero, allele 1 scored 15. Alleles on Glu-B1 and Glu-D1 also were given their individual scores. The scores for the three loci were added and doubled for homozygous, and added for heterozygous. The HMW-GS score of both sets of GXE and F4 material were used for their correlations and regressions to the quality parameters.

### *VI Statistical analysis*

Statistical analysis was made by the computer statistics software Statgraphics Plus, Minitab and SAS for Windows.

## *4.4 Results and Discussion*

The GXE material used in these comparisons is described in Table 4-2, further details including full descriptions of population means and variability are provided in Tables 3-5 (genotype values), 3-4 (treatment values), 5-3 (parental values) and 5-5 (progeny values).

**Table 4-2.** Description of the quality parameters for GXE material (168 samples)

| Source | Glutenin Quantity (mg/100mg flour) | HMW-GS Quantity (mg/100mg flour) | HMW-GS Score | LMW-GS Quantity (mg/100mg flour) | Whole-meal Flour Protein (%) | Flour Protein (%) | Hard-ness | Pelsh-enke (min) | SDS Sedimen-tation (ml) | Mid-line Peak Time (min) | Mid-line Peak Value (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MEAN | 4.3 | 1.2 | 54.3 | 3.2 | 11.8 | 10.9 | 70 | 145 | 52 | 3.7 | 47 |
| MIN | 2.6 | 0.6 | 25.0 | 2.0 | 9.5 | 7.9 | 42 | 32 | 26 | 2.0 | 37 |
| MAX | 6.9 | 2.2 | 80.0 | 5.5 | 17.2 | 17.0 | 100 | 254 | 79 | 7.9 | 72 |
| STDEV | 0.7 | 0.3 | 17.4 | 0.6 | 1.3 | 1.5 | 13 | 55 | 11 | 1.0 | 6 |

### *4.4.1 Correlations of Glutenin, HMW-GS & LMW-GS to the measured quality parameters*

As a first step towards determining whether there were consistent relationships between the quality parameters, correlations were determined between the parameters and glutenin amounts. These correlations were determined in three ways.

(1) A single correlation was determined across all 168 values for each pairing of parameters. This value determines the robustness of the correlation across both genotype and environmental variations and is indicated by G+T in Table 4-4.

(2) It is also known that there are differences in the glutenin alleles between cultivars and it is known that these differences affect some quality characteristics (Chapter 5). Thus there is the possibility of confounding of the effects of glutenin type and quantity in these correlations. Consequently the effects of glutenin amounts would be masked by glutenin type. To avoid this possibility correlations were also determined within cultivars and the average correlation presented across the 14 genotypes. This value determines the robustness of the correlation within a range of genotypes across environmental variations and is indicated by G in Table4- 4.

(3) Different environments may also affect the quality of the glutenins produced as well as their quantity and any indirect relationships and thus confound the effects of amount in the correlations. To avoid this possibility correlations were also determined within environments and the average correlation presented across the 6 environments. This value determines the robustness of the correlation within a range of environments across genotypic variations and is indicated by T in Table 4-4.

Wholemeal flour protein and, flour protein produced very similar correlations throughout, which is not surprising considering their strong correlation to each other (Table 4-5, >0.95) and their similar analytical basis. With approximately 40% of the total flour protein existing as glutenins, predominantly LMW-GS (Table 4-4, approximately 30%) the relatively high, significant correlations between the FP/WFP values and total/LMW-GS are not unexpected (Saxena *et al.*, 1997). Equally the relatively poorer relationship between FP/WFP and HMW-GS is also as expected, though Zhu *et al.* (1996) found a stronger relationship, as HMW-GS represented only 10% of the total protein (Table 4-4). The highly significant correlation between Pel and HMW Score indicates that predicting Pelshenke value very much lies with allele effects rather than glutenin quantity.

The remaining correlations are of greater interest in attempting to separate and understand the differential effects of glutenin amounts and type. It needs to be repeated however, that these are all only correlation values and as such do not necessarily indicate cause and effect nor applicability beyond this trial. Mid-line Peak Values and sedimentation, for example, are significantly and strongly correlated with glutenin levels irrespective of treatments and genotypes. Their correlations with HMW score are weak (MPV) or non existent (SED). In addition the removal of glutenin type from the correlations by testing correlations within varieties has relatively little effect on the magnitude of the correlations except for HMW-GS. This suggests these are responding primarily to quantity and only slightly to form of the glutenins. For sedimentation values this is consistent with the results of Payne *et al.*, (1987) and Gupta *et al.*, (1989). However, more usually MPT rather than MPV has been related to glutenin amount (Singh *et al.*, 1990). Mid-line peak times do not correlate well with any glutenin quantity measured indicating that neither amount nor type (at least as indicated by HMW score) of glutenin strongly influences this value. Hardness is only significantly related to glutenin amounts after the effect of genotype (glutenin type) is taken out. It is also not related to HMW score. Hardness would appear to be influenced by amount of (particularly LMW-GS) glutenins, but is also influenced by genotype either due to allelic differences or other factors differing with genotype. The Pelshenke test correlates very strongly with HMW score indicating the type of the HMW-GS is most important with very little effect of amount of glutenin. This is further indicated by the complete absence of a correlation with amount once the effect of genotype is removed.

Taken as a whole and ignoring possible cross correlations between quality parameters, the data have been summarised in Table 4-3. They indicate that amount of glutenin may influence protein amount, SED, MPV and possibly HAR, whereas type of glutenin has the greatest influence on Pelshenke value. MPT value is unrelated to either. This is consistent with Table 5-13, which indicated MPT and Hardness were the characters least influenced by changes in glutenin alleles whereas Pelshenke was strongly influenced, particularly by HMW-GS allele changes, which has the largest mean range between alleles of more than 16%. Table 5-13 also indicated WFP was strongly influenced by allele type suggesting that allele type may influence both quantity and type of the glutenins present. In general, (except for MPT) the quantity of glutenin subunits, HMW-GS & LMW-GS presented a positive correlation with the measured quality parameters. This supports previous observations (Saxena *et al.* 1997; Zhu *et*

*al.* 1996; Singh *et al.* 1990; Payne *et al.* 1987; Gupta *et al.* 1989). Chapter 5 of this thesis will further investigate whether the quality of HMW-GS & LMW-GS, (*i.e.* allelic differences rather than other unrelated cultivar effects) are influencing quality.

**Table 4-3.** Summary of the effects by glutenin quantity and glutenin type on measured quality parameters using data from Table 4-4

| | Whole-meal FP | Flour Protein | Sedimen-tation | Mid-line Peak Value | Pelshenke | Hardness | Mid-line Peak Time |
|---|---|---|---|---|---|---|---|
| Parameter Abbreviation | WFP | FP | SED | MPV | PEL | HAR | MPT |
| Glutenin Quantity | +++# | +++ | +++ | +++ | - | ++ | - |
| Glutenin Type | + | ++ | + | ++ | +++ | (++?) | (?) |
| HMW-GS | + | + | + | ++ | ++ | + | - |
| LMW-GS | ++ | ++ | ++ | ++ | - | + | - |

* this could include glutenin allele and other variety differences

# additional (+'s) indicate stronger effect, (-) indicates no obvious effect, (?) indicates effect is unclear or is likely to be due to variety differences unrelated to glutenin alleles.

**Table 4-4.** Mean values for Pearson correlation coefficients (r), significance probabilities (p) and standard deviations (STDEV) of the correlations for the measured quality parameters with glutenin, HMW-GS and LMW-GS, from the GXE trial (n=168)@

| | | | WFP* | FP | HAR | SED | PEL | MPV | MPT |
|---|---|---|---|---|---|---|---|---|---|
| Glutenin | G+T | r | 0.520 | 0.524 | 0.141 | 0.411 | 0.221 | 0.505 | -0.106 |
| Quantity | | p | **0.000** | **0.000** | 0.068 | **0.000** | **0.004** | **0.000** | 0.173 |
| | G | r | 0.457 | 0.416 | 0.344 | 0.336 | -0.070 | 0.411 | -0.123 |
| | | p | **0.000** | **0.000** | **0.000** | **0.000** | 0.371 | **0.000** | 0.111 |
| | | STDEV | 0.227 | 0.258 | 0.410 | 0.297 | 0.245 | 0.322 | 0.443 |
| | T | r | 0.488 | 0.494 | 0.075 | 0.395 | 0.233 | 0.467 | -0.087 |
| | | p | **0.000** | **0.000** | 0.335 | **0.000** | **0.002** | **0.000** | 0.262 |
| | | STDEV | 0.183 | 0.176 | 0.207 | 0.173 | 0.100 | 0.177 | 0.111 |
| HMW-GS | G+T | r | 0.245 | 0.314 | -0.025 | 0.190 | 0.327 | 0.214 | -0.033 |
| Quantity | | p | **0.001** | **0.000** | 0.747 | **0.013** | **0.000** | **0.006** | 0.674 |
| | G | r | 0.381 | 0.363 | 0.243 | 0.265 | -0.046 | 0.329 | -0.180 |
| | | p | **0.000** | **0.000** | **0.002** | **0.001** | 0.557 | **0.000** | **0.019** |
| | | STDEV | 0.359 | 0.373 | 0.409 | 0.315 | 0.223 | 0.309 | 0.347 |
| | T | r | 0.184 | 0.267 | -0.089 | 0.161 | 0.332 | 0.160 | -0.014 |
| | | p | **0.017** | **0.001** | 0.254 | **0.037** | **0.000** | **0.039** | 0.853 |
| | | STDEV | 0.116 | 0.144 | 0.140 | 0.107 | 0.077 | 0.122 | 0.130 |
| LMW-GS | G+T | r | 0.524 | 0.494 | 0.190 | 0.415 | 0.108 | 0.521 | -0.115 |
| Quantity | | p | **0.000** | **0.000** | **0.014** | **0.000** | 0.163 | **0.000** | 0.139 |
| | G | r | 0.396 | 0.353 | 0.318 | 0.298 | -0.066 | 0.364 | -0.073 |
| | | p | **0.000** | **0.000** | **0.000** | **0.000** | 0.397 | **0.000** | 0.345 |
| | | STDEV | 0.249 | 0.305 | 0.403 | 0.306 | 0.257 | 0.381 | 0.415 |
| | T | r | 0.509 | 0.474 | 0.139 | 0.406 | 0.117 | 0.495 | -0.100 |
| | | p | **0.000** | **0.000** | 0.073 | **0.000** | 0.131 | **0.000** | 0.196 |
| | | STDEV | 0.214 | 0.206 | 0.199 | 0.180 | 0.121 | 0.184 | 0.131 |
| HMW | G+T | r | 0.108 | 0.203 | 0.010 | 0.052 | 0.642 | 0.165 | -0.074 |
| Score | | p | 0.162 | **0.008** | 0.894 | 0.505 | **0.000** | **0.033** | 0.339 |
| | T | r | 0.125 | 0.232 | 0.011 | 0.054 | 0.646 | 0.180 | -0.075 |
| | | p | 0.107 | **0.002** | 0.888 | 0.489 | **0.000** | **0.020** | 0.336 |
| | | STDEV | 0.152 | 0.112 | 0.142 | 0.043 | 0.057 | 0.093 | 0.148 |

*Abbreviations as used in Table 4-3

**Probability $p < 0.05$ are in **bold**, G+T (n=168): correlation across all treatments and cultivars; G (n=14) mean correlation values across individual genotypes (cultivars), T (n=6): mean correlations across individual treatments

@The 14 NZ wheat lines and cultivars having various baking quality characteristics are listed in Table 3-5. The six N /S fertiliser treatments are listed in Table 4-1

### *4.4.2 Correlations among the measured quality parameters*

#### *I For GXE material*

All the measured quality parameters in the GXE material were significantly correlated to each other, except for two pairs of parameters: Pelshenke-hardness and MPT-Pelshenke, as shown in Table 4-5. Not unexpectedly WFP and FP were highly positively correlated, which is consistent with Bushuk's (1969) observation. WFP; FP; SED and MPV, which were all shown to be related to glutenin quantity, were all highly correlated with each other as would be expected. These cross correlations prevent reaching any firm conclusions as to whether there are direct or only indirect effects of glutenin quantity on these parameters. With these parameters in all instances the correlations were increased when the genotype effect was removed (e.g. increasing from 0.35 to 0.78 for MPV-SED). This suggests that genotype differences change the quantitative relationship between the parameters (i.e. the parameters rose and fell in concert with environmental changes but were not linked during genetic changes). Such a result is to be expected if several independent factors contribute to producing each quality parameter value. All the measured quality parameters, except hardness showed a greater correlation to FP than to WFP. The higher correlation between hardness and WFP suggests that bran is important for consistent hardness evaluation, supporting the observation of Obuchowski *et al.* (1980-b).

