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Journal of Cereal Science 41 (2005) 317–326

Journal of CEREAL SCIENCE

www.elsevier.com/locate/jnlabr/yjcrs

Novel genes on chromosome 3A influencing breadmaking quality in wheat, including a new gene for loaf volume, *Lvl 1*

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Received 10 July 2004; revised 4 November 2004; accepted 22 November 2004

Abstract

It is proposed that a single gene, *Lvl 1*, can explain variation in Chorleywood Bread Process loaf volume, crumb colour and score in bread wheat (*Triticum aestivum* L.). The gene is located on the long arm of chromosome 3A about 30–40 cM from the centromere. It is possible that the *Lvl 1* gene also determines gel-protein weight, although this could be the effect of a closely associated gene. A quantitative trait locus, which is likely to be a single gene controlling Hagberg Falling Number, was also located close to the centromere. Finally, the location of a gene (or genes) affecting gel-protein G' (elastic modulus) could not be established but it may be located distally on the long arm, but proximal to the marker *Xgwm 480*.

Keywords: Wheat; Breadmaking; Chromosome 3A; Gene *Lvl 1*)

1. Introduction

The high molecular weight glutenin subunits coded for by genes on the homoeologous group 1 chromosomes of wheat are now generally acknowledged to be the major contributors to breadmaking quality in wheat (Garcia-Olmedo et al., 1992; Payne et al., 1984). To a lesser extent, low molecular weight glutenin subunits, determined by different genes on the group 1 chromosomes, and gliadin proteins, determined by separate genes on the same chromosomes as well as on the group 6 chromosomes, also influence breadmaking quality. It is thought that, overall, the gluten fraction in wheat could account for up to 60% of the variation in breadmaking quality. This still leaves a substantial amount of variation unaccounted for and determined by non-gluten factors. A large number of such factors have been implicated, for example, lipids (Pomeranz and Chung, 1978), pentosans (D'Appolonia et al., 1970), hydrolytic enzymes and low molecular weight 'soluble' proteins within the albumin and globulin fractions (Pogna et al., 1991; Zawitowska 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).

It has been suggested that the identification of genes influencing breadmaking quality other than those controlling the gluten fraction might be a useful way of recognising such factors. This approach was advocated by Law and Krattiger (1987) who used the Cappelle-Desprez (Bezostaya 1) single chromosome substitution lines to show that chromosomes in addition to those from homoeologous groups 1 and 6 influenced loaf volume. This has been confirmed in other studies with substitution lines. Mansur et al. (1990) using the Cheyenne into Chinese Spring set of lines observed that, apart from the group 1 chromosomes, chromosomes 3A, 3B and 7B also influenced loaf volume.

Abbreviations: CBP, Chorleywood Bread Process; d*f*, degrees of freedom; MS, mean square; QTL, quantitative trait locus.

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doi:10.1016/j.jcs.2004.11.003

As yet, however, the number and types of genes involved in determining these additional 'breadmaking quality' effects is unknown. Here we report a study of such chromosomal effects, notably associated with chromosome 3A, and the location of the genes responsible.

2. Materials and methods

2.1. The Cappelle-Desprez (Bezostaya 1) chromosome substitution lines

The development of these lines is described in detail in Law and Worland (1996). All the lines have undergone at least eight backcrosses to the recipient variety Cappelle-Desprez and were developed as duplicate lines to check for background variation. Molecular, cytological and morphological markers were used to check whether the substituted chromosomes from Bezostaya 1 were present and intact. Three of the lines, 4B, 5BL–7BL and 7D, were shown to be incorrect.

Eighteen substitution lines along with their recipient and donor varieties were grown as drilled trials without replication in 1999 at the Morley Research Centre, UK, and at two sites in Germany, University of Halle and Plant Breeding International (Germany). Unfortunately, the harvest at the UK site was destroyed due to bad weather so that only the material from the two sites in Germany was available for breadmaking quality assessments.

2.2. Recombinant substitution lines for Cappelle-Desprez (Bezostaya 1 3A)

Following the procedure outlined by Law (1966), the 3A substitution line was hybridised with the recipient variety Cappelle-Desprez, and the resulting hybrid backcrossed as male to monosomic 3A of Cappelle-Desprez. A number of monosomic progeny were obtained from this cross. These were selfed and disomic progeny selected. These represent homozygous recombinant lines for this chromosome. Forty-eight lines were grown in drilled trials in 2000 along with the 3A substitution line and Cappelle-Desprez at three sites in the UK, the Morley Research Centre, John Innes Centre Church Farm and Nickerson (UK) Ltd, Woolpit. Each line was grown as duplicate plots, each plot being 1.2×6.0 m.

