PROJECT REPORT No. 276

CHARACTERISATION OF A NOVEL GENETIC CONTRIBUTION TO THE BREADMAKING QUALITY OF WHEAT

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CHARACTERISATION OF A NOVEL GENETIC CONTRIBUTION TO THE BREADMAKING QUALITY OF WHEAT

by

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CONTENTS

ABSTRACT

The molecular basis for the role of protein and non-protein components of breadmaking wheat in determining the processing properties is not fully understood. Previous work performed at John Innes Centre on single chromosome substitution lines had identified chromosome 3A as having influence on the flour parameters related to breadmaking quality. However, the biochemical factors that mediated the observed chromosomal effects were not identified. Studies have suggested that 'soluble' or non-prolamin proteins, i.e. mainly albumins and globulins, which influence breadmaking quality are linked with chromosome 3A. Biochemical studies using single chromosome substitution and recombinant lines to determine the relationship between specific soluble proteins and quality parameters that are influenced by chromosome 3A could be used as a basis for identifying suitable markers for breadmaking quality of wheat.

During mechanical mixing a number of physical and chemical processes occur that result in the modification of the gluten structure. In particular, disulphide-sulphydryl interchange reactions occur reducing the stress within the dough. The inclusion of an oxidising agent creates new reaction possibilities and tends to favour the formation of inter-glutenin disulphide bonds thus stabilising the dough. At the molecular level, mixing creates and breaks bonds within high molecular weight glutenins: de-polymerisation forms smaller glutenin subunits which then reform to some extent. The rheological properties of bread dough, therefore, play an important role in the behaviour of dough during processing. They have been shown to exert a major influence on the quality of the final baked product and on the mixing energy required to produce optimal breadmaking performance. Rheological properties predict the ability to retain the gas produced during fermentation and ensure a satisfactory balance between viscosity (i.e. flow during expansion) and elasticity (i.e. the ability to retain shape). For this reason, the rheology of wheat dough is routinely tested by millers and bakers to check against flour specifications, classify flour samples into broad quality groups, i.e. ensure general "fitness for purpose", and thus identify gross flour faults which would result in severe processing problems within a plant. However, correlations with breadmaking quality are relatively poor and such measurements cannot replace breadmaking quality assessment.

In this study the genetic contribution to the breadmaking quality of wheat was evaluated in two breadmaking systems; the Chorleywood Bread Process (in which the dough is mixed to fixed energy) and a spiral mixer no-time dough (in which the dough is mixed to fixed time). Sets of Cappelle (Bezostaya) single chromosome substitution lines of wheat, together with the two parent wheats, and a population of 48 chromosome 3A recombinant lines were grown for the 1998 harvest at the JIC site at the Morley Research Centre. The growing of these sets was repeated for the 1999 harvest at three sites in the UK and two in Eastern Germany. Two of the UK sites were JIC sites at Morley and in Bawburgh, and the third was a Nickerson UK Ltd site at Bury St Edmunds. One of the German sites was at the University of Halle and the other was owned by PBI Ltd. The set of chromosome 3A recombinant lines, derived from Cappelle-Desprez (Bezostaya 3A) line, plus the parent wheats, were grown for the 2000 harvest at the two JIC sites and the Nickerson UK Ltd site.

AIMS

- To increase knowledge of the relationship controlled by chromosome 3A between biochemistry and breadmaking quality in wheat, in the context of near-commercial milling and baking techniques.
- To characterise genetically the novel 3A influence on breadmaking quality.
- To identify a tagging diagnostic marker (possibly DNA based, but could be biochemical or morphological), for easy selection of breadmaking quality, for use by wheat breeders.
- To identify the biochemical mechanism through which chromosome 3A affects breadmaking, and establish this as a flour quality test, if appropriate.

CONCLUSIONS

- A DNA map of chromosome 3A has been developed within this project. A useful map of six microsatellite markers was obtained, but the degree of polymorphism found between Capelle-Desprez and Bezostaya I was disappointing. This was particularly the case for the long arm around the site of the putative loaf volume gene (***Lvl 1***) deduced from this work.
- A single gene designated ***Lvl 1*** (after loaf volume) appears to be responsible for controlling loaf volume, crumb colour and crumb score. It is located on the long arm of chromosome 3A about 30 to 40 map units from the centromere. It is closely associated with a gene determining gel protein weight. It is possible that this gene could still be ***Lvl 1*** rather than a separate gene.
- A Quantative Trait Locus, which is likely to be a single gene controlling Falling Number, is located on the short arm very close to the centromere.
- A gene (or genes) affecting gel protein G' is located on a chromosome other than 3A and is a consequence of too few backcrosses in the development of the substitution line.
- The analysis of the single substitution lines between the donor Bezostaya I and recipient Capelle-Desprez has confirmed that Group 1 chromosomes of Bezostaya I improve many dough rheological properties compared with those of Capelle-Desprez.
- Single chromosome substitution studies confirmed the adverse effects of 3A and identified a similar influence of 3B on protein strength parameters. It appears that the effect of 3B matches that of 3A but in some cases exceeds its potency.
- Group 7 chromosomes also appear to be contributors to protein quality. These may be candidates for further detailed study.
- This study has added further information to the genetic basis of breadmaking quality but has also shown that environment exerts a major effect (i.e. the 'German effect' where the poor quality recipient Capelle-Desprez grown in eastern Germany produced significantly better loaf volume than the UK-grown equivalent.

IMPLICATIONS and BENEFITS

- The DNA marker map of chromosome 3A produced by this project will be useful to breeders concerned with any quality traits controlled by this chromosome. The traits include an influence on loaf volume which means that the results will be of value to the wheat production and usage chain.
- A new gene has been found which contributes to the regulation of *alpha*-amylase activity in grain. Control of this factor is important to the supply of UK wheat and its use.

Although not discussed in this project, benefit to the UK breeding and farming communities has been generated in terms of resistance to ear blotch since a single-gene resistance to *Septoria tritici* was located on chromosome 3A of Bezostaya I during the course of the study.

1. INTRODUCTION

The molecular basis for the role of protein and non-protein components of breadmaking wheat in determining the processing properties is not fully understood. The high molecular weight glutenin subunits (HMW-G, coded by loci on chromosomes 1A, 1B and 1D) are believed to account for about 50% of the variation in breadmaking quality. The low molecular weight glutenin subunits (LMW-G) and gliadins (coded by loci on chromosomes 1A, 1B, 1D and 6A, 6B, 6D), contribute to about 10% of the variation. Other non-gluten factors may account for a substantial unexplained remainder of the variation. These include: redox enzymes and substrates, such as thioredoxin (Wong *et al*., 1993) and hydroquinones (Koh and Lim, 2000), tyrosine linkages (Tilley *et al*., 2001), lipids (Pomeranz and Chung, 1978), pentosans (D'Appolonia *et al*., 1970), 1B/1R translocation (Martin and Stewart, 1986), hydrolytic enzymes, and low molecular weight 'soluble' proteins within the albumin and globulin fractions (Pogna *et al*., 1991; Arakawa *et al*., 1977; Zawistowska *et al*., 1986). Some of these proteins are enzymes, involved in metabolic processes, while others are amylase and protease inhibitors playing protective roles in plants (Wrigley and Bietz, 1988).

Identification of additional genes determining non-gluten factors and breadmaking quality might help to explain the nature of the relationships between such factors and quality. Such an approach was urged by Law and Krattiger (1987) using the Cappelle-Desprez (Bezostaya I) single chromosome substitution lines as an example.

Further work performed at JIC on these same substitution lines had identified chromosome 3A as having an influence on the flour parameters related to breadmaking quality (I Foot of Nickerson (UK) and D Feuerhelm of Elsoms Seeds Ltd, personal communication). Another set of single chromosome substitution lines in bread wheat had also shown an influence of chromosome 3A on loaf volume (Mansur *et al*., 1990). In addition, a set of recombinant inbred lines of soft and hard bread wheats had shown association between chromosome 3A and flour yield and mixograph tolerance value (Campbell *et al*., 2001), and a set of recombinant inbred lines of durum wheat had revealed an influence of chromosome 3A on wholemeal SDS sedimentation volume (Blanco *et al.*, 1998). The number of genes involved has, however, not been identified nor have the precise biochemical factors that mediated these observed chromosomal effects. Some of biochemical endosperm components which have effects on wheat quality and which are controlled by group 3 chromosomes have been reported. Starch-granule size distribution is influenced by group 3 and group 1 chromosomes (Batey *et al*., 1996). Some studies have suggested that 'soluble' or non-prolamin proteins, i.e. mainly albumins and globulins, which influence breadmaking quality are linked specifically with chromosome 3A (Mansur *et al*., 1990; Blanco *et al*., 1998; Garcia-Olmedo *et al*., 1982).

To answer some of these questions genetic and biochemical studies were carried out using single chromosome substitution and recombinant lines to identify some of the genes responsible for the reported effects and by doing so determine the relationship between specific soluble proteins and quality parameters.

Suitable proteins thus identified could then be used as markers for breadmaking quality of wheat. Similarly, the pentosans (arabino-xylans) have been linked to the group 3 chromosomes, most particularly the 3R chromosome of rye, which is homologous to the group 3 chromosomes of wheat (Cyran *et al*., 1996). Since the soluble pentosan fraction in rye contributes positively to loaf volume (Weipert, 1995), the soluble and total pentosans were surveyed in the available 3A recombinant lines.

During processing, bread dough is subjected to various stresses and strains. In particular careful control of the mixing conditions, as is usual in a typical high speed mixing Chorleywood Bread Process (CBP), is critical to the production of high quality bread products. In such systems, mixing disperses and hydrates the ingredients and mechanical energy converts the flour-ingredient-water mixture into a smooth visco-elastic dough. The inclusion of an oxidising improver and crystalline fat respectively stabilise the dough rheological characteristics, i.e. the ability to be extended into a thin, continuous sheet and improve the gas retention properties. In addition to the above features, breadmaking processes such as CBP have the capacity to more fully utilise protein content and their adoption by UK industry has resulted in reduced requirement for high protein wheat flour. Legislative changes in 1990 resulted in the withdrawal of potassium bromate, a slow acting oxidising agent, previously used in combination with ascorbic acid to improve dough machining and moulding properties. Since April 1990 ascorbic acid has been used as the sole oxidising improver. Although not an oxidising improver in its own right, ascorbic acid is converted into the oxidising agent dehydroascorbic acid in the presence of oxygen. This imposed change in bread improver formulation resulted in an increase in resistance to deformation in bread dough making dough more vulnerable to damage during machining and moulding. New commercial process conditions, such as pressure-vacuum mixing, were developed in order to enhance the oxidising performance of ascorbic acid. Such systems beat in extra oxygen from the air during the pressure part of the mixing cycle and then remove excess air during the vacuum phase to create the fine, even bubble structure required for UK pan bread. The mixing action of the lower energy spiral mixer brings the advantage of increased air occlusion (Marsh, 1999) thus increasing the oxygen available for transformation of ascorbic acid to dehydroascorbic acid and hence potential for oxidation in dough and increased average bubble size compared with CBP. The dough obtained from these two quite different mixing systems also varies in terms of rheology and density resulting in potential handling differences. Challenging Cappelle-Desprez (Bezostaya) single chromosome substitution and recombinant populations using the same recipe, but involving two different mixing processes (CBP and spiral as in this study), will permit assessment of their overall performance in relation to UK breadmaking processes.

During mechanical mixing a number of physical and chemical processes occur that result in the modification of the gluten structure. In particular, disulphide-sulphydryl interchange reactions occur reducing the stress within the dough (Williams & Pullen, 1999; Bushuk *et al*., 1997). The inclusion of an oxidising agent creates new reaction possibilities and tends to favour the formation of inter-glutenin disulphide bonds, thus

stabilising the dough. Therefore at the molecular level, mixing creates and breaks bonds within HMW glutenins: de-polymerisation forms smaller glutenin subunits which then reform to some extent. Size-exclusion high performance liquid chromatography (SE-HPLC) studies by Borneo & Khan (1999) have identified reductions in protein extractability during the mixing, moulding and fermentation stages of breadmaking. Work by Chamberlain (1985) suggested that approximately 5% of the available mechanical energy was required to break the disulphide bonds and the remainder used to mix the ingredients and break weaker bonds. Whilst the major exposure to stress and strain occurs in the mixing bowl, at every point in the process where dough is worked or allowed to relax, there is some effect on dough structure and rheology.

The rheological properties of bread dough, therefore, play an important role in the behaviour of dough during processing. They have been shown to exert a major influence on the quality of the final baked product (Cauvain *et al*., 1992) and on the mixing energy required to produce optimal breadmaking performance. Rheological properties predict the ability to retain the gas produced during fermentation and ensure a satisfactory balance between viscosity (i.e. flow during expansion) and elasticity (i.e. the ability to retain shape). For this reason, the rheology of wheat dough is routinely tested by millers and bakers to check against flour specifications, classify flour samples into broad quality groups, i.e. ensure general "fitness for purpose" and thus identify gross flour faults which would result in severe processing problems within a plant. However, correlations with breadmaking quality are relatively poor and such measurements cannot replace breadmaking quality assessment.

Basic rheological properties are measured in a number of predictive quality tests such as the Farinograph, Extensograph, Alveograph and Mixograph that use basic flour-water doughs and provide the data in empirical units. The most relevant deformation around an expanding gas bubble is considered to be biaxial extension. Bubble rupture is important in terms of effects on loaf volume and fineness of crumb structure and a number of methods have been developed to measure this. One such methodology is the inflation of a single bubble within a sheet of dough, as carried out in the Alveograph test. Small-scale deformation rheometry, using low strain conditions provides data in fundamental rheological terms and has greatly improved the characterisation of wheat flour dough (Bohlin *et al.*, 1980; Oliver & Pritchard, 1993) and the extracted gel protein fraction (Graveland *et al*., 1979; Pritchard & Brock, 1994). The amount of glutenin that is insoluble in sodium dodecyl sulphate (SDS), the gel protein mass, is related to the genetic background of the variety, correlates strongly with SDS sedimentation volume and can therefore be used to distinguish between broad categories of wheat (Salmon & Anderson, 2001). Gel protein elastic modulus provides a more fundamental understanding of the strength of this extracted protein fraction and hence helps in our understanding of breadmaking performance. Work by Whitworth (1999) compared the performance of a range of breadmaking quality predictors. The best discrimination between flours of very different quality proved to be gel protein rheology and basic stress-strain information, calculated from Alveograph measurements carried out on dough mixed at optimum water levels.

2 PROJECT OBJECTIVES

- To increase knowledge of the relationship controlled by chromosome 3A between biochemistry and breadmaking quality in wheat, in the context of near-commercial milling and baking techniques.
- To characterise genetically the novel 3A influence on breadmaking quality.
- To identify a tagging diagnostic marker (possibly DNA based, but could be biochemical or morphological), for easy selection of breadmaking quality, for use by wheat breeders.
- To identify the biochemical mechanism through which chromosome 3A affects breadmaking, and if appropriate establish this as a flour quality test.

3 MATERIALS AND METHODS

3.1 Cultivation of wheat samples

The Cappelle-Desprez (Bezostaya I) substitution lines were developed as described by Law and Worland (1996) and Korzun *et al.* (1997). The population of chromosome 3A recombinant lines had been generated following the procedures described by Law (1966) and Law, Snape and Worland (1988). Sets of Cappelle-Desprez (Bezostaya I) single chromosome substitution lines of wheat, together with the two parent wheats, and a population of 48 chromosome 3A recombinant lines were grown for multiplication for the 1998 harvest at the JIC site at the Morley Research Centre in Norfolk. The growing of these sets was repeated for the 1999 harvest at three sites in the UK and two in Eastern Germany. Two of the UK sites were JIC sites at Morley and Bawburgh, and the third was a Nickerson UK Ltd site at Bury St Edmunds. One of the German sites was at the University of Halle and the other was owned by PBI Ltd. The set of chromosome 3A recombinant lines, derived from Cappelle-Desprez (Bezostaya I 3A) line, plus the parent wheats and selected substitution lines, were also grown for the 2000 harvest at the two JIC sites and the Nickerson UK Ltd site. In all cases the wheat was grown in randomised block duplicate trial plots measuring 6 x 1.2m, according to standard practice of pest control and fertiliser addition for each site. The quality of the 1999 UK harvest of recombinant and single chromosome substitution lines was destroyed by bad weather, therefore only the two sets of single substitution lines (60 lines in total) grown in Germany in 1999 were available for study. This provided an opportunity to test previous estimates of their breadmaking quality. For biochemical studies, samples retained from the 1998 harvest were utilised. The study of the 3A recombinant lines was based entirely upon the 2000 harvest.

3.2 DNA mapping and statistical analysis

High density DNA mapping of the parent and chromosome 3A recombinant lines was carried out using selected Gartersleben (Germany) microsatellite markers, as described by Korzun *et al.* (1997). Statistical analysis of milling and baking characters was carried out by ANOVA. The map indicating the location of the markers was obtained using the 'JOIN-MAP' improved version of the specialised 'MAPMAKER QTL' software (Lander *et al*., 1987). QTL analyses by marker represssion and interval mapping were accomplished by using the Apple devised jointly by Birmingham and Warwick Universities and JIC and available on the

internet (www.bham.ac/g.g.seaton). Four microsatellite markers were located on the short arm of chromosome 3A and two on the long arm. A number of other microsatellite markers known to be located on the long arm were also tested but all were monomorphic. 24 prime sequence combinations were screened in each of the lines but none were found to be polymorphic. Cluster analyses of the observations were performed using MiniTab version 13 in order to see whether the lines could be classified into distinct groups which would allow a segregational analysis to be undertaken.

3.3 Milling quality

3.3.1 Milling yield (Flour extraction,%)

Wheat samples were cleaned using a Carter Day Dockage tester before conditioning to 16.0 % moisture content (all samples were treated as hard milling even though the majority of lines were of soft endosperm texture). Initial samples from the 1999 harvest were milled using CCFRA TES-CM-0001: Laboratory Bühler milling to provide white flour for quality testing and to measure flour yield or extraction. The following mill settings were used in order to achieve flour yields and starch damage levels as close as possible to current commercial practice:

	Break Roll Gap	**Reduction Roll Gap**
For bread flour	No 1., set at 0.06 mm	No 1., set at 0.03 mm
	No 3., set at 0.04 mm	No 3., set at 0.02 mm

The bran and fine offal fractions were subjected to two passages through a Bühler impact finisher. This device uses vibration to remove some of the endosperm still adhering to these fractions. The flour fractions produced during break, reduction and bran finishing were collected and weighed to enable calculation of the percentage of each fraction. In addition, individual flour fractions from the break, reduction and finisher flours were combined, redressed using a Russell-Finex redresser with a 300 micron sieve and blended for 20 minutes before use. The combined flour fractions were used to produce the total flour extraction as a percentage of total products. All samples within a single site were milled using the same Bühler mill.

Following initial milling tests, it was decided to minimise the potential impact of differences in milling performance by milling to a fixed extraction rate of 76+/-1%. This was achieved by keeping the bran and fine offal finisher flour separate then adding the offal flour to meet the extraction rate requirements, followed if necessary, by bran finisher flour.

3.4 Flour quality

All tests indicated as Flour Testing Working Group (FTWG) originate from a set of industry agreed and collaboratively tested methods contained in "Guideline No 3: Manual of methods for wheat and Flour Testing" (Salmon, 1999).

3.4.1 Flour protein (% at 14% moisture content)

Flour protein and moisture contents were measured by Near Infrared Reflectance (NIR) according to FTWG method 0014: Determination of Protein and Moisture contents by Near Infrared Reflectance. Flour protein content (N x 5.7) was then corrected to a 14% moisture basis. The NIR calibration used was based on Dumas protein values as used by the UK milling industry from September 1999.

3.4.2 Falling Number (s)

Flour Falling Number was measured according to FTWG method 0006: Determination of Falling Number. The weight of flour being adjusted according to the moisture content of the flour. Using a graph relating Falling Number values and cereal *alpha*-amylase activity, the cereal *alpha*-amylase activity of each flour was estimated and this value used to calculate the amount of fungal *alpha*-amylase supplement required in CBP and Spiral test baking procedures (see 3.5.1 and 3.5.2).

3.4.3 50 g Farinograph Water Absorption (600 line, %)

The water absorption capacity of flour samples was measured according to FTWG method 0004: Determination of Water Absorption using a Brabender Farinograph. The 50 g bowl version of this instrument is used routinely at CCFRA for measurement of flour water absorption as it uses less flour, but is not used to examine other flour quality attributes. This test provides a measure of the amount of water required to mix a dough to a fixed consistency. The level of water addition indicated by this test was used subsequently in both CBP and Spiral test baking procedures (see 3.5.1 and 3.5.2).

3.4.4 300 g Farinograph (dough rheology : 600 line-water absorption, %; development time, min; stability, min; degree of softening, BU)

Using a 300 g bowl and FTWG method 0004: Determination of Water Absorption and Rheological properties of doughs using a Brabender Farinograph the characteristics of development time, stability and degree of softening were measured. The Farinograph records torque on the mixer blades when flour and water are mixed at low rpm. The added water hydrates the flour to form a dough and under the conditions of the test, the dough must be developed to a fixed consistency, i.e. 600 Brabender Units. The time between the start of mixing and the first point of weakness was taken as the development time and recorded in minutes. In order to assess protein strength, mixing was continued for a period of 12 minutes after the first signs of dough weakening. By measuring the position of the centre of the curve at dough development and the final mixing point, it was possible to obtain a measure of dough weakening as determined by the ability of the flour to withstand continued mixing. This measure was recorded as degree of softening in Brabender Units (BU). The time during which the top of the curve remains above the 600 line, i.e. interval between the curve crossing this line, was measured as dough stability in minutes. The shape of the trace is primarily related to the quality of the gluten protein and, thus, these Farinograph characteristics provide information on dough strength. Any evidence of dough weakening under the gentle mixing conditions in the Farinograph are likely

to occur more rapidly under standard CBP mixing conditions and may be expected to influence final breadmaking performance.

3.4.5 Brabender Extensograph (dough rheology: resistance, BU; extensibility, cm)

Using a 300 g bowl Farinograph and FTWG method 0003: Determination of Rheological properties of doughs using a Brabender Extensograph, the characteristics of resistance to stretching and extension properties of a flour-water-salt dough were measured. The prepared dough is allowed to rest at 30^{o}C in a controlled humidity cabinet for 45 minutes before the sausage shaped dough piece is stretched until it breaks. The height of the curve, or Resistance, is related to dough strength and measured in arbitrary Brabender units and stretching ability of the dough, or Extensibility, is measured in centimetres.

3.4.6 Chopin Alveograph (Dough Rheology: W value, Joules x10^{-4}; P value, mm; L value, mm; P/L ratio)

Flour-water-salt are mixed into a dough under standard conditions using ICC Standard No 121. A fixed amount of 2.5% salt solution, based on the moisture content of the flour, is used to produce a standard dough. The dough is then extruded into a thin sheet, allowed to rest for 20 minutes under conditions of controlled temperature and humidity before being blown into a bubble until it bursts. The amount of work or energy required to blow the bubble until it bursts is measured as the area under the curve, W. The maximum pressure or resistance to expansion is related to the height of the curve and measured as P while the stretching capacity of the dough is measured as L. The P/L ratio is used to indicate the balance of dough rheological properties. In limited studies carried out on the Cappelle-Desprez recipient and selected substitution lines from the 2000 harvest, Alveograph tests were carried out using the Farinograph water absorption in order to remove the effect of differences in water requirement on dough rheology.

3.4.7 Total and soluble pentosan content

The methodology for pentosan determination (CCFRA TES-CM-0022) is based on that of Douglas (1981). For total pentosan content, flour (~0.005g) is weighed and added to water (2ml) followed by a solution of acetic acid, hydrochloric acid, phloroglucinol and glucose (10ml). The suspension is boiled for 25 minutes and forms a coloured product, the absorbance of which is recorded at 552 and 510nm. The pentosan content is measured using a standard curve constructed against µg of xylose. For soluble pentosans, flour (~1.0g) is weighed and suspended in 10ml of distilled water at room temperature by shaking for 30 minutes. The suspension is centrifuged at 2000 rpm for 10 minutes before removal of a 0.2ml aliquot of supernatant for measurement as per total pentosan samples. Pentosans are expressed as a percentage of total flour.

