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Sustainability of UK-grown wheat for breadmaking

by

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CONTENTS

1. ABSTRACT

The project aimed to establish how the interaction of genetic and environmental factors affect protein deposition during grain development and, in particular, to compare varieties which show grain protein deviation (GPD) with varieties which do not. It included comparisons of the protein content and composition of varieties under different growing seasons, locations and nitrogen (N) inputs; analysis of the gluten protein subunit and glutenin polymer profiles in relation to the dough mixing properties and breadmaking performance, and the identification of gene transcripts which are associated with the stability of wheat quality under reduced nitrogen inputs. Such tools will facilitate the development of new varieties that have high N-use efficiency and consistently good breadmaking quality.

The project focused on six varieties (Cordiale, Istabraq, Hereward, Malacca, Marksman and Xi19). Material was grown at multiple sites (Rothamsted in 2009, 2010 and 2011; RAGT, Limagrain, Syngenta and KWS in 2010 and 2011) and analysed for nitrogen, protein composition and processing quality, including milling and baking tests performed in four independent laboratories (Campden BRI, Warburtons, Premier Foods and Allied Technical Centre (ATC)). In addition, developing grain was harvested from the Rothamsted (2009, 2010 and 2011) and RAGT (2010 and 2011) sites at 21 days after anthesis (mid-grain filling) to measure gene expression using Affymetrix wheat microarrays.

Wheat yields and grain %N were responsive to N application at most sites, although little GPD was observed in 2011. An inverse relationship between yield and grain %N was consistent in all trials. Transcriptome data for N-responsive genes were compared for 2009 and 2010 (Rothamsted site). This showed that the same set of genes responded to N in the two years, but that the response was greater in 2010. Further transcriptome data were collected in 2011 for the plots receiving 200 kg/ha N at Rothamsted and RAGT. 159 significantly N-responsive genes were identified.

A detailed analysis of gluten protein gene expression was undertaken. For both 2009 and 2010, gliadin genes were responsive to N-application. A previously uncharacterised γ-gliadin gene was identified as strongly N-responsive. Amino acid sequences for this γ-gliadin from different wheat sources were compared and expression patterns across the six varieties analysed. The patterns of expression of monomeric and polymeric gluten proteins in all varieties at all N-levels were analysed by SDS-PAGE and SE-HPLC, respectively. Functionality testing was performed at Campden BRI and breadmaking at Campden BRI, Warburtons, Premier Foods and ATC.

All datasets were subject to multivariate data analysis, which allowed us to identify key parameters that predicted baking performance. In particular, specific genes were identified as related to GPD.

2. SUMMARY

2.1. Introduction/Background and aims

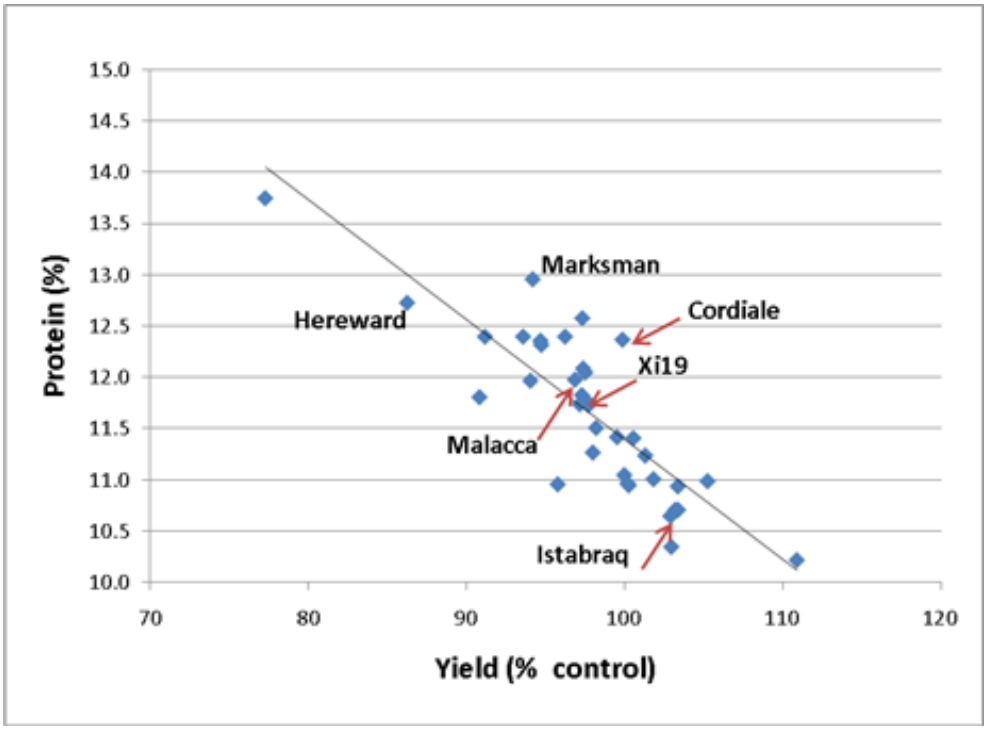


Figure 2.1 Grain protein deviation (deviation from a linear relationship between grain protein content and yield) in UK wheats, taken from the HGCA 2007 harvest survey covering east, west and north regions of the UK. The six selected varieties are indicated. Sixteen samples of each selected variety were analysed, except for Xi19 (12) and Hereward (6).

Wheat is the most important crop in the UK, giving average yields of about 8 tonnes per hectare and being used for food, distilling and livestock feed. However, high yields and the high protein contents required for breadmaking require high inputs of nitrogen fertiliser, which is not sustainable in terms of cost, energy requirement for fertiliser production and environmental footprint.

Furthermore, year to year variation in the weather conditions results in considerable variation in grain processing quality, which may necessitate the import of high volumes of wheat in some years with impacts on the cost of bread and other foods. It is, therefore, crucial that UK wheat production and quality are maintained to guarantee food security and maintain prosperity of the farming and food processing sectors. Data from field trials show significant variation in the response of currently grown wheat varieties to N fertiliser, and in particular in their ability to produce grain with high protein content at the same levels of N application, with Marksman and Cordial showing higher contents of grain protein (called Grain Protein Deviation, GPD) in 2007 (Figure 1.1). Furthermore, they also differ in the extent to which the composition and quality of the grain are affected by environmental fluctuations, with Hereward being recognised as showing unusually high stability.

We have, therefore, investigated the molecular basis for these differences:

1. By growing varieties known to differ in their response to N fertilisation and stability of quality in replicate field trials over several sites in the UK and three harvest years.
2. By performing transcriptome analysis of developing grain across germplasm, nitrogen input, site and year.
3. By collecting corresponding final grain samples for protein and functional analysis
4. By examining processes in canopy tissue contributing to yield/N remobilisation in selected lines.
5. By studying the expression of selected genes in greater depth.
6. By integrating all data sets to correlate functionality with underlying processes/genes.

Project aims:

The overall aim was to compare the expression of genes and the synthesis and accumulation of gluten proteins in the developing grain with the final composition and processing properties, and to relate this to wider aspects of nitrogen use efficiency in the whole plant.

1. To determine how the interactions of genetic and environmental factors affect the timing and spectrum of protein deposition during grain development, by conducting field trials of 6 selected UK wheat varieties, sampling developing grain during grain filling.
2. To determine how key functional proteins accumulate within wheat varieties under different growing seasons, locations and nitrogen inputs, using SDS-PAGE and HPLC analysis to separate the component proteins and polymers.
3. To determine how the assembly of protein polymers affect the dough mixing properties and breadmaking performance, by relating HPLC separated fractions to dough functionality and baking test results.
4. To identify and characterise transcripts which are associated with the stability of wheat quality under reduced nitrogen inputs by using transcriptome approaches (evaluation of level of expression of all genes) and correlating gene expression with the traits measured.

This should result in the identification of genes and proteins whose expression correlates with grain nitrogen content and composition and with processing quality (including stability of quality from year to year). Some of these genes and proteins may be directly involved in determining the traits of interest and hence the work will lead to better scientific understanding. Other genes and proteins may not be directly involved but could nevertheless be developed as markers which can be used by plant breeders to select for improved wheat varieties. The project, therefore, contributes to the target of more sustainable production of wheat in the UK.

2.2. Materials and methods

The project was based on multiple field trials over three years, extensive analysis of harvested grain, from N determination to protein and gene expression profiling, functionality testing and breadmaking by multiple bakers (Figure 2.2). All data have been cross-correlated to relate traits to one another and to specific patterns of gene expression.

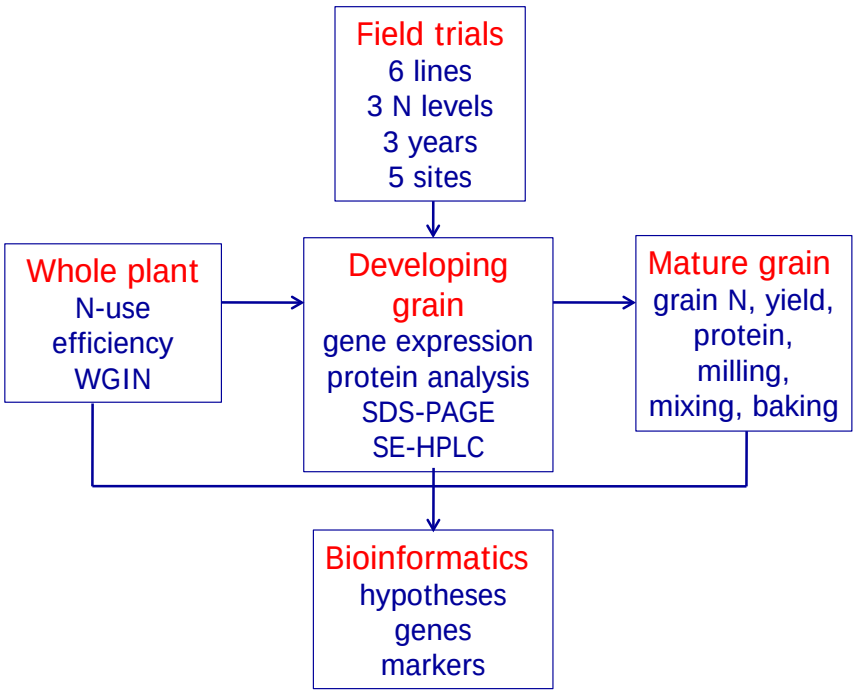


Figure 2.2 Practical components of the project.