**Table 4-5.** Mean values for Pearson correlation coefficients (r), significant probabilities (p) and standard deviations (STDEV) of the correlations among the measured quality parameters (n=168)@

| | | | Flour Protein | Hardness | Sedimen-tation | Pelshenke | Mid-line Peak Value | Mid-line Peak Time |
|---|---|---|---|---|---|---|---|---|
| Whole-meal FP | G+T | r | 0.960 | 0.416 | 0.524 | 0.192 | 0.744 | -0.211 |
| | | p | **0.000** | **0.000** | **0.000** | **0.013** | **0.000** | **0.006** |
| | G | r | 0.956 | 0.610 | 0.676 | 0.066 | 0.832 | -0.288 |
| | | p | **0.000** | **0.000** | **0.000** | 0.395 | **0.000** | **0.000** |
| | | STDEV | 0.048 | 0.332 | 0.291 | 0.348 | 0.167 | 0.298 |
| | T | r | 0.948 | 0.314 | 0.493 | 0.212 | 0.717 | -0.183 |
| | | p | **0.000** | **0.000** | **0.000** | **0.006** | **0.000** | **0.018** |
| | | STDEV | 0.035 | 0.278 | 0.078 | 0.175 | 0.136 | 0.188 |
| Flour Protein | G+T | r | | 0.365 | 0.553 | 0.277 | 0.806 | -0.266 |
| | | p | | **0.000** | **0.000** | **0.000** | **0.000** | **0.001** |
| | G | r | | 0.562 | 0.723 | 0.085 | 0.862 | -0.305 |
| | | p | | **0.000** | **0.000** | 0.272 | **0.000** | **0.000** |
| | | STDEV | | 0.346 | 0.192 | 0.315 | 0.117 | 0.296 |
| | T | r | | 0.255 | 0.524 | 0.309 | 0.791 | -0.246 |
| | | p | | **0.001** | **0.000** | **0.000** | **0.000** | **0.001** |
| | | STDEV | | 0.248 | 0.071 | 0.176 | 0.102 | 0.141 |
| Hardness | G+T | r | | | 0.209 | -0.126 | 0.228 | -0.375 |
| | | p | | | **0.007** | 0.104 | **0.003** | **0.000** |
| | G | r | | | 0.386 | 0.019 | 0.473 | -0.326 |
| | | p | | | **0.000** | 0.803 | **0.000** | **0.000** |
| | | STDEV | | | 0.490 | 0.321 | 0.346 | 0.378 |
| | T | r | | | 0.154 | -0.137 | 0.156 | -0.364 |
| | | p | | | **0.047** | 0.076 | **0.044** | **0.000** |
| | | STDEV | | | 0.147 | 0.066 | 0.279 | 0.204 |
| Sedimen-tation | G+T | r | | | | 0.472 | 0.354 | 0.162 |
| | | p | | | | **0.000** | **0.000** | **0.036** |
| | G | r | | | | -0.002 | 0.784 | -0.278 |
| | | p | | | | 0.977 | **0.000** | **0.000** |
| | | STDEV | | | | 0.429 | 0.114 | 0.303 |
| | T | r | | | | 0.490 | 0.658 | 0.134 |
| | | p | | | | **0.000** | **0.000** | 0.084 |
| | | STDEV | | | | 0.133 | 0.086 | 0.259 |
| Pelshenke | G+T | r | | | | | 0.683 | 0.100 |
| | | p | | | | | **0.000** | 0.198 |
| | G | r | | | | | 0.039 | -0.129 |
| | | p | | | | | 0.611 | 0.096 |
| | | STDEV | | | | | 0.398 | 0.344 |
| | T | r | | | | | 0.368 | 0.165 |
| | | p | | | | | **0.000** | **0.032** |
| | | STDEV | | | | | 0.172 | 0.158 |
| Mid-line Peak Value | G+T | r | | | | | | -0.411 |
| | | p | | | | | | **0.000** |
| | G | r | | | | | | -0.499 |
| | | p | | | | | | **0.000** |
| | | STDEV | | | | | | 0.187 |
| | T | r | | | | | | -0.400 |
| | | p | | | | | | **0.000** |
| | | STDEV | | | | | | 0.141 |

*Probability $p < 0.05$ are in **bold**, G+T (n=1): correlation across all treatments and cultivars; G (n=14) mean correlation values across individual genotypes (cultivars), T (n=6): mean correlations across individual treatments

@The 14 NZ wheat lines and cultivars having various baking quality characteristics are listed in Table 3-5. The six N /S fertiliser treatments are listed in Table 4-1.

By considering the results presented in Table 4-4 and Table 4-5 together, it can be seen that hardness, sedimentation, MPT and MPV all showed greater correlations to WFP and FP than to glutenin (or LMW-GS; HMW-GS) quantity. This result suggests that hardness, sedimentation and MPV are more influenced by the overall amount of protein than by glutenin alone. In both Tables 4-4 and 4-5, Pelshenke showed stronger correlations with all measured quality parameters when the genotype effect was included (T) than when it was excluded (G). This suggested that Pelshenke value is mainly influenced by genetic factors which tend to increase Pelshenke and the other parameters together, (potentially including allele type) consistent with Table 5-4. As expected therefore, Pelshenke had poor correlations with the parameters related to protein quantity (WFP, FP, SED). It did however, have a good correlation with MPV, except when the genotype effect was excluded. This is consistent with the significant effect of HMW score for both of these parameters indicated in Table 4-4.

Hardness showed relatively poor correlations to the other parameters except when the genotype effect was excluded (G) for WFP, FP, SED & MPV. This suggests that different genotypes have different relationships between these values but that within a genotype these values rise and fall together with hardness.

MPT and MPV tended to have moderate negative correlations with each other. Otherwise, MPT tended to only have weak relationships to the other characters.

*II For F4 material*

**Table 4-6.** Pearson correlation coefficient (r) and its probability (p) among the measured quality parameters for the F4 material (52 samples, 50 samples for MPV & MPT)

| | | WFP | Hardness | Pelshenke | Sedimen-tation | Mid-line Peak Value | Mid-line Peak Time |
|---|---|---|---|---|---|---|---|
| Whole-meal FP | r | | 0.231 | -0.013 | 0.197 | 0.553 | -0.453 |
| | p | | 0.099 | 0.357 | 0.162 | **0.000** | **0.001** |
| Hardness | r | | | -0.195 | 0.411 | 0.446 | -0.018 |
| | p | | | 0.167 | **0.003** | **0.001** | 0.902 |
| Pelshenke | r | | | | 0.135 | -0.198 | 0.685 |
| | p | | | | 0.340 | 0.168 | **0.000** |
| Sedimen-tation | r | | | | | 0.538 | 0.324 |
| | p | | | | | **0.000** | **0.022** |
| Mid-line Peak Value | r | | | | | | -0.343 |
| | p | | | | | | **0.015** |
| HMW-GS Score | r | -0.312 | -0.145 | 0.098 | -0.356 | -0.521 | 0.348 |
| | p | **0.027** | 0.314 | 0.500 | **0.011** | **0.000** | **0.013** |

(Probability $p < 0.05$ are in **bold**)

The F4 materials in Table 4-6 had varied genetic backgrounds and were all grown in the same environment; so the measured relationships between the quality parameters should have arisen primarily from genotypic differences within this one environment. This material has a different genetic background from the 14 cultivars used in the GXE trial. Therefore where a correlation is significant among both genotypes and environments in the GXE trial and among genotypes in the F4 trial, it would suggest a genuine relationship existed. A number of correlations fell into this class: MPT-MPV, WFP(or FP)-MPV, SED-MPV. Hardness gave relatively poor correlations in Table 4-5 when genotype effects were included (T) and generally gave poor correlations with this F4 material. The higher correlation values between hardness and SED and hardness and MPV may have resulted from the narrower genetic base of the F4 material. Some correlations only existed in one of the data sets, e.g. MPV and PEL in Table 4-5 and MPT and PEL in Table 4-6.

MPT was not substantially influenced by any of the glutenin quantity measures (Table

4-3), but it was correlated with other quality parameters both in Tables 4-5 & 4-6, which suggests that MPT can be modified by both genetic and environmental factors. Genetic factors other than glutenin have great influence on MPT, particularly grain hardness.

These results contrast with those found by Dong *et al.* (1992) and Preston *et al.* (1992), for Pelshenke and MPT. Hardness was not highly correlated to WFP in the F4 material, in contrast to the GXE trial material, which showed a highly significant correlation between hardness and WFP/FP. This supports the report by Newton *et al.* (1927) that no correlation exists between WFP and hardness. The highly significant correlations of MPV/MPT - WFP, and MPV - hardness are consistent with the report of Khatkar *et al.* (1996) and Martinant *et al.* (1998).

MPV is highly correlated to sedimentation in both Tables 4-5 & 4-6, the sedimentation and MPV value are also highly correlated to all quantities of glutenin/HMW-GS/LMW-GS, MPV also to HMW Score (Table 4-4). This suggests that MPV and sedimentation can be greatly improved by genetic selection, as well as fertiliser treatment (Table 3-4).

### *4.4.3 Prediction of the quality parameters*

The SDS sedimentation test gives good correlations with loaf volumes, and is also easier to perform than the Pelshenke test (Axford, 1979). Both Pelshenke and mixograph tests are relatively more labour intensive and time consuming. It would be an advantage to breeders if the easier tests, such as WFP, FP and hardness, could be used to predict SDS sedimentation, Pelshenke and mixograph results in order to provide an initial selection strategy. Using the simple correlations as a guide, multiple regression equations were therefore derived to see if a generally applicable relationship existed. These equations are given in Table 4-7. The parameter abbreviations are as listed in Table 4-4, with the addition that HMWS = HMW-GS score of Branlard *et al.* (1992).

The best two regression equations were chosen from 1, 2 and 3 predictor models respectively. WFP, HAR and HMWS were used as predictors for F4 material to provide predictions for SED, PEL, MPV and MPT. For PEL, SED was also included as one of the predictors to provide a reasonable prediction. It was possible to include SED as the Pelshenke test is much more labour intensive and time consuming than the sedimentation test. For the GXE materials, WFP, FP, HAR and HMWS were used to predict SED, PEL, MPV and MPT.

When choosing the best equations for prediction, the R square of the regression was used as a guide. If the R square was increased by more than 2% by involving more predictors, the regressions with higher R square were chosen, otherwise, regressions with fewer predictors would be chosen as best equations.

**Table 4-7.** Regression of F4 and GXE material, correlation coefficient (r) and probability (p) of Pearson correlation between the measured value and predicted value by the equations

| Source of Regression | Regression Equation | r | p |
|---|---|---|---|
| F4 material | SED = 48.552-0.0818*HMWS - 0.1051*WFP+0.2291*HAR | 0.532 | **0.000** |
| | MPT = 3.3972+.02*HMWS-0.1592*WFP+0.0121*HAR | 0.999 | **0.000** |
| | MPV = 52.0795-0.247*HMWS+0.8413*WFP+0.0853*HAR | 0.913 | **0.000** |
| | PEL = 153.9 + 0.2147*HMWS - 2.2175*WFP - 0.9873*HAR + 1.7266*SED | 0.335 | **0.018** |
| GXE material | SED = 9.4629+3.9367*FP | 0.551 | **0.000** |
| | MPT = 4.7553+0.6974*WFP-0.6615*FP-0.0299*HAR | 0.468 | **0.000** |
| | MPV = 11.4392+3.2877*FP | 0.806 | **0.000** |
| | PEL = 13.3125+1.8748*HMWS +8.4876*FP - 0.9024*HAR | 0.690 | **0.000** |

The regression equations from both sets of GXE and F4 material were used to calculate the predicted values of SED, PEL, MPV and MPT for both sets of material respectively. The correlation coefficient and probability between the measured value and predicted value given by their respective equations are also shown in Table 4-7. The data indicate that the regression equations were highly correlated to the measured results. However, the generality of the regression equations must be questioned as the regressions produced were very different for the two data sets.

From Table 4-7, the best regression equations suggested that SDS sedimentation result is mainly a function of FP and/or WFP. The Pelshenke result was mainly influenced by HMW score, hardness, FP or WFP. MPV can be better predicted than MPT.

**Table 4-8.** Correlation coefficient (r) and probability (p) of Pearson correlation between the measured value of each treatment and predicted value using the optimal equation derived for each treatment

| Treatment | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No | T1 | | T2 | | T3 | | T4 | | T5 | | T6 | |
| Time of Application | | | early | | late | | early | | late | | late | |
| Applied Ferlizer | | | S | | S | | N | | N | | N&S | |
| Correlation | r | p | r | p | r | p | r | p | r | p | r | p |
| SED | 0.579 | **0.001** | 0.487 | **0.009** | 0.474 | **0.011** | 0.613 | **0.001** | 0.630 | **0.000** | 0.482 | **0.009** |
| PEL | 0.717 | **0.000** | 0.762 | **0.000** | 0.722 | **0.000** | 0.602 | **0.001** | 0.685 | **0.000** | 0.779 | **0.000** |
| MPT | 0.623 | **0.000** | 0.378 | **0.048** | 0.288 | 0.138 | 0.478 | **0.010** | 0.635 | **0.000** | 0.673 | **0.000** |
| MPV | 0.674 | **0.000** | 0.819 | **0.000** | 0.844 | **0.000** | 0.869 | **0.000** | 0.836 | **0.000** | 0.806 | **0.000** |

Separate regressions for each of the GXE treatments of the overall GXE material, using HMW score, WFP, FP and hardness as predictors were also calculated. The correlations between measured and predicted values and their significance are given for each treatment in Table 4-8. Among all the correlations for the different treatments, all the measured and predicted values were significantly correlated, except for MPT in treatment 3. MPT correlation for treatment 2 between measured and predicted value also appeared relatively weak, compared to the other quality parameters. This suggested that fertiliser application, especially lack of N combined with late S application may reduce the predicability of MPT by WFP, FP and Hardness. The correlation coefficient for sedimentation prediction appeared relatively lower than PEL, MPT and MPV, except MPT under S treatment; MPV had the greatest correlation coefficient for all the treatments except Treatment 1. For some of the treatments, the prediction of SED and MPV only had FP involved. This confirmed that SDS sedimentation result is mainly influenced by flour protein content.

**Table 4-9.** Correlation coefficient (r) and probability (p) between predicted and measured value for both GXE and F4 material, 8 sets of equations all used to predict GXE (n=168) and two sets of equations were used to predict F4 material (n=52)

| | | For GXE Material | | | | | | | | For F4 Material | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Source of Equations | | F4 | GXE | GXET1 | GXET2 | GXET3 | GXET4 | GXET5 | GXET6 | F4 | GXE |
| SDS | r | 0.130 | 0.551 | 0.551 | 0.549 | 0.556 | 0.520 | 0.554 | 0.555 | 0.532 | 0.176 |
| | p | 0.094 | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | 0.222 |
| PEL | r | 0.125 | 0.690 | 0.602 | 0.663 | 0.652 | 0.653 | 0.680 | 0.688 | 0.335 | 0.106 |
| | p | 0.106 | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.018** | 0.464 |
| MPT | r | -0.109 | 0.468 | 0.347 | 0.445 | 0.195 | 0.397 | 0.462 | 0.466 | 0.999 | 0.315 |
| | p | 0.160 | **0.000** | **0.000** | **0.000** | **0.012** | **0.000** | **0.000** | **0.000** | **0.000** | **0.026** |
| MPV | r | 0.081 | 0.806 | 0.781 | 0.806 | 0.807 | 0.806 | 0.806 | 0.800 | 0.913 | 0.132 |
| | p | 0.299 | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | 0.359 |

The equations from each treatment were used to predict the value for the whole set of GXE materiala. The correlations with the measured values were all very high, even for MPT of treatment 2, which in Table 4-8 had a lower correlation with its own material. This result indicates that the regression equations from GXE material across all 6 different fertilisation environments can apply to each other. This is consistent with the earlier data of Table 4-7, that the quality parameters SED, PEL, MPV and MPT can be predicted with the overall GXE regression equations.