3.4.8 Gel protein quantity and quality

Flour (15g) was de-fatted with 40ml petroleum ether (b.p. 40-60^{o}C) for 1 hour, filtered and dried. Five grams of de-fatted flour was stirred with 75ml of 1.8% sodium dodecyl sulphate for 10min. at 10^{o}C and then

centrifuged at 40000rpm for 35min. The gel protein layer was removed and weighed. The weight of gel protein represents the amount of functional protein present in the flour. It consists, principally, of glutenin and is genetically controlled. In general, breadmaking wheats have higher levels than feed or biscuit-making varieties. A typical range for breadmaking would be 9-12g / 5g of flour (wet-weight basis).

The elastic modulus (G') and viscous modulus (G") of gel protein were measured using a Rheometrics rheometer. The former can be used to distinguish between varieties in terms of quality for UK breadmaking. Studies by Pritchard & Abel (1993) suggested that samples with a G' of less than 15 and greater than 40 Pascal may not give optimum performance in the CBP. Excessive gluten strength may also be exhibited as poor performance in the Spiral mix baking test.

3.5 Breadmaking Quality

Breadmaking quality was assessed using two specific processes namely CBP and Spiral mix which represent more than 80% and approximately 10% of UK breadmaking production. In each experiment and each baking process, a control breadmaking white flour was baked alongside test samples.

3.5.1 Chorleywood Bread Process (CBP) : 800 g four-piece

A standard recipe and procedure (CCFRA TES-BCP-314) was used to produce laboratory-scale, unlidded 800g four-piece CBP bread. The recipe is intentionally lean, including minimal enzyme and oxidising improvers (i.e. a low level fungal *alpha*-amylase supplement and ascorbic acid respectively) and aims to test the ability of a single variety flour to produce acceptable loaf volume and crumb structure in a standard 11 watt hours kg^{-1} CBP process. In this process high speed mixing is used to develop the dough which is then proved for a constant time of 45minutes at 43^{o}C in an approximation of commercial practice. The following recipe was used:

Flour	1680g
Yeast	42g
Salt	33.6g
Water	as determined by Brabender Farinograph (50g bowl, 600 line)
Fungal *alpha*-amylase	the cereal *alpha*-amylase content is estimated from the Falling Number and a fungal *alpha*-amylase supplement added to equalise amylase levels at a total of 40 Farrand Units.
Ascorbic acid	0.17g

Dough was mixed at a standard speed of 300 rpm and to a final dough temperature of 30.5+/- 1^{o}C.

3.5.2 Spiral mix baking procedure, 400g single piece

Using the same basic recipe as for CBP (3.5.1 above), the Spiral mix system (CCFRA TES-BCP-329) uses slower mixing and fixed time, i.e. 2 minutes at slow speed followed by fast speed for 6 minutes to develop the dough. Dough pieces were proved for 35 minutes at 43°C.

3.5.3 Loaf volume

Loaf volume was determined by seed displacement using CCFRA TES-BCP-303: Test Method – Loaf Volume Determination. Before use, the instrument was calibrated using a dummy fibreglass loaf of nominal volume 2609ml or 1213ml manufactured to represent 800g or 400g loaves respectively. The weight of seed displaced by the test loaves was measured and converted to loaf volume. Two loaves, representing each mix, were measured and the mean result reported.

3.5.4 Crumb colour measurement

Crumb colour measurements were made using a Minolta CR310 colorimeter according to CCFRA TES-BCP-301. The Tristimulus XYZ space is used to measure the whiteness of bread crumb with the Y-value being taken as a measure of whiteness. High Y-values indicate whiter crumb colour. The measuring head was placed directly on the exposed crumb of the cut slice. For each mix, two crumb colour measurements were taken and these were averaged to provide a single crumb colour value.

3.5.5 Visual assessment of crumb structure

The traditional method of evaluating crumb structure is to utilise the experience of a trained baker. The assessor ignores single faults which may not be representative of the loaf (e.g. caused by moulding or position of the slicing operation) and disregarding loaf volume provides a single score which reflects the average quality over the entire slice cross-section. 800g four-piece loaves were sliced approximately in the centre of the second piece of the four-piece bread format. A crumb structure score (maximum 10) was assigned on the basis of the visual appearance of the cut surface.

- Crumb structure scores below 5 reflect totally unacceptable bread structure, i.e. crumb structure is very uneven and coarse, and cells are thick walled. Bread in this category normally exhibits poor oven lift and lacks volume, i.e. is rather dense.
- Scores of 5 and 6 are given when the baked product is just bread-like. Crumb structure is poor with large areas of coarse and thick walled cells, visible cores or streaks.
- A crumb score of 7 is assigned to a sample that produces a loaf of acceptable appearance, but exhibits some faults in crumb structure terms, i.e. some areas of coarse, thick walled cells or streaks are evident.
- A score of 8 is assigned to a loaf that exhibits good quality characteristics. The crumb is relatively uniform and the majority of cells are thin walled. No major faults such as cores or streaks are evident.

- A score of 9 is given to a loaf with very good crumb structure i.e. uniform with thin cell walls and no evidence of major faults.
- A score of 10 is only assigned to the perfect CBP loaf, i.e. where texture consists only of small, uniformly distributed cells in the crumb cross-section and cells are thin walled.

3.5 Biochemical Studies

Analysis of the water- and salt-soluble fractions of wheat grain samples was performed using three modes of gel electrophoresis:

1) Laemmli SDS-PAGE (polyacrylamide gel electrophoresis) method using a BioRad Mini-Protean II gel system, with 7.5-25% linear gradient polyacrylamide gels (Laemmli, 1970);
2) acid-PAGE using the Pharmacia Vertical Gel Electrophoresis GE 2/4 System, featuring 18 x 14 x 0.27 cm gels (Salmon & Burbridge, 1985);
3) 2-D electrophoresis using the Pharmacia Biotech Multiphor II featuring isoelectric focussing (IEF) Immoboline DryStrip (pH 3-10) in the first dimension and ExcelGel XL SDS (8-18% polyacrylamide) gradient gels, 24.5 x 11 cm in the second dimension (Amersham Pharmacia Biotech Ltd).

The water-soluble and salt-soluble proteins were extracted sequentially according to the method reported by Singh and Skerritt (2001).

The Laemmli SDS-PAGE gels were stained using a colloidal Coomassie Brilliant Blue staining procedure (Neuhoff *et al.,* 1988). The SDS- and acid-PAGE gels were documented as images using the GDS 8000 system video camera documentation system (UVP Ltd). The densitometric analyses were performed using Phoretix 1D Advanced Version 4.00 software (Phoretix International Ltd). A 14-step optical density strip (Kodak Control Scale T-14) was used to calibrate band intensities, and to correct for variations in staining and image capture processes. 2-D gels were kindly analysed by Dr Andy Borthwick of Phoretix International Ltd, using Phoretix 2D Version 5.01 software (Phoretix International Ltd).

Sulphur analysis of flour samples was carried out by Adrian Crosland at IACR-Rothamsted. The samples were digested with a mixture of nitric acid and perchloric acid, and the level of S in solution was measured using inductively coupled plasma atomic emission spectroscopy (Zhao *et al*., 1994).

4 RESULTS AND DISCUSSION

4.1 Milling and breadmaking quality assessment

4.1.1 Single substitution lines from German sites

19 characters of potential influence on milling and baking quality were scored for each of the lines at the two sites. The means over sites for each of these characters are given in Table 1. Because only one sample was available per site, the site/line interaction was used as the estimate of error. Those lines showing significant

differences across sites from Cappelle-Desprez are indicated in Table 1 as well as the analysis of variance (ANOVA) testing the significance of the lines and sites overall.

Table 1 (a) Anova of baking quality characters of 18 Cappelle-Desprez (Bezostaya I) chromosome substitution lines plus the recipient variety, Cappelle-Desprez grown at two sites in Germany

		Gel Protein			Alveograph				
Item	**Df**	**Gə**	**Gəə**	**Gel protein wt**	**P**	**L**	**G**	**W**	**P/L**
Line MS	18	67.08***	3.96***	0.37*	123.9***	300	3.36	987.5***	0.032 **
Site MS	1	4.52	0.16	5.69***	68.4	51	0.44	160.1	0.007
Error MS	18	3.79	0.21	0.16	16.8	208	2.23	92.2	0.009

Table 1 (b) Mean scores for each of the subsitution lines, Cappelle-Desprez and Bzostaya I. Indicated are those lines giving mean scores significantly different from Cappelle-Desprez

	Gel Protein			Alveograph				
Chromosome	**Gə**	**Gəə**	**Gel protein wt**	**P**	**L**	**G**	**W**	**P/L**
1A	25.1*	7.30*	12.25	42.0	130	25.3	138.0**	0.330
1B	26.3**	7.30*	11.20	43.5	116	23.9	133.5**	0.405
1D	41.4***	11.15***	10.90	49.5*	99	22.2	150.5***	0.500
2A	17.5	5.40	11.10	36.0	112	23.5	92.0	0.315
2B	21.6	6.40	11.95	35.0	137	26.0	108.0	0.255
2D	16.6	5.05*	10.95	40.0	112	23.5	105.0	0.355
3A	15.2*	4.85*	12.00	34.5	123	24.7	91.5	0.285
3B	14.5*	4.50**	11.00	35.0	123	24.6	84.5*	0.290
3D	19.7	5.75	11.60	38.0	117	24.0	105.5	0.330
4A	18.5	5.55	11.80	37.5	129	25.3	112.0	0.285
4D	19.8	5.80	11.35	36.5	101	22.4	92.5	0.355
5A	18.6	5.70	11.70	37.0	112	23.4	94.0	0.335
5D	21.4	6.15	11.75	67.5***	84	20.3	156.0***	0.835***
6A	20.6	6.05	11.50	37.0	111	23.4	99.5	0.335
6B	19.7	5.85	12.10	41.5	120	24.3	121.5	0.350
6D	19.0	5.70	11.75	42.5	125	24.8	124.5	0.340
7A	19.1	6.00	12.21	32.0	120	24.3	80.0*	0.280
7B	17.2	5.40	11.25	33.5	115	23.3	88.0	0.295
Cappelle	20.5	6.05	11.70	39.0	106	22.9	104.5	0.370
Bezostaya	66.6	15.15	10.20	114.0	84	20.3	322.0	1.450

*P 0.05-0.01 **P0.01-0.001 ***P<0.001

Table 1 (a continued) Anova of baking quality characters of 18 Cappelle-Desprez (Bezostaya I) chromosome substitution lines plus the recipient variety, Cappelle-Desprez grown at two sites in Germany

Item	Df	CBP Vol	CBP Score	CBP Crumb Colour	Spiral Vol	Spiral Score	Spiral Colour
Line MS	18	21085	0.588	3.87	2679	0.991*	3.042**
Site MS	1	9792	0.026	24.27**	708	0.237	0.009
Error MS	18	11166	0.471	2.17	2070	0.348	0.664

Table 1 (b continued) Mean scores for each of the substitution lines, Cappelle-Desprez and Bezostaya I. Indicated are those lines giving mean scores significantly different from Cappelle-Desprez

Chromosome	CBP Vol	CBP Score	CBP Crumb Col	Spiral Vol	Spiral Score	Spiral Colour
1A	2954*	5.0	52.30	1582*	4.5**	50.16**
1B	3033*	5.5	51.30	1636	6.5	53.04
1D	3010*	5.5	54.90	1664	6.5	54.81*
2A	3154	6.0	51.10	1662	6.0	53.07
2B	3184	5.0	53.40	1680	6.0	53.23
2D	3129	5.5	53.30	1643	5.5	53.53
3A	3174	5.0	50.70	1616	5.0*	51.64
3B	3018*	5.0	50.50	1657	5.0*	51.81
3D	3077	6.0	50.50	1634	6.0	50.91
4A	3251	6.0	52.90	1670	6.0	53.09
4D	3210	6.0	51.60	1707	6.0	51.86
5A	3316	6.5	53.10	1675	7.0	54.08
5D	3232	6.5	53.30	1726	6.5	54.34
6A	3056	5.5	51.60	1637	5.5	52.66
6B	3231	6.0	53.10	1700	6.0	53.26
6D	3155	6.0	52.90	1726	6.5	53.35
7A	3016*	4.5	49.30	1639	4.5**	50.77**
7B	3172	6.0	53.50	1673	6.0	52.51
Cappelle	3266	5.5	52.80	1680	6.5	53.06
Bezostaya	3210	7.5	54.54	1680	7.0	55.76

*P 0.05-0.01 **P0.01-0.001 ***P<0.001

Table 1 (a continued) Anova of baking quality characters of 18 Cappelle-Desprez (Bezostaya I) chromosome substitution lines plus the recipient variety, Cappelle-Desprez grown at two sites in Germany

		Farinograph				
Item	**Df**	**W.Abs**	**Stab.**	**Dev. Time**	**Degree of Softening**	**Falling No.**
Line MS	18	1.87***	0.59***	11.9	370***	309***
Site MS	1	58.38***	1.60***	11.0	3760***	195
Error MS	18	0.34	1.02	11.4	60	112

Table 1 (b continued) Mean scores for each of the substitution lines, Cappelle-Desprez and Bezostaya I. Indicated are those lines giving mean scores significantly different from Cappelle-Desprez

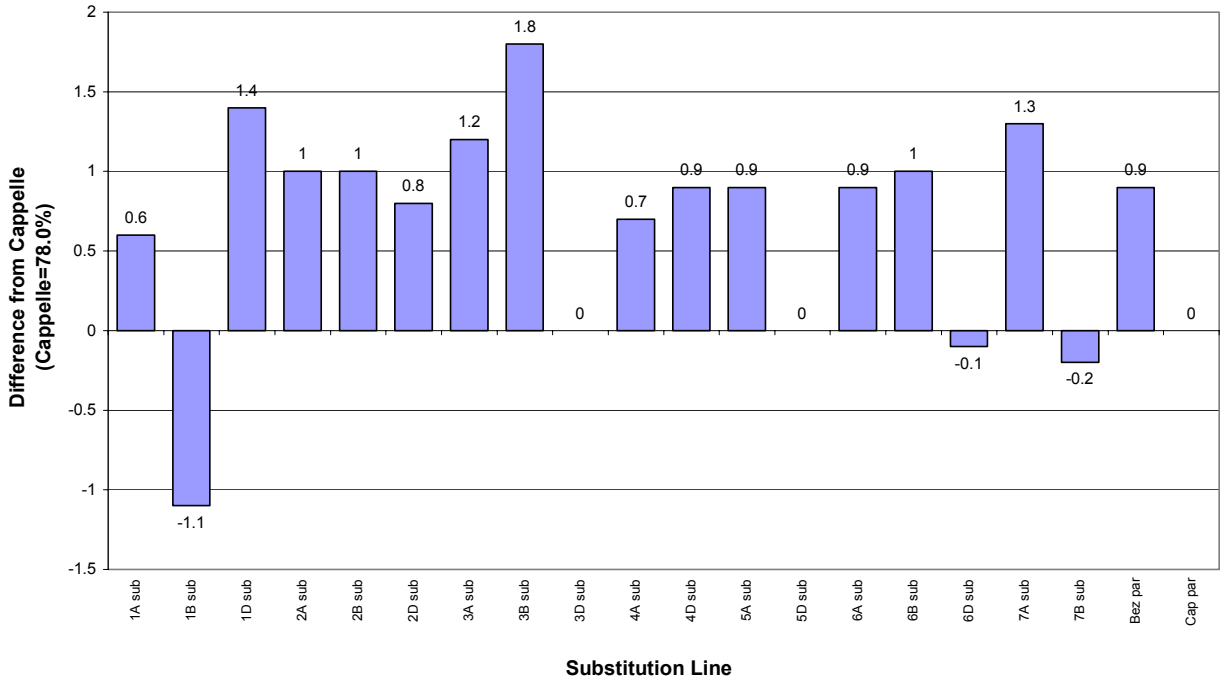
	Farinograph				
Chromosome	**W.Abs**	**Stab.**	**Dev. Time**	**Degree of softening**	**Falling No.**
1A	50.65	2.90	2.2	152.0	311.5*
1B	51.00	3.15*	2.4	147.0*	284.0
1D	50.25	4.05***	2.4	125.5***	312.0
2A	50.65	2.30	2.2	167.5	304.0
2B	50.80	2.25	2.0	160.0	312.5*
2D	51.15	2.25	2.0	170.5	304.0
3A	50.80	1.90*	2.30	171.5	289.5
3B	51.55	1.50*	2.0	187.5**	286.0
3D	51.70	2.40	2.0	168.0	286.0
4A	50.80	2.30	1.9	172.5	300.0
4D	50.50	2.50	2.1	162.0	301.5
5A	50.65	2.10	1.9	174.0	299.5
5D	54.35***	2.70	2.5	152.5	326.5
6A	51.30	2.25	2.7	166.5	284.0
6B	50.65	2.30	1.9	149.0	308.0*
6D	51.50	2.45	2.3	162.5	296.0
7A	51.30	1.80**	2.1	177.0	296.5
7B	49.55	2.25	1.9	169.0	282.5
Cappelle	50.15	2.55	2.0	163.5	284.0
Bezostaya	58.20	6.60	4.0	94.0	344.5

*P 0.05-0.01 **P0.01-0.001 ***P<0.001

13 out of the 20 characters gave significant line differences. A few of these characters also showed significant site differences. The most interesting were gel protein elastic modulus or G'; gel protein viscous modulus or G''; Chopin Alveograph (P and W); Brabender Farinograph (Stability and degree of softening); Hagberg Falling Number; and quality characteristics Spiral crumb colour and crumb score. Unfortunately, the most important of the bread-making attributes, CBP and Spiral loaf volume, gave insignificant differences between the lines.

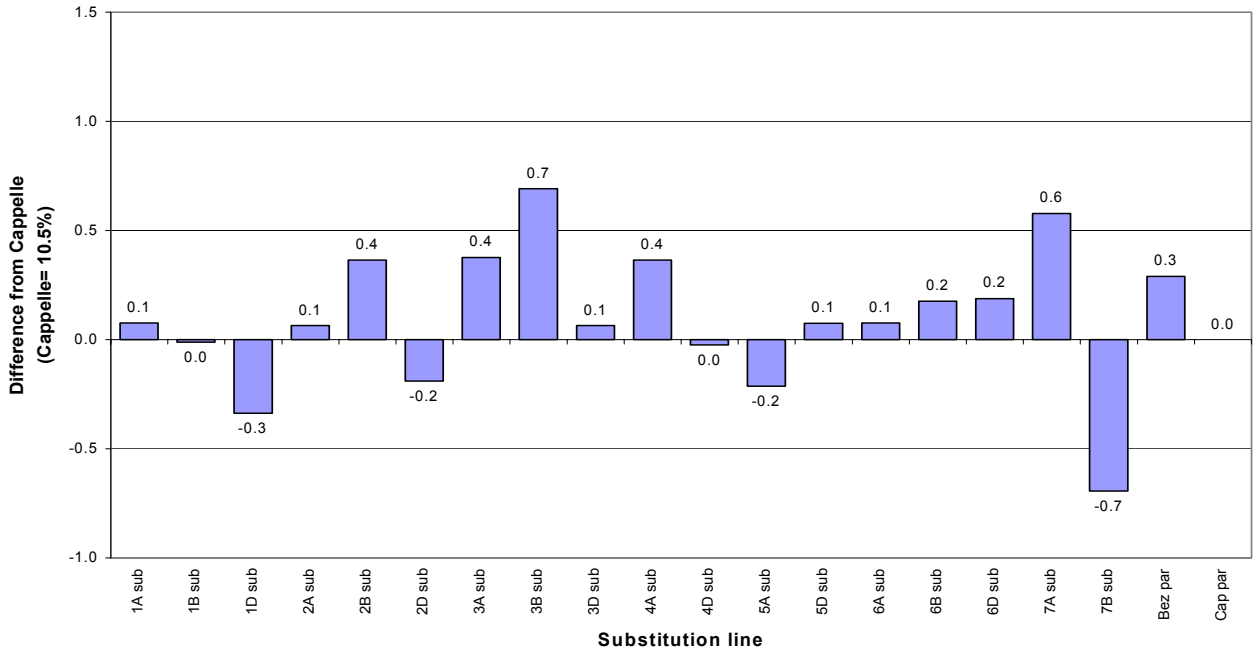
The milling and breadmaking quality of samples from the PBI/John Innes Centre and University of Halle sites grown in Germany were broadly similar and therefore only the Halle site data are presented in Figures 1-7. In each case the data are presented as the difference between the substitution lines and the recipient parent, i.e. Cappelle-Desprez. Due to the lack of duplication of test results, it was not possible to indicate significance on site plots.

Figure 1 Total flour extraction (%) from Cappelle-Desprez and Bezostaya parent and single substitution lines



Despite the soft endosperm texture of the Cappelle-Desprez parent line, a high total flour extraction rate of 78% was achieved under the fixed laboratory milling system used in this part of the study. Most of the single substitution lines had a positive effect on this milling quality parameter at the Halle site (Figure 1): this effect was not repeated at the JIC site and therefore no significant effects of flour extraction were observed. In order to remove any possible interference of milling quality on breadmaking performance, in all subsequent work samples were milled to a fixed flour yield of 76%.

Figure 2 Flour protein content (N x 5.7) @ 14 %mc from Cappelle-Desprez and Bezostaya parent and single substitution lines.



Consistent increases in protein content occurred for Bezostaya substitutions of 3B and 7A across both sites whilst decreases were associated with chromosome 7B (Figure 2). The Cappelle-Desprez flour was just acceptable for CBP breadmaking purposes with a protein content of 10.5% on a 14% moisture basis and therefore any substitution with a negative impact on flour protein content is likely to have a detrimental effect on breadmaking performance in both Spiral and CBP processes.

The differences between individual substitution lines and Cappelle-Desprez for gel protein G', Alveograph W and Farinograph stability have been selected to illustrate effects on protein strength (see Figures 3-5).

Gel protein elastic modulus provides a measure of the functional protein in individual lines and Figure 3 presents results for this quality parameter for the Halle site. The recipient variety Cappelle-Desprez produces a G′ value of above 20 Pascal at both the Halle and JIC site. This value is within the normal range of protein strength, i.e. between 15 and 40 Pascal shown to produce satisfactory performance in UK breadmaking systems (Pritchard & Abel, 1993). In other words Cappelle-Desprez would be classified as a standard breadmaking and not as a weak gluten variety on the basis of its gel protein elastic modulus. As anticipated, the Bezostaya parent had a G′ value in excess of 50 Pascal that would put the donor parent into the "extra strong" category. Consistent increases in G′ were observed for the Group 1 chromosomes whilst 3A produced a consistent decrease in G′.

Figure 3 Gel protein elastic modulus (G′, Pascal), Pa from Cappelle-Desprez and Bezostaya parent and single substitution lines.