2.2.1. Field trials and crop analysis

Six varieties were grown at Rothamsted in 2009, 2010 and 2011 and at sites run by the breeder partners in 2010 and 2011 (RAGT, Ickleton, Cambridge; Limagrain, Woolpit, Suffolk; Syngenta, Whittlesford, Cambridge; KWS-UK, Thriplow, Hertfordshire). Three N levels were used: 100kg/ha as a “low input” level, 200kg/ha to reflect modern practice for breadmaking wheats in the UK and 350 kg/ha as an extreme high input to achieve high grain protein. Nitrogen was applied in ‘splits’ as solid ammonium nitrate prills. All plots were randomised with three replicates.

2.2.2. Transcriptomics

Microarrays were used to profile gene expression. A specific time point of 21 dpa (days post anthesis) was chosen as a key developmental stage (mid-grain filling) in which grain storage proteins are being synthesised. Ears were tagged at anthesis and caryopses harvested 21 d later. Gene expression was determined by profiling RNA extracted from this material against a gene chip containing 55,000 elements. This is not a full genome chip but represents a large proportion of wheat genes and known alleles. Data from the profiling are semi-quantitative giving a good

indication of the relative levels of expression of all RNAs in the sample simultaneously. Data were collected for three years at Rothamsted and for 2010 and 2011 at the RAGT site, for the three N levels in 2009 and 2010, and for the 200 kgN/ha treatment in 2011.

2.2.3. **Protein analysis**

The protein composition of mature and developing grain samples was analysed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and size-exclusion high pressure liquid chromatography (SE-HPLC). For SDS-PAGE analysis, protein was extracted from samples (35mg) of wholemeal flour and separated using gel electrophoresis. Proteins were visualised by staining with Coomassie Brilliant Blue and then scanned and quantified using Total Lab TL120 version 2006F (Nonlinear Dynamics, Newcastle-upon-Tyne, U.K.) with an optical density curve calculated from a Kodak T14 control scale (Tiffen LLC Rochester, NY). The bands were divided into three groups: high molecular weight subunits of glutenin, low molecular weight subunits of glutenin and gliadins. For SE-HPLC analysis (Morel *et al*., 2000), proteins were extracted from white flour (mature samples) or wholegrain flour (developing samples). The polymer size distribution was calculated from the chromatogram which has five identifiable peaks (Figure 2.3). The first peak to elute from the column is referred to as F1 and consists of high molecular weight (HMW) polymers enriched in HMW subunits. The F2 peak comprises low molecular weight (LMW) polymers and is enriched in LMW subunits. The F3 and F4 peaks are comprised principally of ω-gliadins and α-, β-, and γ-gliadins, respectively, while the F5 peak comprises low molecular weight proteins including albumins and globulins. The overall area under the trace is a measure of the total protein content of the flour and is termed AT.

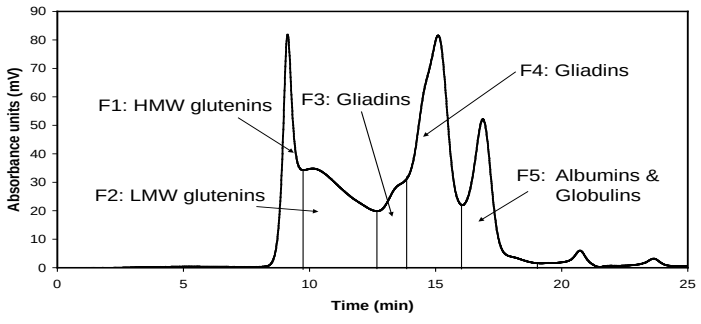


Figure 2.3 Typical SE-HPLC chromatogram of HMW and LMW glutenin polymers (F1 and F2, respectively), monomeric gliadins (F3 and F4) and smaller albumin and globulin proteins (F5).

2.2.4. **Functionality**

Milling

White flour was produced using a Bühler Laboratory Flour Mill MLU 202 at Campden BRI according to an internal Campden BRI method. Where replicates were pooled for analysis, wheat grain from each replicate was combined and blended together thoroughly prior to milling.

NIR analysis of grain and flour

Wholegrain and flour protein and moisture content were measured by NIR according to the Manual of methods for wheat and flour testing Guide Line No. 3 (Campden BRI).

Hagberg Falling number

The Hagberg Falling Number was determined according to the Manual of methods for wheat and flour testing Guide Line No. 3 (Campden BRI).

Dough rheology

Flour water absorption was measured using a Brabender Farinograph according to the Manual of methods for wheat and flour testing Guide Line No. 3 (Campden BRI). The extensibility and resistance of the dough was measured using a Brabender Extensograph according to the Manual of methods for wheat and flour testing Guide Line No. 3 (Campden BRI). Small scale dough rheology was determined using a Reomixer according to an internal method.

2.2.5. **Baking tests**

Bake tests were performed at Campden BRI using no time dough process (spiral mixer). A standard lean recipe was used to produce 800g, four piece loaves in unlidded tins proofed to time (Millar *et al*., 2008). Loaf volume was measured and crumb structure was analysed using a C-Cell imaging system (Calibre Control International Ltd). C-Cell uses high definition imaging and controlled illumination to ensure optimum image quality and consistent analysis. An image is analysed to provide data values which can provide valuable information about cells, circulation, cell elongation, faults and shape and size information. Bake tests were also performed by Warburtons, Allied Technical Centre (ATC) and Premier Foods according to their own procedures.

2.2.6. **Statistical validation**

Univariate statistical analysis was performed on all the data to verify their significance related to the experimental design consisting of the genotypic and the environmental factors and their interactions. The p-values were adjusted for multiple comparisons by False Discovery Rate using a statistical rotation test.

1.2.7. **Data integration**

The data were integrated by the multivariate data regression approach Partial Least Squares Regression as implemented in the software Unscrambler.

2.3. Results

Six varieties were selected for comparison based on the HGCA data shown in Figure 2.1. Marksman and Cordiale are Group 2 wheats which showed GPD (Figure 2.1), Hereward has been

the “gold standard” breadmaking wheat for over 20 years and shows high stability, Malacca and Xi19 are Group 1 breadmaking wheats and Istabraq is a Group 4 feed wheat. The varieties were grown and harvested at multiple sites (Rothamsted in 2009, 2010 and 2011; RAGT, Limagrain, Syngenta and KWS in 2010 and 2011) and analysed for nitrogen, protein composition and processing quality, including milling and baking tests performed in four independent laboratories (Campden BRI, Warburtons, Premier Foods and ATC). In addition, material from the Rothamsted (2009, 2010 and 2011) and RAGT (2010 and 2011) sites was sampled at 21 days post-anthesis (dpa) to determine gene expression using the Affymetrix wheat GeneChip microarray.

2.3.1. **Field trials**

Yields and grain N

In 2009, the yields at Rothamsted varied between 8.2 and 12.7 t/ha (at 85% dry matter), with grain %N varying from 1.4 to 2.4. All varieties responded positively to applied N in terms of yield and grain %N. Istabraq had the highest yields and lowest %N.

In 2010, all months had below average rainfall, with the exception of August which was very wet. The yield at Rothamsted varied from 7.3 to 10.2 t/ha., substantially lower than 2009, and grain %N varied from 1.4 to 2.8. Yield and grain %N both responded to applied N, with %N responding most strongly. Yield and grain %N also responded to N application at Limagrain, RAGT and Syngenta but not at KWS. Yields were lowest at RAGT and highest at KWS and Syngenta.

In 2011, March to May was characterised by exceptionally low rainfall followed by a relatively wet summer. Yields at Rothamsted ranged from 7.6 to 11.5 t/ha and grain %N from 1.6 to 3.2. Responses to applied N were generally poor for all sites except Rothamsted and Limagrain. The lowest yields were at RAGT, Syngenta and KWS, which were close geographically and had low rainfall.

Grain protein deviation

In 2009, Hereward showed a positive deviation at all N levels, Cordiale at 100 and 350, Marksman at 200 and Xi19 at 350 kg/ha (Figure 2.4). In 2010, the inverse relationship between yield and grain %N was consistent in all trials with Cordiale and Marksman showing positive GPD at the KWS site; Marksman and Hereward at Rothamsted; Hereward, Marksman and Cordiale at RAGT and Cordiale alone at Limagrain and Syngenta. Little GPD was observed in 2011 with the exceptions of positive GPD for Hereward at Syngenta and KWS, Xi19 and Hereward at KWS, the two lowest yielding locations

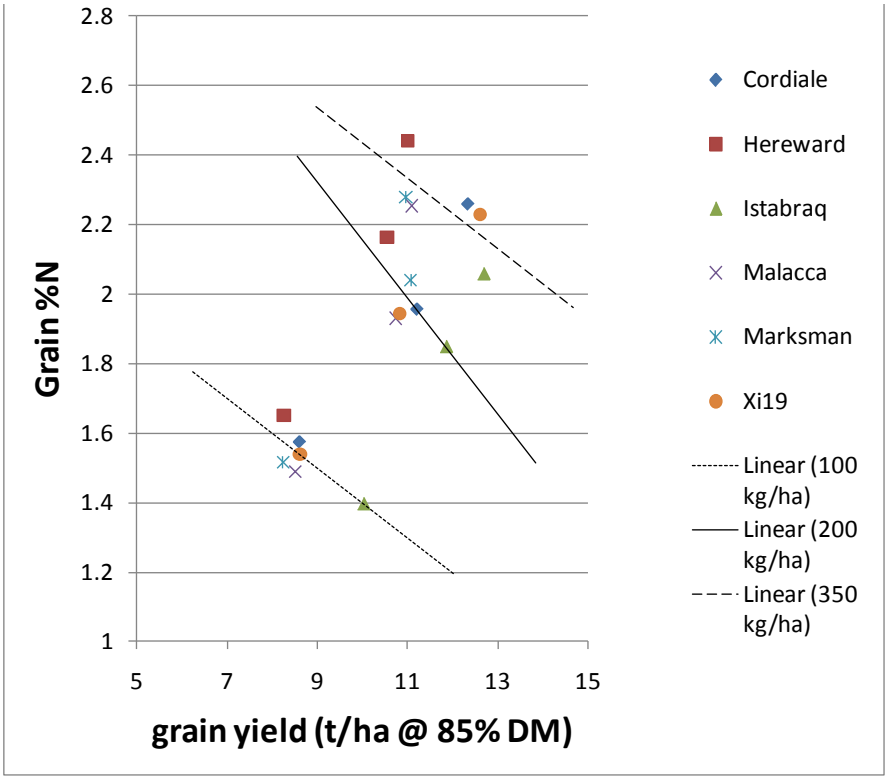


Figure 2.4 Relationship between grain yield and grain N for the six varieties grown at Rothamsted in 2009