The lines were screened with a number of microsatellite markers developed at IPK, Gatersleben, Germany (Korzun et al., 1997). Of the several that were used in the screening, only seven were found to be polymorphic between the 3A chromosomes of Cappelle-Desprez and Bezostaya 1, *Xgwm 369*, *Xgwm 218*, *Xgwm 779*, *Xgwm 2*, *Xgwm 674*, *Xgwm 720* and *Xgwm 480*. Four of these were located on the short arm and three on the long. Six of the markers showed differences between Cappelle-Desprez and the substitution line and, as would be expected, segregation between the recombinant lines. The seventh and most distal marker, *Xgwm 480*, on the long arm showed no differences. This indicated that the line had recombined during the course of its development and part of the long arm of the substitution line was derived from Cappelle-Desprez rather than Bezostaya 1. Without further markers it is not possible to be certain how much of the long arm of Bezostaya 1 is missing but *Xgwm 480* is located between 94 cM (GrainGenes, http://wheat.pw.usda.gov, 2001) and 148 cM (Osa et al., 2003) from *Xwgm 720* so that a substantial part of the Bezostaya 1 long arm could still be present.

2.3. Flour and breadmaking quality assessments

All rheological and baking tests performed in assessing the quality of the flour and its breadmaking potential originate from a set of industrially agreed and collaboratively tested methods described by Salmon (1999). These tests are listed below with brief descriptions of the properties they are designed to measure.

1. *Hagberg falling number*. This measures *alpha*-amylase activity through changes in starch viscosity. Excessive activity has a deleterious effect on breadmaking quality.
2. *Brabender Farinograph*. This measures water absorption or the amount of water required to mix dough to a fixed consistency. The Farinograph also provides three other measurements of dough strength-development time, stability and degree of softening.
3. *Chopin Alveograph*. Provides four measurements of dough rheology. These are the amount of work or energy (W) required to a burst a bubble created in a thin sheet of dough, the maximum resistance to expansion (P), the stretching capacity (L) of the dough and the ratio P/L.
4. *Gel-protein quantity and quality*. Gel-protein was prepared and removed from a sample of 15 g of flour and weighed (Graveland et al., 1979). This represents the amount of functional protein and consists, principally, of glutenin. In general, breadmaking wheats have higher levels of this protein than feed or biscuit-making wheats. Two further measurements were made on this protein, the elastic modulus (G') and the viscous modulus (G'') (Pritchard, 1993). These were obtained using a Rheometer (Rheometrics Scientific Fluid Analyzer ARES-FA-Le, Piscataway, NJ, USA).
5. *Two baking processes were used to assess breadmaking quality*. These were a standard Chorleywood Bread Process (CBP) in which mixing was carried out on a Morton Z-Blade using 11 W h kg^{-1} and the No Time Spiral Mix Baking Procedure. In each case, three parameters were measured, loaf volume, crumb colour and a visual assessment or score of crumb structure on a scale of 1–10, a value of 10 being excellent. Loaf volume was determined by seed displacement and crumb colour using a colorimeter (Minolta Chrome Meter CR-310, Osaka, Japan).

2.4. Genetic and statistical analyses

Two approaches were used to identify genes responsible for controlling the characters listed above. First, QTL analyses were made by marker regression and interval mapping. This was achieved using the Software Package 'QTL Café' (www.bham.ac.uk/g.g.seaton). Secondly, attempts were made to classify the lines into distinct groups to perform a conventional segregational analysis. This was undertaken using a cluster analysis package provided by version 13 of Minitab. The marker map was calculated using JoinMap (Lander et al., 1987). Differences in means were established by ANOVA.

3. Results

3.1. The performance of the substitution lines

Several characters of potential influence on flour and breadmaking quality were scored for each of the lines at the two sites. Because only one sample was available per site, the site/line interaction was used as the estimate of error. Of the characters showing significant overall line 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 the breadmaking quality characteristics, Spiral crumb colour and crumb score. Unfortunately, the most important of the breadmaking attributes, CBP and Spiral loaf volumes, gave no significant differences between the lines. Almost certainly, this reflects the low heritabilities of these characters and the need to increase replication if statistically significant results are to be obtained.

The means for each of the lines, their significance levels from Cappelle-Desprez for each of these characters are given in Table 1 and the results for gel-protein G', Alveograph W and Farinograph stability are depicted in Fig. 1 to illustrate effects on protein strength.