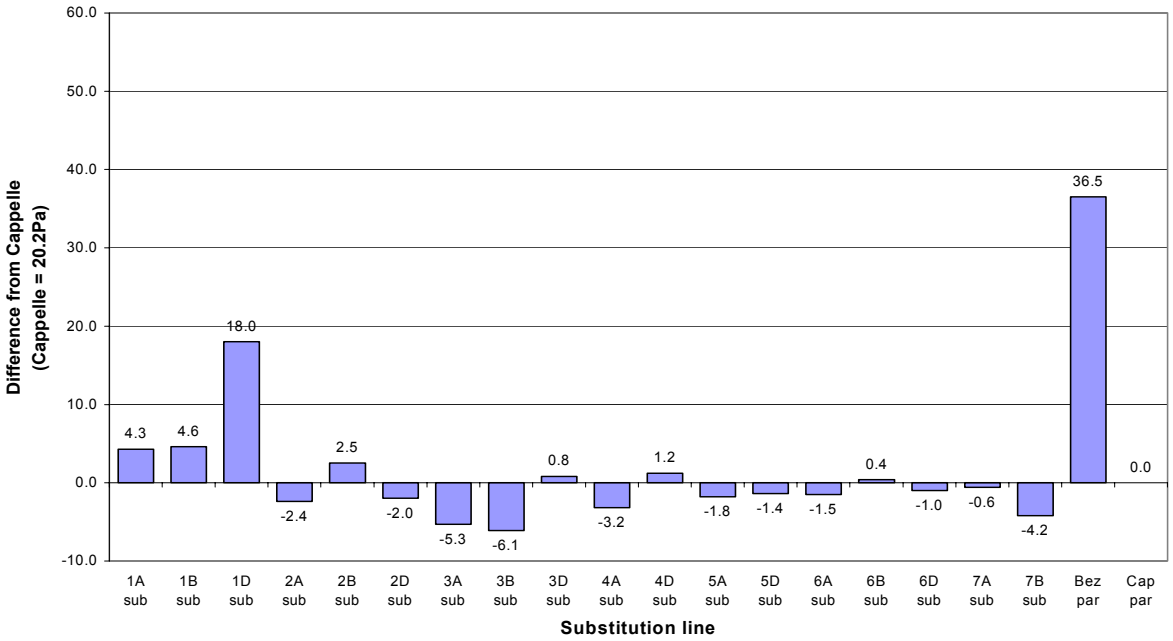
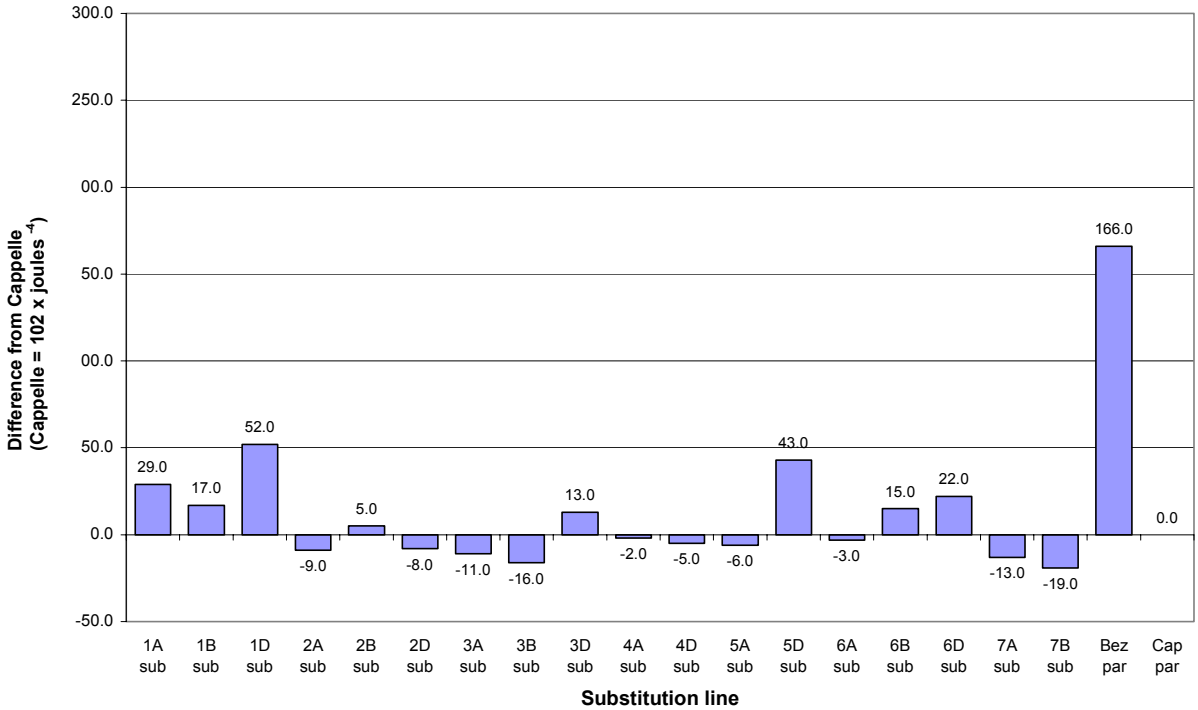
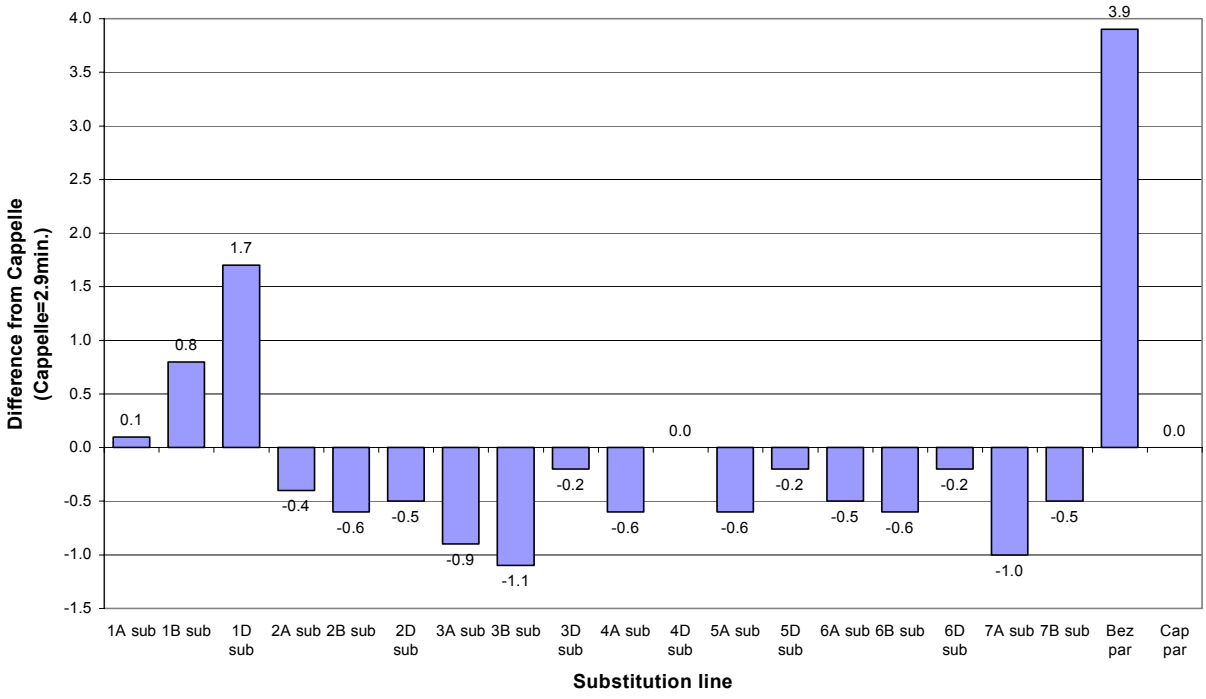


Figure 4 Alveograph W value (Joules x 10^{-4}) from Cappelle-Desprez and Bezostaya parent and single substitution lines.



Results for Alveograph W are shown in Figure 4. The recipient parent Cappelle-Desprez has produced W values typical of a strong biscuit or weak bread wheat whereas the donor Bezostaya gave values typical of a strong bread wheat. As for G', positive effects are observed for the Group 1 chromosomes with 1D being the most significant. The 5D substitution line has also produced an increase in Alveograph W. This line is hard as it expresses the phenotype of Bezostaya I. As the Alveograph test uses a fixed dough water level of 43% regardless of the water requirement of the flour, this sample has produced a tougher dough and hence, a higher P value with consequent effect on the energy required to blow the bubble until it bursts i.e. the area under the curve or W value. Chromosome 3A consistently has a negative effect on Alveograph W value.

Figure 5 Farinograph stability (minutes) from Cappelle-Desprez and Bezostaya parent and single substitution lines.



Farinograph stability provides another indicator of protein strength and a direct comparison with the data produced by Elsoms Seeds and Nickerson (UK) in earlier studies of the impact of chromosome 3A. Once again the positive impact of the Group 1 chromosomes and the negative effect of 3A can be seen (Figure 5). In this case 5D does not have any significant effect as the Farinograph measures dough rheology at variable water levels depending on the water requirements of the sample and therefore the influence of endosperm texture is removed for 5D.

Consistent negative effects were also indicated for 3B and 7A across the two German sites studied. Both 3B and 7A substitution lines produced increased protein content, but this appears to have been balanced by a weakening of the gluten strength.

All three measures of protein strength (G, Alveograph W and Farinograph stability) provide confirmation of the effects of the group 1 chromosomes, 1A, 1B and 1D i.e., increased values compared to Cappelle-Desprez and towards the higher value of the donor variety, Bezostaya 1 indicated in Table 1. A similar agreement occurs for the group 3 chromosomes, particularly 3A and 3B, but in this case the effect of the substitution is to give reduced values compared to either of the parents. It was the direction of this difference, which attracted interest in the earlier experiments at Nickersons/Elsoms and was the justification for the current investigation. However, the present experimental results suggest that it is the 3B substitution and not that for 3A that is the more potent. Farinograph stability and the Alveograph W tests agree in pinpointing the group 7 chromosomes, most strikingly chromosome 7A but also and to a lesser extent, 7B. As for the group 3 effects, these effects are also in the direction of reducing the character expression.

Measurements of Alveograph W and Farinograph stability were obtained from grain harvested from a trial of Cappelle-Desprez (Bezostaya 1) substitution lines grown at Elsoms in 1993. These values were plotted against measurements made on the related lines in the present study. The results of this comparison are shown in Figure 6. In both cases the regressions are highly significant, the extremes of the relationship being represented by the substitution lines, 1A, 1B and 1D, at one end, and 3A and 3B at the other. There is thus a good agreement between the two series of tests carried out in 1993 and 2000.

Figure 6 Comparison of protein quality assessment carried out in 1993 and 2000.

(a) Farinograph Stability

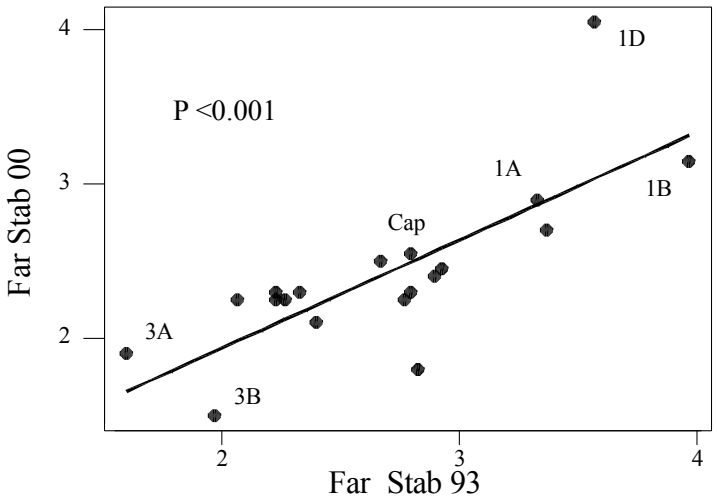


Figure 6 (b) Alveograph W.

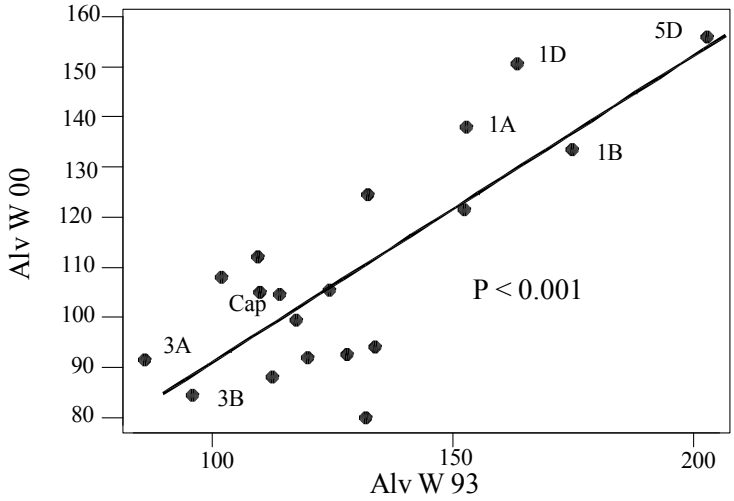


Figure 7 CBP loaf volume (ml) from Cappelle-Desprez and Bezostaya parent and single substitution lines.

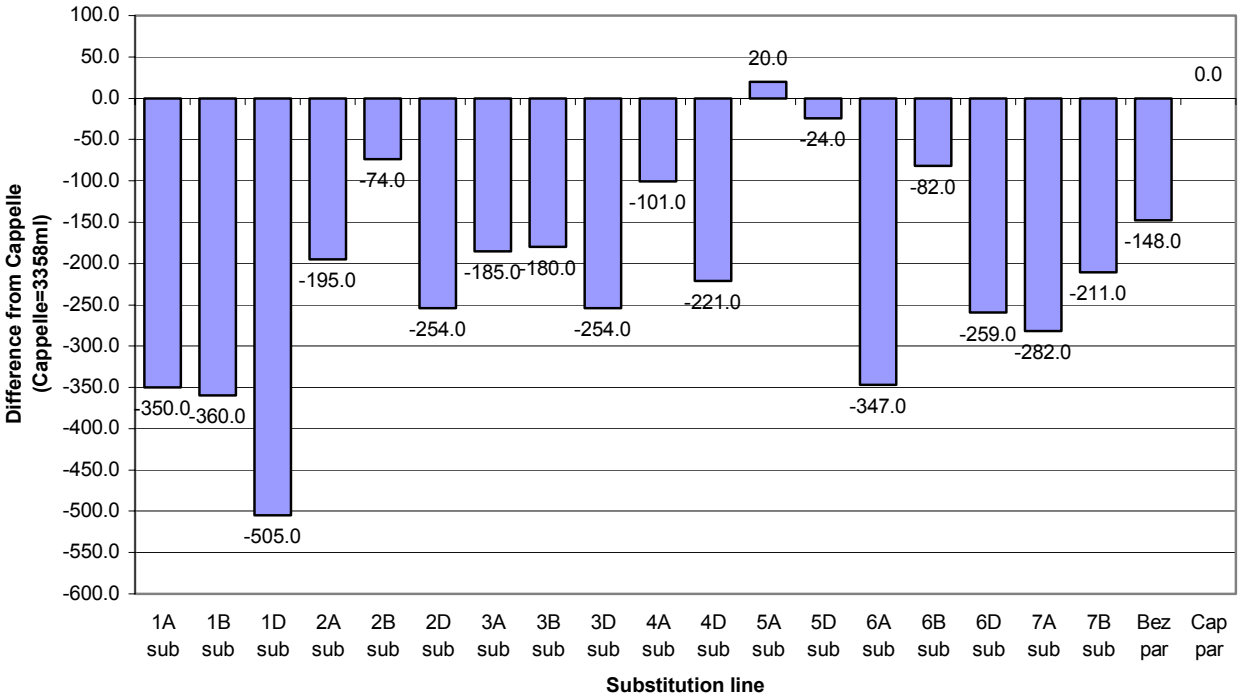
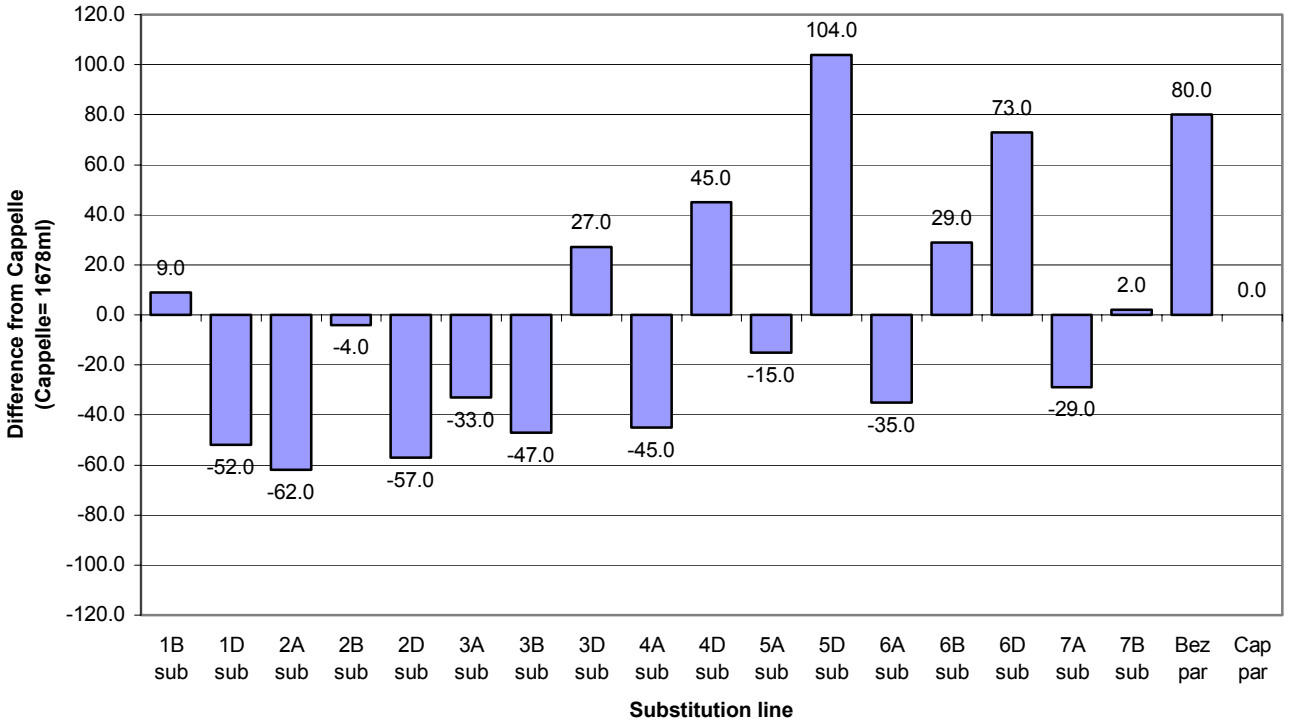


Figure 8 Spiral loaf volume (ml) from Cappelle-Desprez and Bezostaya parent and single substitution lines.

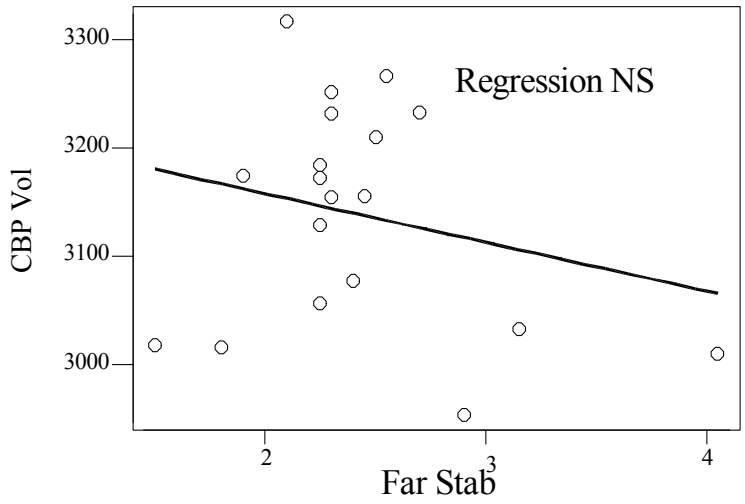


CBP and Spiral loaf volume data for the substitution lines grown in Germany produced rather unexpected results for both sites. Previous work by Krattiger *et al*. (1987) had identified positive effects of high molecular weight glutenin subunits coded by the Group 1 chromosomes. However, in this study the Group 1 chromosome substitutions produced an overall negative effect on loaf volume in both CBP and Spiral mix systems (Figures 7 & 8). In particular, the Cappelle-Desprez parent performed above expectations, frequently producing loaf volumes greater than the strong gluten Bezostaya donor parent, and key substitutions failed to produce a positive or significant response.

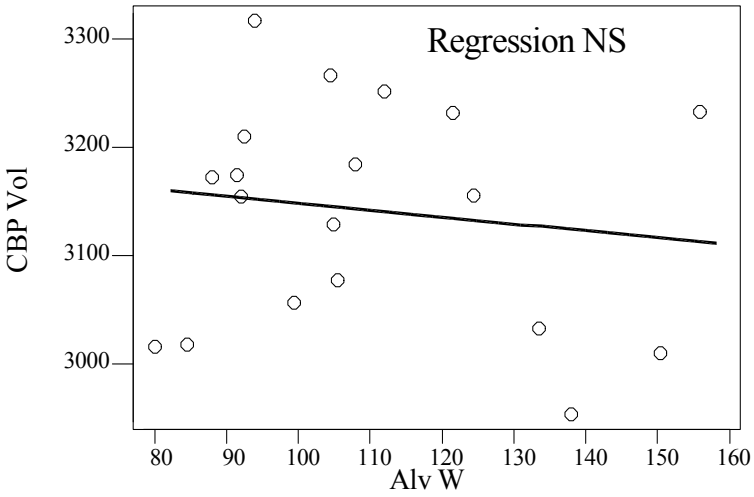
It is perhaps therefore not surprising that none of the correlations between these two characters and any of the other 18 characters measured was significant. Examples of this lack of correlation are shown in Figure 9 for Alveograph W and Farinograph stability. Correlations between empirical measures of dough rheology and breadmaking quality are known to be relatively poor and therefore it is not totally surprising that significant effects of chromosome 3A on protein strength are not translated into differences in loaf volume. As mentioned earlier, it is the good performance of supposed 'poor' breadmaking recipient Cappelle-Desprez that surprised the research team. A much higher level of replication is therefore necessary before it is possible to establish the true relationships between what may be referred to as sub-characters and the final character, loaf volume.

Figure 9 Regression of protein quality parameters and CBP loaf volume

(a) Farinograph stability



(b) Alveograph W



Test baking studies carried out at the Plant Breeding Institute (Krattiger, 1988) using Cappelle-Desprez and Bezostaya intervarietal chromosome substitution lines established significant improvements in baking performance due to the 1D, 6D and 1A chromosome substitutions from Bezostaya. Hence the observation of no significant effect of any of these substitutions within the lines grown in Germany was unexpected and a matter of some concern to the project team. A number of differences in the baking processes used currently

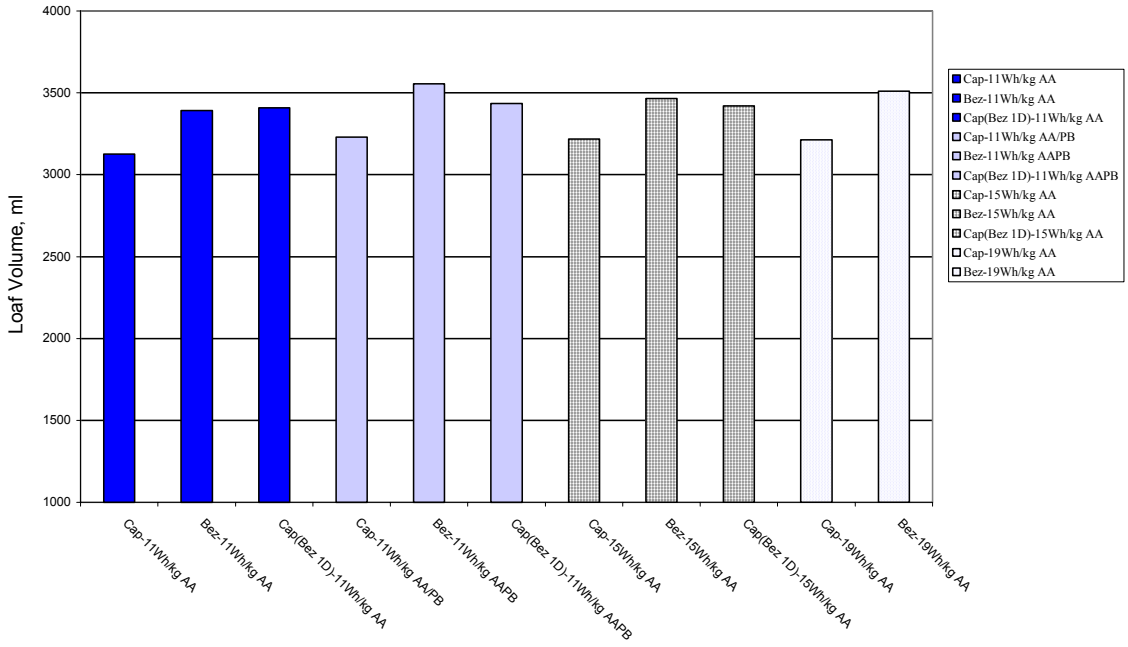
and in the late 1980's are known to exist. Firstly oxidising improvement was achieved using a mixture of potassium bromate (22ppm) and ascorbic acid (75ppm) in the Krattiger studies compared with ascorbic acid alone in 2000. Secondly, private discussions with PBI during the assessment of Fresco in the late 1980's had indicated that work input was being registered at approximately half that measured at FMBRA, Chorleywood and therefore a difference in actual work input may be implicated. Finally, current knowledge of the optimum protein strength for good breadmaking performance (Pritchard & Abel, 1993) suggested that the Bezostaya parent may be overstrong for standard UK processes.