2.3.2. Transcriptomics

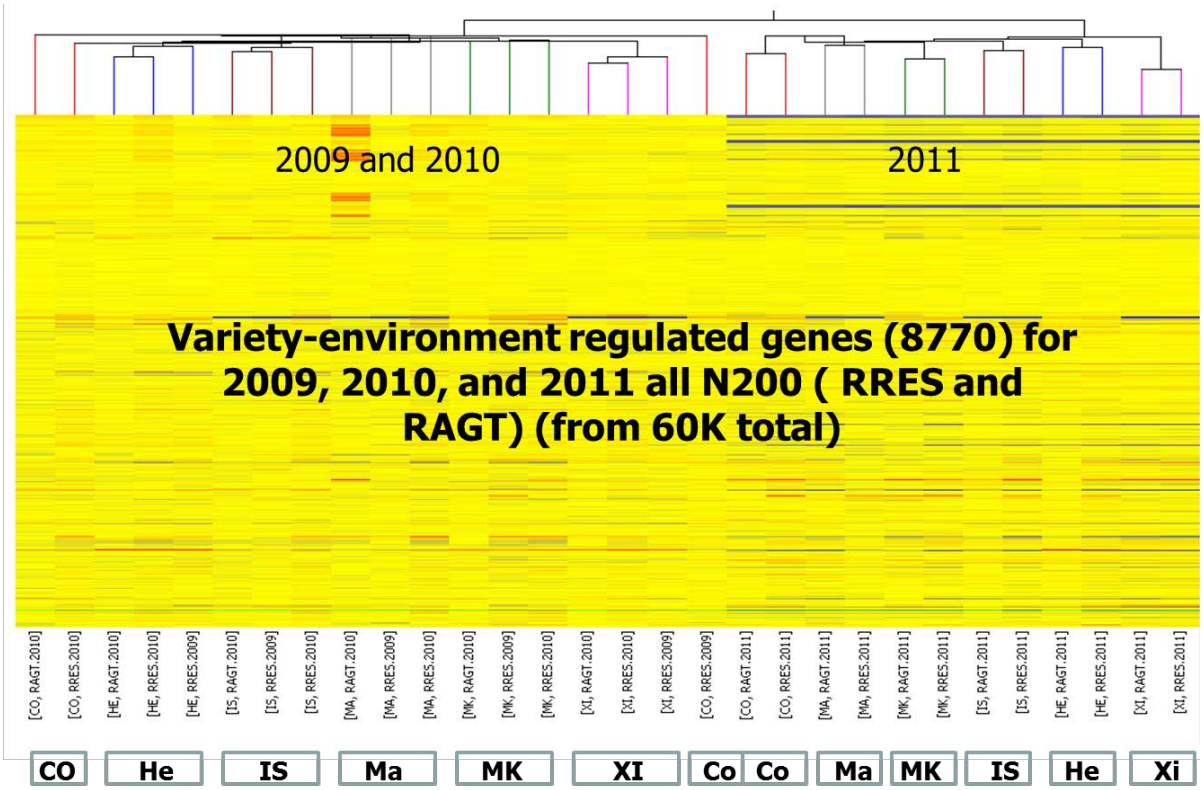


Figure 2.5 Transcriptomics overview: hierarchical cluster analyses of transcriptome profiles for 6 varieties in 3 years at the Rothamsted and RAGT sites at 200 kgN/ha only.

The gene expression profiles determined for whole caryopses at 21 days after flowering showed effects of year, site and variety as well as nitrogen (Figure 2.5). 8,770 of the 60,000 total features (i.e. different genes and alleles) represented on the microarray responded to the year, environment and genotype when comparing the 200 kg/ha treatment alone. The data for 2009 and 2010 were quite similar with the effect of variety overriding the differences due to year and site. The data for 2011 differed, showing strong effects of year.

By averaging the responses of varieties and comparing the profiles for the three nitrogen levels in 2009 and 2010, it was possible to identify multiple nitrogen-regulated genes: 107 genes were up-regulated significantly in both 2009 and 2010 in response to increasing N application, with a greater responsiveness being apparent in 2010; 52 genes were down-regulated in response to increasing N-application in both 2009 and 2010. Of the 107 up-regulated genes: 13 encoded storage proteins, 6 defence-related genes, 5 photosynthetic genes and 81 were un-annotated 'unknown' genes.

Relating GPD to the transcriptome

Firstly a value for GPD was determined for each variety and for each year (for all N-fertilisation rates), after normalisation for effects of N-fertiliser input and yield dilution effects (examples shown in Figure 2.6).

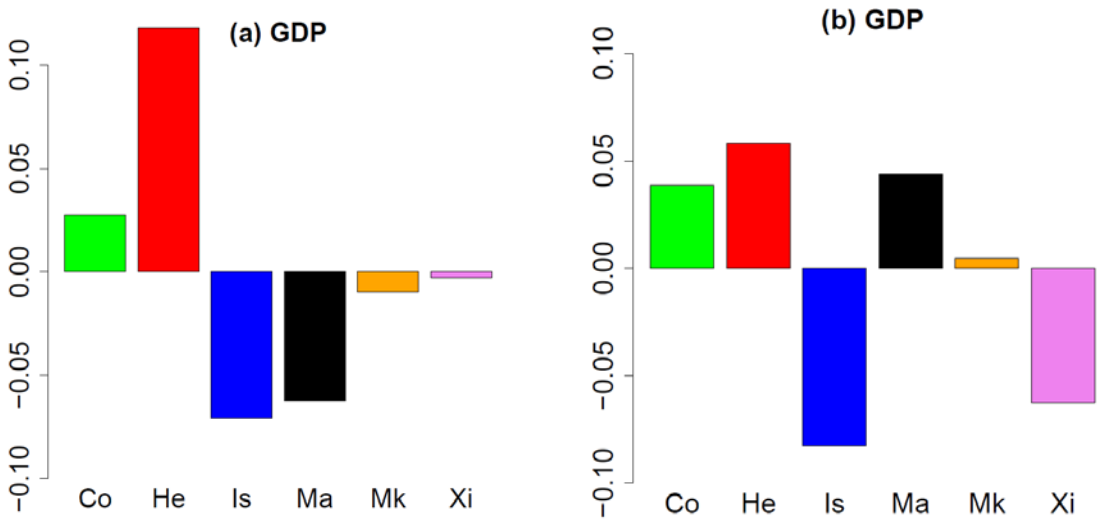


Figure 2.6 A measure of GDP for the 6 varieties along the x-axis; Cordiale (Co), Istabraq (Is), Hereward (He), Malacca (Ma), Marksman (Mk) and Xi19 (Xi)) grown at Rothamsted in 2009 (a) and 2010 (b) found by adjusting the protein content first by the direct effect of N-fertiliser level and secondly for the indirect effect of yield.

To identify genes related to the GPD, principal component analysis (PCA) was first performed on the gene expression data (the first 15 PCs are shown), and PCs related to GPD but not to yield

were identified (see Table 2.1). This table indicates which principal components in the analysis correlate most significantly with the various parameters (yield, grain N and GPD).

Table 2.1 Results of ANOVA (FDR adjusted p-values) showing the effect of the scores of PCA of the gene expression data (input of the model) on the phenotypic characteristics (output of the model), (a) for 2009, (b) for 2010.

(a)

	Yield	Protein	Yield corr N	Protein corr N	Protein corr N and Y
mean PC1	0.078	**0.031**	0.740	0.527	0.591
mean PC2	0.065	0.998	0.053	**0.005**	**0.042**
mean PC3	0.152	0.453	**0.008**	**0.006**	0.126
mean PC4	0.736	0.682	0.139	0.911	0.412
mean PC5	0.362	**0.036**	**0.000**	**0.014**	0.653
mean PC6	0.357	0.195	0.823	0.572	0.412
mean PC7	0.853	0.062	0.199	**0.000**	**0.000**
mean PC8	0.071	**0.034**	0.431	0.642	0.285
mean PC9	0.314	0.444	0.080	0.067	0.299
maen PC10	**0.000**	**0.000**	0.447	0.089	**0.010**
maen PC11	**0.024**	0.379	**0.000**	**0.000**	0.099
mean PC12	0.088	**0.018**	0.414	0.641	0.276
mean PC13	0.525	0.716	0.445	0.437	0.152
mean PC14	**0.000**	**0.000**	0.427	0.252	0.398
mean PC15	0.191	0.179	0.263	0.600	0.174

(b)

	Yield	Protein	Yield corr N	Protein corr N	Protein corr N & Y
mean PC1	**0.001**	**0.000**	0.293	0.392	0.756
mean PC2	0.603	**0.000**	0.170	**0.012**	**0.038**
mean PC3	0.942	**0.021**	0.285	**0.030**	0.062
mean PC4	0.966	0.085	0.449	0.081	0.111
mean PC5	0.143	0.255	0.369	0.837	0.708
mean PC6	**0.013**	**0.000**	0.860	0.244	0.120
mean PC7	0.313	**0.005**	0.641	0.491	0.608
mean PC8	0.283	**0.295**	0.740	0.084	0.061
mean PC9	**0.026**	**0.245**	0.136	0.087	0.301
mean PC10	0.836	0.948	0.704	0.561	0.329
mean PC11	0.204	0.014	0.812	0.483	0.306
mean PC12	0.840	0.384	0.762	0.549	0.604
mean PC13	0.639	0.798	0.380	0.752	0.317
mean PC14	0.966	0.916	0.963	0.855	0.849
mean PC15	0.135	0.338	0.221	0.913	0.473

For 2009, PC2 and PC7 showed a significant relation to GPD but not to the yield or the N-fertilisation level. And for 2010, PC2 and PC3 showed significant relation to GPD, but not to yield. The scores of these selected PCs are shown in Figure 2.7 as means for each of the cultivars.