The best regression equations derived for each of the GXE and F4 material were also used to predict the value for the other data set, as presented in Table 4-8. In this case, the predicted values were not correlated to the measured value. This result suggested that regression equations from different sets of material, GXE or F4, cannot apply to each other, and that the predictions are limited in utility to their own environment. This was expected as the relationships were different between the measured quality parameters from these two sets of material (Tables 4-4 & 4-5).

The cultivars used as GXE material were all homozygous, and thus much more stable than F4 material, which still had a large number of heterozygous. The above results suggested that the prediction of quality is limited to within the particular genetic environment in which it was produced and is not sufficiently universal to use across data sets. However, under their

own genetic environment, the predictions could be highly reliable.

## *4.5 Conclusion*

The relationships between different quality parameters identified by this study have confirmed that baking quality is a very complex character.

From 168 samples in the GXE trial, the quantity of glutenin, HMW-GS and LMW-GS are all significantly correlated to the quality parameters: WFP, FP, SDS sedimentation volume, and mid-line peak time value of mixograph.

Both sets of materials, GXE and F4, presented highly significant correlations for the parameters: WFP-MPV/MPT; SDS sedimentation-hardness/MPV; MPV-hardness/MPT; and MPT-Pelshenke.

Quality predictions are made for both F4 and GXE materials by the equations from both sets of materials. Quality parameters that are more difficult to measure, such as SDS sedimentation, Pelshenke and mixograph, can be regressed from basic tests of WFP, FP and hardness, plus the information of the HMW score. For F4 material, SED was involved as a predictor for Pelshenke prediction. The predicted values in both GXE and F4 materials, mostly have highly significant correlation with their respective measured values, and can provide some guide for breeders, growers and traders to modify their plans and strategies. However, the regression equations from F4 materials can not predict the results for GXE materials, and vice versa.

Whether and how the gluten quality (ie. allele differences of HMW-GS & LMW-GS) is affecting the quality parameters, will be tested in Chapter 5 of this thesis.

# Chapter 5

# Comparison of LMW and HMW Wheat Glutenin Allele Effects on Flour Quality


## *5.1 Abstract*

Five crosses were made using a set of New Zealand wheat cultivars to measure the effect of glutenin allele differences on baking quality parameters. The alleles involved were: Glu-A1 (2*, 1 & n), Glu-D1 (5+10, 2+12), Glu-A3 (c, d & e), Glu-B3 (Sec-12, Sec-13, b & g), Glu-D3 (a & b). The allelic variation of F3 individual plants was identified by SDS-PAGE, and plants with the same HMW-GS and LMW-GS patterns were grouped. Quality parameters were then measured on the grouped F4 bulks. Quality parameters measured for this study were wholemeal flour protein content (WFP), grain hardness (HAR), SDS sedimentation volume (SED), Pelshenke time (PEL), mid-line peak value (MPV) and mid-line peak time (MPT) of a mixograph. The results showed there were significant quality differences within most populations associated with the possession of a particular allele reaching magnitudes of up to 42% for the range between populations differing at a particular allele. Most glutenin allelic comparisons showed significant differences for at least one of the resultant measured quality parameters. Allelic differences of Glu-A1 significantly influenced all characters but MPT with the null allele apparently inferior; possession of 5+10 at Glu-D1 significantly increased Pelshenke time and SED volumes relative to allele 2+12; WFP, SED & MPV were significantly affected by the Glu-A3 alleles tested. Glu-B3 alleles significantly affected all characters except hardness and Glu-D3 alleles tested significantly affected all characters other than hardness and SDS sedimentation volume.



**Keywords**: baking quality; allele, glutenin, HMW-GS; LMW-GS; SDS-PAGE; quality parameters; wholemeal flour protein (WFP); hardness; Pelshenke test; SDS sedimentation (SED); mid-line peak value (MPV) and mid-line peak time (MPT) of mixograph; recombinant inbred lines (RILs)

## *5.2 Introduction*

Two major classes of glutenin polypeptides have been identified in wheat endosperm: these are designated as HMW-GS and LMW-GS, both classes occur in flour as cross-linked proteins resulting from inter-polypeptide disulphide linkages. The genes coding for HMW-GS subunits are located at the long arms of chromosomes 1A, 1B and 1D at the Glu-A1, Glu-B1 and Glu-D1 loci respectively (Payne, 1987). The genes coding for LMW-GS occur on the short arms of group-1 chromosomes at the Glu-A3, Glu-B3 and Glu-D3 loci (Singh and Shepherd, 1988) which are tightly linked to the Gli-1 locus (Singh and Shepherd, 1988; Pogna *et al.*, 1990). It is generally accepted that glutenins are mainly responsible for bread-making quality.

The work of Payne *et al.* (1980) provided evidence of a strong association between the presence of certain alleles coding for HMW-GS and bread-making quality. Branlard and Felix (1994) observed 18% to 55% variation of strength, tenacity and Pelshenke result could be explained by HMW-GS, and less than 20% by LMW-GS. Sontag-Strohm *et al.* (1996) found that progeny carrying alleles Glu-A1b (ie. 2*) had significantly greater SDS sedimentation volumes than the null (n) allele, and that adding a HMW-GS affected extensograph dough strength more than adding a LMW-GS, although both increased the sedimentation volumes. Griffin (1989) found that HMW-GS played only a minor role in regulating environmental variability for bread-making, the whole gluten protein fraction appearing to be important and not just the HMW-GS. Other studies have also shown that allelic variation of HMW-GS and LMW-GS are both associated with differences in technological qualities of wheat flour (Payne, 1987a; Autran *et al.*, 1987; Gupta *et al.*, 1989c; Nieto-Taladriz *et al.*, 1994).

As LMW-GS are present in much greater amount than HMW-GS, great effort has been made to establish their role in bread-making quality (Payne *et al.*, 1987; Gupta and Shepherd, 1987, 1988; Gupta *et al.* 1989; Boggini and Pogna, 1989; Pogna *et al.* 1990; Metakovsky *et al.*, 1990). LMW-GS have a pronounced effect on dough viscoelastic properties in both bread wheat and durum wheat. Largely additive effects of individual Glu-3 alleles (Gupta *et al.*, 1989c; Pogna *et al.*, 1990), and significant interactions (Gupta *et al.*, 1994) have been found.

Several alleles at the Glu-3 loci have been ranked with respect to their effect on dough resistance and extensibility (Gupta and Shepherd 1988; Gupta *et al.*, 1989, 1990, 1991, 1994; Metakovsky, E. V. *et al.* 1990). In Australian wheat cultivars, LMW-GS provided better

predictions than HMW-GS for Rmax (maximum dough resistance). For Rmax, the alleles of Glu-A3: b>d>e>c; the alleles of Glu-B3: i>b=a>e=f=g=h>c; the alleles of Glu-D3: e>b>a>c>d. In particular, allele b of Glu-B3 was shown to increase dough strength when compared to allele c, and allele b of Glu-A3 and Glu-D3 was present in all the more extensible wheats. It was shown that dough strength could be improved without increasing grain protein levels, therefore, without reducing grain yield. They concluded that HMW-GS alone are insufficient to account for differences in quality, and that breeding lines should not be selected or discarded based only on their HMW-GS compositions. LMW-GS must also be taken into consideration.

Cornish *et al* (1993) catalogued information about the gluten alleles of nearly 600 wheats in GENEJAR and summarised that null alleles were detrimental to extensibility; the Glu-3 pattern b b b gave the best extensibility, particularly in combination with Glu-1 alleles b b a. Glu-3 b b c also had excellent extensibility. Glu-A3 e is a null allele; Glu-B3 c, d and g alleles had medium to weak dough properties, and should be avoided at the early stages of a breeding programme. For Glu-3, the best combinations are b b b, b b c, and c b c.

Vazquez *et al.* (1996) reported that the allelic variation at the Glu-A3 locus did not have a significant influence on gluten strength, whereas the allelic variation at the Glu-B3 locus did significantly affect gluten strength, measured by sedimentation volume. Null alleles also did not negatively affect quality despite their presence implying a lower level of glutenin polymerisation (Payne *et al.*, 1987).

Biochemical (Autran *et al.*, 1987) and genetic (Pogna *et al.*, 1990) studies indicated that the positive effects associated with the Gli-1/Glu-3 complex were due to Glu-3 alleles. However, the influence of the omega gliadins, encoded at Gli-1 loci was evidenced for dough extensibility (Branlard and Felix, 1994). The alleles at the Glu-A3 locus affected both quantity and the size of the polymers. The positive effects of the glutenin subunits could be attributed primarily to their capacity to form inter-molecular disulphide linkages. LMW-GS affected the quantity and /or size distribution of the polymers due to differences in the amounts and type their subunits.

The relative quantity of total glutenin is a prime factor determining dough strength and the positive effect of HMW-GS & LMW-GS might be due to increased total glutenin rather than qualitative superiority of specific subunits. Using near-isogenic bread wheat lines, Lawrence *et al.* (1988) showed that the percentage of densitogram area under HMW-GS (in SDS-PAGE gels) was strongly associated with dough strength. There are many exceptions to

the qualitative basis of allele superiority. Some bread wheat cultivars contain HMW-GS 5+10, but produce weak doughs. Furthermore, 1BL-1RS wheat-rye translocation lines consistently produced weak-sticky doughs irrespective of their HMW-GS composition. In contrast, many good bread-making quality cultivars possess HMW-GS 2 +12.

The effects of the Glu-1 and Glu-3 alleles in a wider range of genotypes are needed before their use in predicting dough properties can be fully justified. A better understanding of the effect of individual alleles on quality parameters will provide clearer information for the bread-making quality breeders.

The major objective of this work was to develop a set of recombinant inbred lines (RILs) showing allelic variation at Glu-1 and Glu-3 loci in a set of NZ wheat populations with varing bread-making qualities. These RILs presented an opportunity to study the allelic effect on bread-making quality parameters in a common genetic background.

The aim of this study was to (1) find out the effect of the glutenin alleles on the baking quality parameters; and (2) clarify differences between the alleles for different quality parameters, in order to provide information for wheat breeders and complement the general information relating specific glutenin alleles to bread-making quality.

## *5.3 Materials and Methods*

### *5.3.1 Material - Crossing Progeny*

From 14 New Zealand lines, 10 crosses were made in 1995 and their progenies grown at Crop & Food Research Institute under the supervision of Dr. W. B. Griffin, From the 10 crosses, 5 F3 populations were selected and analysed for individual plant variation by SDS-PAGE. The F3 SDS-PAGE results were used to determine the banding patterns in the resulting F4 families. The F4 families were then grouped for analysis. The F4 progeny families which had the same band patterns were bulked for quality tests. 52 F4 bulks originating from 193 F4 families were tested for wholemeal flour protein (WFP), hardness, SDS sedimentation and Pelshenke, and 50 by mixograph analysis. The SDS-PAGE analyses of F3 plants and quality tests of F4 materials were carried through in 1997&1998, at INRA, Clermont-Ferrand, France.

Table 5-1 lists the HMW-GS and LMW-GS alleles of the parental lines, and table 5-2

lists the 5 crosses which had their progeny quality tested.

**Table 5-1.** Glutenin Alleles of Parental Cultivars

| Parent | Quality | GluA1 | GluB1 | GluD1 | GluA3 | GluB3 | GluD3 |
|---|---|---|---|---|---|---|---|
| Tui | D | 2* | 7+9 | 5+10 | e | Sec-12 | b |
| Morahi | A | 2* | 7+9 | 5+10 | d | Sec-13 | b |
| Rongotea | B | 2* | 7+9 | 5+10 | e | b | b |
| Otane | A | 2* | 7+8 | 2+12 | d | b | a |
| Pernel | D | 1 | 7+8 | 5+10 | c | g | b |
| Karamu | C | n | 7+8 | 2+12 | c | b | b |
| Involved Allele No. | | 3 | 2 | 2 | 3 | 4 | 2 |

*The name of alleles for HMW-GS based on Payne and Lawrence (1983), and LMW-GS on Gupta and shepherd (1990c). Sec-12 and Sec-13

**Table 5-2.** Glutenin Alleles in the Progeny of the Crosses and the Number of analysed F3 Plants and F4 bulks

| Cross | GluA1 | GluB1 | GluD1 | GluA3 | GluB3 | GluD3 | F3 plant | F4 group# | Possible groups |
|---|---|---|---|---|---|---|---|---|---|
| Morahi x Tui (Pop5) | 2* | 7+9 | 5+10 | e/d | Sec-13/-12 | b | 34 | 7 | 9 |
| Morahi x Rongotea (Pop6) | 2* | 7+9 | 5+10 | e/d | Sec-13/b | b | 31 | 6 | 9 |
| Otane x Karamu (Pop7) | 2*/n | 7+8 | 2+12 | d/c | b | a/b | 33 | 14 | 27 |
| Otane x Pernel (Pop9) | 2*/1 | 7+8 | 2+12/5+10 | d/c | b/g | a/b | 51 | 23 | 243 |
| Rongotea x Tui (Pop10) | 2* | 7+9 | 5+10 | e | Sec-12/b | b | 44 | 3 | 3 |

# The possible number of F4 groups that would result from the cross to give a fully balanced factorial design including all the glutenin profiles

### *5.3.2 Analysing methods*

Analyses of the materials were carried out in INRA, Station D'Amelioration des Plantes, Clermont-Ferrand, France.

*I* ***Protein separation by SDS-PAGE***

193 F3 plants were tested by SDS-PAGE. The protocol is based on Singh *et al.* (1991). In order to have better resolution for both HMW-GS and LMW-GS, the acrylamide/bisacrylamide concentration was constant and the gel concentration (T) and the cross linker (C) were modified as follow: T = 12.8%, C = 0.99%, by the laboratory of Dr. G. Branlard, INRA, Clermont-Fd, France.

*II* ***Allele reading of HMW-GS & LMW-GS on SDS-PAGE***

The bands of HMW-GS & LMW-GS on SDS-PAGE were read with the standardised HMW-GS methodology and nomenclature described by Payne and Lawrence (1983), and for LMW-GS using the methodology and nomenclature described by Gupta and Shepherd (1990a).