As mentioned, gel-protein elastic modulus, G', provides a measure of functional protein. Cappelle-Desprez gave a mean G' value of about 20 Pa. This would be regarded as falling within the normal range of wheats giving satisfactory performance in UK breadmaking (Pritchard and Abel, 1993). On this basis, Cappelle-Desprez would be classified as a standard breadmaking variety and not, as anticipated, a weak gluten variety. Bezostaya 1, on the other hand and as expected, had a G' value in excess of 60 Pa, putting it into the 'extra strong' category. All three group 1 substitution lines gave significantly higher G' values than Cappelle-Desprez, reflecting almost certainly the effects of the different glutenin alleles present in Bezostaya 1. The biggest difference was due to the 1D chromosome of Bezostaya 1 known to be carrying the allele *Glu-D1d*, responsible for the good quality glutenin subunits 5 and 10 (Krattiger et al., 1987). By contrast, the 3A and 3B substitution lines decreased G' compared to Cappelle-Desprez. This is despite the fact that Bezostaya 1 exceeds Cappelle-Desprez by a large margin with respect to this character and is also the better quality parent.

The results for Alveograph W and Farinograph stability, measuring aspects of protein strength, were similar to each

Table 1
Mean scores for each of the substitution lines, Cappelle-Desprez and Bezostaya 1 for characters giving significant overall line differences

Chromosome	G' elas. mod	G'' visc mod	Alv P	Alv W	Far stab	Far Dg Sft	HFN	Spiral score	Spiral colour
1A	25.1*	7.30*	42.0	138.0**	2.90	152.0	311.5*	4.5**	50.16**
1B	26.3**	7.30*	43.5	133.5**	3.15*	147.0*	284.0	6.5	53.04
1D	41.4***	11.15***	49.5*	150.5***	4.05***	125.5***	312.0*	6.5	54.81*
2A	17.5	5.40	36.0	92.0	2.30	167.5	304.0	6.0	53.07
2B	21.6	6.40	35.0	108.0	2.25	160.0	312.5*	6.0	53.23
2D	16.6	5.05*	40.0	105.0	2.25	170.5	304.0	5.5	53.53
3A	15.2*	4.85*	34.5	91.5	1.90*	171.5	289.5	5.0*	51.64
3B	14.5**	4.50**	35.0	84.5*	1.50**	187.5**	286.0	5.0*	51.81
3D	19.7	5.75	38.0	105.5	2.40	168.0	286.0	6.0	50.91*
4A	18.5	5.55	37.5	112.0	2.30	172.5	300.0	6.0	53.09
4D	19.8	5.80	36.5	92.5	2.50	162.0	301.5	6.0	51.86
5A	18.6	5.70	37.0	94.0	2.10	174.0	299.5	7.0	54.08
5D	21.4	6.15	67.5***	156.0***	2.70	152.5	326.5***	6.5	54.34
6A	20.6	6.05	37.0	99.5	2.25	166.5	284.0	5.5	52.66
6B	19.7	5.85	41.5	121.5	2.30	149.0	308.0*	6.0	53.26
6D	19.0	5.70	42.5	124.5	2.45	162.5	296.0	6.5	53.35
7A	19.1	6.00	32.0	80.0*	1.80**	177.0	296.5	4.5**	50.77*
5BS–7BS	17.2	5.40	33.5	88.0*	2.25	169.0	282.5	6.0	52.51
Cappelle	20.5	6.05	39.0	104.5	2.55	163.5	284.0	6.5	53.06
Bezostaya	66.6***	15.15***	114.0***	322.0***	4.00***	94.0***	344.0***	7.0	55.76**

Indicated are those lines giving mean scores significantly different from Cappelle-Desprez. *P=0.05–0.01; **P=0.01–0.001; ***P<0.001.