4.1.2 Additional CBP breadmaking studies

The project team considered that one or other of these differences may account for the unexpected lack of breadmaking response of substitutions known to contribute positively to quality. In order to investigate this further, additional CBP breadmaking studies were undertaken on selected lines (the Cappelle-Desprez and Bezostaya parents plus the 1D substitution line) grown in the UK at the Morley site in 1998. Samples were milled according to the standard protocol and baked approximately 1 week after the completion of milling. Due to limitations of sample size, minimal flour quality assessment was carried out, i.e. the Falling Number and water absorption measurements required for test baking. The experiment was designed to identify the effects of different levels of work input and the inclusion of potassium bromate along with ascorbic acid to provide slower acting oxidising improvement in CBP baking. Samples were tested at three different work inputs, namely 11, 15 and 19 watt hours kg $^{-1}$. Potassium bromate (45ppm) was included in the recipe of doughs mixed at the standard work input of 11 watt hours kg $^{-1}$ only. In all other respects the recipe and conditions of CBP test baking were as shown in 3.5.1.

Results are presented in Figure 10. In contrast to the samples from the German sites, the Cappelle-Desprez parent line produced the expected poor loaf volume when baked using the standard 11 watt hour kg^{-1} recipe and an improvement of 284 ml was observed in the Cappelle Bezostaya 1D substitution line. Thus the UK grown material performed as expected and confirmed the positive influence of the 1D Bezostaya substitution on breadmaking quality. This suggests that the German samples produced above average quality in the Cappelle parent line due to some climatic or husbandry factor. One hypothesis was that the sulphur status of the German grown material may be very different to that in the UK as the growing sites in Eastern Germany were known to be in an area of high natural sulphur supply. In this work, an 11% protein content Cappelle-Desprez flour produced mediocre loaf volume of 3125 ml at 11 watt hours kg^{-1} with ascorbic acid only. Thus, the high loaf volume (3358 ml) obtained for the German grown samples of this variety are within the variation one might expect to see in this variety.

Figure 10 Effect of work input ascorbic acid and potassium bromate on CBP loaf volume of Cappelle-Desprez (Cap) and Bezostaya (Bez) parents plus the 1D substitution line (Cap-1D) for samples grown at the Morley site in 1998



The data also indicate that increasing the work input level from 11 to 19 watt hours kg^{-1} produces an increase in loaf volume of 115ml in the "strong" Bezostaya parent line but only 87 ml in the Cappelle-Desprez parent. Work carried out by Collins *et al* (1992) showed that the requirement in the Chorleywood Bread Process (CBP) can vary from 5 watt hours kg^{-1} for Riband (soft, weak gluten biscuit variety) up to 20 watt hours kg^{-1} for hard "extra-strong" varietal types such as Fresco. Clear differences were also observed between varieties in their tolerance to high work input levels and rates of work input. Fresco performed best at high work input/mixer speed combinations, and performed poorly in mixing regimes equivalent to current commercial practice (i.e. 300 rpm and work inputs less than or equal to 11 watt hours kg^{-1}). The Bezostaya donor parent clearly requires extra work input to achieve optimum performance in a CBP baking system.

It is certainly surprising that a weak gluten, poor breadmaking variety such as Cappelle-Desprez should respond in a positive manner at such high work inputs. Annual assessments of Cappelle-Desprez samples in Recommended List trials (Harrison & Greer, 1967) had suggested significant variation in breadmaking performance (the volume of 360g bread varied by 30% over 9 sites and two harvest years). Significant effects of environmental inputs on breadmaking quality have been reported by many other workers and the difference between German and UK grown Cappelle-Desprez (~8%) observed would not be inconsistent with reported views. Early work by Dodds (1974) investigated the performance of Cappelle-Desprez in a standard CBP baking system using the combined oxidising influence of ascorbic acid and potassium bromate. In this study loaf volume in Cappelle-Desprez was found to respond very positively to an increase in protein content of 4%. Thus, considerable variation in breadmaking quality had been observed in this

variety over a range of breadmaking processes and it had been classified as a 3 in terms of breadmaking value, i.e. a variety producing poor volume and texture (Greer & Stewart, 1967).

Due to sample limitations it was only possible to test the 1D substitution line at 11 and 15 watt hours kg^{-1}, but this comparison indicated little change in loaf volume (12ml) as a result of increasing the work input level. The inclusion of the oxidant potassium bromate in the recipe resulted in an increase in loaf volume in all three lines with the biggest improvement to be found in the Bezostaya parent line.

4.1.3 Sulphur Analysis

It is well known that sulphur nutrition of wheat influences yield, composition and quality of grain (Randall and Wrigley, 1986). More specifically, the breadmaking quality of wheat is affected by sulphur deficiency through decreased extensibility and increased elasticity of dough (Moss *et al*., 1981; Moss *et al*., 1983; Zhao *et al*., 1999). One possible explanation for the exceptional breadmaking performance of the German grown Cappelle-Desprez parent may be due to differences in N:S.

The nitrogen and sulphur content of flour produced from UK grown (Morley, 1998) and German grown (Halle and PBI, 1999) wheat are presented in Table 2. Effects of sulphur deficiency only become apparent at N:S ratios above 17:1 in Australian studies and about 16:1 in the UK. This level is above that found for almost all the samples analysed in the current work as shown in Table 2.

There were insignificant differences in the sulphur contents of the samples of Bezostaya I, Cappelle-Desprez, Cap(Bez 3A) and Cap(Bez 1D) between the three sites. Also, the nitrogen : sulphur (N/S) ratios were similar for all the sites.

The measured sulphur levels for the selected samples from the German sites were unlikely to account for the marked discrepancy between the baking performances of the wheat samples grown in Germany (University of Halle and PBI sites) in 1999 and those of the UK-grown wheat in 1998. However, this does not rule out other environmental influences.

The marked discrepancy between the baking performances of the wheat samples grown in Germany in 1999 and those of the UK-grown wheat in 1998 (JIC Morley) was most likely to be due to environmental factors.

Table 2 Nitrogen and Sulphur Analysis of Bezostaya I, Cappelle, and the single chromosome substitution lines Cap(Bez 3A) and Cap(Bez 1D).

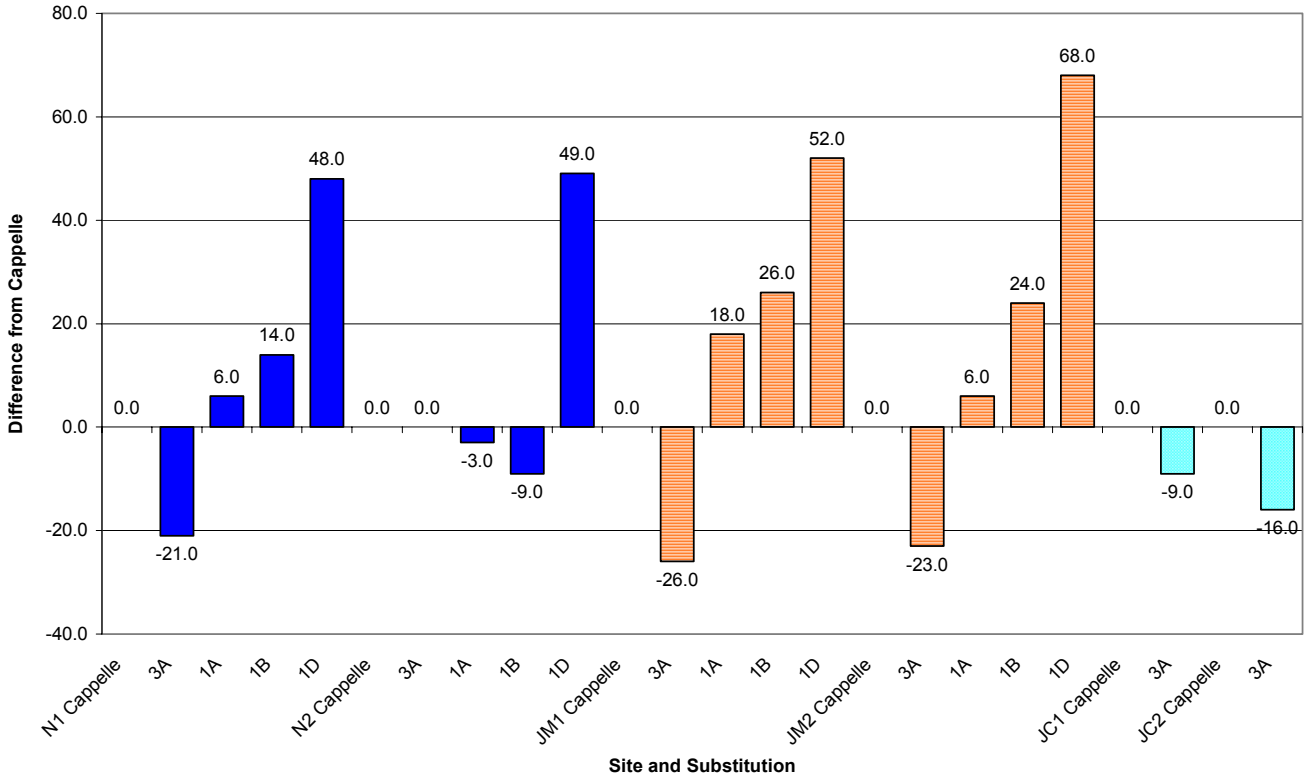
	Morley (1998)			Halle, Germany (1999)			PBI, Germany (1999)		
	S * g/100g	N * g/100g	**N/S (ratio)**	S * g/100g	N * g/100g	**N/S (ratio)**	S * g/100g	N * g/100g	**N/S (ratio)**
Bezostaya I	0.16	2.70	**16.65**	0.15	2.16	**14.32**	0.17	2.63	**15.91**
Cappelle	0.15	2.24	**15.32**	0.14	2.12	**14.66**	0.16	2.27	**14.41**
Cap(Bez 3A)	-	-	**-**	0.15	2.20	**14.65**	0.16	2.33	**14.71**
Cap(Bez 1D)	0.13	2.00	**15.11**	0.14	2.06	**14.46**	0.15	2.19	**15.02**

S*, N* - dry matter basis

4.1.4 Recombinant and selected substitution lines from 2000 UK sites

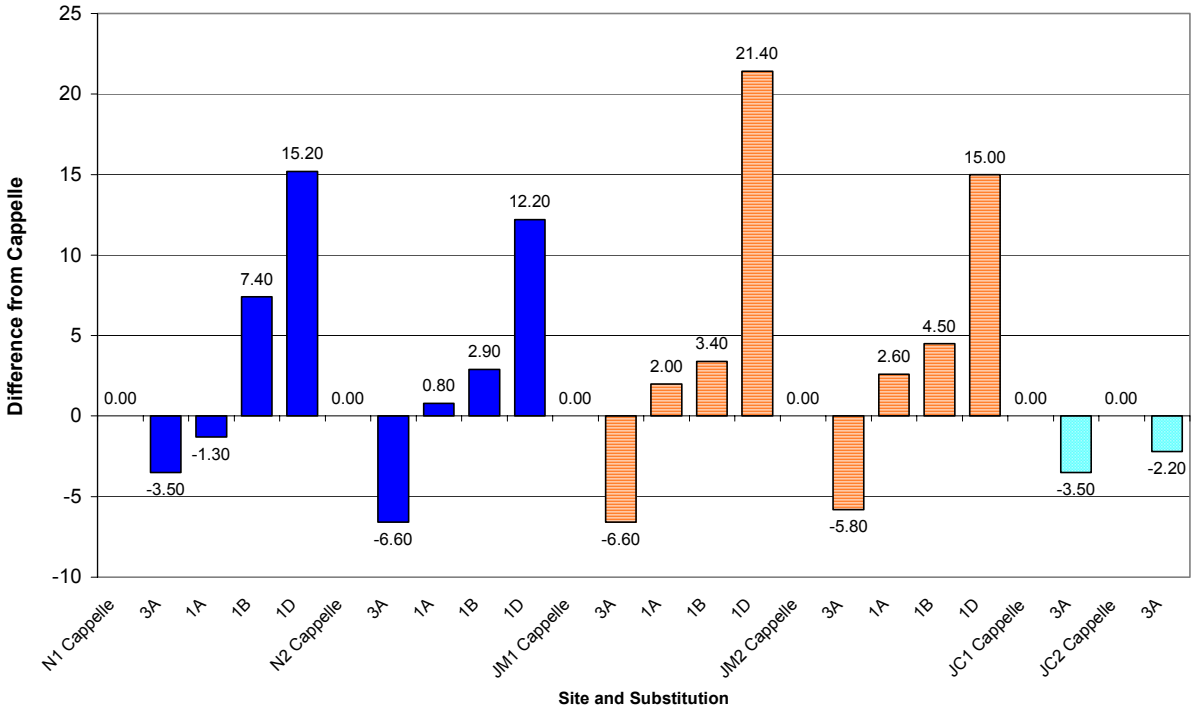
Samples of Cappelle-Desprez plus the 1A, 1B,1D and 3A Bezostaya substitution lines were milled to a fixed extraction rate of 76% and tested for basic breadmaking quality attributes as follows: protein content, water absorption, Alveograph using the Farinograph water absorption levels, gel protein (mass and rheology) and CBP baking only.

Figure 11 Alveograph W (Joules x 10^{-4}) for duplicate plots of Cappelle-Desprez and selected substitution Bezostaya lines for Nickerson UK (N), Morley (JM) and John Innes (JC) sites.



Results for Alveograph W are shown in Figure 11 and confirm that chromosome 3A has a overall negative effect on this measure of protein strength when the complication of differing water requirements are removed by using the appropriate Farinograph water levels. Where the Group 1 chromosome substitutions from Bezostaya were available, these had an overall positive effect with 1D being the most influential.

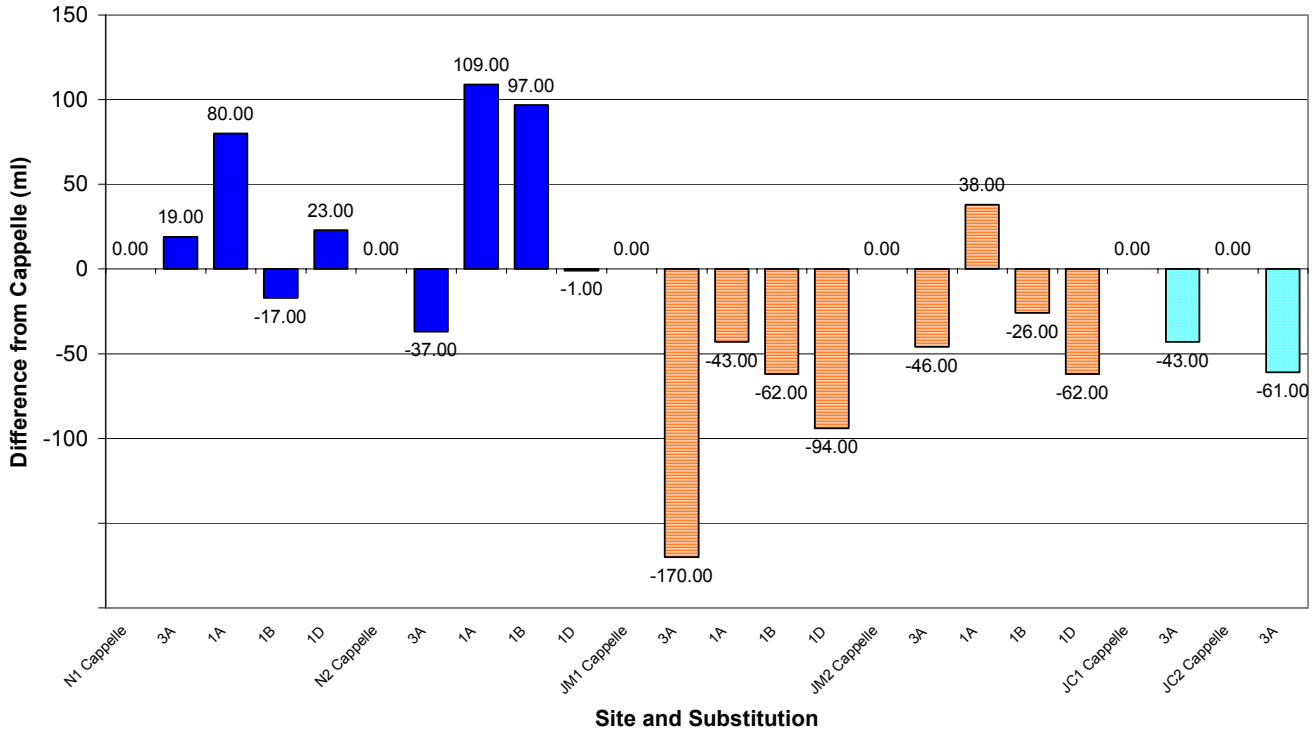
Figure 12 Gel protein elastic modulus (G', Pascal) for duplicate plots of Cappelle-Desprez and selected substitution Bezostaya lines for Nickerson UK (N), Morley (JM) and John Innes (JC) sites



Gel protein elastic modulus data is shown in Figure 12. The values obtained for the Cappelle-Desprez parent lines were typical of that expected for this variety (range 13.8-19.2 Pascal) placing at the bottom end of the range in terms of protein strength for CBP baking. Once again, substitution of chromosome 3A from Bezostaya resulted in a further reduction in this measure of protein strength confirming previous findings in this and Elsoms/Nickersons studies while, overall, the Group1 chromosomes from Bezostaya increased protein strength.

Figure 13 presents CBP loaf volume data. The Cappelle-Desprez parent line consistently produced poor loaf volume across all 3 sites (values ranging from 3060 to 3188ml) with the 3A chromosome substitution lines having an overall negative effect. The picture for the Group 1 substitutions is less clear.

Figure 13 CBP loaf volume (ml) for duplicate plots of Cappelle-Desprez and selected substitution Bezostaya lines for Nickerson UK (N), Morley (JM) and John Innes (JC) sites.

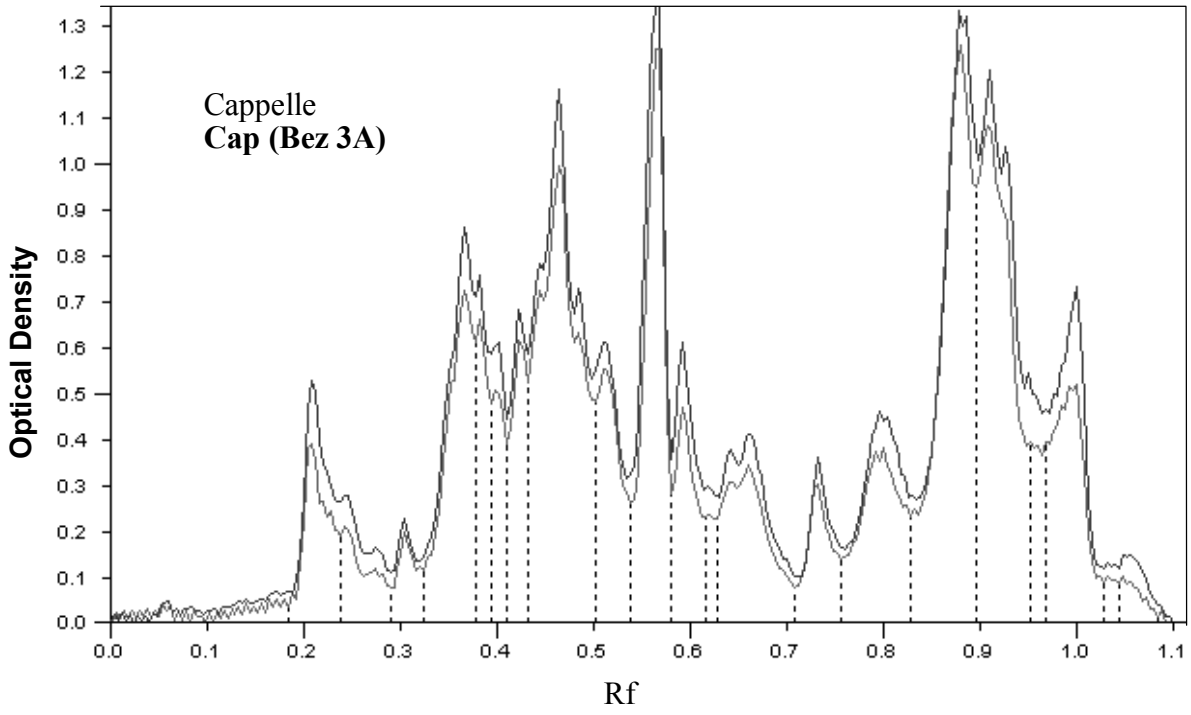


4.2 Biochemical Studies

4.2.1 Gel Electrophoresis

With the aim of identifying suitable protein markers for quality which could be attributed to genes on chromosome 3A, a systematic analysis of soluble proteins within a limited number of samples - Cappelle-Desprez and Bezostaya I parents and selected single chromosome substitution and recombinant lines - was performed with various electrophoretic techniques. The first of these was the basic-PAGE method used by Pogna *et al*. (1991), who had described the analysis of the albumin-globulin fraction ('s' proteins) of Italian bread and durum wheats. These authors had reported the presence of three major protein bands with MW of between 14-16 kDa, and suggested that one of these components is coded by chromosome 3D. This strategy was employed to examine the presence of similar components that could be coded for by chromosome 3A. Attempts to run basic-PAGE gels of the water-soluble material were unsuccessful. While three corresponding putative bands were observed on some gels (data not shown), repeat results were inconsistent and hence it was not possible to reach any conclusion. Better resolution of the water-soluble fractions was obtained using SDS-PAGE, but the comparison of the profiles of the single substitution line Cap (Bez 3A) and the parent Cappelle-Desprez indicated little or no differences between them (Figure 14).

Figure 14 Comparison of the band density profiles of SDS-PAGE of albumin fractions of samples of Cappelle and single substitution line Cap (Bez 3A)



The examination by SDS-PAGE of the salt-soluble fractions of the single chromosome substitution line Cap (Bez 3A) and Cappelle-Desprez showed that they possessed nearly identical profiles except for a peak, corresponding to a MW of 30 kDa, which was higher in Cappelle-Desprez than in Cap (Bez 3A) (see Figure 15). Interestingly, this peak is most prominent within the profile of the albumin extract of the Bezostaya I sample (Figure 16).