*III* ***Quality tests***

A Near Infrared Reflectance (NIR) instruments (Inframatic 8620 Perten Instruments, Hamburg, Germany) was used to estimate the wholemeal flour protein content and hardness, white flour protein content and moisture level. This result was also used for calculating the amount of added water to the flour for the mixograph test (Martinant *et al.* 1998).

For the SDS sedimentation test, 5g of wholemeal flour was used, according to the procedure described by Axford *et al.* (1979).

The Pelshenke test was carried out by using 10g of wholemeal flour. The original procedure (Pelshenke, 1933) was employed for determining the dough swelling time. A temperature controlled cabinet was designed to better control the condition of dough swelling.

A 10g mixograph was performed according to the American Association of Cereal Chemist (AACC) approved method AACC 54-40A (1988). Dough hydration took into account flour protein content, flour moisture and grain hardness (Martinant *et al.* 1998). The mixograph curves were computed by Mixomart ® software. Mid-line Peak Value (MPV) and Mid-line Peak Time (MPT) were the major parameters used in this study.

Percentage ranges were calculated to determine the effect of a change in a particular allele on the value of each quality parameter. The formula used for each population was;

$$100 * \frac{\{(\text{maximum mean allele value}) - (\text{minimum mean allele value})\}}{\{[(\text{maximum mean allele value}) + (\text{minimum mean allele value})]/2\}}$$

### *IV Statistical analysis*

Statistical analysis was by the computer statistics software Minitab and SAS 6.12 for Windows. ANOVA contrast models in SAS were used for the allelic comparison, the data were grouped and analysed for each pair of alleles on each locus (Table 5-6). It can be seen from Table 5-2 that the data did not consist of full factorials for every allele combination. In most instances several possible combinations of alleles were missing. This was a consequence of the random nature of the assortment of the alleles into the F3 and F4 populations. Therefore the statistical model used for the analysis was required to take into account the unbalanced nature of some of the designs. SAS is well set up to handle this type of unbalanced ANOVA's using it's General Linear Models procedure. A main effects only model was therefore used for each family with interactions ignored but all main effects included for each population analysis. As a consequence all means are least squares means in the results tables. These main effects models were then used to establish contrasts between the means that were consistent with a variety of genetical expression models (dominant/recessive, additive, overdominant). Because there were generally only two degrees of freedom available for the contrasts and generally 6 models were tested, the actual p values determined by the models will be underestimates though their relative values will be consistent. Equally the overdominant model will underestimate the level of overdominance because the heterozygosity was established in the F3 seed and therefore the F4 seed would consist of 25% of each allele in the homozygous form and 50% of the heterozygote.

## *5.4 Results*

### *5.4.1 Quality differences among the F4 populations*

**Table 5-3.** Quality test results for all the parents used in the crosses

| Parent | Wholemeal Flour Protein (%) | Hardness | Sedimentation (ml) | Pelshenke (min) | Mid-line Peak Time (min) | Mid-line Peak Value (%) |
|---|---|---|---|---|---|---|
| Tui | 11.83 | 83 | 43.1 | 156.8 | 2.8 | 44.2 |
| Morahi | 12.19 | 49 | 50.8 | 193.1 | 3.7 | 49.3 |
| Rongotea | 12.25 | 78 | 48.8 | 156.2 | 3.0 | 51.5 |
| Otane | 10.85 | 58 | 52.2 | 159.8 | 4.4 | 46.9 |
| Pernel | 12.33 | 73 | 58.3 | 175.2 | 3.9 | 48.0 |
| Karamu | 10.85 | 70 | 57.3 | 38.0 | 2.2 | 45.8 |

*The results for each cultivar are the average of 12 samples from the same environment.

**Table 5-4.** The population@ mean values for the measured quality parameters for all crosses listed in Table 5-2

| Rank | Wholemeal Flour Protein | Hardness | Sedimen-tation | Pelshenke | Mid-line Peak Time | Mid-line Peak Value |
|---|---|---|---|---|---|---|
| I | 6 | 7 | 7 | 6 | 9 | 7 |
| II | 7 | 9 | 9 | 9 | 6 | 6 |
| III | **5 (I)** | 10 | **6 (I-II)** | 5 | **10 (I)** | **9 (I)** |
| IV | **10 (I-II)** | 6 | **10 (I-III)** | **10 (I-III)** | **5 (I)** | **10 (I)** |
| V | **9 (I-II)** | 5 | **5 (I-III)** | **7 (I-IV)** | **7 (I)** | **5 (I-II)** |

***Bold ( )** indicates the population mean was significantly different from the ranked population(s) enclosed within the brackets.

**@ The population numbers are the same as those listed in Table 5-2.**

**Table 5-5.** Descriptive statistics of the measured quality parameters for all the populations

| Quality parameters | Population | Mean | Std Dev | Min | Max |
|---|---|---|---|---|---|
| Wholemeal Flour Protein (%) | 5 | 14.8 | 0.5 | 14.1 | 15.5 |
| | 6 | 16.0 | 0.4 | 15.5 | 16.5 |
| | 7 | 15.5 | 1.2 | 13.5 | 17.6 |
| | 9 | 13.9 | 1.3 | 11.9 | 16.8 |
| | 10 | 14.2 | 0.7 | 13.4 | 14.6 |
| Hardness | 5 | 61.3 | 13.0 | 36 | 76 |
| | 6 | 71.4 | 6.8 | 64 | 81 |
| | 7 | 82.2 | 9.0 | 68 | 97 |
| | 9 | 75.6 | 9.3 | 54 | 94 |
| | 10 | 72.0 | 7.6 | 64 | 79 |
| Sedimen-tation (ml) | 5 | 51.7 | 3.0 | 48 | 56 |
| | 6 | 57.2 | 3.5 | 53 | 62 |
| | 7 | 64.1 | 6.9 | 55 | 82 |
| | 9 | 63.0 | 4.0 | 54 | 70 |
| | 10 | 52.0 | 2.7 | 49 | 54 |
| Pelshenke (min) | 5 | 172 | 23 | 151 | 212 |
| | 6 | 190 | 14 | 174 | 206 |
| | 7 | 119 | 24 | 92 | 172 |
| | 9 | 178 | 35 | 123 | 251 |
| | 10 | 150 | 26 | 125 | 177 |
| Mid-line Peak Time (min) | 5 | 2.22 | 0.06 | 2.12 | 2.28 |
| | 6 | 2.34 | 0.20 | 2.16 | 2.63 |
| | 7 | 1.97 | 0.24 | 1.68 | 2.51 |
| | 9 | 3.12 | 0.65 | 2.31 | 4.37 |
| | 10 | 2.25 | 0.39 | 1.97 | 2.69 |
| Mid-line Peak Value (%) | 5 | 59.1 | 1.9 | 55.5 | 60.3 |
| | 6 | 64.3 | 0.8 | 63.4 | 65.4 |
| | 7 | 67.7 | 6.1 | 58.4 | 82.9 |
| | 9 | 60.4 | 4.0 | 55.4 | 68.7 |
| | 10 | 59.6 | 1.3 | 58.2 | 60.8 |

There were significant differences among the population means for all quality parameters measured except hardness (Table 5-4). Population 7 had the greatest value for hardness, sedimentation and MPV, even though both of its parents did not have obviously higher MPV and sedimentation values (Table 5-3). Its sedimentation value and MPV were significantly higher than the populations ranked III-V below. This implies that the recombination may have had an additive effect for these two parameters, especially for MPV. The relatively low Pelshenke time of population 7 could be the result of inheritance from one of the parents, Karamu, whose Pelshenke time was exceptionally low. Population 9 ranked 1st for MPT, and 2nd for hardness, sedimentation and MPV, but its whole flour protein content was the lowest (Tables 5-4&5-5). This result further confirmed that greater protein quantity does not necessarily mean better quality of bread-making. The greatest MPT of population 9 could result from both parents, Otane and Pernel (Table 5-3) as they were both high for this character. Population 6 had the greatest WFP, consistent with its lineage as both of its parents, Morahi and Rongotea, had relatively high WFP.

The original design of the experiments was to produce completely balanced sets of progeny, however, the segregation of the F3 plants for the alleles was not entirely balanced. According to the populations' allelic segregation, the appropriate populations were chosen for the particular allelic comparisons as indicated in Table 5-6.

**Table 5-6.** Population and allele's code used for allele's contrast model

| Locus | | Glu-A1 | | Glu-D1 | Glu-A3 | | Glu-B3 | | Glu-D3 |
|---|---|---|---|---|---|---|---|---|---|
| Population | | 7 | 9 | 9 | 5+6 | 7+9 | 5+6 | 9 | 7+9 |
| Allele Name | | 2*, n | 2*, 1 | 5+10, 2+12 | d, e | d, c | b, Sec-12, Sec-13 | b, g | a, b |
| In Model | x | n | 1 | 2+12 | e | c | see **Table 5-7** | g | a |
| | y | 2* | 2* | 5+10 | d | d | | b | b |

*The data used for population (5+6) were strictly balanced, the rest of the analyses used incompletely balanced data.

**Table 5-7.** The ANOVA contrast models which were tested for significance. The x/y designations correspond with different alleles in different populations as indicated in Table 5-6

| Two Allele Models | | Three Allele Models | |
|---|---|---|---|
| Model | Allele Designation (x, y) | Model | b, Sec-12 (S12) & Sec-13 (S-13) |
| Over Dominant | xy>y>x | 1 Recessive | b>S12=S13=S12S13=bS13 |
| Over Dominant | xy>x>y | Two Equal Alleles | S12>bS13=S12S13=S12=S13 |
| Over Dominant | xy>y=x | | S13>bS13=S12S13=S12=b |
| Dominant | y=xy>x | 1 Dominant | b=bS13>S12=S12S13=S13 |
| Dominant | x=xy>y | Two Equal Recessive | S12=S12S13>b=S13=bS13 |
| Additive | y>xy>x | Alleles | S13=S12S13=bS13>b=S12 |
| | | One Allele Over Dominant | S12>b=S13>bS13=S12S13 |
| | | Three Additive Alleles | b>bS13>S13>S12S13>S12 |
| | | Two Equal Alleles Additive | b>bS13>S13=S12S13=S12 |
| | | with the Third Allele | S12>S12S13>b=bS13=S13 |
| | | | S13>bS13=S12S13>b=S12 |

*The direction indicators could apply either way (> or <) in a model as long as they are all consistently in the same direction in any contrast model. All models are simplified by assuming in models with more than one difference that all neighbouring differences within a model were of the same size.

### *5.4.2 Comparison of HMW-GS alleles n, 1 & 2* on Glu-A1*

**Table 5-8a.** Comparison of effects produced by Alleles at the Glu-A1 Locus on Measured Quality Parameters; **Alleles 2* & n** (see methods for calculation of range %)

| Quality Parameters | | Wholemeal Flour Protein (%) | Hardness | SDS Sedimentation (ml) | Pelshenke (min) | Mid-line Peak Time (min) | Mid-line Peak Value (%) |
|---|---|---|---|---|---|---|---|
| Allele Value | 2* | 15.2 | 77.6 | 66.3 | 130.4 | 2.02 | 68.7 |
| | n | 15.6 | 80.5 | 60.4 | 97.3 | 1.86 | 65.2 |
| | 2*/n | 15.8 | 89.3 | 68.1 | 138.7 | 2.04 | 70.2 |
| LSD | | 1.2 | 15.6 | 9.4 | 29.8 | 0.34 | 5.5 |
| Range % | | 3.9 | 14.2 | 11.9 | 33.9 | 9.1 | 7.4 |
| Best fitted Models (Better on top) | | | | | | | |
| Model | | No | Over dominant | Over dominant | Dominant | No | Over dominant |
| Allele | | significant | 2*/n>n>2* | 2*/n>2*>n | 2*=2*/n>n | significant | 2*/n>2*>n |
| p value | | difference | 0.081 | 0.095 | **0.013** | difference | 0.072 |
| Model | | | Over dominant | Dominant | Over dominant | | Dominant |
| Allele | | 2*=n | 2*/n>n=2* | 2*=2*/n>n | 2*/n>2*>n | 2*=n | 2*=2*/n>n |
| p value | | | 0.094 | 0.097 | **0.014** | | 0.083 |

**Table 5-8b.** Comparison of effects produced by Alleles at the Glu-A1 Locus on Measured Quality Parameters; **Alleles 2* & 1**

| Quality Parameters | | Wholemeal Flour Protein (%) | Hardness | SDS Sedimentation (ml) | Pelshenke (min) | Mid-line Peak Time (min) | Mid-line Peak Value (%) |
|---|---|---|---|---|---|---|---|
| Allele Value | 2* | 13.1 | 71.1 | 63.1 | 178.9 | 3.17 | 52.8 |
| | 1 | 15.4 | 72.1 | 63.1 | 164.2 | 3.14 | 61.1 |
| | 2*/1 | 12.9 | 76.8 | 60.8 | 167.5 | 3.16 | 58.4 |
| LSD | | 1.5 | 14.8 | 4.4 | 28.5 | 0.93 | 4.4 |
| Range % | | 18.1 | 7.8 | 3.7 | 8.6 | 1.0 | 14.5 |
| Best fitted Models (Better on top) | | | | | | | |
| Model | | Over dominant | No | No | No | No | dominant |
| Allele | | 1>2*>2*/1 | significant | significant | significant | significant | 1=2*/1>2* |
| p value | | **0.014** | difference | difference | difference | difference | **0.033** |
| Model | | dominant | | | | | Over dominant |
| Allele | | 1>2*=2*/1 | 2*=1 | 2*=1 | 2*=1 | 2*=1 | 2*/1>1>2* |
| p value | | **0.016** | | | | | **0.041** |

* **Bold** represents a significant genetic model for the parameter as indicated in the table.

The results in Table 5-8 represent a comparison of results produced from two pairs of alleles at locus Glu-A1. The comparisons were carried out separately in different populations but both pairs had the 2* allele involved.

For the pair wise comparison of the 2* and null (n) alleles, no significant differences were found between families possessing these two alleles for WFP and MPT. However, families containing the 2* allele showed greater values for all the other quality parameters except possession of allele n resulted in families with higher hardness. These increases were generally not significant in direct comparisons using the LSD. The genetic models 'over dominant' or 'dominant' for the 2* allele having a greater value were, however, generally applicable and significant at the 10% level for this pairing. For Pelshenke time significance with these models was even greater.