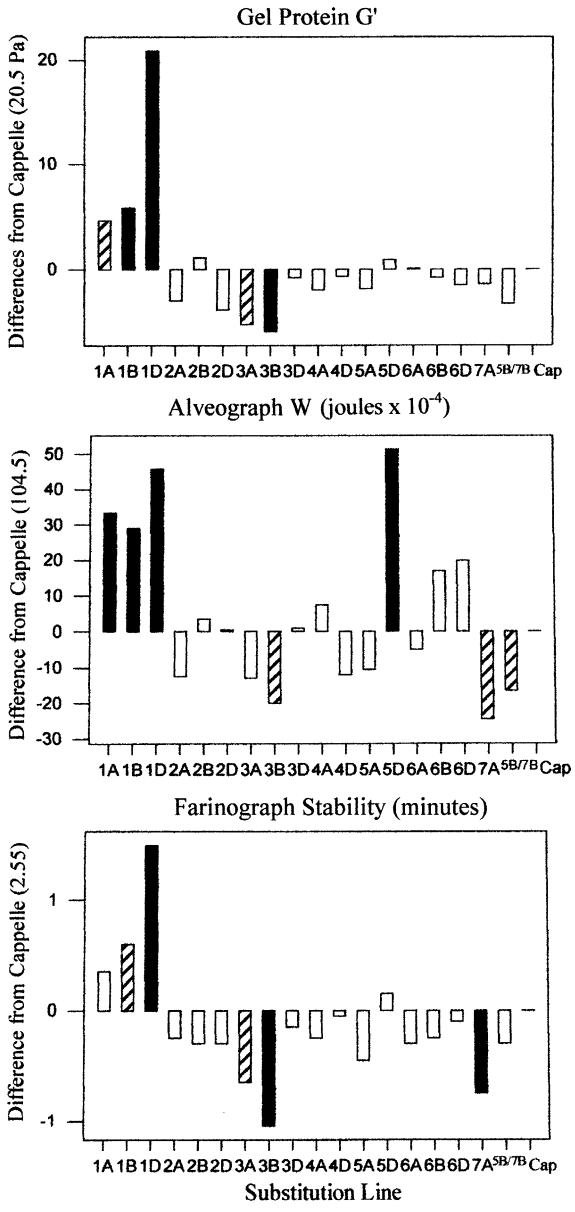

Fig. 1. Differences between the substitution lines and Cappelle-Desprez for Gel Protein G', Alveograph W and Farinograph stability. Hatched histograms indicate significance at the $P=0.02$–0.01 level, solid histograms at $P<0.01$.

other and G'. The substitution lines for group 1 showed significantly greater strengths than Cappelle-Desprez whereas substitution of 3A and 3B from Bezostaya 1 again gave reduced values. In addition, both tests identified 7A and 5BS–7BS of Bezostaya 1 as also having a reducing effect on protein strength.

The major effect of 5D in Alveograph W is due to the hardness gene, *Ha*, carried by the 5D chromosome of Bezostaya 1 (Krattiger, 1988; Law and Krattiger, 1987). The Alveograph test uses a fixed dough water level of 43% irrespective of the water requirement of the flour, so that this line will produce a much tougher dough, requiring greater energy to burst the dough bubble. The Farinograph measurement on the other hand measures dough rheology at variable water levels depending upon the water requirements of the sample, so that the influence of endosperm texture controlled by *Ha* is removed.

The screening of the Cappelle-Desprez substitution lines highlighted the expected effects of the group 1 chromosomes on breadmaking quality but they also revealed effects of chromosomes 3A and 3B as well as 7A and possibly 5BS–7BS. The fact that 3A and 3B, and 7A and 5BS–7BS have been pinpointed might suggest that homoeoalleles are involved. Although these chromosomes have previously been cited as influencing breadmaking quality (Mansur et al., 1990), the number and type of genes that might be responsible has not been studied previously.

3.2. The genetic map of chromosome 3A

Forty-six of the 48 3A recombinant lines were screened using the seven microsatellite markers, *Xgwm 369*, *Xgwm 218*, *Xgwm 779*, *Xgwm 2*, *Xgwm 674*, *Xgwm 720* and *Xgwm 480*. The latter, as mentioned earlier, showed that all the lines were identical to Cappelle-Desprez indicating that the distal part of the long arm of 3A had recombined during the course of the development of the substitution line. The remaining six markers however segregated between lines, and in every case the markers fitted the expected 1:1 ratio. This information was subjected to analysis using the JoinMap programme to produce the best fitting map (see Fig. 2). The position of the centromere is not exactly known but it is thought to reside in the region between the markers *Xgwm 2* and *Xgwm 720*. The position of *Xgwm 369* is reported as being about 20 cM from the end of the short arm (GrainGenes, http://wheat.pw.usda.gov, 2001).

3.3. Analysis of the 3A recombinant lines

The material obtained from the field trials of these recombinant lines was scored for eight quality related characters, CBP loaf volume, CBP crumb colour, CBP crumb score, Hagberg falling number, gel-protein elastic modulus (G'), gel-protein weight, Farinograph water absorption and protein content. The results were analysed

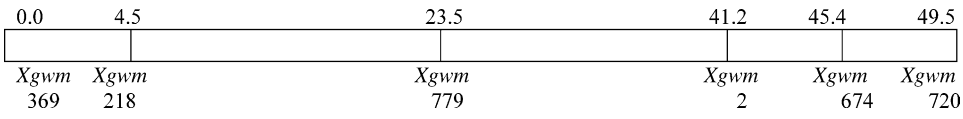

Fig. 2. The best fitting map of chromosome 3A showing the position of the six markers.