Figure 15 Comparison of the lane density profiles of SDS-PAGE of salt-soluble extracts of samples of Cappelle and single substitution line Cap (Bez 3A)

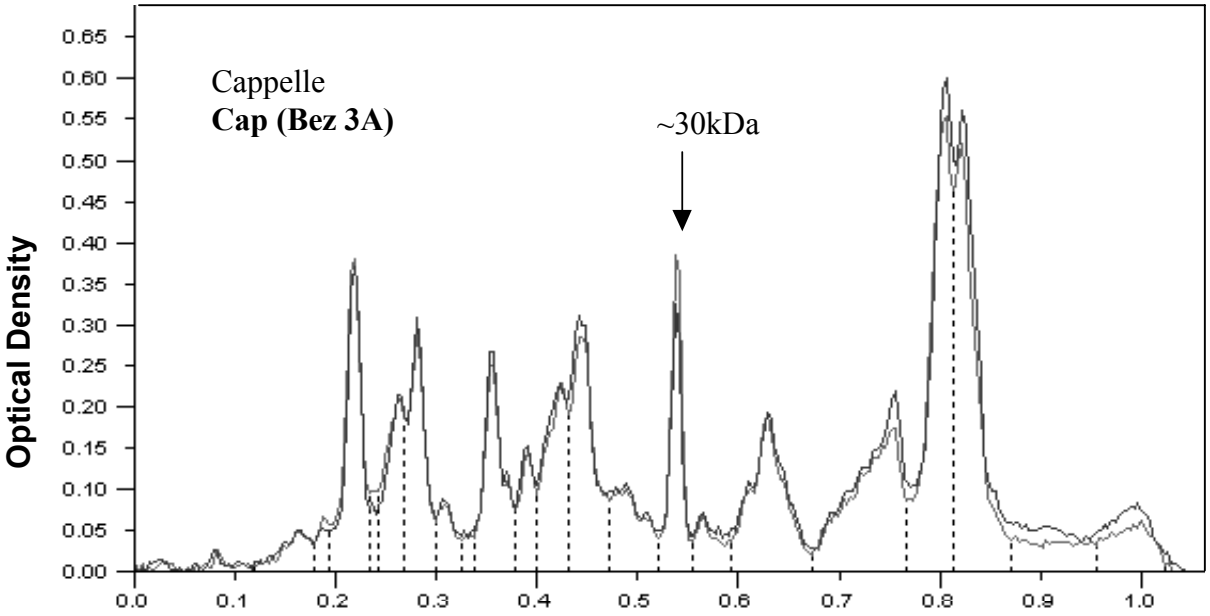


Figure 16 Lane density profiles of SDS-PAGE of albumin extract of samples of Bezostaya, with the 30 kDa protein component identified with an arrow.

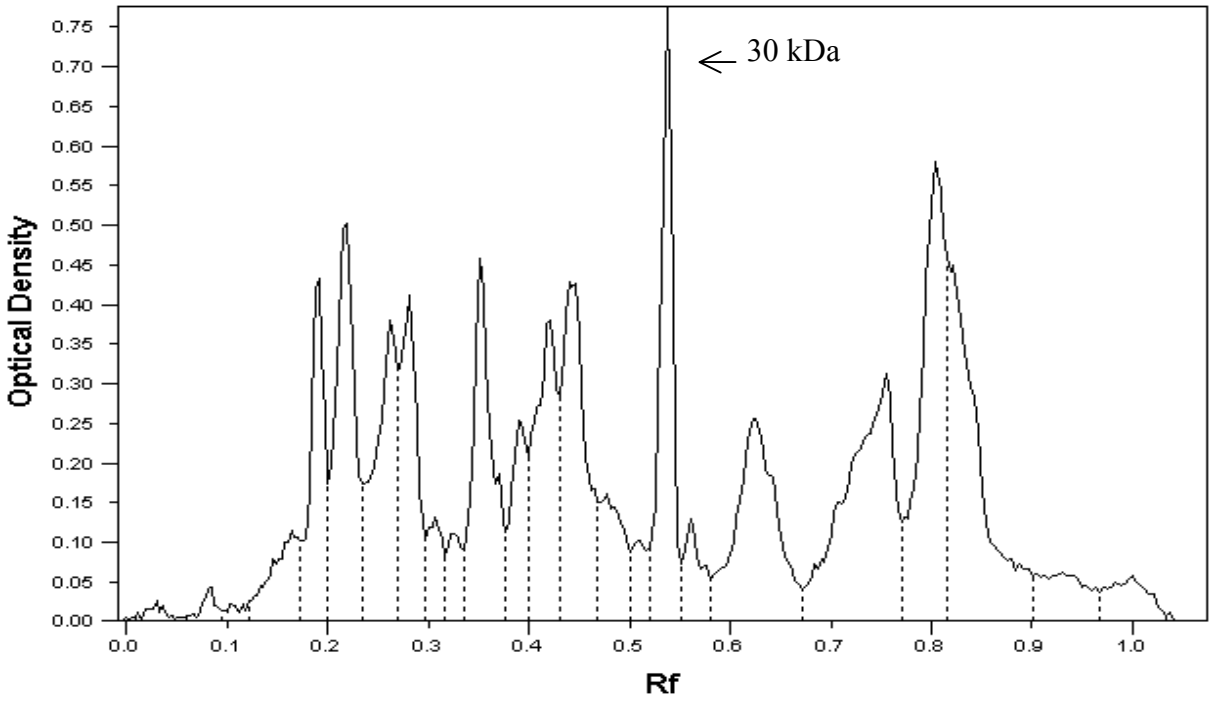
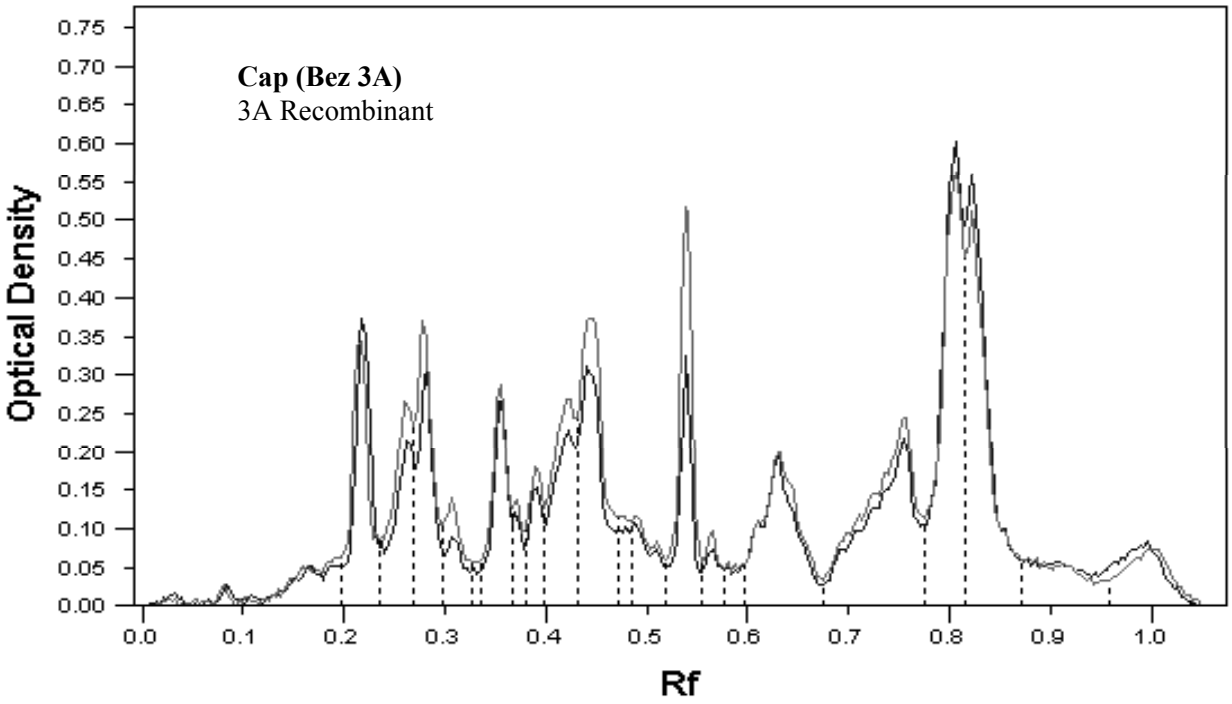


Figure 17 Comparison of the lane density profiles of SDS-PAGE of salt-soluble extracts of a recombinant 3A sample and the parent single substitution line Cap (Bez 3A). The 30 kDa protein peak is identified with an arrow.



Inspection of the SDS-PAGE profiles of salt-soluble proteins from 44 of the single chromosome recombinant lines indicated that in some cases there was also a noticeable difference in the intensity of this 30 kDa protein compared to the parent Cap (Bez 3A) sample (an example is given in Figure 17). These limited results suggest that the expression of this 30 kDa protein may be linked to chromosome 3A.

The water-soluble proteins were analysed by 2-D gels. The protein maps of the extracts from Cappelle-Desprez and the single chromosome substitution line Cap (Bez 3A) are shown in Figures 18 and Figure 19, respectively. A difference map between Cappelle-Desprez and Cap (Bez 3A) revealed numerous minor differences. The shaded spots in Figure 20 depict these differences. Unfortunately, the poor repeatability experienced in both running and staining the 2-D gels compromised the reliability of this highly sensitive technique and made the results inconclusive.

Figure 18 2-D gel of water-soluble extracts of Cappelle

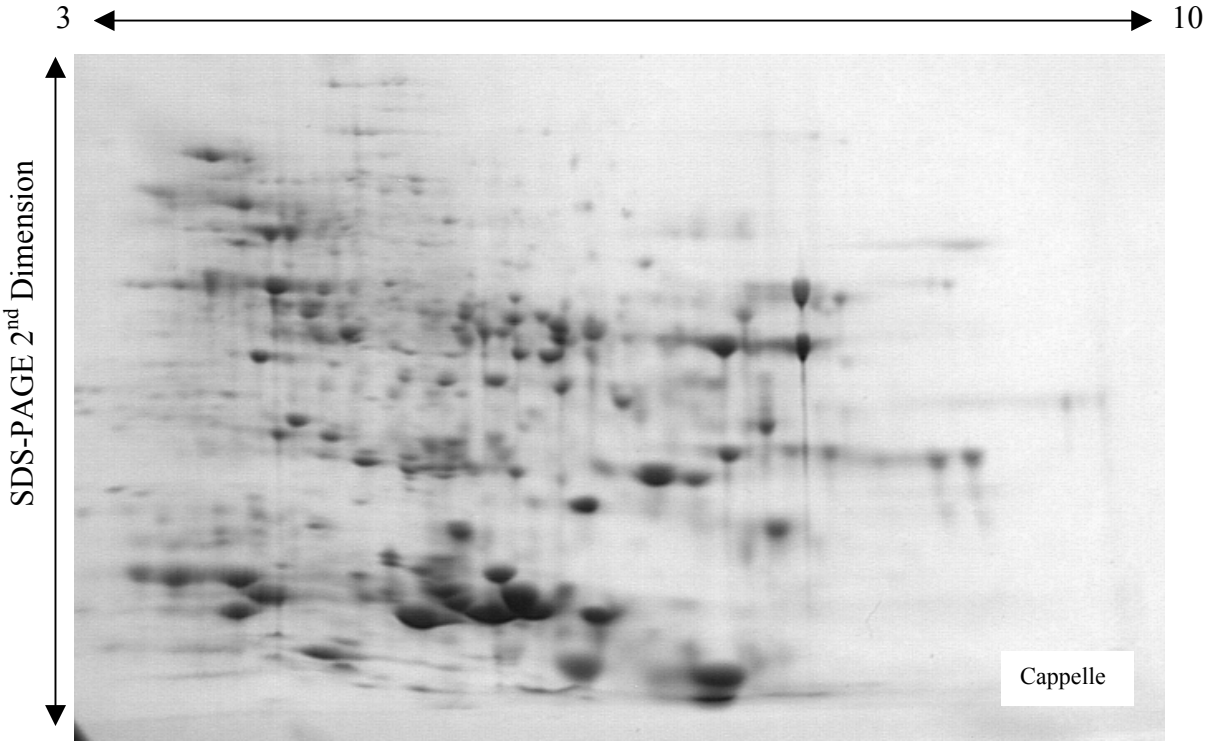


Figure 19 2-D gel of water-soluble extracts of the single substitution line Cap (Bez 3A)

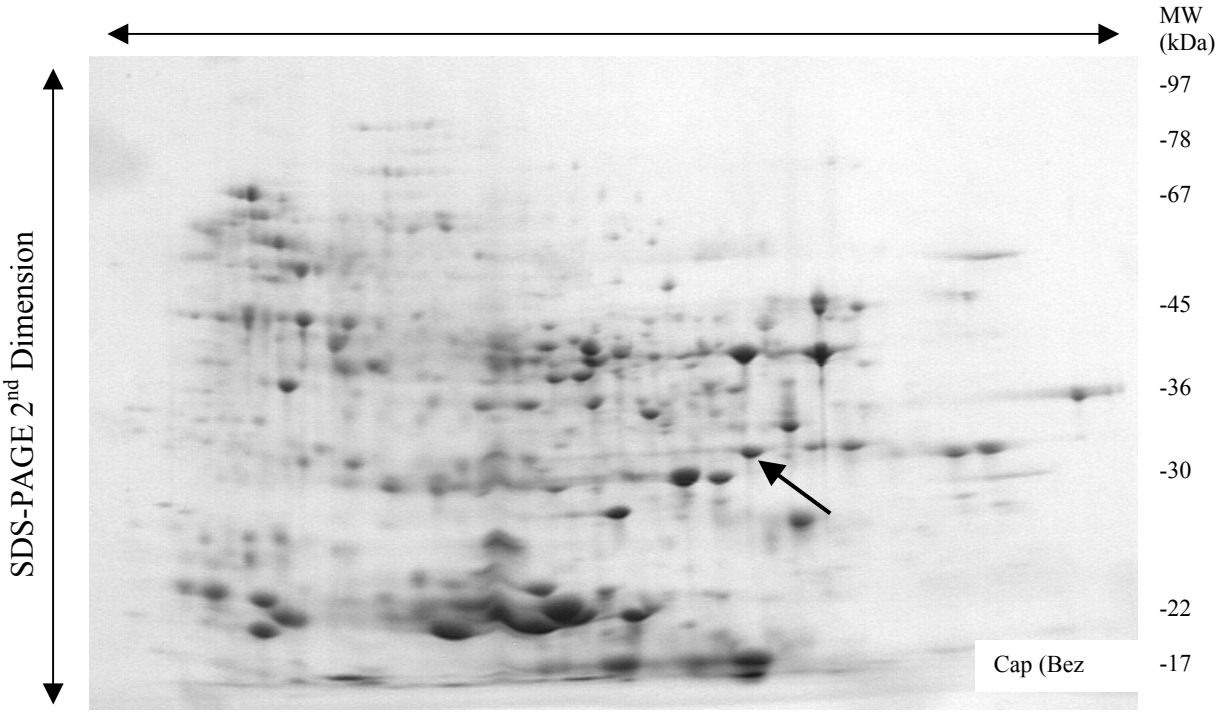
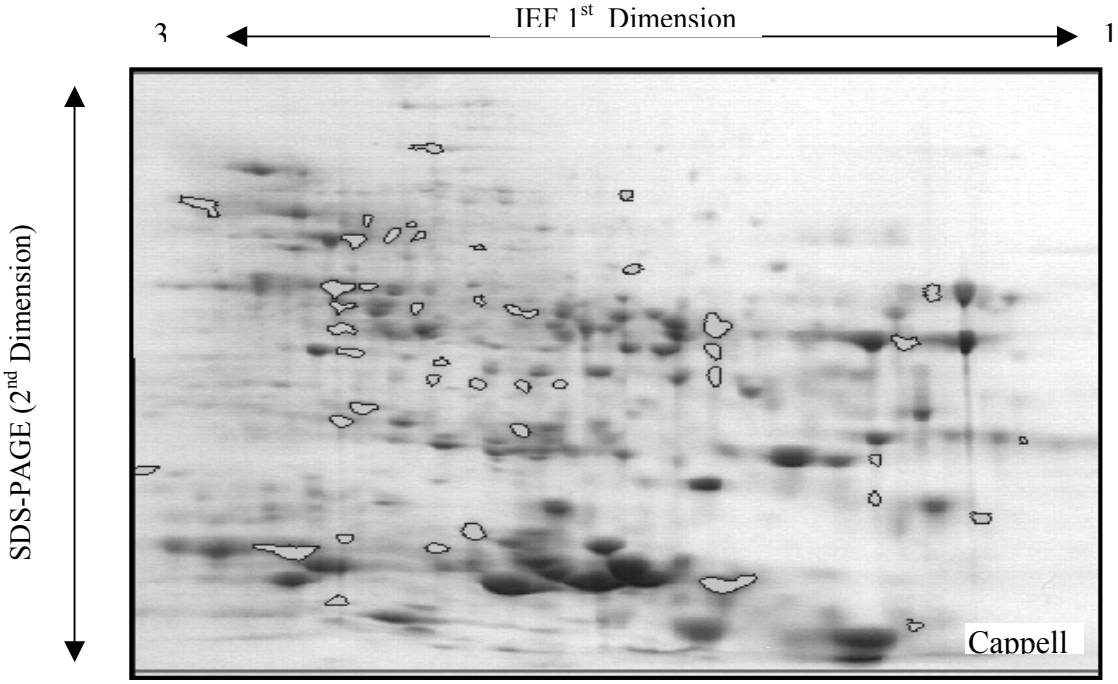


Figure 20 2-D difference map between Cappelle and Cap (Bez 3A) water-soluble fractions shown as outlined shaded spots



A number of electrophoresis techniques were employed to seek differences within the albumin and globulin (water- and salt-soluble) fractions of Cappelle-Desprez, Bezostaya I and the single chromosome substitution or recombinant lines. There was little or no evidence of any differences within the albumin fractions of the various samples examined using either 1-D SDS-PAGE or 2-D gels. However, a component of the salt-soluble fraction (30 kDa) appeared to be present in varying amounts within the population of chromosome 3A recombinant lines that was examined. Singh and Skerritt (2001) have recently reported the mapping of a 32 kDa protein of pI 7.20 on the long arm of the chromosome 3A, using 2-D gels and isoelectric focusing techniques. These authors found the 32 kDa protein in the water-soluble protein fraction, and have detailed its location on a 2-D analysis map. The 2-D gel map as shown in Figure 19 is comparable to that reported by Singh and Skerritt and a spot, possibly corresponding to the 32 kDa protein is indicated with an arrow. The presence of the 30/32 kDa protein component in both the salt-soluble and water-soluble fractions is probably due to inevitable contamination between these two similar fractions, and the division between globulins and albumins is not always clear (Shewry and Miflin, 1985). In conclusion, this preliminary study has identified a putative protein marker, ~30 kDa, for chromosome 3A. Further work will be required to confirm that it varies systematically between Cappelle-Desprez and Bezostaya, through a more comprehensive quantitative determination of this protein by SDS-PAGE, using the complete set of 48 recombinant line samples. If the difference in the 30 kDa protein within the 3A recombinant population were then found to map close to the putative bread quality gene on chromosome 3A, then a detailed characterisation of the protein and its biochemical properties would be justified to elucidate its possible causal role in the breadmaking quality of wheat.

4.3 Pentosan Analysis

The soluble pentosans appeared to be a simple normal distribution with a mean value of 0.443% (Figure 21). The Cappelle-Desprez and Cap (Bez 3A) parents and the Bezostaya I donor produced flour soluble pentosan levels of 0.48, 0.41 and 0.43% respectively which are within the range typically found in current UK wheat varieties (Whitworth, 1999). The total flour pentosan content and hence the insoluble pentosan fraction, which is calculated by difference between the total and soluble values, suggested a bimodal distribution. This double peak implied that different levels of insoluble pentosan may be associated with contrasting alleles in 3A of Cappelle- Desprez and Bezostaya I. The mean insoluble pentosan content for the 3A recombinant lines was 1.32% and the corresponding values for the Cappelle-Desprez, Cap (Bez 3A) parents and Bezostaya I donor from this experiment were 1.28, 1.23 and 1.41% respectively. The complete set of pentosan data was analysed for QTL (Quantitative Trait Loci) using the six microsatellite markers identified in other molecular mapping studies to detect significant differences in pentosan content at each molecular marker. No significant differences in pentosan concentration were detected suggesting that a gene controlling insoluble pentosan content is not carried on chromosome 3A. However, due to sample limitations it was not possible to carry out pentosan determinations on duplicate plots and that may have contributed to this lack of effect. In order to confirm this, screening of duplicate samples of the Cappelle-Desprez parent plus selected Group 1 and the 3A substitution lines from all three UK sites harvested in 2000 was carried out to look for differences in pentosan content. Figures 22 and 23 illustrate the data for the soluble and insoluble pentosan contents of Bühler milled flour for all three sites. Large "between" and "within" site differences were observed for soluble and insoluble pentosan content of Cappelle and no consistent effect of the chromosome 3A substitution was detected. For this reason, no further pentosan work was carried out on the recombinant lines from the 2000 harvest. Against this background and based on the limited data available for Group 1, chromosome 1A appeared to increase the insoluble and 1B increase the soluble pentosan levels in this experiment.