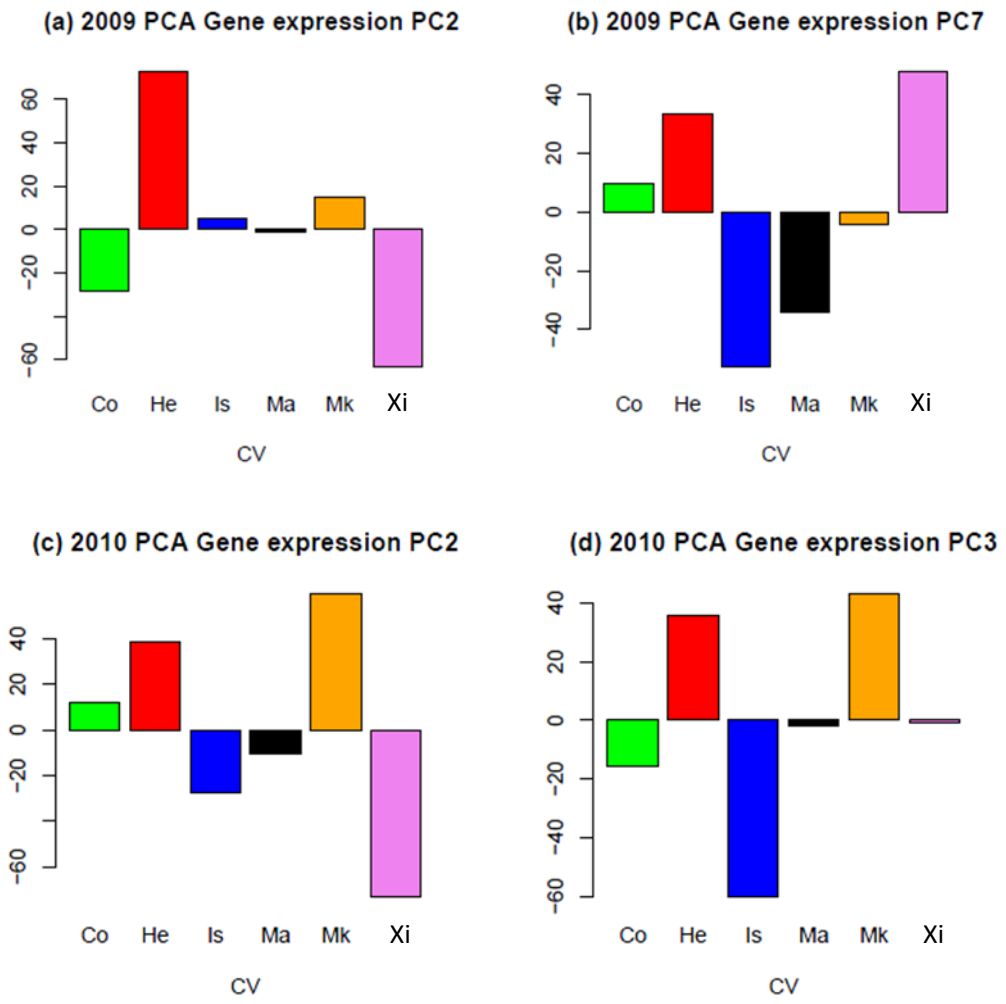


Figure 2.7 Cultivar means of scores of 4 selected principal components (PC2: a and c; PC3, b and d) PCA of the gene expression data obtained in 2009 (a and b) and 2010 (c and d) at Rothamsted. The PCs shown were those significant for GPD but not for grain yield.

Comparing Figures 2.6 and 2.7, there is a striking similarity in the patterns of GPD and the specific PC analysis of gene expression. For example, Hereward is positive and Istabraq is negative in both 2009 and 2010. The other cultivars vary in their position.

Genes do not usually act as individually, but as orchestrated multivariate patterns. The PC found to be significantly related to GPD reflects a multivariate pattern of genes related to GPD. All the genes will have a loading along each of these PCs. However, some genes are more important than others in spanning this PC with a consistent expression pattern across the biological replicates. To identify these genes an analysis of variance was performed using the means of the scores of the biological replicates as input in the model and the gene expression values as the response. By this approach the most important genes contributing to each of the selected PCs were identified. The different growth years were analyzed separately and consistent patterns across the years were regarded as the most relevant. To further zoom into a fewer selection of genes we also looked for genes significant in all the selected PCs.

Selection of significant genes across the growth year and PCs gave7669 genes that were significant for both PC2 and PC7 in 2009, and 9300 genes that were significant for both PC2 and PC3 in 2010. The total number of genes being identified in both years was 3207. These genes were therefore selected as good candidates for GDP, and the two dataset were combined. Partial Least Squares regression analysis was then performed to obtain a visual overview of the results, and to further focus on the most relevant genes for future studies.

Gene expression levels of selected genes with positive or negative relation to GPD are shown in Figure 2.8. The selection of the genes was performed on the data set from Rothamsted in 2009 and 2010. For the selected genes the gene expression profile in Figure 2.8 displays the gene profiles for all the three growth years (indicated by different symbols in the plot) and for both sites where gene expression pattern was available (Rothamsted and RAGT). Figure 2.8 shows that the genes selected are cultivar specific with a consistent pattern across growth environments. The genes in the upper rows of Figure 2.8, which were positively related to GDP, were genes with low expression in Istabraq (in blue) and high expression in Hereward (in red). Conversely, the genes in the lower row of Figure 2.8, which were negatively related to GDP, had low expression in Hereward (in red) and high expression in Istabraq (in blue).

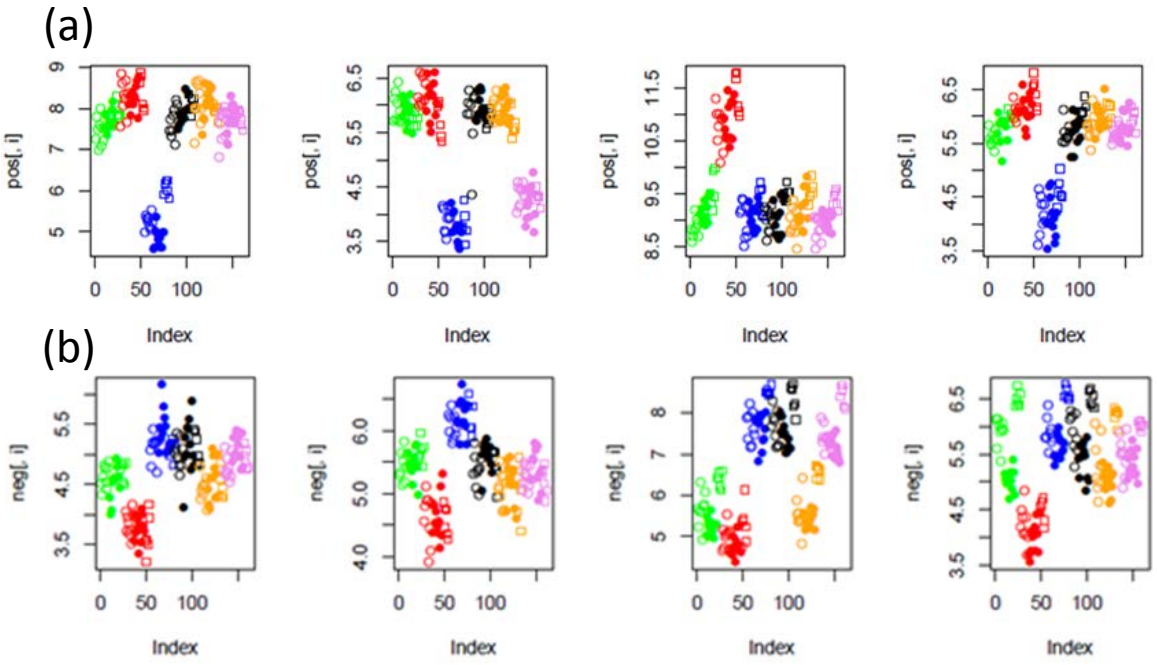


Figure 2.8 A gene expression profile of 4 of the genes with (a) the most positive and (b) 4 of the genes with the most negative relation to GDP in 2009 (open circles), in 2010 (closed circles) and 2011 (open squares). The samples are sorted along the x-axis according to the cultivars for clarity. Green is Cordiale, red is Hereward, blue is Istabraq, black is Malacca, yellow is Marksman and purple is Xi19.

2.3.3. **Protein analysis**

SDS-PAGE

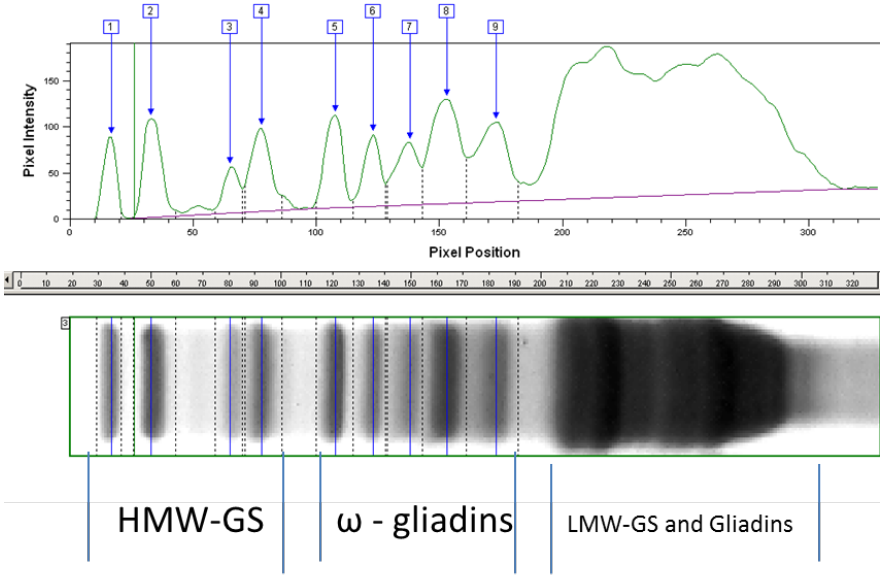


Figure 2.9 SDS-PAGE of storage proteins

Protein composition

SDS-PAGE analysis (Figure 2.9) was carried out on individual replicates of milled whole developing (21 dpa) and mature grain while SE-HPLC analysis was carried out on pooled replicates of ground whole developing grain and white flour from mature grain. Developing grain samples were from Rothamsted Research for 2009 and 2010 and mature grain was from Rothamsted Research alone in 2009 and from Rothamsted Research plus four additional sites in 2010 and 2011.