Table 2
ANOVA for loaf volume, colour, score, falling number, gel protein G', G protein weight, water absorption and %protein

Item	df	Loaf volume MS	Colour MS	Score MS	Falling number MS	Gel protein G' MS	Gel protein weight	Water absorption	Protein (%)
Line	47	9274*	6.21**	1.30 NS	906***	6.79***	0.494***	1.58 NS	0.121 NS
Site	2	311293***	184.28***	8.46***	44758***	11.04***	30.243***	201.07***	21.37***
Interaction	94	4782 NS	3.22 NS	0.50 NS	314 NS	2.77 NS	0.195 NS	1.60 NS	0.139*
Error	144	5833	3.67	1.04	318	2.67	0.204	1.72	0.094
Xgwm369	1	1332 NS	22.44 NS	3.78 NS	813 NS	16.45 NS	1.64 NS	0.16 NS	0.02 NS
Residual	44	9738*	5.99*	1.30 NS	906***	6.70***	0.47***	1.64 NS	0.13 NS
Xgwm218	1	0 NS	10.47 NS	1.37 NS	2026 NS	16.00 NS	1.86 NS	0.02 NS	0.00 NS
Residual	44	9768*	6.26**	1.35 NS	879***	6.71***	0.46***	1.64 NS	0.13 NS
Xgwm779	1	37872*	3.07 NS	0.43 NS	11909***	15.60 NS	0.41 NS	0.83 NS	0.00 NS
Residual	44	8910*	6.43**	1.37 NS	654**	6.72***	0.50***	1.62 NS	0.13 NS
Xgwm2	1	101928***	15.24 NS	6.37*	17206***	6.68 NS	0.72 NS	1.719 NS	0.04 NS
Residual	44	7452 NS	6.17**	1.24 NS	534**	6.92***	0.49***	1.60 NS	0.12 NS
Xgwm674	1	101940***	12.27 NS	7.05*	19416***	14.08 NS	0.21 NS	4.79 NS	0.00 NS
Residual	44	7452 NS	6.22**	1.22 NS	484*	6.76***	0.50***	1.54 NS	0.13 NS
Xgwm720	1	110928***	4.64 NS	7.05*	16151***	25.58 NS	0.46 NS	4.48 NS	0.0 NS
Residual	44	7248 NS	6.40**	11.22 NS	558**	6.49***	0.49***	1.54 NS	0.13 NS
Factor1 (*Lvl* 1)	1	246960***	136.78***	30.65***	5259***	6.43 NS	6.52***		
Residual	44	4158 NS	3.37 NS	0.69 NS	805***	6.93***	0.36*		

Includes the analysis of means for all six molecular markers and Factor 1 (See Later). *P=0.05–0.01; **P=0.01–0.001; ***P<0.001.

by ANOVA and are presented in Table 2. Included in the analysis are the comparisons between the marker means, i.e. *Xgwm 369 Cap* vs *Xgwm 369 Bez*. Since the analysis of the means is based upon 46 lines, each of these comparisons uses 1 df and will leave a residual item for 44 df measuring the variation within each of the two marker classes combined. The magnitude of the residuals is a measure of how close the marker is to the gene(s) affecting the character being studied.

Five of the characters, loaf volume, crumb colour, Hagberg falling number, gel-protein G' and gel-protein weight, gave highly significant differences between the lines. 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 the three sites.

For the comparisons between the marker means, significant differences were found for loaf volume, crumb score, and Falling Number but not for crumb colour, gel-protein G', gel-protein weight, protein content or water absorption. However, for crumb colour, gel-protein G' and gel-protein weight the overall line differences are highly significant even though none of the six marker comparisons are. The residuals on the other hand are significant. This indicates that the genes responsible for these characters are located independently of the two extreme markers, *Xgwm 369* and *Xgwm 720*. Since the markers available are restricted to the short arm and the proximal part of the long arm of 3A, the genes could be located distally and outside these regions. However, it has been established that an unknown region of the long arm beyond and including the location of *Xgwm 480* is effectively homozygous. As already pointed out, this could still leave a region in excess of 90 cM between *Xgwm 720* and *Xgwm 480* which would be sufficient to allow an independent location of the genes affecting these characters. Alternatively, the genes could be located on another chromosome. This point will be discussed later.

3.4. CBP 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 for this character is therefore 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.

3.5. CBP crumb score

Crumb score gave no overall line effect but the comparisons involving the three markers *Xgwm 2*, *Xgwm 674* and *Xgwm 720*, were just significant. On the other hand, none of the residuals was significant, again suggesting a gene(s) location outside the markers.

3.6. Hagberg 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.

3.7. QTL analysis

Each of the characters was analysed using marker regression and interval mapping procedures. In every case, the assumption was made that only one QTL was involved for each character. A summary of these results for each character is provided in Table 3.