Figure 21 Frequency distribution of soluble ( ) and insoluble (■) pentosan contents of 3A recombinant lines

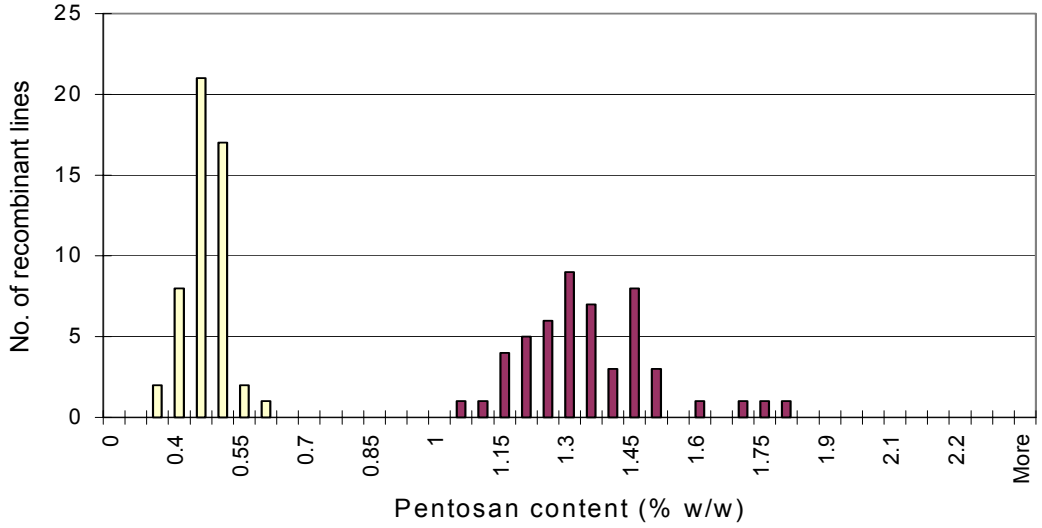


Figure 22 Soluble Pentosan content (%) for duplicate plots of Cappelle-Despresz and selected substitution Bezostaya lines for Nickerson UK (N) Morley (JM) and John Innes (JIC) sites

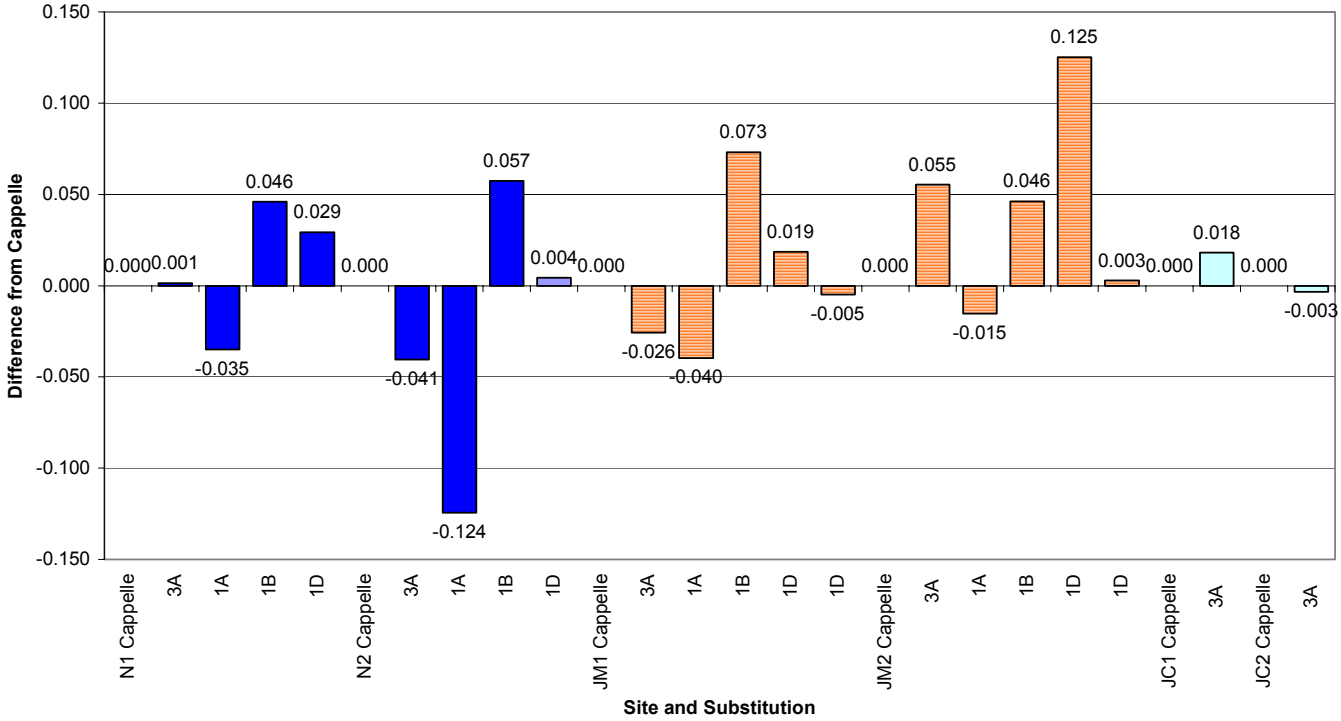
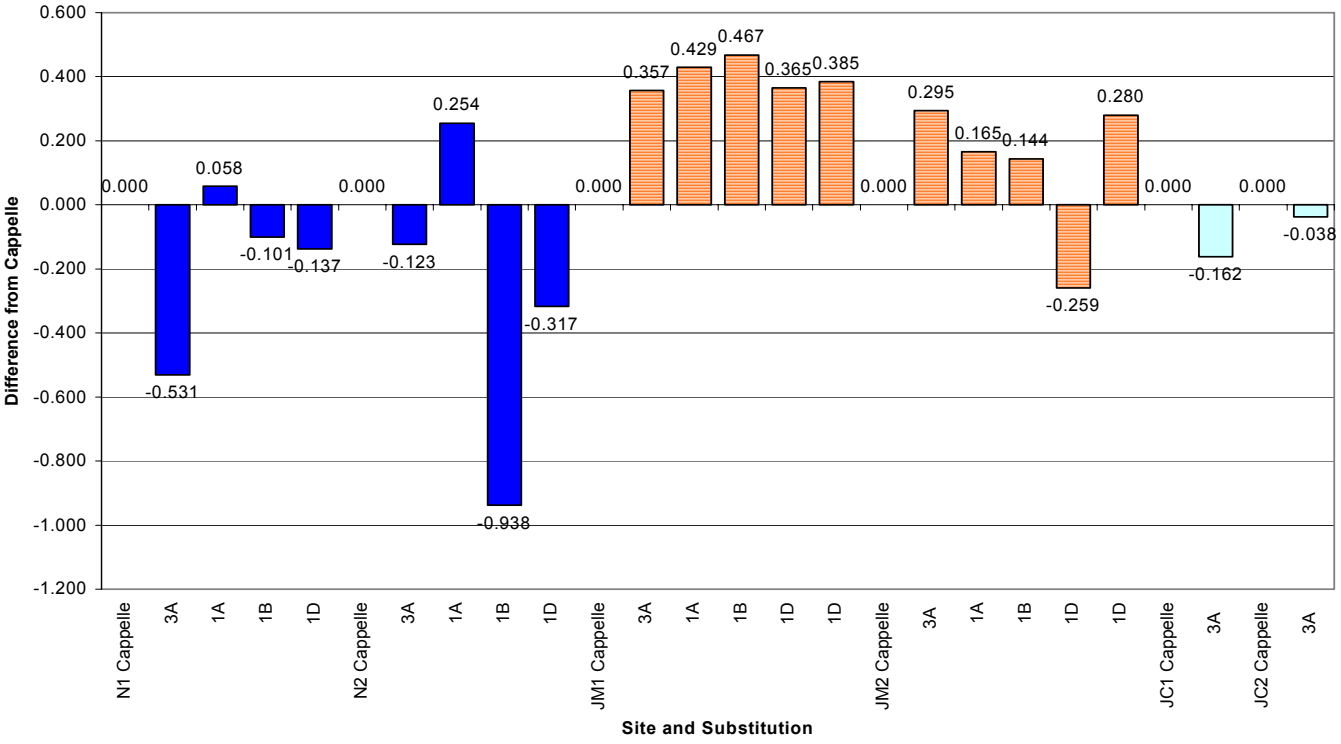


Figure 23 Insoluble Pentosan content (%) for duplicate plots of Cappelle-Despresz and selected substitution Bezostaya lines for Nickerson UK (N) Morley (JM) and John Innes (JIC) sites



4.4 Genetic analysis of the 3A recombinant lines

4.4.1 The genetic linkage map

The classification of 46 of the 48 recombinant lines with respect to the six microsatellite markers is shown in Table 3. Unfortunately, two of the lines were not available for screening so that the full classification could not be accomplished. The linkage map obtained by using the computer programme Join-Map (see Methods and Materials) is shown in Figure 24, along with the existing published map of chromosome 3A. Comparisons between the two maps indicate that the order of these markers (Xgwm 218, 2 and 674) in this investigation is identical to that given on the published map, although the distances between them vary a little. The biggest difference is the rather restricted range of markers segregating amongst the recombinant lines. These are confined predominantly to the short arm of 3A and only the marker, Xgwm 720, is possibly located on the proximal part of the long arm. As mentioned in the Methods and Materials, several microsatellites located on the long arm were tested to see if they segregated between the lines, but all of them proved to be monomorphic.

Figure 24 Comparison of published map of chromosome 3A (GrainGene database) and Join-Map generated within this study

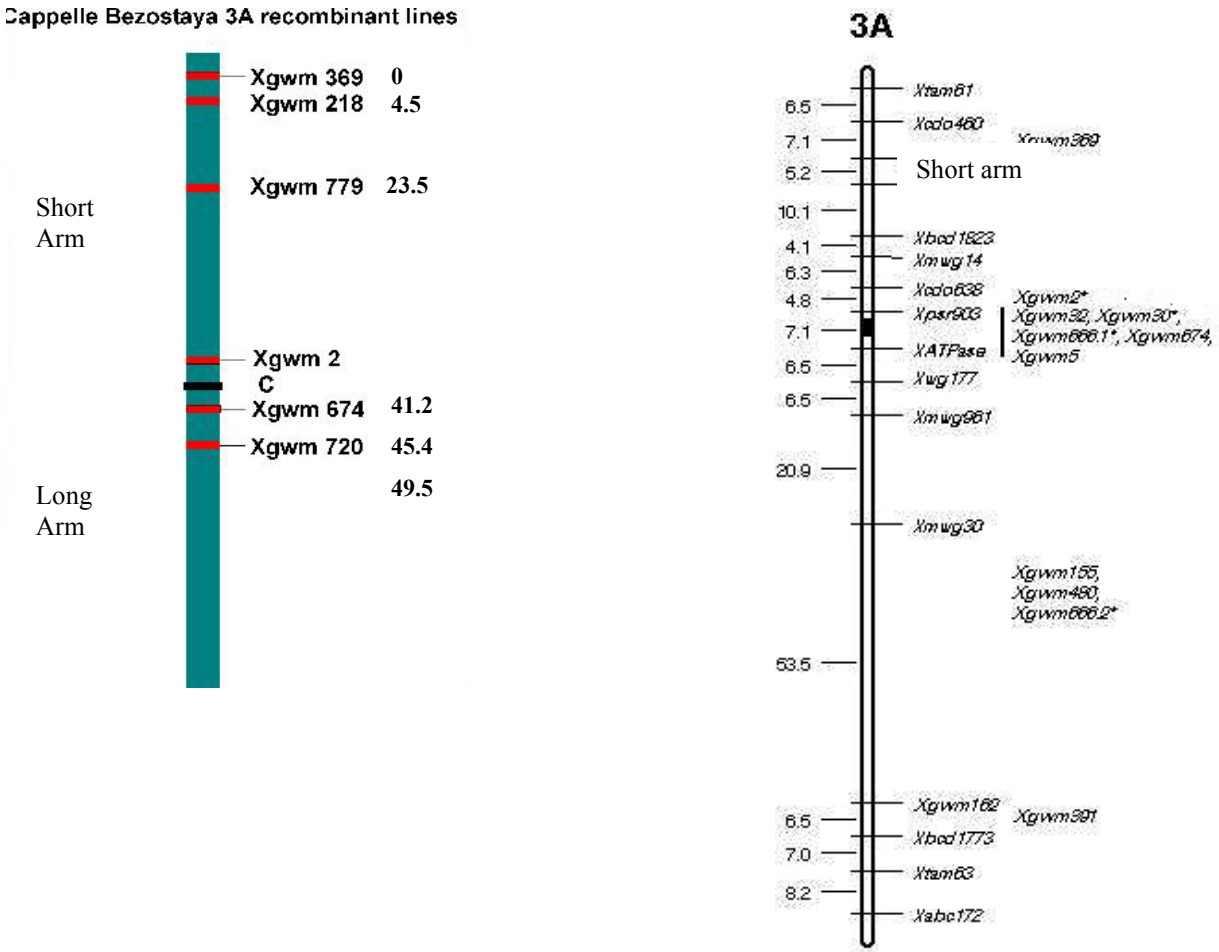


Table 3 Classification of the recombinant lines with respect to the six microsatellite markers. -1 refers to the Bezostaya 1 allelic marker; t1 to the Cappelle-Desprez marker

Line	Marker					
	369	**218**	**779**	**2**	**674**	**720**
1	-1	-1	1	-1	-1	-1
2	-1	-1	1	1	1	1
3	1	1	1	1	1	1
4	-1	-1	-1	-1	-1	-1
5	-1	-1	-1	1	1	1
6	-1	-1	1	1	1	1
7	1	1	1	1	1	1
8	-1	-1	-1	-1	-1	-1
9	-1	-1	-1	-1	1	1
10	-1	-1	-1	-1	-1	-1
11	-1	-1	1	1	1	1
12	-1	-1	1	1	1	1
13	-1	-1	-1	-1	-1	-1
14	1	1	-1	-1	-1	-1
15	1	1	1	1	1	1
16	-1	-1	-1	-1	-1	-1
17	-1	-1	-1	-1	-1	-1
18	1	1	1	1	1	1
19	1	1	1	1	1	1
20	-1	-1	1	1	1	1
21	1	1	1	1	1	-1
22	-1	-1	-1	1	1	-1
23	1	1	1	-1	-1	-1
24	-1	-1	-1	-1	1	1
25	1	1	1	1	1	1
26	-1	-1	-1	-1	-1	-1
27	-1	-1	-1	1	1	1
28	1	1	1	1	1	1
29	-1	-1	-1	-1	-1	1
30	1	1	1	-1	-1	-1
31	1	1	1	1	1	1
32	1	1	1	1	1	1
33	1	1	1	1	1	1
34	1	1	1	1	1	1
35	1	-1	-1	-1	-1	-1
36	1	1	1	-1	-1	-1
37	1	1	1	1	1	1
38	-1	-1	-1	-1	-1	-1
39	-1	-1	-1	-1	-1	-1
40	1	1	1	-1	-1	-1
41	1	1	1	1	1	1
42	-1	-1	-1	-1	1	1
43	-1	-1	-1	-1	-1	-1
44	1	1	-1	-1	-1	-1
45	-1	-1	-1	-1	-1	-1
46	-1	-1	-1	-1	-1	-1

4.4.2 The analysis of the recombinant lines.

The eight characters scored for each of the lines grown as duplicates at three sites were analysed by ANOVA and the results of these overall analyses are given in Table 4. Included in the Table are the comparisons between each of the marker means, i.e., Xgwm 369 Cap vs Xgwm 369 Bez. Each of these comparisons uses 1 degree of freedom and leaves a residual item for 44 degrees of freedom since it is based on only 46 lines and not the 48 in the overall analysis. The residual item measures the variation within each of the two marker classes combined. If the character being analysed is controlled by a single gene and this gene is closely linked to a marker or is in fact the marker itself, then the comparison between the means will remove a major part of the variation and the residual will be negligible. On the other hand, if the gene is loosely linked to the marker then the comparison between the means may still be significant but the residual will almost certainly be significant because it will reflect recombination that has occurred between the marker and the gene in question.

Five of the eight characters, loaf volume, crumb colour, Falling Number, gel protein G' and gel protein weight gave highly significant line differences. Also, all of the characters showed highly significant site effects and only one, protein content, gave a marginally significant interaction between line and site. Those characters showing significant differences between lines therefore behave consistently across sites.

Comparisons between the marker means showed some differences which are significant for loaf volume, crumb colour, crumb score, and Falling Number but not for gel protein G', gel protein weight, protein content or water absorption. For the latter two characters there was little point in continuing the analysis further. Neither showed significant overall line differences; only protein content gave any hint that there might be a small line effect through the marginally significant line/site interaction. However, the position was different for gel protein G' and gel protein weight. Both characters gave line MS's that were highly significant but none of the six marker comparisons was significant, the residuals on the other hand were highly significant. This suggests that the genes responsible for these two characters are located independently of the two extreme markers. Since the markers available are restricted predominantly to the short arm of chromosome 3A, this could suggest that they are located at least 50 map units away from the most extreme marker, Xgwm 720, out on the long arm. The reported length of the long arm is about 110 map units (see Figure 24), so that there is ample room for these genes to be located somewhere on this arm. The alternative explanation is that they are located on another chromosome or chromosomes. This point will be discussed later.

Table 4 Analysis of variance (mean squared, MS) for loaf volume, crumb colour, crumb score, Falling Number, gel protein G', gel protein weight, water absorption and protein content. Includes the analysis of means for all six molecular markers

	df*	**Loaf volume MS**	**Crumb colour MS**	**Crumb Score MS**	**Falling Number MS**	**Gel Protein G' MS**	**Gel Protein Weight**	**Water Absorption**	**Protein content**
Line	47	9274*	6.21**	1.30NS	906***	6.79***	0.494***	1.58NS	0.121NS
Site	2	311293***	184.28***	8.46***	44758***	11.04***	30.243***	201.07***	21.37***
Interaction	94	4782NS	3.22NS	0.50NS	314NS	2.77NS	0.195NS	1.60NS	0.139*
Error	144	5833	3.67	1.04	318	2.67	0.204	1.72	0.094
Marker 369	**1**	**1332NS**	**22.44NS**	**3.78NS**	**813NS**	**16.45NS**	**1.64NS**	**0.16NS**	**0.02NS**
Residual	44	9738*	5.99*	1.30NS	906***	6.70***	0.47***	1.64NS	0.13NS
Marker 218	**1**	**0NS**	**10.47NS**	**1.37NS**	**2026NS**	**16.00NS**	**1.86NS**	**0.02NS**	**0.00NS**
Residual	44	9768*	6.26**	1.35NS	879***	6.71***	0.46***	1.64NS	0.13NS
Marker 779	**1**	**37872***	**3.07NS**	**0.43NS**	**11909*** **	**15.60NS**	**0.41NS**	**0.83NS**	**0.00NS**
Residual	44	8910*	6.43**	1.37NS	654**	6.72***	0.50***	1.62NS	0.13NS
Marker 2	**1**	**101928*** **	**15.24NS**	**6.37***	**17206*** **	**6.68NS**	**0.72NS**	**1.719NS**	**0.04NS**
Residual	44	7452NS	6.17**	1.24NS	534**	6.92***	0.49***	1.60NS	0.12NS
Marker 674	**1**	**101940*** **	**12.27NS**	**7.05***	**19416*** **	**14.08NS**	**0.21NS**	**4.79NS**	**0.00NS**
Residual	44	7452NS	6.22**	1.22NS	484*	6.76***	0.50***	1.54NS	0.13NS
Marker 720	**1**	**110928*** **	**4.64NS**	**7.05***	**16151*** **	**25.58NS**	**0.46NS**	**4.48NS**	**0.0NS**
Residual	44	7248NS	6.40**	11.22NS	558**	6.49***	0.49***	1.54NS	0.13NS

*P 0.05-0.01; **P 0.01-0.001; ***P<0.001

* df = degrees of freedom

4.4.3 Loaf volume

The marker MS's for this character were significant for each of the markers Xgwm 779, Xgwm 2, Xgwm 674 and Xgwm 720. The residual item for marker Xgwm 779 was significant but not for any of the other markers. The variation in this character is thus highly associated with the three markers located at positions 41.4, 45.4 and 49.5 on the map of chromosome 3A. The gene(s) responsible must therefore be located near to this region.

4.4.4 Crumb colour

This character was similar to gel protein G' and gel protein weight. The overall line difference was significant but none of the marker MS's are, the residual items being only just significant. Therefore this suggests that the variation associated with crumb colour may be located towards the end of the long arm of Chromosome 3A where no microsatellite markers have been found.

4.4.5 Crumb score

Crumb score gave no overall line effect but had significant associations with the three markers Xgwm2, Xgwm 674 and Xgwm 720. For this character none of the residuals was significant. Again this combination tends to suggest that the variation in crumb score may be located towards the long arm of Chromosome 3A.

4.4.6 Falling number

Falling Number gave a highly significant overall line effect and similar levels of significance for the markers Xgwm 779, Xgwm 2, Xgwm 674 and Xgwm 720. All the residual items were significant but gave a lower level of significance where the marker comparisons were also significant. These results indicate that Falling Number is controlled by a gene(s) located somewhere in the region delimited by the map positions 23.5 and 49.5.

4.5 QTL analysis.

Each of the six characters was analysed using the marker regression and interval mapping procedures. In every case, the assumption was made that only one QTL is involved for each character. A summary of these results for each character is provided in Table 5.

The two methods agreed closely, although not for crumb colour where the locations for the proposed QTL, one obtained by the marker regression approach, the other by interval mapping, are at opposite ends of the marker map. For loaf volume, a single QTL maps at 48cM which is close to the most extreme marker, Xgwm 720. The residual item in the regression analysis is just significant suggesting that the model of a single QTL at that position is not quite adequate. Similar conclusions can be made for crumb colour and score where the residual items were also significant. In these two cases, however, the magnitude of the effects as measured by the regression was insignificant and was not different from zero. This contrasts with

the analysis of loaf volume where the regression was significant. For Falling Number, the QTL was located in the same region but in this case the residual item was insignificant, supporting the adequacy of the model. The measurement of effect was significant. The suggested positions of the QTLs for the two remaining characters, gel protein G' and gel protein weight are at the opposite end of the marked region, i.e., distally on the short arm.

Table 5 Summary of the results obtained from the QTL Analysis by means of marker regression and interval mapping.

Character	Marker Regression			Interval Mapping	
	Location(cM)	**Effect**	**Residual**	**Location(cM)**	**Effect**
Loaf Volume	48.0	18.52	P 0.04	48.0	20.68
Crumb colour	48.0	0.123NS	P 0.01	4.0	0.27
Crumb score	48.0	0.118NS	P 0.00	47.0	0.168
Falling Number	46.0	8.456	NS	45.0	8.494
Gel protein G'	14.0	0.361NS	NS	7.0	0.345
Gel protein wt.	0.0	0.81NS	NS	0.0	0.08

The results of this QTL analysis based upon six markers indicated that the gene(s) determining the six characters are most likely to be found at the extremes of the marked region. The only possible exception to this might be the gene(s) for Falling Number which is located near to Xgwm 674. It is also the only character to give a good fit with the model.

4.6 Correlations between the characters.

The QTL analysis established a number of possible positions for the genes responsible for the six characters. Many of the positions were identical suggesting strongly that some of the characters are due to the pleiotropic activities of the same genes. If this is the case, then some of the characters should be closely correlated.

A correlation matrix for the six characters is given in Table 6. This shows that four of the characters, loaf volume, crumb colour, crumb score and gel protein weight were positively and, from the levels of significance, strongly correlated. Both crumb colour and score are properties influenced by loaf volume so that they would be expected to be functionally related and therefore to be correlated. The reasons for the correlation with gel protein weight will be discussed later. Falling Number was also correlated with loaf volume but in this case negatively. It showed marginal significance with colour and score but not gel protein

weight. There is some rationale for low Falling Number i.e., high *alpha* amylase activity, to relate to increased loaf volume but any potential effect due to high Falling Number and hence lack of enzyme activity should have been removed by the addition of fungal amylase in the test baking recipe. Gel protein G' gave no correlation with any of the other characters.

Table 6. Correlation coefficients and their probabilities based upon the means of 48 lines between the six characters.

	Loaf volume	**Crumb colour**	**Crumb score**	**Gel Protein weight**	**Falling Number**
Crumb colour	0.612 0.000				
Crumb score	0.704 0.000	0.714 0.000			
Gel protein weight	0.381 0.009	0.385 0.008	0.342 0.020		
Falling Number	-0.439 0.002	-0.268 0.072	-0.289 0.052	-0.062 0.685	
Gel protein G'	0.033 0.826	0.144 0.339	0.210 0.162	0.155 0.305	0.208 0.166

4.7 Cluster Analysis.

The close correlation between several of the characters supports therefore the notion of pleitropy or tight linkage. To pursue this further, it is necessary to extend the analysis to cover more than two variables. Cluster analysis is a way of combining several variables to see whether they can be formed into meaningful groupings.

It may be possible, for instance, to classify the lines on the basis of such a multivariate analysis into two or more groups. This is, after all, the basis of Mendelian Genetics where the estimation of the numbers of genes and their locations are based upon the grouping of segregants into discrete classes. This is a different approach from QTL analysis which relies entirely upon statistical properties to arrive at a location and never considers the possibility that the lines being studied may be classifiable into groups. This is largely because most QTL analyses are based upon recombinant lines obtained from crosses involving many QTL's. To classify such lines into groups would be impossible apart from those rare situations where only one or two QTL's were segregating, or where one of the QTL's has a large effect. Recombinant lines derived from a single chromosome substitution line are on the other hand a different proposition. They are likely to be segregating for one or perhaps two QTL's at the most, because steps have been taken already to remove or reduce any QTL variation in the background by sequential backcrossing to Cappelle-Desprez in the development of the substitution line.

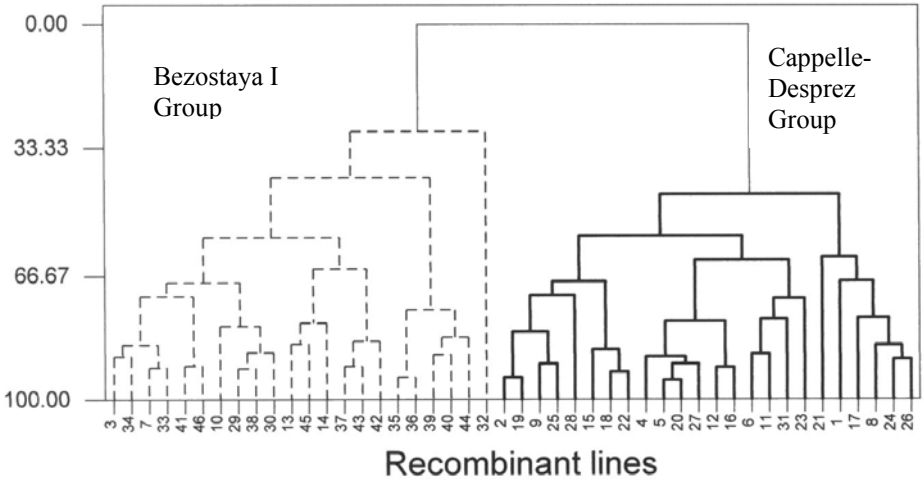
Two types of cluster analysis have been used. The first asks the analysis to group the lines into two groups as if a single gene was segregating amongst them. A test of the reasonableness of this grouping is whether or not it fits the expected 1:1 ratio for a single gene. The second analysis uses the parental values, i.e., the values for Cappelle-Desprez and the substitution line, Cappelle-Desprez (Bezostaya 3A), the two parents used to develop the recombinant lines, as controls in classifying each of the lines as being like one parent or the other. This is the classification that would be expected if only one gene was segregating because the segregants should be identical to the two parents. Again a further test of reasonableness will be the fit or otherwise with a 1:1 ratio. However, perhaps a better test of reasonableness is whether the two types of cluster analysis agree. The values for Cappelle-Desprez and the substitution line are given along with the mean values of the recombinant lines in Appendix A.