SDS-PAGE showed that in developing grain, the proportions of HMW subunits were not affected by N fertilisation, but the proportions of LMW subunits and gliadins increased and decreased, respectively, with increasing levels of N application. With increasing days after anthesis, the gliadins tended to accumulate at the expense of HMW subunits. Similarly, in mature grain the proportions of HMW subunits were generally not affected by N fertilisation, while LMW subunits decreased with increasing N fertilisation and gliadins increased. These patterns were consistent across the three years of the trial. Hence, it can be concluded that increasing fertilisation resulted in a higher content of grain protein but decreased intrinsic quality due to disproportional increases in gliadins (which contribute to dough extensibility rather than strength)..

SE-HPLC analysis (see Figure 2.3 for explanation of fractions F1-F5) showed that, in general, the proportion of polymeric proteins increased (fractions F1 and F2) during development, while that of the monomeric proteins decreased. The proportions of the the α- and γ-gliadins (F4) and non-

gluten protein (F5) fractions only were affected by the level of nitrogen fertilisation, where the proportion of the F4 fraction increased with increasing nitrogen addition and that of the F5 fraction decreased. For mature grain, only the the monomeric gliadins (F3, F4) and F5 fractions were affected by the level of nitrogen application, where the proportions of the F3 and F4 fractions increased with increasing nitrogen application, and the proportion of the F5 fraction decreased.

Both SDS-PAGE and SE-HPLC analysis showed differences between varieties that were consistent across different sites and years. Taken together, the results of the protein analysis show effects of nitrogen on protein composition that varied with variety.

2.3.4. **Functionality**

Sufficient samples for milling were not available for samples grown at 100N and 350N from the RAGT site in 2010. Both functionality and baking tests were undertaken on material from three sites in 2010 (Rothamsted Research, Limagrain and Syngenta), and functionality testing was additionally made on material from KWS.

NIR analysis of grain and flour

Determination of total grain N showed that the response to nitrogen fertilisation was dependent on the site. For samples grown at Rothamsted Research, the grain N increased with increasing levels of N fertilisation. The degree of response was lower at the Syngenta site, and lower still at the Limagrain site, while the samples grown at KWS showed no apparent response to N fertilisation in total grain N.

Hagberg Falling number

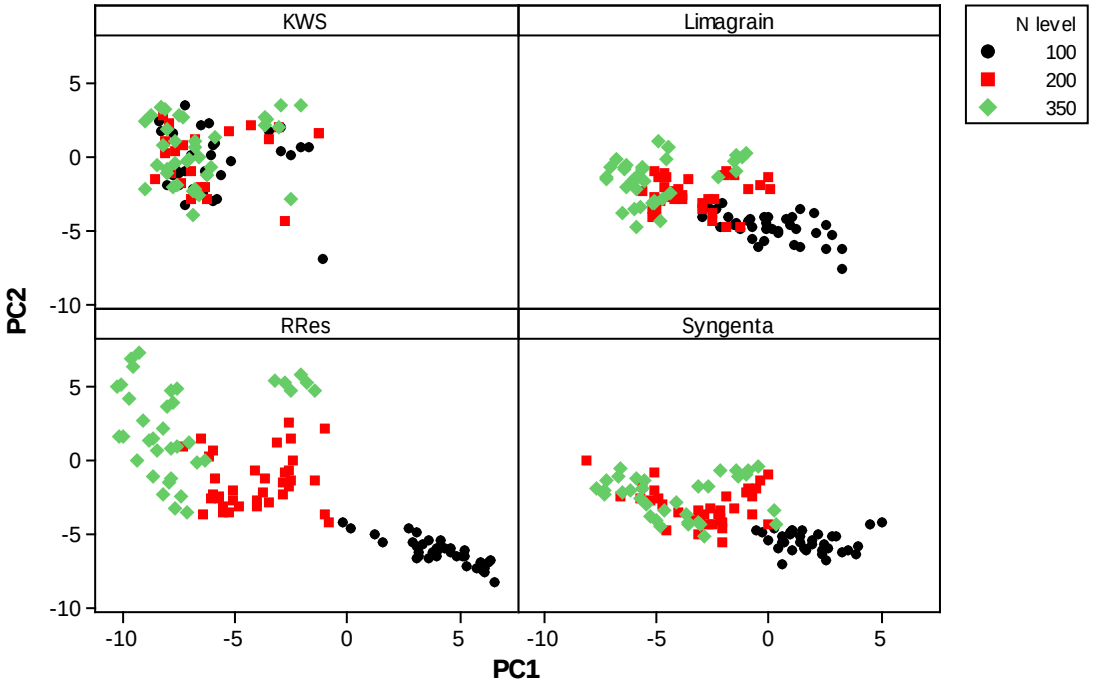
There was no effect of N fertilisation on Hagberg Falling Number (HFN). The HFN was generally lowest for Istabraq at each site, and overall, HFN values were low indicating the presence of pre-harvest sprouting for all varieties grown at Rothamsted Research in 2010.

Dough rheology

Dough rheology was determined on white flour samples made from pooled replicates. In general, Istabraq showed the lowest values for water absorption, development time, stability, resistance and extensibility. For samples grown at Rothamsted Research, there was an effect of nitrogen fertilisation, with increases in nitrogen fertilisation resulting in increases in water absorption, development time, stability, resistance and extensibility. There were also differences between varieties in their response to nitrogen fertilisation. A similar trend was observed for samples grown at the Limagrain and Syngenta sites, but not for the samples grown at KWS. Reomixer traces were collected in duplicate for each biological replicate and the traces reduced to two principal co-ordinates (PC1 and PC2) from which a quality map can be plotted. In this map negative PC1

values reflect higher dough strength, development time and consistency; negative PC2 values reflect greater dough stability. Plotting the data for all of the samples on the quality map shows a clear separation on the level of nitrogen fertilisation (Figure 2.8), which was most clearly seen for the samples grown at Rothamsted Research. By contrast, the samples grown with different levels of nitrogen fertilisation at KWS overlapped, showing that the applied fertiliser had less effect on grain quality.

A



B

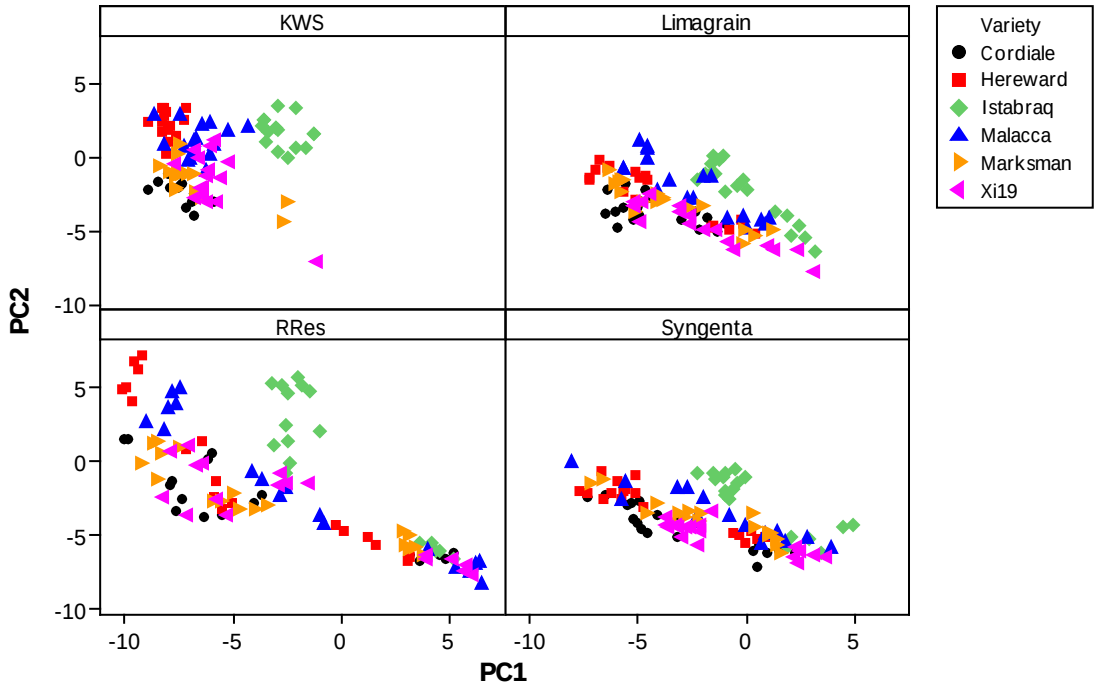


Figure 2.8 Reomixer quality map for white flour milled from wheat grown at four sites in 2010 at different levels of nitrogen fertilisation showing the effect of nitrogen fertilisation (A) and variety (B)

Taken together, the results for total grain N (protein) and flour rheology show no effect of N fertilisation at the KWS site, this suggests that the extra nitrogen applied at the KWS site was not taken up by the crop or that the residual N in the soil was high.. The results also show differences between the varieties in their response to nitrogen fertilisation.

2.3.5. **Baking tests**

The loaf volume of bread baked at Campden BRI generally increased with an increasing level of nitrogen (Figure 2.9). Bread made from Istabraq consistently had the lowest loaf volume and poorest crumb structure compared with bread made from the other varieties, which was expected as Istabraq is not a breadmaking variety. For samples grown at Rothamsted Research, there was an increase in the loaf volume with an increase in nitrogen fertilisation. This trend was seen for both 2009 and 2010 (in 2011, only samples from the 200N treatment were baked). There was also an increase in crumb score (subjective measurement). There was also evidence of a similar effect for bread baked from samples grown at Syngenta and Limagrain in 2010, but not for bread baked from samples grown at KWS.