The two methods agree 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 48 cM 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 colour and score where the residual items are also significant. In these two cases, however, the magnitudes of the effects as measured by marker regression are insignificant and are not different from zero. This contrasts with the analysis of loaf volume where the effect is significant. For Falling Number, the QTL is located in the same region and the measurement of effect is significant but in this case the residual item is insignificant, supporting the adequacy of the model. 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.

The results of this QTL analysis based upon the six markers indicates 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.

3.8. Correlations between characters

The QTL analysis has established a number of possible positions for the genes responsible for the six characters.

Table 3
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
Colour	48.0	0.12 NS	$P=0.01$	4.0	0.27
Score	48.0	0.12 NS	$P=0.00$	47.0	0.17
Falling number	46.0	−8.46	NS	45.0	−8.49
Gel-protein G'	14.0	0.36 NS	NS	7.0	0.35
Gel-protein weight	0.0	0.81 NS	NS	0.0	0.08

The effects refer to the Cappelle-Desprez allele.

Table 4
Correlation coefficients between the six characters and their significance levels, based upon the means of the 48 recombinant lines

	Loaf vol.	Colour	Score	Gel-prot. weight	Falling number
Colour	0.612***				
Score	0.704***	0.714***			
Gel-prot. weight	0.381**	0.385**	0.342*		
Falling number	−0.439**	−0.268 NS	−0.289 NS	−0.062 NS	
Gel-prot. G'	0.033 NS	0.144 NS	0.210 NS	0.155 NS	0.208 NS

*$P=0.05$–0.01; **$P=0.01$–0.001; ***$P<0.001$.

Many of the positions are 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 4. This shows that four of the characters, loaf volume, colour, score and gel-protein weight are positively and, from the levels of significance, strongly correlated. Both colour and score are properties influenced by loaf volume so that they would be expected to be functionally related and therefore to be correlated. Falling Number is also correlated with loaf volume but in this case negatively. It shows marginal significance with colour and score but not gel-protein weight. Gel-protein G' gives no correlation with any of the other characters.

3.9. Cluster analysis

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

Two types of cluster analysis were used. The first grouped the lines into two as if a single gene was segregating amongst them. In this analysis each variable is standardized and then, by a process of agglomeration, a hierarchical classification depicted as a dendrogram is produced which shows the relationships of each of the lines to each other. The analysis then splits the classification into two groups. The second analysis achieves a non-hierarchical clustering of observations by using 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 classification would be expected if only one gene was segregating because the segregants should be identical to the two parents.

Four of the variables, loaf volume, crumb colour, crumb score and gel-protein weight, were strongly correlated so

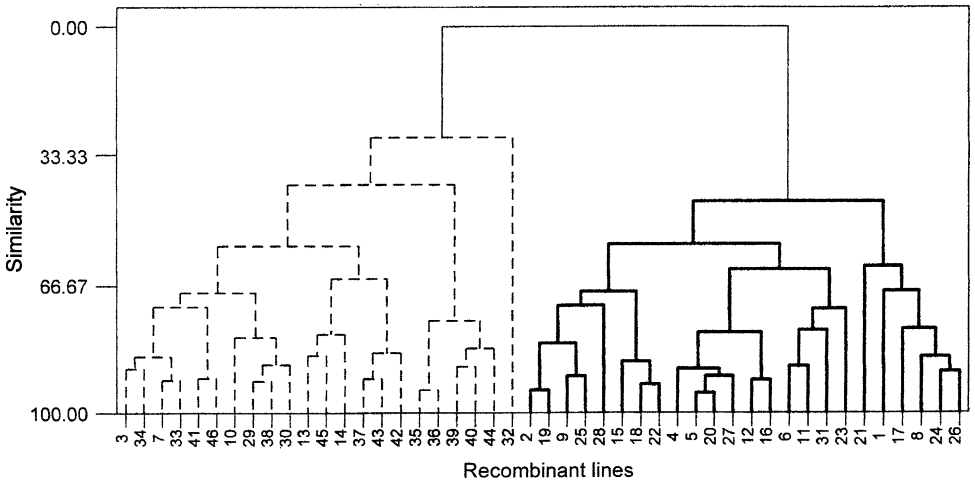

Fig. 3. Cluster analysis, dashes refer to the Bezostaya 1 group, unbroken lines to the Cappelle-Desprez group.

that these four characters were used in the two analyses. The results of the first analysis are presented as a dendrogram in Fig. 3. This shows that the 46 lines can be placed into two groups, 24 in one and 22 in the other, agreeing closely with the 1:1 segregation expected for a single gene. The result of the second analysis is even better, the classification giving two groups, each of equal size. More importantly though, the classification in the first analysis agrees very closely with that in the second, only differing for line 16. In the second analysis, this line is predicted not to have involved a crossover with the nearest marker, *Xgwm 720*, so on the assumption that a non-crossover is the more likely, this classification, designated Factor 1, was used in the further analyses.