4.7.1 Cluster analysis 1

Four of the variables, loaf volume, crumb colour, crumb score and gel protein weight, are strongly correlated so that these four characters were used in the first cluster analysis. Briefly in this analysis each variable is standardised and then by a process of agglomeration, a hierarchical classification visualised as a dendrogram is produced which shows the relationships of each of the lines to each other. The analysis will also split the classification into groups depending upon the number of groups which are logical for the data. In this case, the simplest grouping is two.

The results of this analysis are presented in Figure 25. This shows that the 46 lines can be placed into two groups, 24 in one and 22 in the other. This of course fits closely the 1:1 segregation expected for a single gene. The classification of each of the lines into the two groups is given in Table 7 and is designated Factor 1.

Figure 25 Cluster analysis of 46 recombinant lines using loaf volume, crumb colour, crumb score and gel protein weight



4.7.2 Cluster Analysis 2.

The second cluster analysis achieves a non-hierarchical clustering of observations when it is possible to make good starting cluster designations. As mentioned above, this means using the parental values for the four characters to designate the two initial cluster centroids. Each recombinant line is then assigned to the cluster to whose centroid it is closest. The results of this analysis are again shown in Table 7 as Factor 1*. The two groups are of equal size, each having 23 lines. This is of course an exact agreement with a 1:1 ratio. More importantly though, the classification of Factor 1* is almost exactly the same as Factor 1, differing only for line 16.

Using these classifications it is possible to estimate the map position of both Factor 1 and Factor 1* with respect to the other markers. However, since the difference between these Factors only involves line 16 and since for Factor 1* this line is predicted not to have involved a crossover with the nearest marker, Xgwm 720, it is proposed to use the classification for Factor 1* in the further analyses.

In combination with the full marker classification given in Table 3, it is evident that Factor 1* must be located distally to Xgwm 720 and that just 16 of the lines are recombinants. The map distance between Xgwm 720 and Factor 1* is therefore 16/46 or 0.348 ± 0.070. A new map covering the marker genes and Factor 1* is therefore as follows: -

0.0---4.5----------23.5----------41.2----45.4----49.5----------84.3 map distance

Xgwm 369	Xgwm 218	Xgwm 779	Xgwm 2	Xgwm 674	Xgwm 720	Factor 1*	**marker genes**

4.8 Analysis of Variance for Factor 1*.

To test whether Factor 1* can account for the variation amongst the 46 lines for the four characters used in the Cluster Analysis, the analysis of variation presented in Table 8 can be extended to include the comparison between the means of the groups defined by this factor. This complete analysis is shown in Table 8 for all the six characters. For loaf volume, crumb colour, crumb score and gel protein weight, the variation due to Factor 1* was highly significant. Indeed, in all cases the MS's were much greater than the largest of the other marker MS's, in some cases by a factor of 6. Apart from gel protein weight, the residuals were also insignificant. For Falling Number although both Factor 1* and the residual were significant, the level of significance for the Factor 1* marker was much lower than for the markers more proximally placed. This fits with a median location for the gene affecting this character. On the other hand, Factor 1* for Gel Protein G' was insignificant and the residual significant, again suggesting that the gene(s) responsible is distally located on the long arm, or is on a chromosome other than 3A.

This analysis therefore confirms that Factor 1* or the activities of a single gene can explain the variation in loaf volume, crumb colour and score and probably also gel protein weight. The caution about the latter character is due to the significant residual item in the analysis which might suggest that the gene(s) for this character is separate but still linked to Factor 1*.

Table 7 The classification of the recombinant lines into 2 groups. (1 refers to the Cappelle-Desprez allele and –1 to the Bezostaya 1 allele. The result of the 1st Cluster Analysis is designated Factor 1, the 2nd Factor 1*)

Line	Factor 1	Factor 1*		Line	Factor 1	Factor 1*
1	1	1		24	1	1
2	1	1		25	1	1
3	-1	-1		26	1	1
4	1	1		27	1	1
5	1	1		28	1	1
6	1	1		29	-1	-1
7	-1	-1		30	-1	-1
8	1	1		31	1	1
9	1	1		32	-1	-1
10	-1	-1		33	-1	-1
11	1	1		34	-1	-1
12	1	1		35	-1	-1
13	-1	-1		36	-1	-1
14	-1	-1		37	-1	-1
15	1	1		38	-1	-1
16	1	-1		39	-1	-1
17	1	1		40	-1	-1
18	1	1		41	-1	-1
19	1	1		42	-1	-1
20	1	1		43	-1	-1
21	1	1		44	-1	-1
22	1	1		45	-1	-1
23	1	1		46	-1	-1

Table 8 Analysis of variance (mean squared, MS) for loaf volume, colour, score, Falling Number, gel protein G' and gel protein weight. Includes the analysis of the means for all six molecular markers and Factor 1*.

Item	df	Loaf volume MS	Crumb colour MS	Crumb score MS	Falling Number MS	Gel protein G' MS	Gel Protein Weight MS
Line	47	9274*	6.21**	1.30NS	906***	6.79***	0.494***
Site	2	311293***	184.28***	8.46***	44758***	11.04***	30.243***
Interaction	94	4782NS	3.22NS	0.50NS	314NS	2.77NS	0.195NS
Error	144	5833	3.67	1.04	318	2.67	0.204
Marker 369	**1**	**1332NS**	**22.44NS**	**3.78NS**	**813NS**	**16.45NS**	**1.64NS**
Residual	44	9738*	5.99*	1.30NS	906***	6.70***	0.47***
Marker 218	**1**	**0NS**	**10.47NS**	**1.37NS**	**2026NS**	**16.00NS**	**1.86NS**
Residual	44	9768*	6.26**	1.35NS	879***	6.71***	0.46***
Marker 779	**1**	**37872***	**3.07NS**	**0.43NS**	**11909*****	**15.60NS**	**0.41NS**
Residual	44	8910*	6.43**	1.37NS	654**	6.72***	0.50***
Marker 2	**1**	**101928*****	**15.24NS**	**6.37***	**17206*****	**6.68NS**	**0.72NS**
Residual	44	7452NS	6.17**	1.24NS	534**	6.92***	0.49***
Marker 674	**1**	**101940*****	**12.27NS**	**7.05***	**19416*****	**14.08NS**	**0.21NS**
Residual	44	7452	6.22**	1.22NS	484*	6.76***	0.50***
Marker 720	**1**	**110928*****	**4.64NS**	**7.05***	**16151*****	**25.58NS**	**0.46NS**
Residual	44	7248NS	6.40**	1.22NS	558**	6.49***	0.49***
Factor 1*	**1**	**246960*****	**136.776*****	**30.653*****	**5259***	**6.426NS**	**6.520*****
Residual	44	4158NS	3.372NS	0.688NS	805.2***	6.930***	0.356***

P 0.05-0.01; P 0.01-0.001; P<0.001

4.9 QTL Analysis with Factor 1*.

The validity of Factor 1* can be further tested by adding it as a marker to a QTL analysis by marker regression and interval mapping. The results of this analysis are presented in Table 9 and also in Figure 26 where the positions of each of the QTL's obtained by both methods are shown on the same linkage map for each character in turn.

Table 9 Summary of the results obtained from the QTL Analysis by means of marker regression and interval mapping but including marker 1* in the analysis.

Character	Marker Regression			Interval Mapping	
	Location(cM)	**Effect**	**Residual**	**Location(cM)**	**Effect**
Loaf volume	78.0	33.46	NS	73.0	40.41
Crumb colour	84.0	0.50	P 0.02	84.0	0.71
Crumb score	84.0	0.29	P 0.01	78.0	0.39
Falling Number	46.0	8.44	NS	45.0	8.49
Gel protein G'	16.0	0.31NS	NS	50.0	0.31
Gel protein weight	0.0	0.07NS	P 0.02	84.0	0.15

Once again the two methods agreed closely but in contrast to the earlier analyses given in Table 5, the estimates for the character crumb colour are also in agreement. Furthermore, the measure of effect was now significant for all the characters apart from gel protein G' and gel protein weight. Likewise the residual item testing the robustness of the model was no longer significant for loaf volume. The disappointing feature of the analysis was the large disagreements for the two gel protein characters. Gel protein G' behaved in a similar manner to the earlier analysis, but the locations for gel protein weight, which agreed in the earlier analysis, are now at opposite ends of the proposed map. The method of interval mapping supports the evidence of the analysis of means but not so the prediction from marker regression.

Overall there was a strong case for accepting the hypothesis that Factor 1* is a single gene controlling loaf volume, crumb colour and score and possibly gel protein weight. The case is based upon the twin Cluster Analyses, which agree very closely in their classifications, the highly significant associations between this classification and the variation in the four characters, plus the results of the two QTL analyses. It is the significant residual variation after removing the mean differences for gel protein weight combined with the anomalous QTL analysis that makes the inclusion of this character less certain than for the other characters.

The analysis of Falling Number has been consistent. It has shown strong association with the markers situated in the middle part of the map under consideration, even when Factor 1* was introduced into the

Figure 26 QTL analysis of six quality traits, 1 = loaf volume, 2 = crumb colour, 3 = crumb score, 4 = Falling Number, 5 = gel protein G' and 6 = gel protein weight, and their location on the linkage map of chromosome 3A

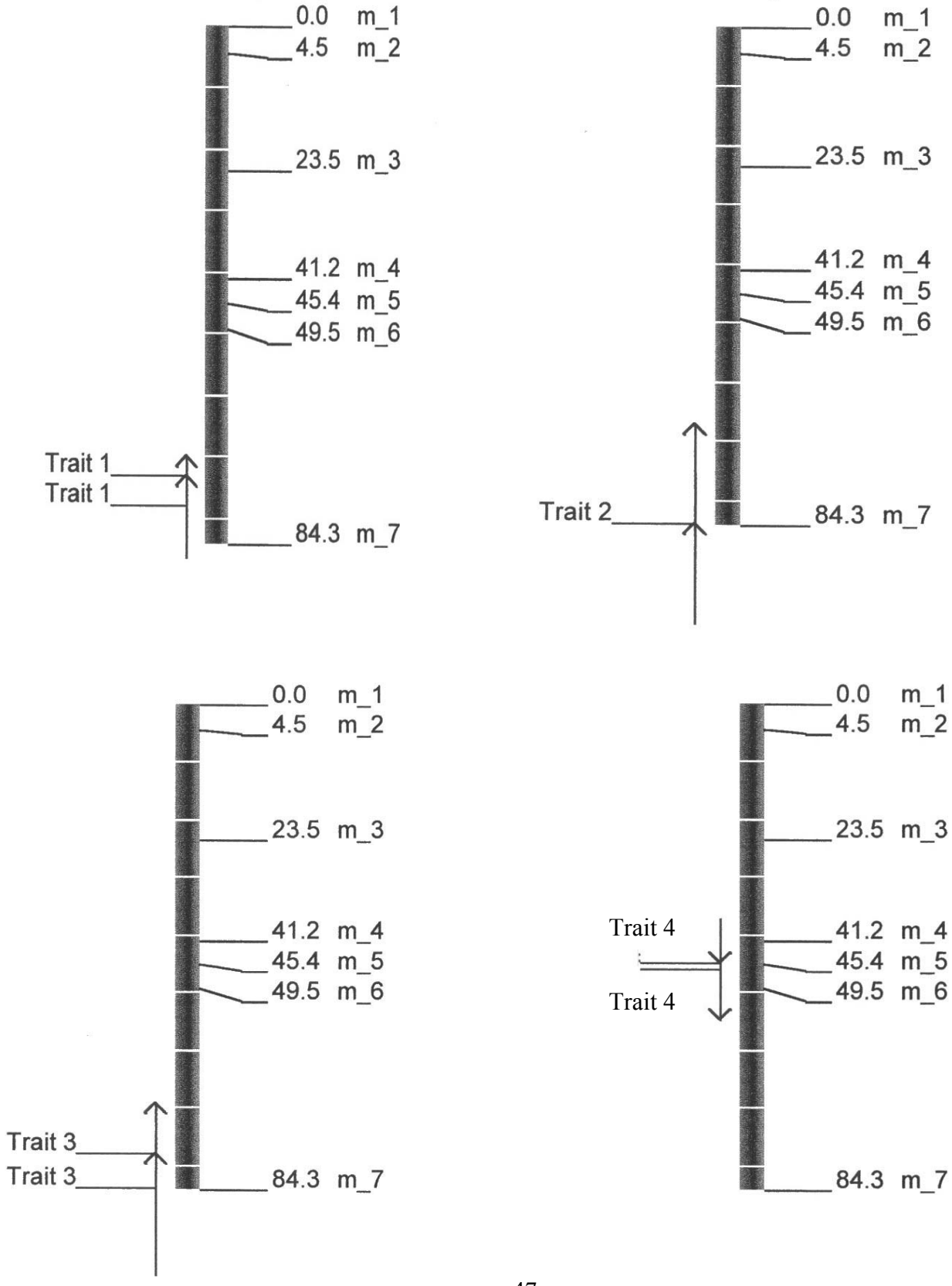
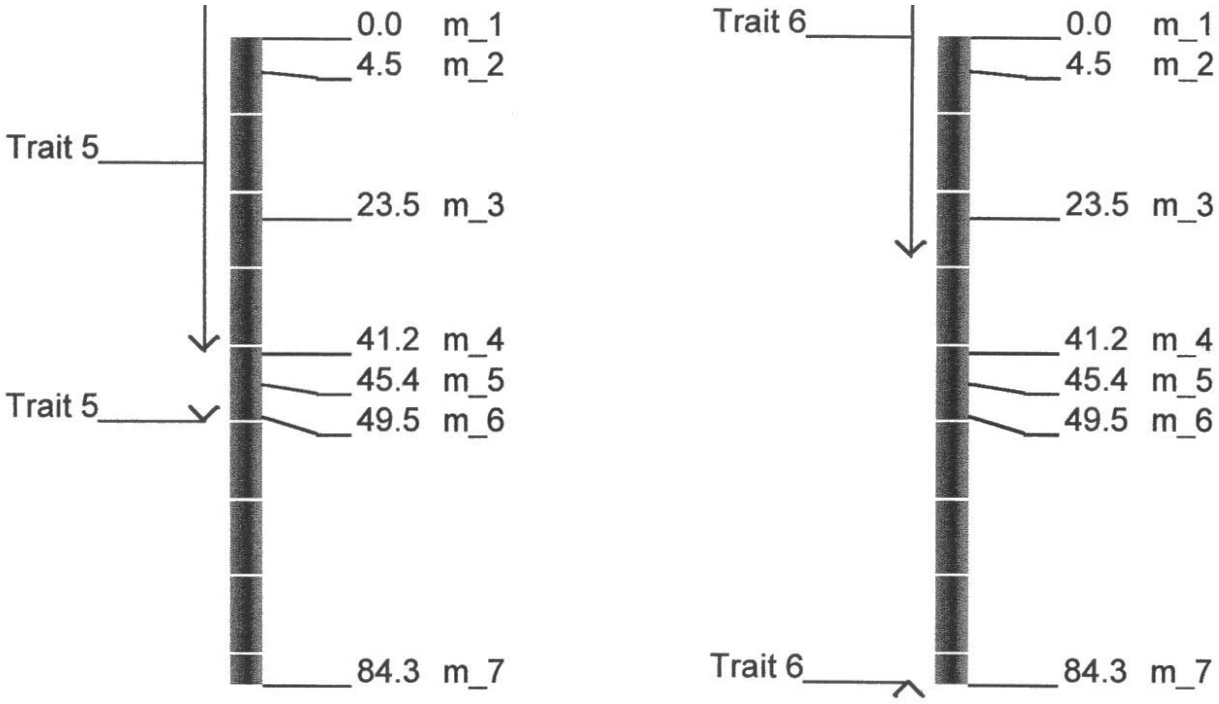


Figure 26 continued…



analysis. The QTL analyses have also produced the same results. The effects have been the same and significant but more importantly the residuals were insignificant. This supports strongly the position of this QTL affecting Falling Number.

4.9.1 The location of the gene(s) affecting gel protein G' .

Although there are highly significant differences between the lines for this gel protein G', it has not been possible to show that it is associated with any of the markers on Chromosome 3A, including Factor 1*. Neither the analysis of marker means, nor the QTL analyses have been able to pinpoint a location for the gene(s) responsible. It has been suggested that this could mean that the location is outside the area of the map being considered. Certainly there is sufficient room on the distal part of the long arm of the chromosome for this to be so.

Another possibility is that the gene(s) is found on another chromosome. Although the development of the substitution line requires several backcrosses to convert the background to that of the recipient variety, Cappelle-Desprez, there is still a possibility that some genes from Bezostaya I were fixed in the background. This possibility is difficult to test using the present material and marker classifications. However, as argued earlier the similarity between the 3A and 3B substitution lines, for a range of characters including G' suggests that they have genes in common which affect these characters. If this is so, then the gene(s) responsible for the G' variation amongst the 3A recombinent lines must therefore be present on this chromosome rather than in the background. How could such segregation be detected? Without a marker system this is not easy.

One possible reason for the variation in gel protein G' between the lines was considered to be inhomogeneity in the HMW- G subunit content or composition of the recombinant population.

A set of four single chromosome recombinant lines were selected for examination by SDS-PAGE to determine whether there was any variation in the HMW-glutenin content and composition of the fraction which would account for the differences in gel protein G' observed within the population for gene mapping purposes. Samples 8 and 19 were selected as representatives of high G' values, and samples 6 and 46 selected as representatives of low G' values (Figures 27 and 28). Table 10 (A) contains the results for the duplicate gels (labelled 1 and 3) and Table 10 (B) contains the results of the duplicate gels (labelled 2 and 4). Overall, the HMW-G content is about 7.5% of total extracted protein, and the 3 subunits (2, 7 and 12) are present in similar relative amounts within all the samples. Therefore, there appears to be no relationship between the variations found in the G' values and HMW-G content of these selected recombinant lines.

Table 10 Densitometric analyses of the SDS-PAGE of selected single chromosome recombinant lines % HMW-G and relative peak ratios measured in Gels 1 and 3 (A), and in Gels 2 and 4 (B).

(A)

	% Total HMW-G (Subunits 7, 2+12) in each lane											
Site	N1	N1	N1	N1	N2	N2	N2	N2	JM1	JM1	JM1	JM1
	Line 8	Line 19	Line 6	Line 46	Line 8	Line 19	Line 6	Line 46	Line 8	Line 19	Line 6	Line 46
Gel 1 Lane %	7.0	7.8	7.5	7.5	7.4	7.2	7.7	7.5	7.7	7.5	7.7	7.7
Gel 3 Lane %	8.1	7.7	6.9	7.4	7.5	7.5	7.4	7.6	7.2	7.3	7.1	7.5
Average Lane % of Gels 1 & 3	*7.6*	*7.7*	*7.2*	*7.5*	*7.4*	*7.3*	*7.5*	*7.5*	*7.5*	*7.4*	*7.4*	*7.6*
Gel protein G'	15.4	15.6	8.9	11.4	16.9	14.7	10.9	12.4	13.6	12.2	10.4	11.2
HMW-G Peak	Peak ratios											
1 (Subunit 2)	1	1	1	1	1	1	1	1	1	1	1	1
2 (Subunit 7)	1.4	1.4	1.4	1.3	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
3 (Subunit 12)	1.3	1.6	1.6	1.5	1.5	1.5	1.6	1.5	1.5	1.7	1.5	1.7

(B)

	% Total HMW-G (Subunits 7, 2+12) in each lane											
Site	JM2	JM2	JM2	JM2	JC1	JC1	JC1	JC1	JC2	JC2	JC2	JC2
	Line 8	Line 19	Line 6	Line 46	Line 8	Line 19	Line 6	Line 46	Line 8	Line 19	Line 6	Line 46
Gel 2 Lane %	7.9	7.2	6.7	7.1	7.1	7.3	7.5	7.2	7.2	7.6	7.3	7.7
Gel 4 Lane %	8.1	8.0	7.6	7.7	7.9	8.0	7.8	7.6	7.4	8.0	8.0	8.2
Average Lane % of Gels 2 & 4	*8.0*	*7.6*	*7.1*	*7.4*	*7.5*	*7.7*	*7.6*	*7.4*	*7.3*	*7.8*	*7.6*	*7.9*
Gel protein G'	15.4	16	11.7	12	16.7	16.3	9.6	11.2	15.6	10.8	11.7	9.9
HMW-G Peak	Peak ratios											
1 (Subunit 2)	1	1	1	1	1	1	1	1	1	1	1	1
2 (Subunit 7)	1.5	1.4	1.4	1.5	1.4	1.6	1.7	1.5	1.6	1.5	1.7	1.5
3 (Subunit 12)	1.5	1.5	1.5	1.7	1.4	1.8	1.9	1.6	1.7	1.6	1.8	1.7

N1 -Nickerson site replicate1; N2 -Nickerson site replicate 2.

JM1 - JIC Morley site replicate 1; JM2 - JIC Morley site replicate 2.

JC1 - JIC Church Farm site replicate 1; JC2 - JIC Church Farm site replicate 2.

Figure 27 SDS-PAGE of total protein, extracted from recombinant lines, using Laemmli buffer.
(A) - Gel 1, (B) - Gel 3.
N1- Nickerson site replicate 1, N2- Nickerson site replicate 2, JM1- JIC Morley site replicate 1.