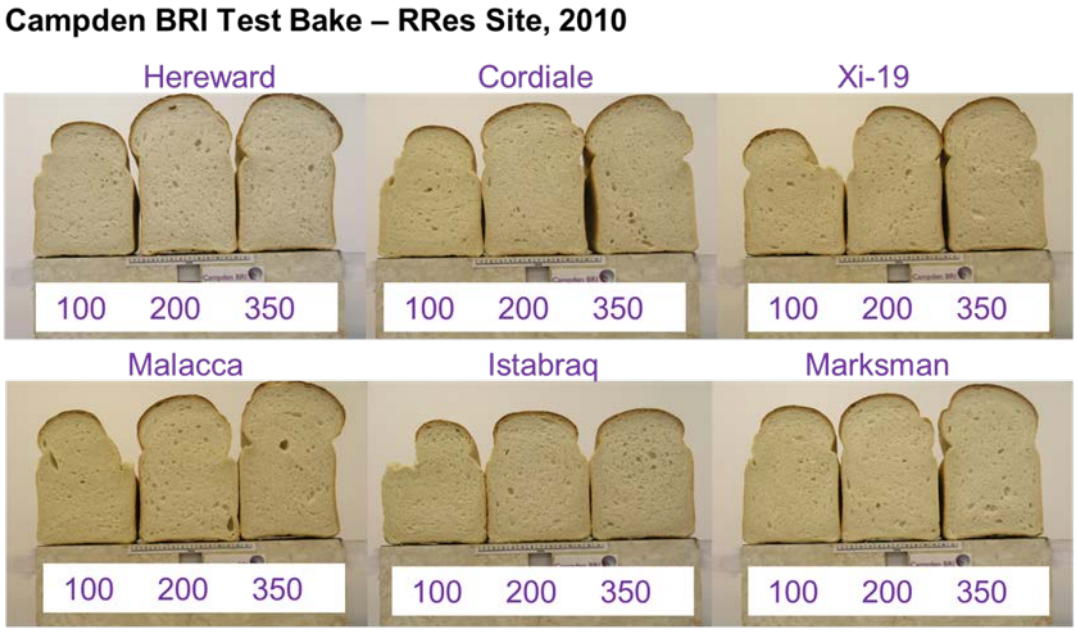


Figure 2.9 Bread baked from flour samples milled from wheat grown at Rothamsted Research in 2010 at three levels of nitrogen fertilisation (100, 200 or 350 kg/ha)

Industry partner data

Bread was baked by industry partners in 2009, 2010 and 2011. In 2010, the sample received from RAGT was not sufficient to allow for baking. The baking assessment was carried out by the industry partners according to their standard protocols and, therefore, the data recorded varied between partners.

The data from the industry partners also showed some differences between each other and from the Campden BRI analyses in relation to varieties and nitrogen treatments. The differences between the results obtained by Campden BRI and the industry partners are probably due to differences between the processes. The spiral bake performed by Campden BRI is a lean recipe and is baked in an unlidded tin, while at ATC and Warburtons the loaves were baked in lidded tins. The quality criteria demanded by industry will also include parameters other than simply a high loaf volume, such as handling properties and slicing.

2.3.6. **Data integration**

An objective of this study was to relate specific genes to function and wheat performance. This is targeted at grain protein deviation but also includes genes responsible for, or at least diagnostic of, baking parameters. It is also possible that these groups of genes may overlap.

The data were integrated by a multivariate data regression approach (Partial Least Squares (PLS) Regression). In the analysis performed here the focus is on genes related to baking quality which overlap the genes responsible for GPD.

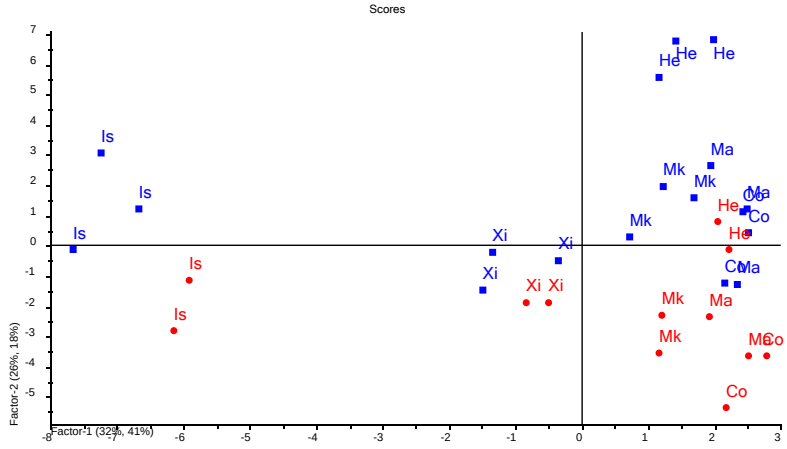
Analysis was first performed on the 2009 dataset which included all three biological replicates for the Campden BRI baking tests.

- Firstly, analysis was performed to study how the design parameters were related to the baking data (data not shown)
- Secondly, analysis was performed for the same growth year using the genes as input in the analysis and the baking data from Campden BRI as output. Only those genes found in the analysis above to be significant for the GPD were included (data not shown)
- Thirdly, genes selected as significant in this analysis performed on growth year 2009, were then subjected to similar analysis for the two other growth years (see Figure 2.10). Thus, we are then performing validation of the selected genes in new datasets.

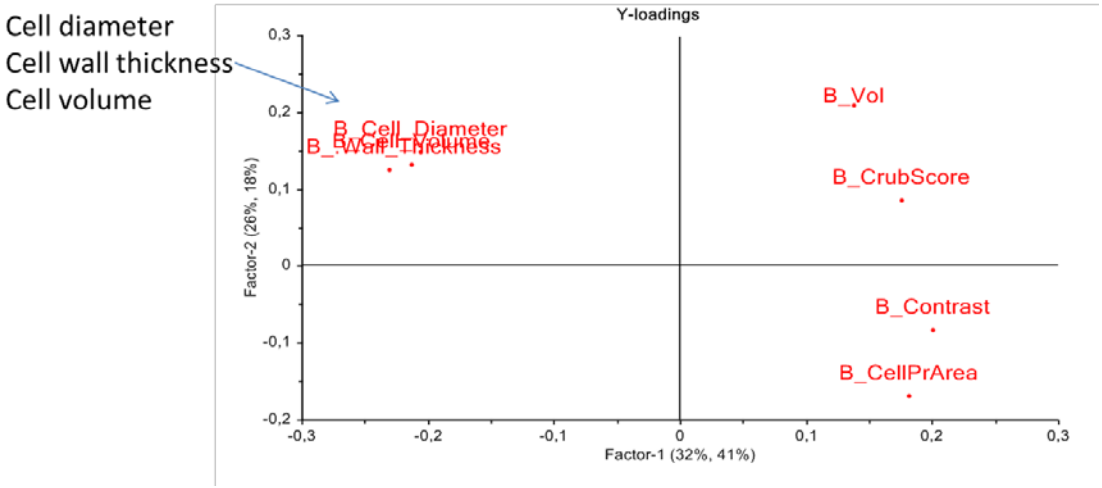
By PLS regression the data are projected down on a few new variables, called PLS factors, describing the main relation between the input data (x-variables) and the output data (y-variables). Visual inspection of plots of the first few PLS factors, viewing both a sample plot (score plot) and a plot of the x and y- variable, then reveal the main pattern of variation relevant for the prediction. The score plot and the loading plot correspond to each other, as the axes are the same PLS factors. Thus, samples located in one direction in the score plot have high value of the parameters in the loading plot located in the same direction, and low value of parameters located in the opposite direction.

An example is shown in Figure 2.10. Genes, chosen only from the GPD selected set (see section 3.2.3 above), and found to be significant in prediction baking performance in 2009, were selected and used in an analysis of the two remaining growth years. When analysing all genotypes, Istabraq was dominating the first PLS factor, located towards the lefthand side of the plot (Figure 2.10.a); this direction was characterised by high cell diameter, high wall thickness and high cell volume, and low value of loaf volume, crumb structure, contrast and cell per unit area (Figure 2.10.b). Genes responsible for this variation are shown in Figure 2.10c. The second PLS factor reflects primarily the growth year, where all the samples from 2010 (in blue) are located in the upper part of the plot, and the samples from 2011 in the lower half (in red). Along the second PLS factor loaf volume is located in the upper part of the loading plot of the y-variables (Figure 2.10.b), which reflects generally higher loaf volume of the 2010 samples compared with the 2012 samples. In the x-loading plot genes significant for the differences seen in the two first PLS factors are circled.

(a)



(b)



(c)

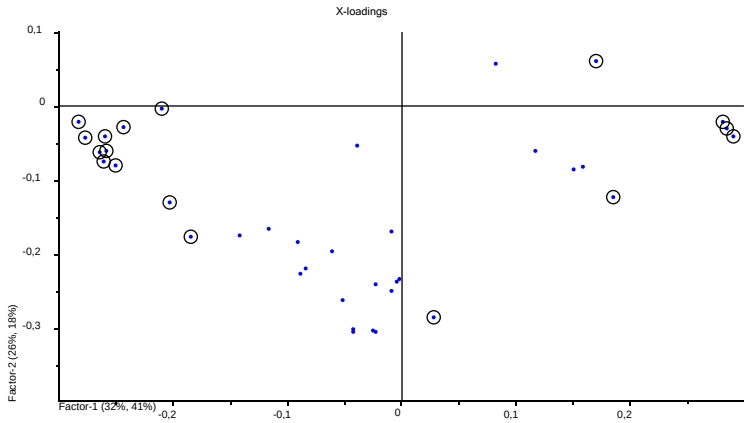


Figure 2.10 PLS regression on data from 2010 and 2011. Genes found in 2009 are used as input, and baking performed at Campden BRI as response, (a) score plot of the samples showing the cultivars (Co) Cordiale, (Is) Istabraq, (He) Hereward, (Ma) Malacca, (Mk) Marksman and (Xi) Xi19), (b) y-loading plot of the response parameters and (c) x-loading plot of the genes where genes circled are significant by statistical significance test.

2.4. Discussion/Conclusions and implications

Key messages to emerge from the project

- The inverse relationship between wheat grain yield and grain N is robust and consistent
- Grain protein deviation (GPD) is a much more variable trait: with Cordiale and Marksman showing consistent positive GPD, and sometimes Hereward (but with a lower yield)
- The three years studied had very different weather conditions, which were reflected in the wide variation between the results obtained. 2010 and 2011 were very dry with negative impacts on N-responses (either yield and/or grain N)
- Variety and nitrogen inputs have defined but complex impacts on dough quality
- N-responsive genes have been identified, including genes encoding gluten proteins
- Genes correlating with GPD were identified including some which also reflect baking performance

Implications for breeders/producers

- Methodologies used in this study and the extensive correlations with quality, compositional and baking traits will provide leads for high throughput selection protocols
- Further screening will identify varieties which make more efficient use of applied N, in terms of translocation to the grain and incorporation into quality-related grain proteins.
- The project has demonstrated the potential for determining the genes and mechanisms responsible for GPD and quality traits, which will facilitate the development of new wheat lines with improved N utilisation

Implications for millers/bakers

- Reducing the use of N on breadmaking wheats is important to reduce costs and environmental/energy footprints
- It is possible to identify varieties with stable and high processing quality when grown at low N inputs using the approaches described.