For the pair-wise comparison of F4 families containing the 2* and 1 alleles, the significant models were also 'over dominant' and 'dominant'. Families possessing allele 1 had a greater value than those possessing allele 2*, both for WFP and MPV. No significant

differences (P=0.1 or below) were found between families different for these two alleles for the other measured quality parameters.

Families containing allele 2* had a significantly longer Pelshenke time than those containing the n allele. Possession of allele 2* also resulted in a longer Pelshenke time than possession of allele 1 (though not significantly). This result supports the belief that allele 2* had a positive effect on the dough strength parameters. The results also suggested that allele 1 is related to high WFP and MPV.

### *5.4.3 Comparison of HMW-GS alleles 5+10 & 2+12 on Glu-D1*

**Table 5-9.** Comparison of effects produced by Alleles at the Glu-D1 Locus on Measured Quality Parameters; **Alleles 5+10 & 2+12**

| Quality Parameters | | Wholemeal Flour Protein (%) | Hardness | SDS Sedimentation (ml) | Pelshenke (min) | Mid-line Peak Time (min) | Mid-line Peak Value (%) |
|---|---|---|---|---|---|---|---|
| Allele Value | 5+10 | 13.8 | 72.4 | 63.0 | 194.7 | 3.59 | 57.9 |
| | 2+12 | 13.4 | 73.4 | 58.5 | 133.2 | 2.81 | 54.0 |
| | 5+10/2+12 | 14.5 | 72.4 | 63.3 | 170.8 | 3.41 | 54.0 |
| LSD | | 1.5 | 15.8 | 4.3 | 28.1 | 1.80 | 13.9 |
| Range % | | 7.9 | 1.4 | 7.8 | 37.0 | 23.8 | 7.1 |
| Best fitted Models (Better on top) | | | | | | | |
| Model | | No | No | Dominant | Additive | No | No |
| Allele | | significant | significant | (5+10)=(5+10/2+12)>(2+12) | (5+10)>(5+10/2+12)>(2+12) | significant | significant |
| p value | | difference | difference | 0.057 | **0.002** | difference | difference |
| Model | | | | Additive | Dominant | | |
| Allele | | 5+10=2+12 | 5+10=2+12 | (5+10)>(5+10/2+12)>(2+12) | (5+10)=(5+10/2+12)>(2+12) | 5+10=2+12 | 5+10=2+12 |
| p value | | | | 0.105 | **0.006** | | |

* **Bold** represents a significant genetic model for the parameter as indicated in the table.

For locus Glu-D1, the possession of alleles 5+10 and/or 2+12 caused no significant differences between their progeny for WFP, Hardness, MPT and MPV. However, F4 families containing allele 5+10 had significantly higher sedimentation volume and longer Pelshenke times than families containing allele 2+12. Possession of allele 5+10 also resulted in greater WFP, MPT and MPV, and lower hardness than possession of allele 2+12, but not at a significant level. These results give some support to the general finding that possession of allele 5+10 is better for baking quality than allele 2+12. The 'dominant' and 'additive' models

were applicable for both SDS sedimentation volume and Pelshenke time, and both of models were highly significant for Pelshenke time.

### *5.4.4 Comparison of LMW-GS alleles c, d & e on Glu-A3*

**Table 5-10a.** Comparison of effects produced by Alleles at the Glu-A3 Locus on Measured Quality Parameters; **Alleles c & d**

| Quality Parameters | | Wholemeal Flour Protein (%) | Hardness | SDS Sedimentation (ml) | Pelshenke (min) | Mid-line Peak Time (min) | Mid-line Peak Value (%) |
|---|---|---|---|---|---|---|---|
| Allele Value | d | 16.0 | 78.4 | 67.3 | 167.8 | 2.52 | 64.6 |
| | c | 14.3 | 76.8 | 66.2 | 164.7 | 2.97 | 64.2 |
| | d/c | 14.5 | 76.4 | 62.1 | 154.8 | 3.09 | 56.5 |
| LSD | | 1.0 | 9.2 | 4.0 | 18.1 | 0.94 | 6.8 |
| Range % | | 11.4 | 2.6 | 8.0 | 8.0 | 19.9 | 14.7 |
| Best fitted Models (Better on top) | | | | | | | |
| Model | | Dominant | No | Over dominant | No | No | Over dominant |
| Allele | | d>c=d/c | significant | d=c>d/c | significant | significant | d=c>d/c |
| p value | | **0.014** | difference | **0.01** | difference | difference | **0.03** |
| Model | | Additive | | Over dominant | | | dominant |
| Allele | | d>d/c>c | d=c | d>c>d/c | d=c | d=c | c>d>d/c |
| p value | | **0.019** | | **0.012** | | | **0.053** |

* **Bold** presents a significant genetic model for the parameter as in the table.

**Table 5-10b.** Comparison of effects produced by Alleles at the Glu-A3 Locus on Measured Quality Parameters; **Alleles e & d**

| Quality Parameters | | Wholemeal Flour Protein (%) | Hardness | SDS Sedimentation (ml) | Pelshenke (min) | Mid-line Peak Time (min) | Mid-line Peak Value (%) |
|---|---|---|---|---|---|---|---|
| Allele Value | d | 15.7 | 72.2 | 54.3 | 181.1 | 2.29 | 63.2 |
| | e | 15.3 | 71.2 | 53.4 | 173.2 | 2.31 | 60.0 |
| | d/e | 14.9 | 62.4 | 53.6 | 190.2 | 2.25 | 60.9 |
| LSD | | 0.9 | 17.9 | 5.8 | 28.0 | 0.21 | 3.8 |
| Range % | | 5.2 | 14.3 | 1.7 | 9.4 | 2.6 | 5.2 |
| Best fitted Models (Better on top) | | | | | | | |
| Model | | Over dominant | No | No | No | No | Additive |
| Allele | | d>e>d/e | significant | significant | significant | significant | d>d/e>e |
| p value | | 0.080 | difference | difference | difference | difference | 0.092 |
| Model | | | | | | | Dominant |
| Allele | | | d=e | d=e | d=e | d=e | d>e=d/e |
| p value | | | | | | | 0.093 |

* **Bold** presents a significant genetic model for the parameter as in the table.

F4 families possessing allele d had higher values than families possessing either of the alleles c or e for all of the measured quality parameters except MPT. F4 families possessing allele d had shorter MPT than F4 families possessing either allele c or e. The higher WFP of families with allele d over those with allele c is significant. In general, possession of allele d was better for wheat quality than possession of either allele c or e. The applicable significant genetic models indicated that allele d may be recessive to allele c.

The results of Table 5-10 suggest that at the Glu-A3 locus, possession of allele d is more desirable for improving wheat quality than possession of either allele c or e. The shorter MPT of flour containing allele d means that less work input would be required in mixing times with genotype containing this allele while still maintaining the higher values for the other measured quality parameters. This result agrees with the conclusion of Gupta *et al.* (1990a) for Rmax in Australian wheat.

### *5.4.5 Comparison of LMW-GS alleles b, g, Sec-12 & Sec-13 on Glu-B3*

**Table 5-11a.** Comparison of effects produced by Alleles at the Glu-B3 Locus on Measured Quality Parameters; **Alleles b & g**

| Quality Parameters | | Wholemeal Flour Protein (%) | Hardness | SDS Sedimentation (ml) | Pelshenke (min) | Mid-line Peak Time (min) | Mid-line Peak Value (%) |
|---|---|---|---|---|---|---|---|
| Allele Value | b | 14.0 | 73.7 | 61.4 | 167.3 | 3.39 | 56.8 |
| | g | 12.7 | 71.6 | 62.5 | 171.1 | 3.49 | 54.2 |
| | b/g | 14.5 | 76.4 | 62.8 | 174.2 | 3.64 | 53.9 |
| LSD | | 1.7 | 17.3 | 5.3 | 33.8 | 1.99 | 14.0 |
| Range % | | 13.1 | 6.5 | 2.2 | 4.0 | 7.1 | 5.3 |
| Best fitted Models (Better on top) | | | | | | | |
| Model | | Over dominant | No | No | No | No | No |
| Allele | | b/g>b>g | significant | significant | significant | significant | significant |
| p value | | **0.034** | difference | difference | difference | difference | difference |
| Model | | Dominant | | | | | |
| Allele | | b=b/g>g | b=g | b=g | b=g | b=g | b=g |
| p value | | 0.060 | | | | | |

* **Bold** presents a significant genetic model for the parameter as in the table.

In Table 5-11a, F4 families containing allele b had greater WFP than those containing allele g. The 'over-dominant' genetic model "b/g >b>g" was significant for this pair of alleles for WFP. The higher WFP of allele b agree with the conclusions of Gupta (1990a) and Cornish's (1993). For all the other parameters, there was no significant difference between alleles b and g.

**Table 5-11b.** Comparison of effects produced by Alleles at the Glu-B3 Locus on Measured Quality Parameters; **Alleles b, Sec-12 & Sec-13**

| Quality Parameters | | Wholemeal Flour Protein (%) | Hardness | SDS Sedimentation (ml) | Pelshenke (min) | Mid-line Peak Time (min) | Mid-line Peak Value (%) |
|---|---|---|---|---|---|---|---|
| Allele Value | b | 16.1 | 69.7 | 58.7 | 195.3 | 2.44 | 64.5 |
| | S12 | 15.0 | 82.2 | 49.2 | 187.3 | 2.23 | 60.7 |
| | S13 | 15.1 | 65.1 | 51.6 | 161.9 | 2.17 | 59.1 |
| | bS13 | 15.6 | 69.3 | 55.3 | 183.3 | 2.32 | 62.3 |
| | S13S12 | 14.9 | 56.7 | 54.0 | 179.7 | 2.26 | 60.1 |
| LSD | | 1.3 | 25.9 | 8.3 | 40.3 | 0.30 | 5.4 |
| Range % | | 7.8 | 36.7 | 17.7 | 18.4 | 11.8 | 8.8 |
| Best fitted Models (Better on top) | | | | | | | |
| Model | | One allele additive with two equal alleles | No | One allele additive with two equal alleles | 1 recessive 2 equal alleles | 1 dominant 2 equal recessive | One allele additive with two equal alleles |
| Allele | | b>bS13>S13=S12S13=S12 | significant | b>bS13>S13=S12S13=S12 | bS13=S12S13=S12=b>S13 | b>bS13>S13=S12S13=S12 | b>bS13>S13=S12S13=S12 |
| p value | | **0.041** | difference | **0.015** | 0.069 | **0.028** | **0.037** |
| Model | | 1 dominant 2 equal recessive | | 1 dominant 2 equal recessive | One allele additive with two equal alleles | 1 recessive 2 equal alleles | 1 recessive 2 equal alleles |
| Allele | | b=bS13>S12=S12S13=S13 | b=S12=S13 | b=bS13>S12=S12S13=S13 | b=S12>bS13=S12S13>S13 | b>S12=S13=S12S13=bS13 | b>S12=S13=S12S13=bS13 |
| p value | | 0.053 | | **0.021** | 0.082 | **0.038** | **0.048** |

* **Bold** presents a significant genetic model for the parameter as in the table.

In Table 5-11b, F4 families possessing allele b had higher values for all the measured quality parameters than families possessing either Sec-12 or Sec-13, with the exception of hardness. Sec-12 appeared to be a high hardness recessive allele. However, its influence was not significant. In general, possession of allele b was better for increasing flour quality characteristics relative to either allele Sec-12 or Sec-13. Genetic models were significant for WFP, SDS sedimentation, MPT and MPV. In the significant genetic models, possession of allele b mainly resulted in higher quality values and was additive with the other two alleles.

Table 5-11a indicates for the F4 families containing alleles b & g on locus Glu-B3, no significant differences were found between any of the measured quality parameters, except that allele b had higher WFP than allele g. Allele b seems to be a better baking quality allele than either allele Sec-12 or Sec-13. Sec-12 seemed to be related to a higher hardness value.

### *5.4.6 Comparison of LMW-GS alleles a & b on Glu-D3*

**Table 5-12.** Comparison of effects produced by Alleles at the Glu-D3 Locus on Measured Quality Parameters; **Alleles a & b**

| Quality Parameters | | Wholemeal Flour Protein (%) | Hardness | SDS Sedimentation (ml) | Pelshenke (min) | Mid-line Peak Time (min) | Mid-line Peak Value (%) |
|---|---|---|---|---|---|---|---|
| Allele Value | a | 14.6 | 77.7 | 64.5 | 151.3 | 3.01 | 58.5 |
| | b | 15.6 | 77.7 | 66.6 | 162.7 | 2.19 | 68.1 |
| | a/b | 14.6 | 76.2 | 64.4 | 173.2 | 3.38 | 58.6 |
| LSD | | 1.2 | 10.5 | 4.6 | 20.7 | 1.07 | 7.8 |
| Range % | | 6.7 | 1.9 | 3.4 | 13.5 | 41.6 | 15.5 |
| Best fitted Models (Better on top) | | | | | | | |
| Model | | Over dominant | No | No | Over dominant | Over dominant | Over dominant |
| Allele | | b>a>a/b | significant | significant | a/b>b>a | a/b>a>b | b>a>a/b |
| p value | | 0.099 | difference | difference | 0.064 | **0.011** | **0.017** |
| Model | | | | | | Over dominant | Dominant |
| Allele | | | a=b | a=b | | a/b>a=b | a=a/b>b |
| p value | | | | | | **0.023** | **0.028** |

* **Bold** presents a significant genetic model for the parameter as in the table.

On locus Glu-D3, possession of allele b lead to higher WFP, SDS sedimentation, Pelshenke and MPV values, though not significantly so. The MPT associated with allele b was significantly shorter than that associated with allele a. The negative relationship between MPT and MPV, is also indicated in Chapter 4 of this thesis, and is generally observed. The higher MPV and short MPT associated with allele b on locus Glu-D3 could be explained by its higher LMW-GS quantity, which resulted in a higher quantity of aggregated proteins. The consequently higher ratio of "Aggregated Protein" (HMW-GS + LMW-GS)/"Non- Aggregated Protein" (Gliadin) provided more polymeric proteins, which can increase the strength, tenacity and the resistance of the dough (ie. MPV). The higher the amount of aggregated glutenin in the dough, the easier or quicker the gluten forms. MPT is the time of maximum resistance, when the gluten network is at its best. Therefore, families containing allele b had a higher MPV, and logically, a shorter MPT than allele a. The 'over dominant' genetic model seemed applicable to this pair of alleles. In general, possession of allele b was associated with better quality characters than possession of allele a.