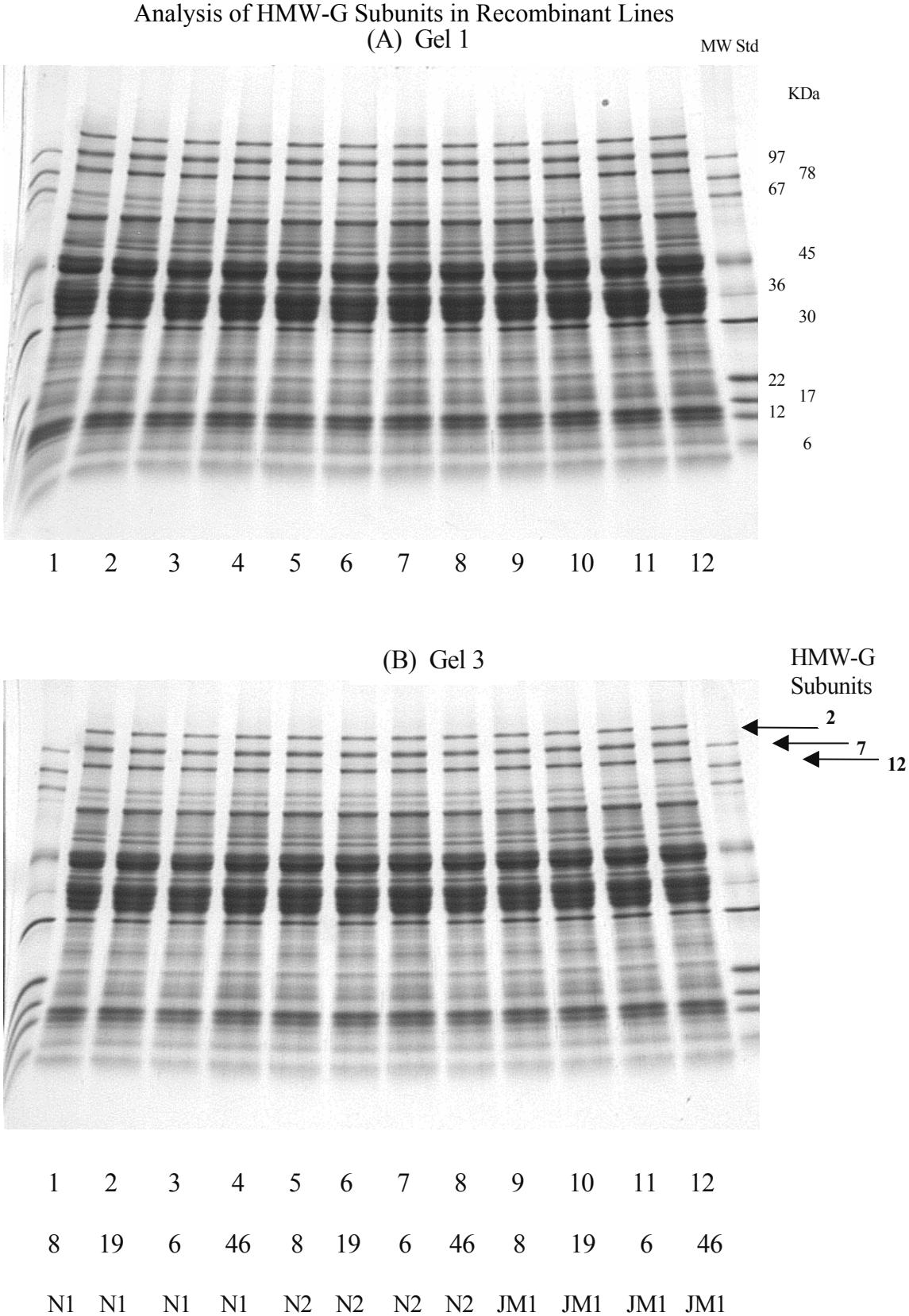
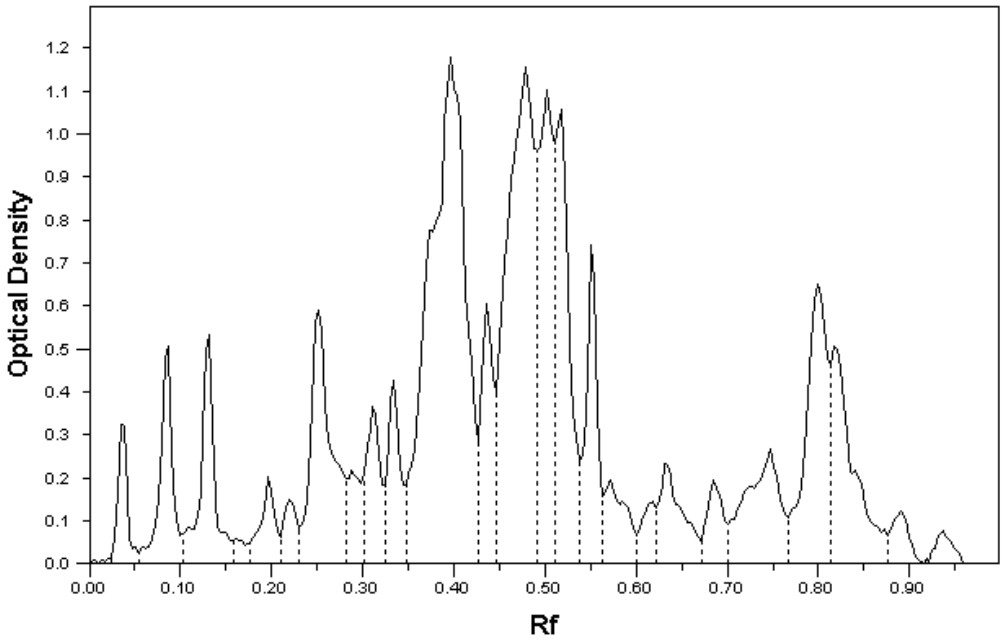


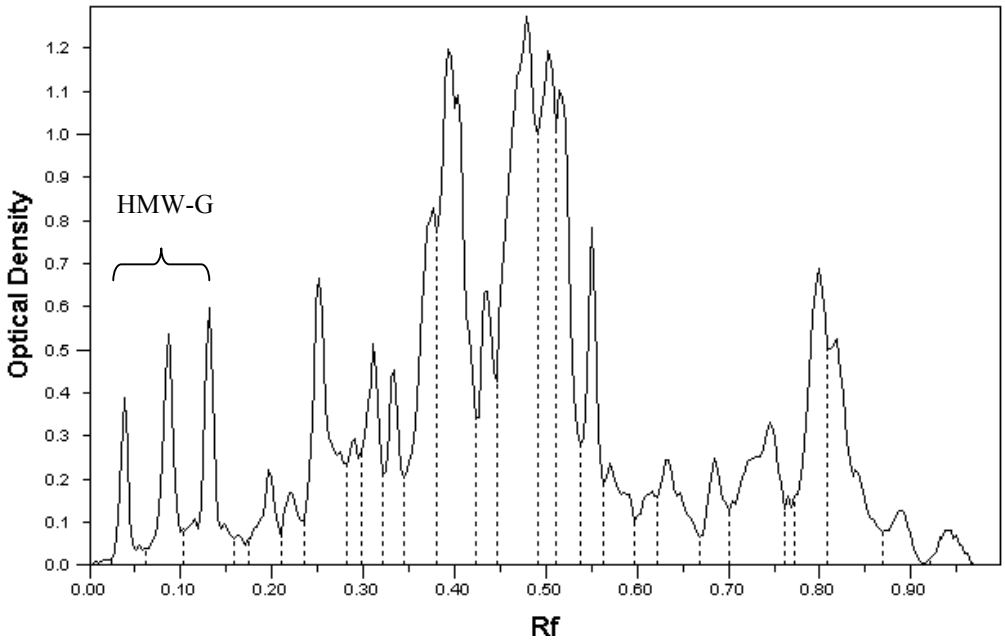
Figure 28 Profiles of SDS-PAGE lanes shown in Gel 3 of Figure 19B

(A) - Lane 2 (N1 Recombinant Line No. 19), (B) - Lane 4 (N1 Recombinant Line No. 46).

(A)



(B)



5 PROJECT OUTCOMES AND AREAS FOR FUTURE STUDY

- A DNA map of chromosome 3A has been developed within this project. A useful map of six microsatellite markers was obtained, but in view of the effort expended with both microsatellite and RFLP probes, the degree of polymorphism found between Cappelle-Desprez and Bezostaya I was disappointing. This was particularly the case for the long arm around the site of the putative loaf volume gene (***Lvl 1***) deduced from this work. In contrast, a contemporary study of a 3A substitution line involving Wichita and Cheyenne wheats and different probes found 3 microsatellite markers and 17 RFLP's (Shah *et al*., 1999 and Shah *et al*., 2000), which were more useful for mapping agronomic traits. In future, therefore, it may be fruitful to re-evaluate the present 3A recombinants with alternative DNA probes. Flour samples produced during this work will be stored as a resource that may be used in future work.

- A single gene which can now be designated ***Lvl 1*** (after loaf volume) rather than Factor 1* is responsible for controlling loaf volume, crumb colour and crumb score. It is located on the long arm of Chromosome 3A about 30 to 40 map units from the centromere. It is closely associated with a gene determining gel protein weight but it is not possible to determine whether this is a separate gene or ***Lvl 1***. In another study, the gene in wheat orthologous to the viviparous gene (Vp1) of maize has been mapped some 30 map units from the centromere on the long arm of chromosome 3A.

- A QTL, which is likely to be a single gene controlling Falling Number, is located on the short arm very close to the centromere (between 23.5 and 49.5 map units).

- Vp1 is a regulator gene affecting many aspects of grain development (Bailey *et al*, 1999). It is possible that ***Lvl 1*** and Vpl are the same gene. Further collaborative work between CCFRA and JIC would establish whether this is so or not.

- A gene (or genes) affecting gel protein G' is probably located on the distal part of the long arm of chromosome 3A.

- The analysis of the single substitution lines between the donor Bezostaya I and recipient Cappelle-Desprez has confirmed that the Group 1 chromosomes of Bezostaya I improve many dough rheological properties compared to those of Cappelle-Desprez as shown in earlier work by Krattiger *et al*. (1988). This is presumed to be a consequence of the different alleles for HMW and LMW glutenins carried by these chromosomes.

- Single chromosome substitution studies also confirmed the adverse effects of 3A and identified a similar influence of 3B on protein strength parameters, as measured by empirical (Alveograph W and Farinograph stability) and fundamental (gel protein elastic modulus) rheology was also evident and justifies the need to investigate further the cause within this study. An encouraging feature of the additional study is the effect of 3B which matches that of 3A but in some cases exceeds it in potency. This suggests that there may be a homoeoallelic series of genes involving these two chromosomes in the two varieties and possibly chromosome 3D in others.

- The other group of chromosomes that emerge from the single substitution work as potentially significant contributors to protein quality belong to Group 7. These have not been highlighted as having an effect in previous studies of breadmaking quality and therefore may be useful candidates for further detailed study.

- No correlation between measurements of loaf volume and any rheological properties were observed within the substitution line study. This is not unexpected as dough rheology is notoriously poor as a predictor of breadmaking quality providing a better estimation of the broad quality classification for a wheat flour or the potential for problems within a specific mixing/baking process.

- Combination of genetic and environmental factors accounts for the majority of variation observed in UK breadmaking wheat. This study has added further information to the genetic basis of breadmaking quality that should lead to an improved understanding and better control of this within new wheat varieties. However, environment has been shown to exert a significant influence on breadmaking quality as demonstrated by the "German effect" where the "poor" quality recipient Cappelle-Desprez grown in East Germany produced significantly better loaf volume than the UK-grown equivalent. The underlying reason for this difference could not be identified in terms of N:S ratios in the flour. Such site-to-site variation currently presents a very real problem to a miller who aims for uniformity in his raw material at intake (i.e. individual varieties with specified quality attributes) in order to provide his customer with a consistent end-product. Better information on the magnitude of the environmental component, possible methods for ameliorating negative effects and improved methods of measuring wheat quality at intake have been identified as priorities for research by the UK flour milling industry. The latter is being addressed within a current HGCA project entitled "Characterising wheat flour protein quality from Mixograph traces" but there work on the impact of environment on wheat quality is not currently being studied.

- Variation in the total and insoluble pentosan content of Quadrumat milled flour was observed within the Chromosome 3A recombinant line population, but QTL analysis failed to relate this to any of the 6

microsatellite markers. As the Chromosome 3A map becomes better defined, there may be further opportunities to seek DNA markers for these quality traits which are assuming greater importance amongst cereal processors.

- Towards the end of the study, biochemical work suggested the presence of a protein component (~30kDa) within the water- and salt-soluble protein fractions which appeared to be present in varying amounts across the 3A recombinant line population. The ideal biochemical marker would be the presence or absence of a particular component. However, provided differences in quantity are significant and consistent they could form the basis of a protein marker for 3A. Unfortunately, insufficient time and resource meant that it was not possible to survey the entire 3A recombinant population within the lifetime of this study. Further investigations would confirm whether this protein could be used as a biochemical marker and provide significant added value to this work..

- Although not discussed in this project report, added benefit to the UK breeding and farming communities has been generated in terms of resistance to ear blotch. Since a single-gene resistance to *Septoria tritici* was located on chromosome 3A of Bezostaya I during the course of the study.

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7 REFERENCES

Arakawa, T., Yoshida, M., Morishita, H., Honda, J., and Jonezawa, D., 1977. Relation between aggregation of glutenin and its polypeptide composition. *Agric. Biol. Chem*., **41**, 995- 999.

Bailey, P.C., McKibbin, R.S., Lenton, J.R., Holdsworth M.J., Flintham, J.E. and Gale, M.D., 1999. Genetic map locations for orthologous Vp1 genes in wheat and rice. *Theor.Appl.Genet*., **98**, 281-284.

Batey, I.L., Curtin, B.M., Abbott, D.C. and Rahman, S., 1996. Starch granule size distribution of Chinese Spring In: *Cereals '96. Proceedings of the 46th Australian Cereal Chemistry Conference* (Ed. Wrigley C.W.). Royal Australian Chemical Institute, North Melbourne, pp. 228-230.

Blanco, A., Bellomo, M.P., Lotti, C., Maniglio, T., Pasqualone, A., Simone, R., Troccoli, A., and Di Fonzo, N., 1998. Genetic mapping of sedimentation volume across environments using recombinant inbred lines of durum wheat. *Plant Breeding*, **117**, 413-417.

Bohlin, L., Carlson, T.L.-G and Backstrom, G., 1980. Cone-plate instrument for stress relaxation measurements. *Journal of Colloid Interface Science*, **73**, 61-65.

Borneo, R. and Khan, K., 1999 Protein changes during various stages of breadmaking of four spring wheats: quantification by size-exclusion HPLC. *Cereal Chemistry*, **76**, 711-717.

Bushuk, W. Hay, R.L. Larsen, N.G. Sara, R.G Simmons, L.D. and Sutton, K.H., 1997. Effect of mechanical dough development on the extractability of wheat proteins from bread dough. *Cereal Chemistry*, **74**, 389-395.

Campbell, K.G., Finney, P.L., Bergman, C.J., Gualberto, D.G., Anderson, J.A., Giroux, M.J., Siritunga, D., Zhu, J., Gendre, F., Roué, C., Vérel, A. and Sorrells, M.E., 2001. Quantitative Trait Loci associated with milling and baking quality in soft x hard wheat cross. *Crop Sci*., **41**, 1275-1285.

Cauvain, S., Collins, B and Pateras, I. 1992 Effects of ascorbic acid during dough processing. *Chorleywood Digest*, 121, 111-114.

Chamberlain, N. 1985 Dough formation and development. In: *The Master Bakers Book of Breadmaking,* 2nd edition, Ed. Brown, J. Turret-Wheatland Ltd, Rickmansworth, pp 1-46.

Collins, T.H., Little, K. and Sang, B.E. 1992 The characteristics and processing requirements of wheat for breadmaking. *HGCA Report No.60, HGCA*, Caledonia House, London.

Cyran, M., Rakowska, M. and Miazga, D., 1996. Chromosomal location of factors affecting content and composition of non-starch polysaccharides in wheat-rye addition lines. *Euphytica*, **89**, No.1, 153-157.

D'Appolonia, B.L., Gilles, K.A. and Medcalf, D.G., 1970. Effect of water-soluble pentosans on gluten-starch loaves. *Cereal Chem.,* **47**, 194-204.

Dodds, N.J.H., 1974. Some English wheat varieties in the CBP. *FMBRA Bulletin*, No 3, 72-80.

Douglas, S.G., 1981. A rapid method for the determination of pentosans in wheat flour. Fd Chem., 1981, 7, No.2, 139-145.

Garcia-Olmedo, F., Carbonero, P., and Jones, B.L., 1992. Chromosomal location of genes that control wheat endosperm proteins. *Advances in Cer. Sci. Technol*., **5**, 1-47.

Graveland, A. Bonger, P. and Bosveld, P., 1979. Extraction and fractionation of wheat flour proteins. *Journal of the Science of Food and Agriculture*, **30**, 71-84.

Greer, E.N. and Stewart, B.A., 1967 A revised classification of home-grown wheat varieties according to their value for milling and baking purposes. *FMBRA Bulletin No 1*, 197-202.

Harrison, K.R. and Greer, E.N., 1967. Notes on the 1966 harvest. *FMBRA Bulletin No 1,* 21-24.

Koh, B.K. and Lim, S.T., 2000. Effects of hydroquinone on wheat gluten extrusion. *Food Science and Biotechnology*, **9**, 341-345.

Korzun, V., Börner, A., Worland, A.J., Law, C.N. and Röder, M.S., 1997. Application of microsatellite markers to distinguish inter-varietal chromosome substitution lines of wheat. *Euphytica*, **95**, 149-155.

Krattiger, A.F., 1988 The genetics and biochemistry of bread-making quality in wheat (*Triticum aestivum* L.) PhD thesis, Wolfson College, Cambridge.

Krattiger, A.F., Payne, P.I. and Law, C.N., 1987 The relative contribution of proteins and other components to the bread-making quality of varieties determined using chromosome substitution lines. In *Proceedings 2nd International Workshop on Gluten Proteins* (Eds. Lasztity, R. and Bekes, F.). World Scientific, Singapore, pp 254-265.

Laemmli U.K., 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature*, **227**, 680-685.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E. and Newburg, L., 1987. MAPMAKER: an interactive computer package for constructing primary linkage maps of experimental and natural populations. *Genomics*, **1**, 174-181.

Law, C.N., 1966. The location of genetic factors affecting a quantative character in wheat. *Genetics*, **56**, 487-498.

Law, C.N. and Krattiger, A.F., 1987. Genetics of grain quality in wheat, In *'Cereals in a European Context* (I.D. Morton, ed). Ellis Horwoood, Chichester pp. 33-47.

Law, C.N., Snape, J.W. and Worland, A.J., 1988. Aneuploidy in wheat and its uses in genetic analysis in *'Wheat breeding - its scientific basis*. F.G.H Lupton, ed. Chapman and Hall, London, pp 71-108.

Law, C.N. and Worland, A.J., 1996. Intervarietal substitution lines revisited. *Euphytica*, **89**, 1-10.

Mansur, L.M., Qualset, C.O., Kasarda, D.D. and Morris, R., 1990. Effects of 'Cheyenne' chromosomes on milling and baking quality in 'Chinese Spring wheat in relation to glutenin and gliadin storage proteins. *Crop Science*, **30**, 593-602.

Marsh, D., 1999. Mixing and dough processing. In : *Technology of Breadmaking* Blackie Academic & Professional. London, 81-139.

Martin, D.J. and Stewart, B.G., 1986. Dough mixing properties of a wheat-rye derived cultivar. *Euphytica*, **35**, 225-232.

Moss, H.J., Wrigley, C.W., MacRitchie, F. and Randall, P.J., 1981. Sulphur and nitrogen fertiliser effects on wheat. II. Influence on grain quality. *Aust. J. Agric. Res*., **32**, 213-226.

Moss, H.J., Randall, P.J. and Wrigley, C.W., 1983. Alteration to grain, flour and dough quality in three wheat types with variation in soil sulphur supply. *J. Cereal Sci*. **1**, 255-264.

Neuhoff, V., Arnold, N., Taube, D. and Ehrhardt, W., 1988. Improved staining of proteins in polyacrylamide gels, including isoelectric focusing gels with clear background at nanogram sensitivity using the Coomassie Brilliant Blue G-250 and R-250. *Electrophoresis*, **9**, 255-262.

Oliver, G and Pritchard, P.E., 1993. Rheology of the gel protein fraction of wheat flour. In: *Food Colloids and Polymers: Stability and Mechanical Properties*, Cambridge, Royal Society of Chemistry, 255-259.

Pogna, N.E, Redaelli, R. and Baretta, A.M., 1991. The water-soluble proteins of wheat: Biochemical and immunological studies. In: *Gluten Proteins* (W. Busuk and R.Tkachuk, eds). AACC, St Paul, Minnesota USA. Pp 407-413.

Pomeranz, Y. and Chung, O.K., 1978. Interaction of lipids with proteins and carbohydrates in breadmaking. *J. Am. Oil Chem. Soc*., **55**, 285-289.

Pritchard, P.E. and Abel, B., 1993. Characteristics and processing requirements of wheat for white and wholemeal breadmaking. Annual Report of the ÉCLAIR project (Contract No.AGRE 0052). To explore and improve the industrial uses of EC wheat, European Commission, Brussels.

Pritchard, P.E. and Brock, C.J., 1994. The glutenin fraction of wheat protein: the importance of genetic background on its quantity and quality. *Journal of Science of Food & Agriculture* **65**, 401-406.

Randall, P.J. and Wrigley, C.W., 1986. Effects of sulphur fertilizers on the yield, composition, and quality of grains from cereals, oilseeds, and legumes. *Adv. Cereal Sci. Technol*. **8**, 171-206.

Salmon, S.E., 1999. Manual of methods for wheat and flour testing. Guidelines No. 3 CCFRA, Chipping Campden.

Salmon, S.E. and Anderson, C.A., 2001. Flour quality for breadmaking. *Aspects of Applied Biology* **64**, 2001 Wheat Quality, 39-46.

Salmon, S.E. and Burbridge, K.M., 1985. Wheat variety identification by polyacrylamide gel electrophoresis. *FMBRA Bulletin*, **2**, 78-88.

Shah, M.M., Gill, K.S., Baenziger, P.S., Yen, Y., Kaeppler, S.M. and Ariyarathna, H.M., 1999. Molecular mapping of loci for agronomic traits on chromosome 3A of bread wheat. Crop Science, **39**, 1728-1732.

Shah, M.M., Yen, Y., Gill, K.S and Baenziger, P.S., 2000. Comparisons of RFLP and PCR-based markers to detect polymorphism between wheat cultivars. *Euphytica*, **114**, 135-142.

Shewry, P.R. and Miflin, B.J., 1985. Seed storage proteins of economically important cereal seeds. In *'Advances in Cereal Science and Technology* (Y. Pomeranz, ed). American Association of Cereal Chemistry, St. Paul, USA. Pp 1-83.

Singh, J., Sharp, P.J., Blundell and Skerritt, J.H., 1998. Antibody probes for non-storage proteins and mapping of wheat chromosomes. In: *Proceedings of the 48th Australian Cereal Chemistry Conference*, 16-20 August, 1998 (O'Brien, Blakeney, Ross and Wrigley, eds). Cereal Chemistry Division, Royal Australian Chemical Institute, Melbourne, Australia. Pp 46-52.

Singh, J. and Skerritt, J.H., 2001. Chromosomal control of albumins and globulins in wheat grain assessed using different fractionation procedures. *J. Cer. Science*, **33**, 163-181.

Tilley, K.A., Benjmin, R.E., Bagorogoza, K.E., Okot-Kotber, B.M., Prakash, O. and Kwen, H., 2001. Tyrosine cross-links: Molecular basis of gluten structure and function. *J. Agric. Food,Chem*., **49**, 2627-2632.

Weipert, D., 1995. Processing performance of rye as influenced by sprouting resistance and pentosan contents. In *International Rye Symposium: Technology and Products* (Eds. Poutanen, K. and Autio, K.) VTT, Espoo, Finland, pp 39-48.

Whitworth, M. 1999 Increasing the competitiveness of home-grown wheats through improved methods for quality evaluation and optimised milling. *HGCA project No. 195*, HGCA, Caledonia House, London.

Williams, T. and Pullen, G., 1999. Functional ingredients. In : *Technology of Breadmakng* Blackie Academic & Professional, London, 45-80.

Wong, J.H., Kobrehel, K., Nimbona, C., Yee, B.C., Balogh, A., Kiss, F. and Buchanan, B.B., 1993. Thioredoxin and bread wheat. *Cereal Chem*., **70**, 113-114.

Wooding, A.R., Kavale, S., MacRitchie, F., Stoddart, F.L. and Wallace, A., 2000. Effects of nitrogen and sulphur fertiliser on protein composition, mixing requirements, and dough strength of four wheat cultivars. *Cereal Chem*., **77**, 798-807.

Wrigley, C.W., and Bietz, J. A., 1988. Proteins and amino acids. In *Wheat: Chemistry and Technology* (Y. Pomeranz, ed). Amercian Association of Cereal Chemists, St Paul, USA. Pp 159-275,

Zawistowska, U., Bekes, F., and Bushuk, W., 1986. Characterisation of low molecular weight protein with high affinity for flour lipids from two wheat classes. *Cereal Chem*., **63**, 414-419.

Zhao, F., McGrath, S.P. and Crosland, A.R., 1994. Comparison of three wet digestion methods for the determination of plant sulphur by inductively coupled plasma atomic emission spectroscopy (ICP-AES). *Communications in Soil Science and Plant Analysis*, **25**, 407-418.

Zhao, F., McGrath, S.P., Crosland, A.R. and Salmon, S.E., 1995. Changes in sulphur status of British wheat grain in the last decade, and its geographical distribution. *J. Sci. Food Agric*., **68**, 507-514.

Zhao, F., Salmon, S.E., Withers, P.J.A., Monaghan, J.M., Evans, E.J., Shewry, P.R. and McGrath, S.P., 1999. Variation in the breadmaking quality and rheological properties of wheat in relation to sulphur nutrition under field conditions. *J. Cer. Science*, **30**, 19-31.