3. TECHNICAL DETAIL

3.1. Introduction

The ability to grow high quality breadmaking wheats in the UK is of immense economic importance, with up to 5.7m tonnes of home grown wheat being milled annually in the UK (nabim, 2008). It is also likely to increase in strategic importance with growing concern about global food security. However, any increases in production must be viewed against the environmental consequences in terms of agrochemical (i.e. energy) inputs and the environmental footprint.

There is no doubt that UK plant breeders have been highly successful in increasing wheat yield, by an average of about 1% a year. However, increased yield is associated with lower grain protein concentration in grain (Kettlewell, 1996) and the higher levels of protein content (a minimum of 13% db) required for breadmaking means that high inputs of fertiliser N are required. Thus, on average, modern breadmaking varieties require about 35kg N/ha more than older varieties. A recent study showed that 6 out of 16 modern varieties required >280kg N/ha to achieve 13 % db protein, while 4 out 16 required >300kg N/ha (Dampney *et al*., 2006). The sustainability of such farming practices is now being questioned, in terms of economic returns, diffuse pollution and water framework compliance. For example, it can be calculated that a 10 tonne per hectare crop of wheat with 13% db protein contains about 230kg N, meaning that at current application rates about 50kg of N is at risk of being lost. Since the recent price of fertiliser has been as high as £370 per tonne of ammonium nitrate, equating to around £1 per kg of N, this represents a financial as well as an environmental cost. Furthermore, breadmaking wheats are considered to be particularly high risk crops for N leaching. Increased awareness of the carbon footprint of fertiliser use will also add to the pressures on growers as it is estimated that UK agriculture contributes to around 7.5% of global greenhouse gases, and the use of 150kg N/ha results in the production of 171kg carbon/ha.

Climate change projections of increased CO_2 and temperatures suggest that UK wheat yields are likely to rise in the near future, but such increases would require increased N inputs. Furthermore, climate change is also predicted to result in increased fluctuations in conditions between years (Porter and Semenov, 2005; Richter and Semenov, 2005), which is illustrated by the fact that only 11% of the 2007 milling wheat crop met the quality specification for breadmaking compared to over 40% in 2006. There is, therefore, an urgent need to develop new varieties that are efficient and adaptable in their N utilisation and also stable to seasonal variation in growing conditions. This requires a more detailed understanding of the mechanisms that determine the efficiency of N use in the plant, and in particular, the accumulation of N in the grain and its partitioning into the glutenin polymers that determine breadmaking quality. The unique consortium brought together for this proposal (including all of the major UK wheat breeders, milling and baking companies and academics) has focused on N-accumulation and partitioning in the grain, combining novel

molecular approaches with direct measurements of quality parameters. It has built on detailed datasets on varietal variation in N use efficiency generated from the Defra Wheat Genetic Improvement Network (WGIN) field trials.

This study dissected three interacting facets of the relationship between grain N, protein and quality with the aim of identifying the key genetic determinants of these traits.

1. Genetic variation in grain N response- Analyses of grain from the HGCA harvest survey and the WGIN field trials (www.wgin.org.uk/) show that most varieties exhibit a similar negative relationship between grain N (or protein) and yield. However, several varieties show reproducible deviations from this relationship, such as Marksman and Cordiale in which high yield is combined with high grain protein. A similar effect has been shown independently by Monaghan *et al*. (2001), who coined the term 'grain protein deviation'. Nothing is known about the molecular basis for this difference and it could result from differences in source metabolism (i.e. the transport of nitrogenous compounds into the grain during grain filling and leaf senescence), sink activity (i.e. differential expression of genes in the developing grain) or a combination of these two effects. The present project, therefore, relates grain protein content and composition to the efficiency of nitrogen utilisation in the plant, by integrating the work with studies of a larger number of cultivars as part of the Wheat Genetics Improvement Network (WGIN) field trials.

2. The effects of grain N on protein composition and quality- Although total grain nitrogen is widely used as a criterion for breadmaking it is crucial that this is incorporated into proteins that contribute to good breadmaking performance. The wheat gluten proteins are the major determinants of processing quality with the polymeric glutenin fraction being important in conferring the dough strength which is required for breadmaking. Furthermore, one group of glutenin proteins, the high molecular weight (HMW) subunits, is particularly important in this respect, with allelic variation in their composition being related to differences in dough strength. These effects appear to be mediated by direct effects on the size distribution of the glutenin polymers, with 'good quality' subunits being associated with increased proportions of large glutenin polymers (reviewed by Payne *et al*., 1987; Shewry *et al*., 2003). We, therefore, have a good understanding of the molecular basis for the differences in quality associated with allelic variation in the HMW subunits and other gluten proteins (reviewed by Shewry *et al*., 2003; Shewry *et al*., 1995). However, there are also strong environmental effects on quality and we know little about the molecular basis for these. They include the effects of nitrogen fertilisation which is of particular interest to grain producers in the UK (as discussed above) as well as broader effects of climate.

Several studies have shown that increases in grain N result in increases in the proportions of the monomeric gliadins (Jia *et al*., 1996a, b; Panozzo and Eagles 2000; Kindred *et al*., 2008; Zhu and

Khan, 2001) leading to increased dough extensibility, and we have shown similar effects when analysing grain of the cultivar Hereward grown on the Broadbalk long term wheat nutrition experiment at RRes. However, Pechanek *et al*. (1997) showed that the effect of nitrogen on grain protein composition was not consistent but varied between varieties. Even less is known about the effects of nutrition on the glutenin fraction, either on the proportions of the individual subunits or on the size distribution of the glutenin polymers. Thus, both increases (Weiser and Seilmeier 1998) and decreases (Pechanek *et al*., 1997) in the proportions of HMW subunits have been reported while Panozzo and Eagles (2000) and Zhu *et al*., (2001) showed differential effects of N on glutenin polymers and processing properties in cultivars with different HMW subunit alleles.

This project determines the extent to which variation in nitrogen availability affects the proportions of gluten proteins and glutenin polymers, and the extent to which this varies depending on the allelic composition and the genetic background (including cultivars which show 'grain protein deviation').

3. Interactions with other environmental factors- It has been recognised by farmers and millers for many years that some varieties are more consistent in their processing properties from year to year, most notably Hereward which is out-yielded by modern varieties but is still grown to contract as it commands a premium. It is known that glutenin polymers increase significantly in size during the desiccation phase of seed development (Carceller and Aussenac, 1999; 2001; Daniel and Triboï, 2002) and we have recently found similar effects in a detailed study of grain development of cv. Hereward (Toole *et al*., 2010). This could provide a mechanism for effects of the environment during the later stages of grain maturation on grain quality but would not explain well established effects of the environment at earlier stages of grain development. Environmental effects on grain protein composition and quality that are not related to N content have also been reported by Kolster *et al*. (1991).

It is, therefore, clear that more work is required to understand the effects of environment on grain development and quality, and the extent to which these are affected by the availability of nitrogen and genotype.

3.1.1. **Hypotheses**

Wheat cultivars differ in their response to applied N, in total grain protein content and in protein composition and quality. Furthermore, cultivars differ in their stability to year-to-year variation in weather conditions, and this is also reflected in differences in protein amount and composition.
We therefore hypothesise that:

1. The molecular basis for these effects can be identified by detailed comparisons of wheat cultivars grown in multisite trials with varying N levels

2. This knowledge can be exploited, for example, by providing molecular markers and target genes for breeding, to facilitate the development of new breadmaking wheats with reduced N requirements and greater stability of processing properties.

3.2. Materials and methods

3.2.1. Field trials

Selection of cultivars and growth conditions

Table 3.1 Notes on varieties used in this study

Variety	nabim	Source	Expected GPD	Protein content	Other notes
Cordiale	2	KWS	+ ve deviation	Medium protein	Early flowering
Malacca	1	KWS	-ve deviation	Medium protein, outclassed in yield	
Hereward	1	RAGT	+ ve deviation	Stable high protein, outclassed in yield	
Marksman	2	RAGT	+ ve deviation	Medium protein	
Istabraq	4	Limagrain	none	Low protein, suitable for feed and biofuel	
Xi19	1	Limagrain	none	Medium protein	

GPD: grain protein deviation

Six cultivars were selected based on their contrasting properties. Hereward has been the “gold standard” for breadmaking wheats since it was released in 1992. It is low yielding compared to more recent cultivars but is characterised by high stability of quality under varying environmental condition, including N application. Replacement varieties are required to supplant Hereward and in recent years Malacca and Xi19 have been among the most widely grown breadmaking wheats in the UK. However, they are not as stable as Hereward and Xi19 is also know to respond less well in terms of quality to applied N than other modern varieties. Furthermore, Malacca is less popular with bakers due to the undesirable yellow crumb colour, while Xi19 is variable in its breadmaking performance. Marksman and Cordiale are relatively new UK varieties which deviate positively in their grain N content. Finally, Istabraq is a low protein variety used for distilling and animal feed.

Trial sites

All lines were grown at three N levels in multisite trials at Rothamsted Research (Harpenden, Hertfordshire) and by the four breeding partners (RAGT Ickleton, Cambridge), Limagrain (Woolpit,

Suffolk), Syngenta (Whittlesford, Cambridge), KWS-UK, Thriplow, Hertfordshire). The trials at Rothamsted were for all three years (2009–2011) and utilised the wheat N use efficiency field trials funded by Defra as part of the Wheat Genetic Improvement Network (WGIN) project. The trials at the other sites were for 2010 and 2011 only. Three N levels were used: 100kg/ha as a "low input" level, 200kg/ha to reflect modern practice for breadmaking wheats in the UK and 350 kg/ha as an extreme high input to achieve high grain protein. Nitrogen was applied in 'splits' (see Table 3.2) as solid ammonium nitrate prills. All plots were randomised with three replicates. Detailed analyses focused on material from the plots at Rothamsted with the other sites being used to both confirm the validity of the results and investigate the stability of responses across sites.

Table 3.2 Nitrogen application splits (kg/ha N applied as solid ammonium nitrate prills)

Total	March (GS24)	April (GS31)	May (GS39)
50	50		
100	50	50	
200	50	100	50
350	50	250	50

Rothamsted is in southern England (latitude 52^{o} N, longitude 1^{o} W). The soil is a well-drained, flinty silt clay loam (25% clay) overlying clay with flints (50% clay). This soil is designated as 'Batcombe Series' in the UK Soil Classification, 'Aquic Paleudalf in the USDA system and 'Chromic Luvisol' in the FAO system (Avery and Catt, 1995). Annual rainfall at Rothamsted is typically 700mm which is spread evenly over the year. Spring and summer rainfall patterns for the three years of the experiments are given in Table 3.3.