## *5.5 Discussion*

**Table 5-13.** Overall summary of the effects of the different alleles on the quality parameters

| Quality Parameter | | | | | | Locus | Mean Range % |
|---|---|---|---|---|---|---|---|
| WFP | HAR | SED | PEL | MPT | MPV | | |
| Significant (P<0.1) differences between alleles and their direction. | | | | | | | |
| - | (-) | 2*>n | 2*>n | - | 2*>n | A1-HMW | 13.4 |
| 1>2* | - | - | - | - | 1>2* | A1-HMW | 9 |
| - | - | 5+10>2+12 | 5+10>2+12 | - | - | D1-HMW | 14.2 |
| d>c | - | (-) | - | - | (-) | A3-LMW | 10.8 |
| (-) | - | - | - | - | d>e | A3-LMW | 6.4 |
| b>g | - | - | - | - | - | B3-LMW | 6.4 |
| b>Sec13/ Sec12 | - | b>Sec13/ Sec12 | b/Sec12> Sec13 | b>Sec13/ Sec12 | b>Sec13/ Sec12 | B3-LMW | 16.8 |
| b>a | - | - | b>a | a>b | b>a | D3-LMW | 13.8 |
| Number (%) of significant differences for that quality parameter | | | | | | | (P=0.47) ↑ |
| 6 (75) | 1 (12.5) | 4 (50) | 4 (50) | 2 (25) | 6 (75) | | |
| Mean range (%) of values across F4 populations for that quality parameter | | | | | | | |
| 9.2 | 10.7 | 7 | 16.6 | 14.6 | 9.8 | ←(P=0.36) | |

"(-)" Indicates that there was a significant difference (P<0.10) but only for the heterozygote relative to one of the homozygotes. "-" indicates no significant difference.

When considered among all the F4 cross populations, significant differences existed for every measured quality parameter in at least one population. Hardness seemed to be the least responsive to changes in the glutenin alleles, with only one weak (P=0.08) overdominant significant model resulting. However, the average percentage range resulting across all populations of F4 families differing in their glutenin alleles was greater for hardness than for several other characters that showed more significant differences. This can be explained by assuming hardness was more intrinsically variable than several (sedimentation volume in particular) of the other characters. The differences in the mean percentage ranges were not

significant for either the particular quality test or the loci being investigated. Thus these values did not indicate where the most important responses to changes in alleles were likely to occur. A statistical contrast between all the loci representing HMW alleles (12.2%) and LMW alleles (10.8%) for percentage ranges did not indicate a significant statistical difference. This indicated, for the alleles tested, that the average effects on flour quality parameters were about equal for changes in HMW and LMW alleles. This equality of action between HMW and LMW allele changes is consistent with some authors (eg. Payne, 1987a) though others have found HMW glutenins to have a greater effect (eg. Branlard and Felix, 1994). In four instances the percentage ranges associated with a particular allele exceeded 30%. This indicated that there were very strong differences in the quality parameters associated with the possession of a particular allele. In this series of trials it was not possible to measure glutenin quantities for all the F4 bulks. Therefore it is not known whether the changes in quality parameters associated with changes in alleles were a consequence of altered total glutenin quantity or altered quality of the glutenins. MPT had only two significant associations with particular alleles whereas values of the remaining characters were significantly associated with possession of a particular glutenin in 50% or 75% of the comparisons.

Where changes in an allele led to a significant increase in a quality measure then in all but one instance that allele was associated with a higher value for all significant changes in quality parameters. The sole variation was in the Glu-D3 alleles where MPT and MPV changed in inverse directions (though as discussed in the results this may be expected) when changing from possessing allele a to allele b. The best fit genetic model was also consistent for any allele comparison. Most frequently the best fitting model was a dominant or overdominant model rather than an additive model. The most consistent exception was for the Glu-B3 comparison of b, Sec 12 & Sec13 where an additive model dominated. There must be some concern however, about the reality of some of the overdominant models in a genetic sense as 50% of the individual grains should have segregated out in the F4 to be homozygous since the heterozygous condition was determined in the F3. This may indicate that the mixture of alleles creates an overdominant effect even if the mixture is attained by mixing different homozygous lines.

For Glu-A1, 2* appears to be a better baking quality allele (ie. confers better values for the quality parameters measured in the F4 population tested) than allele n. This is consistent with the findings of Sontag-Strom *et al*, 1996. The data presented here suggest that allele 1 may be even better. Studies on Australian wheat cultivars ranked several of the Glu-3 alleles

for their effects on R max (Gupta and Shepherd 1988; Gupta *et al.*, 1989, 1990, 1991, 1994; Metakovsky, E. V. *et al.* 1990). Our rankings, though on different characters, are entirely consistent with their results. For example we both found Glu-A3 d>c & e, Glu-B3 b >g, (Sec12 & Sec13 not mentioned in the Australian data), and Glu-D3 b>a. From among the loci tested it is possible to select a preferred genotype which would be A1:1, B1:?, D1(5+10), A3:d, B3:b, D3:b. It should be stressed that these selections are only from among the alleles tested. The selections also assume that improvements in the quality parameters tested will result in improvements in bread-making quality. Confirmation of this hypothesis will depend on the values of actual bread-making qualities obtained from bulks of these lines in future generations.

In some instances the most significant models showed strong overdominance effects suggesting F1 hybrid wheats may have some advantages in quality (eg. the 2*/n hybrid at the locus Glu-A1 was superior for several characters). Conversely often the overdominance was in the direction of reduced benefit from the hybrid (eg. the 2*/1 hybrid at the locus Glu-A1 was inferior for several characters). In this latter situation early generation testing could overemphasise the negative aspects of a particular cross. Though, as specified earlier, these findings on significant overdominance models must be tempered by the knowledge that many of the F4 grains tested were not heterozygotes but had segregated to produce an F4 mix of heterozygotes and both types of homozygotes.

In conclusion this study provided evidence that some of the glutenin allelic variations in RIL's can significantly improve values for at least some of the quality characters which have been found to be related to bread making. The information given above could therefore be a valuable reference for designing a quality breeding programme for bread making wheat. We also believe that the more extensive testing of the progeny of these RIL's particularly for actual glutenin quantities as well as allelic composition and for actual bread-making quality rather than just for characters given here will be of even greater use to future breeding programmes.

# Chapter 6
# General Discussion

## *6.1 Research Background and Objectives*

Glutens are a group of proteins that are unique to wheat. As a consequence wheat has unique properties suiting it for bread making. Gluten is comprised of two major groups of protein, in approximately equal proportions, gliadin and glutenin. Glutenins are polymeric and form the structure of the gluten. The large number of glutenin alleles, their interactions in the gluten matrix and their response to the environment are not fully understood. However, the extensive allelic variation of glutenins and their interactions with the environment are very important in creating the variation among wheats in bread-making quality. As it is labour intensive and time consuming to quantify some of the selection parameters related to bread making quality, a simple method of predicting these parameters is desirable for wheat quality breeders.

### *6.1.1 Environmental Effects on Glutenin and Quality Parameters*

The quality of wheat for bread-making is affected by many different factors. It has been frequently shown that environment can affect protein quantity (Fowler and de la Roche, 1975; Lelley *et al.*, 1997). The first objective of this thesis was to determine environmental effects on glutenin composition. The environment in this instance was modified by different fertilizer regimes. Subsequently the responses of the measured wheat quality parameters to these changes could be determined.

### *6.1.2 Relationships among Glutenin, Quality Parameters and their Predicability*

Small-scale quality tests are very useful for early selection in wheat breeding programs. There are a variety of these small scale tests which represent different aspects of potential bread-making quality. Some of the tests are much more labour intensive and time consuming than others. The second objective of this thesis was to better understand the relationships among the quality test parameters. The third objective was to explore the predicability among

lines and environments of the more difficult to determine quality tests using the simpler tests. If there are universal and strong relationships among the quality tests, use of these as predictors should make selection among lines much more effective for wheat.

### *6.1.3 Allelic Effects on the Quality Parameters*

In the selection phase of a breeding program, selection of appropriate HMW-GS and LMW-GS types (the two constituents of glutenin), is important to effectively improve bread-making quality (Gupta *et al.*, 1989c & 1990d/e). Allelic effects on some of the quality parameters have been studied for HMW-GS and LMW-GS respectively (Gupta *et al.*, 1990c/d, 1991b & 1992). The effects of favourable variants of HMW-GS and LMW-GS on dough properties can be combined to maximise the genetic potential of the progeny. The last objective of this thesis was, therefore, to study a wide range of alleles and determine their effects on quality parameters. Achieving this objective would complement existing knowledge of allelic difference on quality parameters and provide further selection criteria for breeding programs.

## *6.2 Major Research Procedure*

### *6.2.1 Generation of Research Material*

All research materials were generated at Lincoln, Christchurch, New Zealand. The GXE experiment was carried out at Lincoln University; crossing of the wheat cultivars, and growing of the progenies were carried out at Crop & Food Research, Crown Research Institute of New Zealand. Fourteen New Zealand cultivars were chosen for both the GXE and crossing experiments based on achieving a wide spread of bread-making quality characters and a wide range of glutenin alleles (Payne and Lowrence, 1983; Gupta and Shepherd, 1990c).

#### *I GXE Material*

Fourteen cultivars all received six treatments, which varied in the amount of nitrogen and sulphur fertiliser applied as well as in the timing of the application (full details in Table 3-1). Two replications, ie. 168 samples, were used for later analyses and quality tests.

*II* ***Crossing Progeny***

Ten crosses were made based on the available glutenin allele information in order to create recombinant inbred lines (RILs). Five crosses were chosen for later analyses and quality tests (full details in Table 5-1/2/3).

### *6.2.2 Analyses and Quality Tests*

All analyses and quality tests were carried out in INRA, Clermont-Fd, France.

The analyses included: SDS-PAGE for both the GXE and F3 individual plants of the crosses; glutenin/HMW-GS/LMW-GS quantification of the GXE material by the combined methods of SDS-PAGE, spectrophotometer and densitometer.

Quality parameters determined for both the GXE and F4 material were: WFP, FP (only for GXE material) and hardness by a Near Infrared Reflectance (NIR) instrument; 5 gram SDS sedimentation test results; 10 gram Pelshenke test results and; 10 gram mixograph results. From the mixograph the MPT and MPV values were selected as the most useful (Martinant, 1998).

The HMW scores (Branlard *et al.*, 1992) were calculated for both the GXE and F4 material to determine its utility for predicting SDS sedimentation, Pelshenke time, MPT and MPV.

All data were analysed by the statistics software SAS, Minitab and Statgraphics Plus.

## *6.3 Major Findings and Contributions of this Research*

### *6.3.1 The Effects of N & S Application on HMW-GS, LMW-GS and Quality Parameters*

The quantities of Glutenin/HMW-GS/LMW-GS were found to be primarily genetically determined. The late application nitrogen did increase the quantity of all three of these parameters, but not by a substantial or significant amount (Tables 3-2/3, Figure 3-1).

N application did significantly increase the value of several quality parameters: WFP, FP, hardness, SDS sedimentation, and MPV; it also increased the Pelshenke time but not significantly (Table3-4).

S application did not affect any quality parameters significantly when applied alone, however, when applied together with late N there were a number of significant differences as

indicated above (Table 3-4).

Genotypic differences were the major source of the variation in quality parameters. However, this was complemented by interactions of fertiliser applications and genotypes, which were found to significantly influence WFP, FP, hardness and MPV (Tables 3-5/6).

With respect to obtaining good quality parameters from the harvested grain and restricting the conclusions to the NZ wheat cultivars tested, PBI 3058 and Kotare are recommended for use in specific controlled environments. Domino, Otane and Morahi are recommended for their stable quality performance over more diverse environments (Table 3-6 and Figure 3-2).

### *6.3.2 Relationships among Quality Parameters and HMW-GS/LMW-GS*

When compared within a genotype across environments resulting from replicate and fertiliser treatment variations, the quantities of glutenin/HMW-GS/LMW-GS were all significantly correlated with the following quality parameters: WFP, FP, SDS sedimentation volume, and MPV (Tables 4-4).

Both the GXE and F4 materials presented highly significant correlations for the parameters: WFP-MPV/MPT; SDS sedimentation-hardness/MPV; MPV-hardness/MPT; and MPT/Pelshenke (Tables 4-5/6).

The SDS sedimentation, Pelshenke, MPT and MPV values are more difficult to measure. It was shown that they could be predicted for the GxE material and most of the F4 material from the combined results of the other more basic tests such as: WFP, FP and hardness. When used in conjunction with the HMW score and sedimentation volume it was also possible to predict the Pelshenke time for the F4 material. SDS sedimentation mainly required WFP or FP for it's prediction; Pelshenke time was mainly associated with HMW score, hardness and FP/WFP; MPV was more predicable than MPT and both were predicted from a function of FP/WFP, hardness and/or HMW score (Table 4-7).

Most predicted values for both the GXE and F4 materials had highly significant correlations with the measured values. However, the prediction equations derived from the GXE and F4 materials respectively could not be applied to the other material. The predictions, therefore, are not universal and would only apply to the materials and environments in which they were used. However, given this major limitation, for any particular set of wheat materials, the use of predictive equations should provide an efficient guide for breeders, growers and

traders to modify their plans and strategies (Tables 4-8/9).

### *6.3.3 Allelic Comparison of HMW-GS and LMW-GS*

Significant differences for all the measured quality parameters were found among all five F4 populations (Tables 5-4/5).

For Glu-A1, when possession of the alleles 2* and n were compared, allele 2* appeared to be dominant or overdominant to the null (n) allele and provided greater SDS sedimentation, Pelshenke and MPV values. Possession of the null allele may be associated with higher hardness. However, there was no significant difference between possession of these two alleles for the parameters WFP and MPT (Table 5-8a). When possessions of the alleles 2* and 1 were compared, allele 1 appeared overdominant or dominant to allele 2* for WFP and MPV. Possession of allele 1 provided higher values for these two parameters. There were no significant differences between possession of these two alleles for other quality parameters that were compared (Table 5-8b).

For Glu-D1, possession of allele 5+10 was associated with higher SDS sedimentation and Pelshenke values than possession of allele 2+12. There was no significant difference between possession of these two alleles for the other measured quality parameters (Table 5-9).