In combination with the full marker classification of the lines, it was evident that Factor 1 must be located distally to *Xgwm 720* and that just 16 of the lines were recombinants. The map distance between *Xgwm 720* and Factor 1 is therefore 16/46 or 0.348 ± 0.070. This gives a map location of 84.3 for Factor 1.

3.10. Analysis of variance for actor 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 ANOVA presented in Table 2 was extended to include the comparison between the means of the groups defined by this factor. This additional comparison is given at the bottom of Table 2 for all six characters showing significant line or marker variation. For loaf volume, colour, 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 mean square comparing the means for Factor 1 was much lower than 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' is insignificant and the residual significant, again suggesting that the gene(s) responsible is very 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, colour, score and probably also gel-protein weight. The caution about the latter character is due to the significant residual item in the analysis, albeit at a much reduced level of significance, which might suggest that the gene(s) for this character is separate but still linked to Factor 1.

3.11. QTL analysis with factor 1

The validity of Factor 1 was 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 5 and graphically in Fig. 4. Once again the two methods agree closely but, in contrast to the earlier analyses given in Table 3, the estimates for the character crumb colour are also in agreement. Furthermore, the measure of effect is 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 is no longer significant for loaf volume. The disappointing feature of the analysis is the large disagreements for the two gel-protein characters. Gel-protein G' behaves 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 this is not the case for the analysis by marker regression.

Table 5
Summary of the results obtained from the QTL analysis by means of marker regression and interval mapping but including factor 1 in the analysis

Character	Marker regression			Interval mapping	
	Location (cM)	Effect	Residual	Location (cM)	Effect
Loaf vol.	78.0	33.46	NS	73.0	40.41
Colour	84.0	0.50	$P=0.02$	84.0	0.71
Score	84.0	0.29	$P=0.01$	78.0	0.39
Falling number.	46.0	−8.44	NS	45.0	−8.49
Gel-prot. G'	16.0	0.31 NS	NS	50.0	0.31
Gel-prot. weight	0.0	0.07 NS	$P=0.02$	84.0	0.15

The effects refer to the Cappelle-Desprez allele.

4. Discussion

4.1. Factor 1

Overall, there is a reasonable case for accepting the hypothesis that Factor 1, which can now be designated *Lvl 1* (after loaf volume), is a single gene controlling loaf volume, 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.

Undoubtedly, the small sample size used raises some doubts about the degree of confidence that can be placed on a single gene hypothesis even though it is based on segregational data as well as the more usual QTL analysis. Indeed, the significant residual variation for gel-protein weight after removing the mean effect of *Lvl 1*, plus the anomalous result with the regression analysis, suggests that the control of this character in particular could be a separate but closely linked gene. Additional recombinant lines might help to clarify this but the final resolution will depend upon the identification of gene products and ultimately to the isolation of the gene(s) involved.

4.2. Falling number

The analysis of this character was consistent. It showed strong association with markers situated in the middle part of the map under consideration, even when *Lvl 1* was introduced into the analysis. The QTL analyses have also produced the same results. The effects were the same and significant, but more importantly, the residuals were not significant. This suggests strongly that there is a QTL, which is possibly indicative of a single gene affecting Falling Number, located close to the centromere.

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

Although there were highly significant differences between the lines for this character, it has not been possible to show that it is associated with any of the markers on Chromosome 3A, including *Lvl 1*. Neither the analysis of marker means, nor the QTL analyses have been able to pin point 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. The distal part of the long arm beyond *Xwgm 480* is unlikely to be the location because this part of the substituted chromosome is homozygous. How much of the chromosome proximal to *Xwgm 480* is homozygous is unknown at the moment, but the distance between the position of *Lvl 1* and *Xwgm 480* could be of the order of 60–100 cM. If this were to be