For Glu-A3, possession of allele d was compared with possession of alleles c and e separately. Possession of allele d provided greater WFP and MPV than was provided by both alleles c & e. Possession of allele d was also associated with higher SDS sedimentation values than possession of allele c. Therefore, possession of Glu-A3, allele d could be a valuable high quality predictor in breeding programmes (Tables 5-10a/b).

For Glu-B3, possession of allele b was compared to possession of allele g. No significant differences were found between possession of alleles b and g except that allele b provided higher WFP (Table 5-11a). Possession of allele b was also compared to possession of the secalin alleles Sec-12 & Sec-13 (originating from rye, Field *et al.*, 1983). Allele b was either dominant over or additive to the two secalin alleles, and provided higher WFP, SDS sedimentation, Pelshenke, MPT and MPV values than either of the secalin alleles (Table 5-11b).

For Glu-D3, possession of allele b provided greater WFP, Pelshenke and MPV values than possession of allele a. It also resulted in short MPT values, which confirms the negative correlations between MPT & MPV given in chapter 4. No significant differences were found

between possession of alleles a & b for hardness and SDS sedimentation values (Table 5-12).

### *6.3.4 Conclusions*

This thesis has quantified glutenin/HMW-GS/LMW-GS, as well as several quality parameters for: 1) their associations among a number of wheat lines in several fertiliser environments, 2) their correlations among themselves and the predicability for SDS sedimentation volume, Pelshenke time, MPV and MPT of the mixograph. This thesis has also compared allelic effects on these quality parameters and attempted to find out their allelic relationships (either additive, dominant or overdominant). The opportunity has thus been created to cross check on the relationships among wheat lines, alleles and environments with bread making quality parameters.

In general, the findings of this thesis suggest that bread-making quality can be mainly improved by genetic selection, particularly through the additive inclusion of the best possible glutenin alleles. However, in addition providing the best environment (in this instance N supply) can allow full expression of the genetic potential (Chapter 3). The quantification of glutenin/HMW-GS/LMW-GS relationships with other quality parameters confirmed that not only the quantity but also the quality of the glutenin subunits can make a significant difference to quality parameters which have been shown elsewhere to be related to baking quality (Gupta and Shepherd, 1987, 1988; Payne *et al.*, 1987b; Gupta *et al.*, 1989a&c, 1990c&d, 1994b&c; Metakovsky *et al.*, 1990). However, what is required next is that these results are extended from quality related variables, to actual baking quality.

For instance, the Pelshenke result was strongly associated with HMW-GS score in chapter 4, and this association was also confirmed by the longer Pelshenke time provided by possession of allele 5+10 relative to 2+12 on Glu-D1, one of the HMW-GS loci (Chapter 5). The allelic comparisons have also shown significant influences from LMW-GS, such as:

1) Possession of allele d for Glu-A3 resulting in higher WFP, SDS sedimentation and MPV values. Consistent with this result these three parameters were also found to be highly correlated in Chapter 4;

2) Possession of allele b on Glu-B3 was shown to result in higher WFP than possession of allele g. Higher values for all the measured quality parameters except for hardness were obtained from possession of allele b than possession of the secalin alleles from a rye translocation;

3) Possession of allele b on Glu-D3 resulted in higher WFP, Pelshenke and MPV values. The lower MPT, associated with the higher MPV and lower MPV associated with higher MPT also confirmed the negative correlations found for these two parameters in Chapter 4.

This combined influence of both the quantity and allele type for total glutenins as well as LMW-GS and HMW-GS is consistent with the findings in the literature where different authors have stressed different glutenin values as being important in determining quality (Gupta and Shepherd, 1987, 1988; Payne *et al.*, 1987b; Gupta *et al.*, 1989a&c, 1990c&d, 1994b&c; Metakovsky *et al.*, 1990). For wheat breeders this offers both the opportunity and problem of having a host of possible genes and interactions to deal with as a means of improving wheat quality.

The desire expressed at the start of the thesis to be able to predict the more difficult-to-measure parameters on the basis of the easier to measure parameters, has been shown to be only partially possible. The prediction equations derived for predicting quality parameters that are more labour and time consuming to determine was limited to within a particular set of material (Chapter 4). The equations derived were useful only for either the set of genetically more stable cultivars with various fertiliser condition or the set of segregating crossing progenies. No universally applicable equations have been found from this research.

The findings of this thesis have greatly complemented existing knowledge in a number of areas of wheat quality research:

1) Relationships were found among the quantity and quality of glutenin subunits with several small-scale quality parameters,

2) Relationships were found among these quality parameters themselves, and:

3) Effects were shown on quality parameters induced by allelic differences of glutenin subunits.

Taken together the results provided by this thesis could provide valuable information for: 1) wheat breadmaking quality breeders to modify their breeding program accordingly, 2) wheat grower and trader to adapt their cultivation and selection plans, 3) better general understanding about factors that influence breadmaking quality and associated characteristics.

## *6.4 Suggestions for Future Research*

### *6.4.1 Allelic Effect*

Following RILs into later generations with further segregation of the F4 material could confirm the allelic effects observed in this thesis and provide more definitive information about allelic effects on the quality parameters. Bulking up of this material would also allow the testing of the effects on actual bread-making quality to confirm its relationships to the quality parameters that were measured.

A statistical protocol has been developed that allows comparisons of alleles in earlier generations than is usually the case. With the aid of this protocol more crosses from different genetic resources could be created involving a wider range of glutenin alleles. Thus a more comprehensive comparison could be carried out on the quality effects of a greater range of glutenin alleles. This would also lead to the possibility of testing interactions between alleles at the same and different sites to determine whether such interactions are important.

The additive effects of different glutenin alleles on quality parameters, and the additive effects of glutenin alleles with certain gliadins could also be studied using the protocols used here, to provide a clearer picture of potential interactions for bread-making quality control.

The double-haploid methodology can be adopted to make the allelic comparison more effecient.

### *6.4.2 Environmental Effects on Glutenin Composition*

Further quantification of individual subunit responses to the environment could be determined by using the RILs generated in the crossing program. This could be done with the existing material or better still by taking the material through a few more generations to create more homozygous RILs. This could identify subunits (LMW/HMW) with greater or lesser environmental responses and thus answer the questions: which subunit (s) are more responsive to the environmental variations? and which subunits have more stable performance? This information should provide extremely useful guidelines for wheat quality breeders.

This analysis could be done by further quantification of individual subunits from existing SDS-PAGE gels of the GXE material, as well as by obtaining better separated proteins from two-dimensional gels and use of HPLC to confirm the observations.

### *6.4.3 Other Influential Components on Bread-making Quality*

Apart from glutenins, other components (such as pentosans) in wheat flour or bran could influence the Pelshenke values and MPT. These could be further investigated and their effects on quality parameters could also provide better understanding for wheat quality researchers and breeders. It is possible that inclusion of these components into the prediction equations would considerably improve their utility.

### *6.4.4 Adaptation of HPLC (High Performance Liquid Chromatography)*

HPLC has been widely used for analysis of HMW-GS (Bietz, 1984), and it would appear to be possible to adapt it for quantifying and identifying the LMW-GS as well. This would greatly facilitate many of the suggested future directions for research given above.

## *6.5 Final Statement*

Overall, this thesis has demonstrated that a combined genetic and agronomic approach to wheat quality improvement seems to offer substantial possibilities. However, the area of wheat quality is not simple and the results of this thesis confirm the findings of others that gains will be small and incremental. However, there still appears to be substantial scope for improvement.

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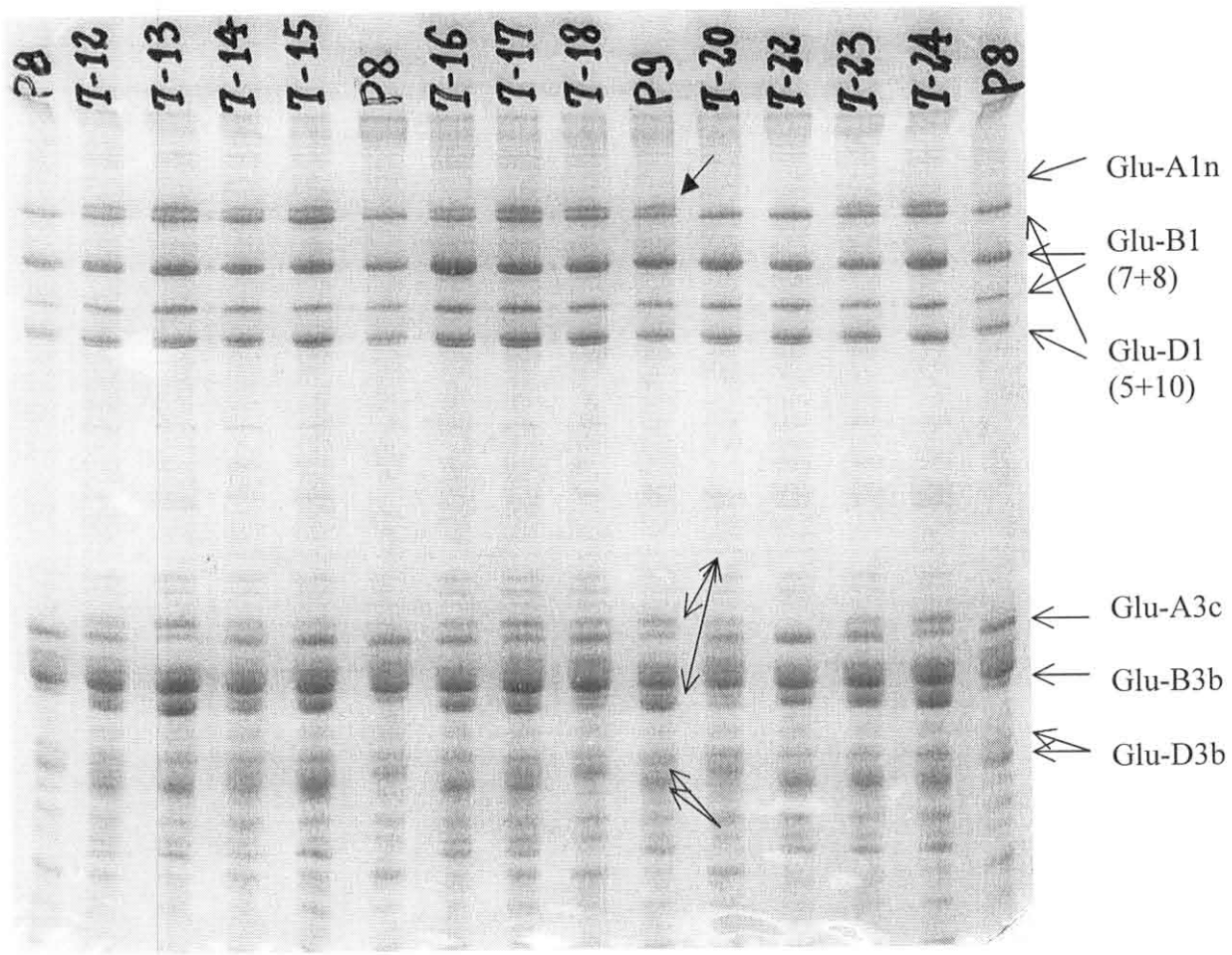
# Appendix I

## Electrophoresis Gels for the Materials

**Figure A-I-1.** SDS-PAGE of individual F3 plants for Population 7 (Table 5-2)




*P8 & P9 are the two parents of Population 7 used as standard, '7-12', 7-13 present plant numbered 12 and 13 of population 7, and so on.

Glutenin alleles of P8 for both HMW and LMW are illustrated as on the right side of the gel

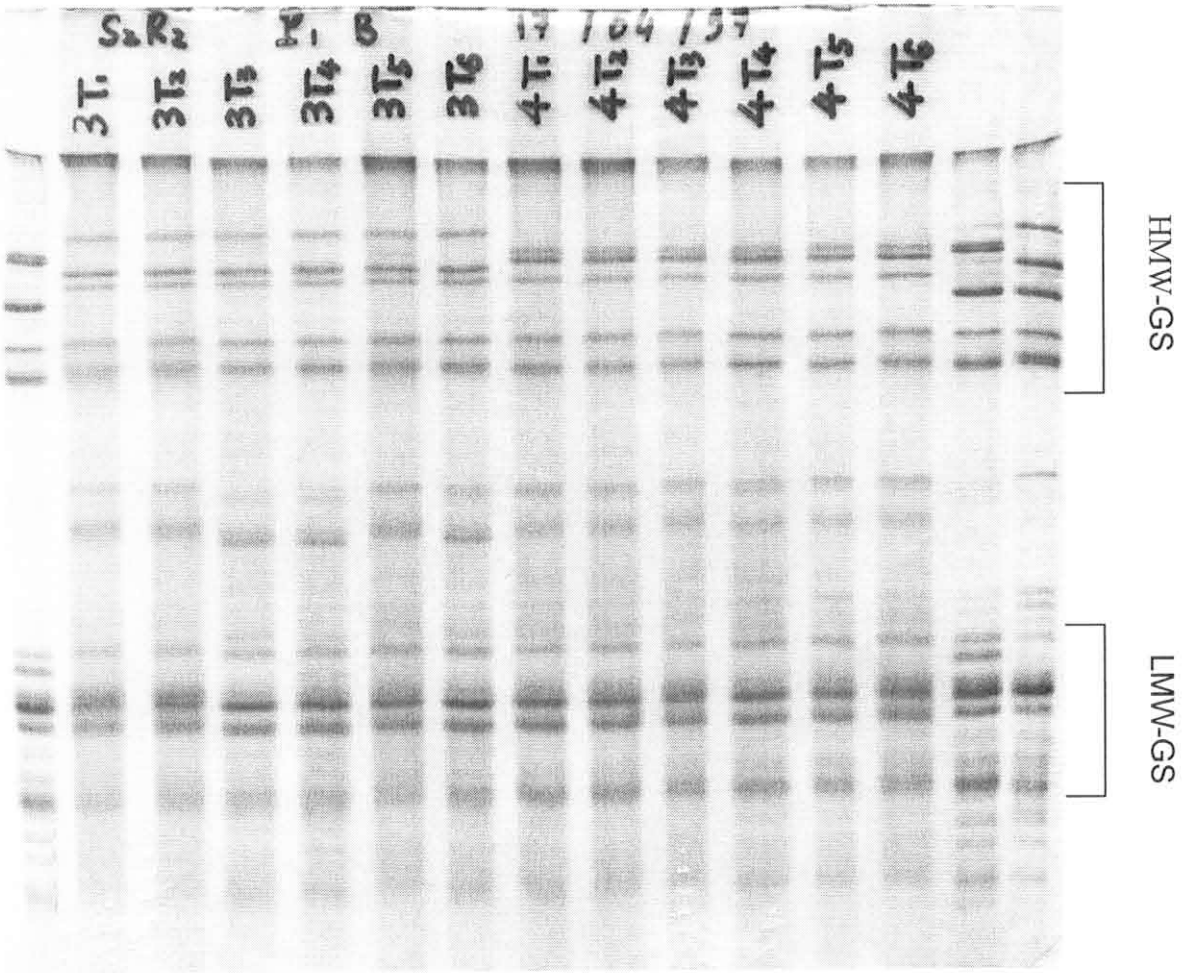
On the gel, for P9 Glu-B1, Glu-D1 and Glu-B3 alleles are the same as P8