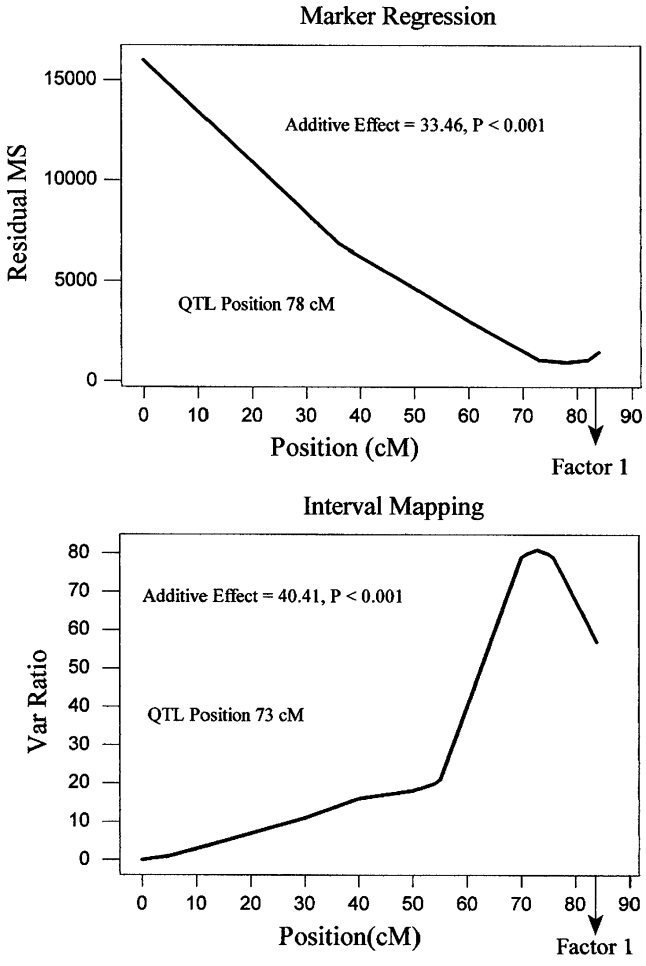

Fig. 4. Marker regression and interval mapping for loaf volume showing the location of a QTL close to Factor 1.

the case then this would still leave room for an additional gene independent of any marker including *Lvl 1*.

Another possibility is that the gene(s) is found on another chromosome. The development of the substitution line requires several backcrosses to convert the background to that of the recipient variety, Cappelle-Desprez. In this case, the line Cappelle-Desprez (Bezostaya 1 3A) had undergone eight backcrosses in its development. This would mean that most of the background was indeed identical to that of Cappelle-Desprez, but it should be emphasised that even eight backcrosses cannot ensure that some genes from Bezostaya 1 were not fixed in the background. 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 recombinant lines must either be present on 3A or possibly chromosome 3B and not on any other chromosome in the background.

4.4. Other reports of genes with possible effects on breadmaking quality

A number of possible candidate genes or QTLs have been cited on the short and long arms of 3A. A QTL (*QPhs.ocs-1*) affecting dormancy on the short arm of 3A has been reported by Osa et al. (2003). This could be the Falling Number QTL located in this study. However, the map locations do not agree. The Falling Number QTL was located close to the centromere whereas *QPhs.ocs-1* was positioned much further out on the short arm although inconsistencies in location were noted between the two experiments described. Homology with the gene viviparous (*Vp 1*) recently located on the 3A long arm and which is associated with dormancy effects should also be considered. *Vp 1* was positioned in the middle of the long arm, 30 cM from the centromere in one investigation (Bailey et al., 1999) and 84.8 cM in another (Osa et al., 2003). Either of these positions will rule out homology with the Falling Number QTL but *Lvl 1* could fit with the first reported location but not the second. It should be possible to test this using the available *taVp1* clone as a probe on the 3A recombinant lines used in this study.

4.5. The group 7 chromosome effects

The substitution line data revealed effects of chromosome 7A and to a lesser extent chromosome 5BS–7BS on dough strength. Mansur et al. (1990), reported a similar effect of 7B. 7A also influenced dough mixing traits and loaf volume in a QTL study of recombinant inbred lines derived from a cross between a soft and a hard wheat (Campbell et al., 2001). Clarke et al. (2002) reported a family of proteins determined by groups 7 and 4 chromosomes which had a marked effect on mixing and breadmaking qualities. Singh and Skerritt (2001) have also identified non-gluten proteins determined by several chromosomes including 7B and 7D, and all of the group 3 chromosomes. In addition, the group 7 chromosomes, along with other chromosomes, were shown to have a regulatory effect on the expression of the HMW glutenin genes (Wanous et al., 2003). There would appear therefore to be ample scope for identifying the genes on these chromosomes, their products and roles in influencing quality aspects of breadmaking.

Acknowledgements

We acknowledge the support of HGCA (Hane Graun Cereal Authority) in completing this work and the help of and use of facilities at Plant Breeding International (IPK), Gatersleben in carrying out the mapping of chromosome 3A. We should also like to dedicate this paper to the memory of Tony Worland who sadly passed away before this work was completed.

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