← presents Glu-A1 allele 2*

↔ present Glu-A3 allele d

→ present Glu-D3 allele a

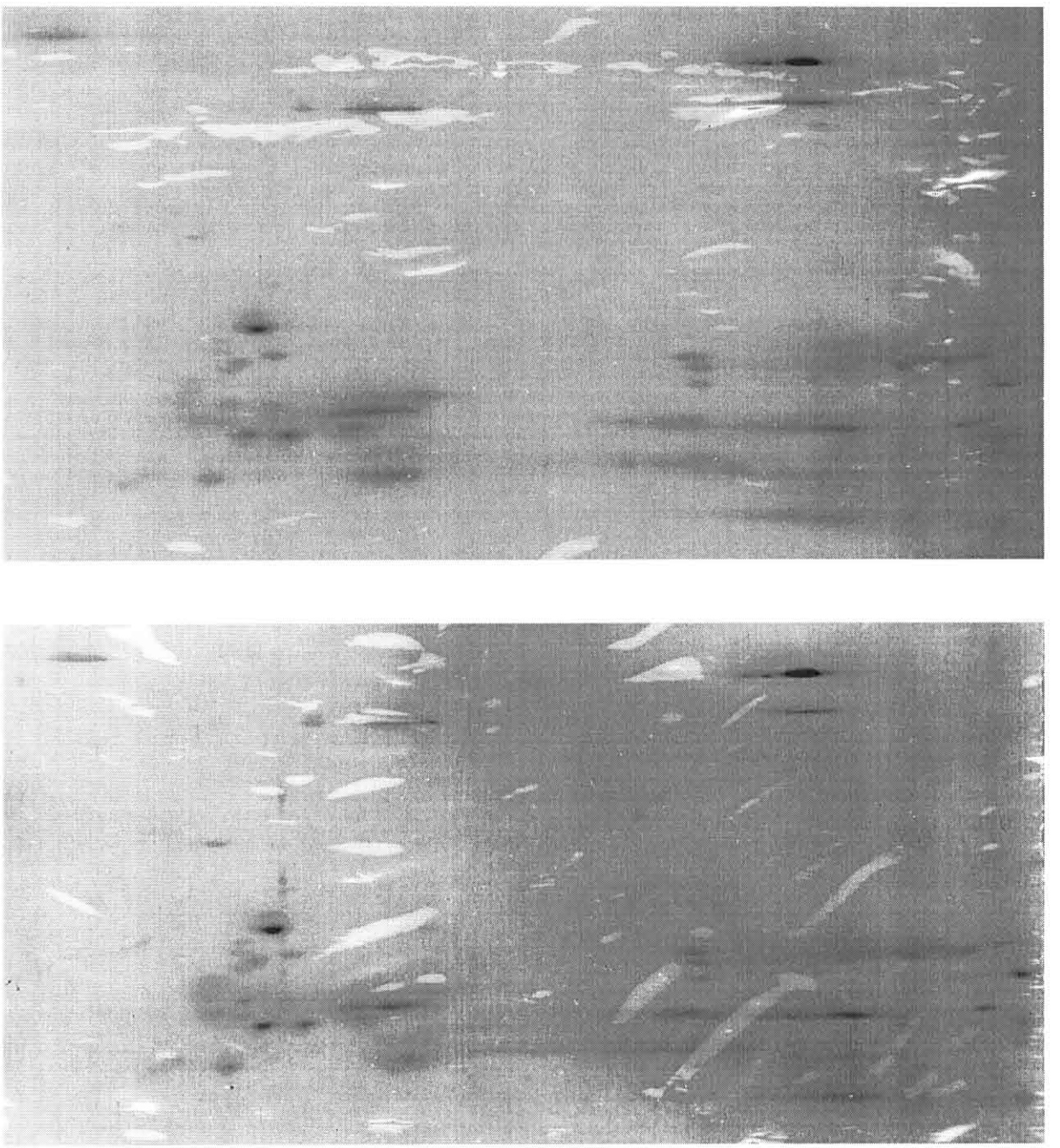
**Figure A-I-2.** SDS-PAGE of GXE material, cultivars No 3-Domino and No 4-PBI3058 with 6 treatments (detail information as shown in Tables 3-1, 3-4 & 3-5)




*French cultivars Curtot (lane 1 & 14) and Gerbier (lane 15) are used as standard.

HMW-GS and LMW-GS band were scanned and their respective areas and percentages were determined by densitometer system Hoefer Scientific Instruments, GS 3.02.

**Figure A-I-3.** 2-Dimentional (IPHGE X SDS-PAGE) gel of line 14 (27.330) (Table 3-5), Treatment 5 (top gel) and Treatment 6 (bottom gel) (Treatments as in Table 3-1)



* 1st dimension (→): immobilised pH gradient gel electrophoresis (IPHGE)

  2nd dimension (↓): sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

# Appendix II

# Quantification of HMW/LMW-GS

The procedure for obtaining HMW/LMW-GS area by Electrophoresis Data Reduction System (GS365w Version 3.02) were shown by the following figures. The data was from the scanned gels of GXE SDS-PAGE as shown in Figure A-I-3.

**Figure A-II-1**. Original scanning band of cultivar PBI3058 with treatment 2 (Table 3-1)

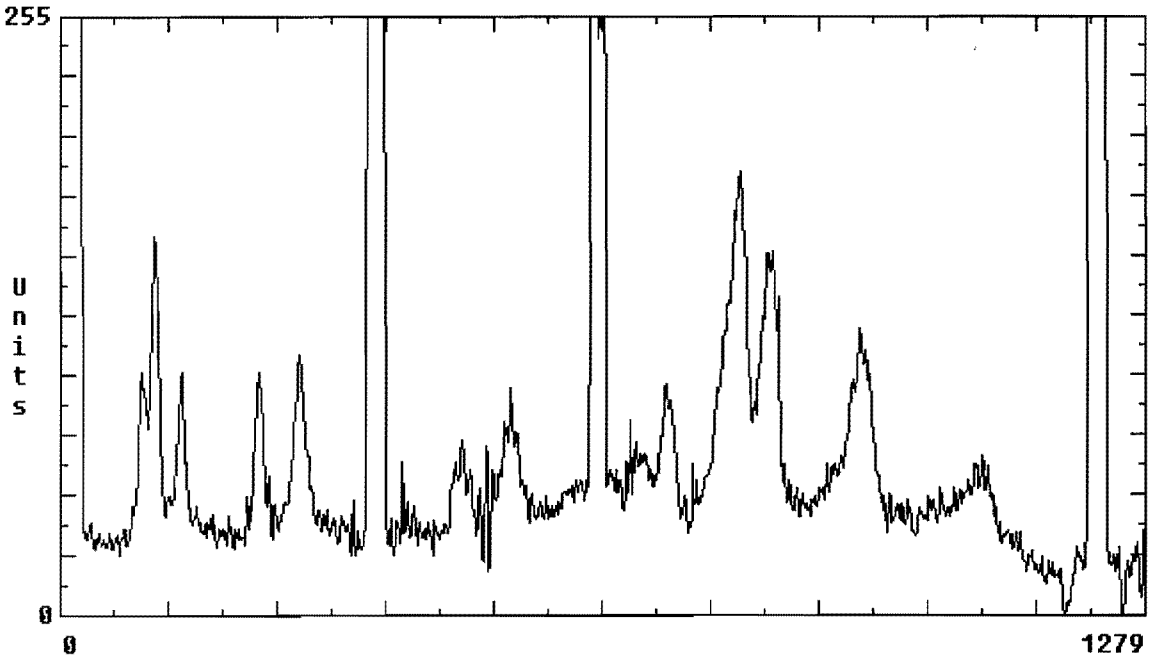


**Figure A-II-2**. Smoothed figure of Figure A-II-1

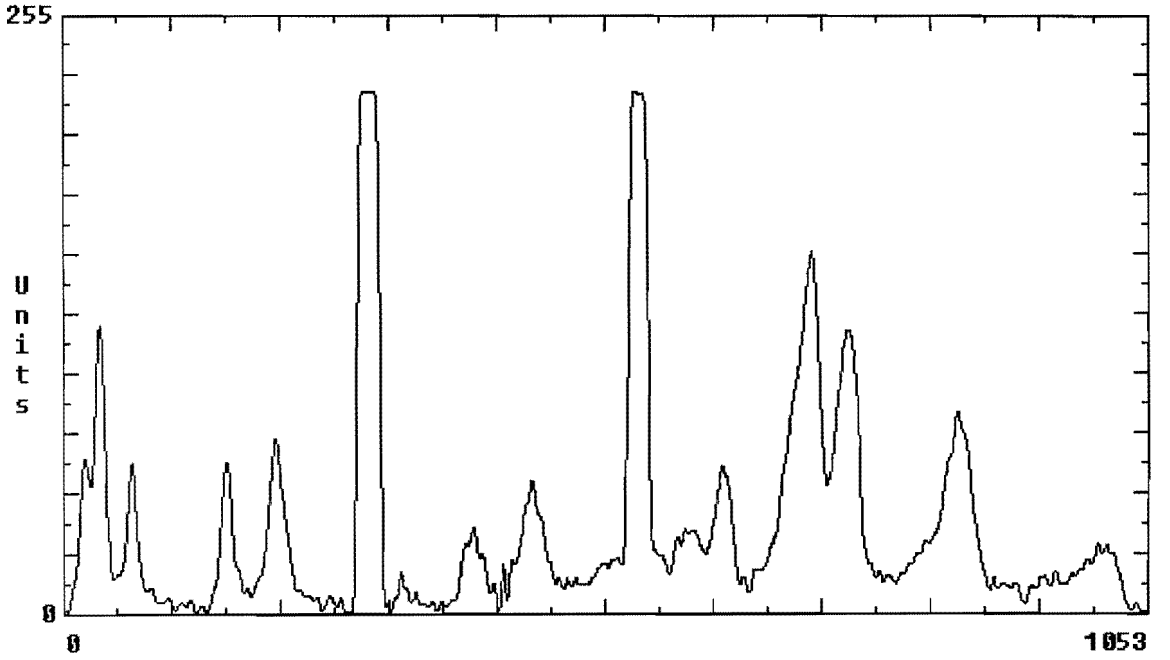


**Figure A-II-3.** After quick integration of Figure A-II-2, the areas of HMW-GS (peak 1) and LMW-GS (peak 2) was shown in Table A-II-1

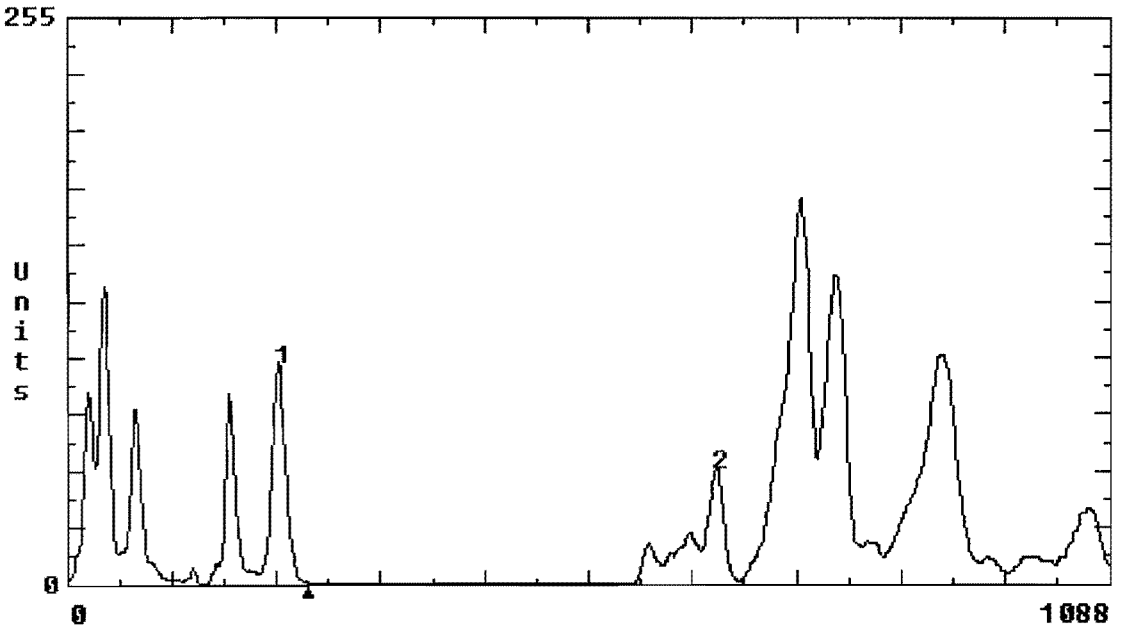



**Table A-II-1.** The areas of peak 1 (HMW-GS), peak 2 (LMW-GS) and Total (Glutenin) were shown by GS365w

| Quick Integration analysis | | | | | |
|---|---|---|---|---|---|
| n | CENTER | HEIGHT | WIDTH | AREA | %AREA |
| 1 | 220 | 99 | 249 | 7130 | 27.2 |
| 2 | 678 | 52 | 839 | 19061 | 72.8 |
| | | | TOTAL | 26191 | |

The quantities of HMW/LMW-GS and glutenin were calculated from the combined results of their areas by GS365w and concentrations of glutenin from spectrophotometry.