Table 3.3 Monthly rainfall in spring and summer (mm) at Rothamsted in the years 2009–2011. Six monthly totals and 30-year averages (1971–2000) are shown.

Year	March	April	May	June	July	August	Total
2009	37.3	46.7	24.8	68.1	73.3	63.4	313.6
2010	45.2	18.7	38.4	23.5	31.6	127.6	285
2011	10	5.2	23.6	83	44.6	81.2	247.6
30-year average (mm)	54	54	50	60	42	54	314

All crops were a first wheat following winter oats to avoid effects from the root disease 'take all' which is prevalent in continuous wheat crops in the UK. The winter oats were given only modest amounts of N-fertiliser which ensured relatively low residual soil N-min levels for the following wheat. All crops were autumn-sown predominantly in mid-October. Seed was precision-drilled at a rate of 350 seeds/m^{2} in 12.5cm rows in plots measuring 3m by 15m. Available soil P, K and Mg

was Index 2 on all fields which is non-limiting to yield. The crops were top-dressed with potassium sulphate in March supplying sulphur at a rate of 20 kg-S/ha. Crops were given growth regulator and protected against weeds, pests and diseases as required. Grain yields were recorded at harvest.

Nitrogen determination

Total N in grain and straw dry matter was determined on oven-dried (80^{o}C overnight) milled samples by the Dumas combustion method (Dumas, 1831) using a 'Leco N-analyser'.

3.2.2. **Transcriptomics**

Tissue sampling

Individual ears (typically 10 per plot per required time point) were tagged at anthesis and caryopses harvested from the middle third of each ear 21 days after anthesis (daa) at the Rothamsted site (2009–11) and RAGT site (2010 and 2011) sites for transcriptome analysis. In addition, in years 2009 and 2010, caryopses were harvested at 14, 21, 28, 35 and 42 daa for protein analysis at the Rothamsted site. These were taken from material grown at all three N levels at Rothamsted in 2009 and 2010 apart from a single N level (200kg/ha) in 2011 from Rothamsted and RAGT.

RNA extraction and cDNA preparation

RNA was extracted using a method based on Chang *et al*. (1993). About 1.5g of whole caryopses were ground in a cooled mill and RNA extracted in CTAB buffer (2% CTAB, 2% PVP K30, 100 mM Tris-HCl, pH8.0, 25 mM EDTA, 2.0 M NaCl, 0.5 g/l spermidine), 2% (w/v) 2-mercaptoethanol) with chloroform:isoamyl alcohol (IAA) (24:1) to remove proteins. RNA was precipitated by 10 M LiCl and incubation on ice overnight, dissolved in buffer (1.0 M NaCl, 0.5% (w/v) SDS, 10 mM TrisHCl pH8.0, 1 mM EDTA) to remove polysaccharides and extracted once with chloroform:IAA. After ethanol precipitation, total RNA was dissolved in DEPC-treated water and stored at -80°C.

Microarray hybridisation and data analysis

Affymetrix GeneChip® Wheat Genome Array microarrays were used to profile gene expression in RNA fractions from endosperms dissected from the developing caryopses (Table 3.4). This platform comprises 55,000 features, including gluten protein genes which comprise multigene families ranging from six copies (HMW subunits) to over 100 copies (for α-type gliadins). The standard one-cycle cDNA synthesis protocol and hybridisation as described in the GeneChip® Expression Analysis Technical Manual was used. Transcriptome data analysis used GeneSpring® version 11 (Agilent Technologies, Inc). The standard workflow was followed for two-factor experiments (nitrogen level, variety). Differences in expression for genes of interest were confirmed by quantitative RT-PCR.

Table 3.4 Microarray data sets

Year	Sites	Varieties	N-levels (kgN/ha)	Reps	Total arrays
2009	Rothamsted	6	100,200,350	3	54
2010	Rothamsted	6	100,200,350	3	54
2010	RAGT	6	200	3	18
2011	Rothamsted	6	200	3	18
2011	RAGT	6	200	3	18

3.2.3. **Protein analysis**

SDS-PAGE (developing and whole grain)

Samples (35mg) of wholemeal flour were extracted with 1 mL 0.0625 M Tris-HCl, pH 6.8, 2% (w/v) sodium dodecyl sulphate (SDS), 1.5% (w/v) dithiothreitol (DTT), 10% (v/v) glycerol, and 0.002% (w/v) bromophenol blue. Prior to electrophoresis, samples were heated at 90°C for 5 min and then centrifuged for 5 min at 13000rpm. The supernatant layer was analysed by SDS-PAGE. Gels (NuPAGE 12% Bis-Tris, Life Technologies, Paisley, U.K.) were stained with Coomassie Brilliant Blue and then scanned and analysed using Total Lab TL120 version 2006F (Nonlinear Dynamics, Newcastle-upon-Tyne, U.K.) with an optical density curve calculated from a Kodak T14 control scale (Tiffen LLC Rochester, NY). The banding patterns were divided into three sections: high molecular weight glutnenin subunits, low molecular weight glutenin subunits and gliadins. Values for band optical density and band per cent as a proportion of the total lane optical density were analysed. Two technical replicates of each gel were run together on the same day and stained and destained together.

SE-HPLC

Size exclusion high performance liquid chromatography (SE-HPLC) was used to determine the protein polymer size distribution of flour samples. The analysis was performed according to the Profilblé method developed jointly by ARVALIS and l'Institut National de Recherche Agronomique (Morel *et al*., 2000). Flour (160 mg) was combined with 20 mL 1% SDS (w/v) in 0.1 M phosphate buffer (pH 6.9) to dissolve the soluble gluten proteins. The solution was sonicated (Misonix Microson XL2000) to solubilise the polymeric gluten proteins, and then centrifuged for 10 min at 5000rpm. An aliquot of the supernatant was sealed in a HPLC vial ready for analysis. The SE-HPLC analysis was conducted using a Jasco system operating with a TSK gel G 4000SW column and a TSK gel SK guard column. The flow rate was 0.7 mL min^{-1}, and detection was performed at 214nm.

The chromatograms were integrated using a combination of automated algorithms and manual rules developed as part of the Profilblé method. The resulting SE-HPLC trace has five identifiable peaks, corresponding to the different protein fractions. The first peak to elute from the column is referred to as F1 and consists of high molecular weight (HMW) polymers enriched in HMW glutenin subunits. The F2 peak comprises low molecular weight (LMW) polymers and is enriched with LMW glutenin subunits. The F3 and F4 peaks are comprised principally of ω-gliadins and α-, β-, and γ-gliadins, respectively, whilst the F5 peak comprises low molecular weight soluble proteins including albumins and globulins. The overall area under the trace is a measure of the total protein content of the flour and is termed AT.

3.2.4. **Functionality**

Milling

White flour was produced using a Bühler Laboratory Mill LM 202 at Campden BRI according to an internal Campden BRI method which includes preliminary sample preparation and treatment of by-products to release adhering flour. Where replicates were pooled for analysis, wheat grain from each replicate was combined and blended together thoroughly prior to milling.

NIR analysis of grain and flour

Wholegrain and flour protein and moisture contents were measured using NIR according to the Manual of methods for wheat and flour testing Guide Line No. 3 (Campden BRI). Samples of cereals may be analysed by NIR reflectance in the ground form or, for whole grains, by either NIR reflectance or NIR transmittance. For both reflectance and transmittance NIR instruments, the analysis is dependent on calibration against a suitable standard method. Such calibration assumes an empirical model in which constituent concentration may be predicted by a linear combination of reflectance or transmittance data at a number of wavelengths in an equation, which includes a non-zero intercept term. Analysis of cereals by NIR is based on absorption of NIR energy at specific wavelengths, by peptide linkages between amino acids of protein molecules, by OH groups in starch molecules and by OH bonds in water molecules. Measurements at reference wavelengths and mathematical manipulation of the data are required for background correction.

Hagberg falling number

The Hagberg falling number was determined according to the Manual of methods for wheat and flour testing Guide Line No. 3 (Campden BRI). The Falling Number method indicates alpha-amylase activity using the starch in the sample as substrate. The method is based on the rapid gelatinisation of an aqueous suspension of the flour or the total milled product of a cereal and the subsequent liquefaction, by alpha-amylase, of the starch contained in the sample.

Dough rheology

Flour water absorption was measured using a Brabender Farinograph according to the Manual of methods for wheat and flour testing Guide Line No. 3 (Campden BRI). The Brabender Farinograph measures and records the resistance of dough to mixing as it is formed from flour and water, developed and broken down. This resistance is called consistency. The maximum consistency of the dough is adjusted to a fixed value by altering the quantity of water added. This quantity, the water absorption, may be used to determine a complete mixing curve, the various features of which are a guide to the strength of the flour.

The extensibility and resistance of the dough was measured using a Brabender Extensograph according to the Manual of methods for wheat and flour testing Guide Line No. 3 (Campden BRI). A flour-salt-water dough is prepared under standard conditions in the Brabender Farinograph and moulded on the Brabender Extensograph into a standard shape. After 45 minutes rest under controlled conditions, the dough is stretched and a curve drawn recording the extensibility of the dough and its resistance to stretching.

Small scale dough rheology was determined using a Reomixer according to an internal Campden BRI method (Anderson, 2003). The Reomixer, with attached computer, records the torque-time trace from a developing dough and requires 10g of white flour as opposed to 300g for the Farinograph.

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3.2.5. **Baking tests**

Baking performance

Bake tests were performed at Campden BRI using no time dough process (spiral mixer). A standard lean recipe was used to produce 800g, four piece loaves in unlidded tins. Proving time for the dough was for 50 minutes (Millar *et al*., 2008). Loaf volume was measured and crumb structure was analysed using C-Cell. Bake tests were also performed by Warburtons, ATC and Premier according to their own procedures.

Data integration

All data have been cross correlated and significant interactions determined, including correlations with gene expression as identified in the transcriptomic study.

3.3. Results

3.3.1. **Field trials**

The project compared six varieties (Cordiale, Istabraq, Hereward, Malacca, Marksman and Xi19) grown and harvested at multiple sites (Rothamsted in 2009, 2010 and 2011; RAGT, Limagrain, Syngenta and KWS in 2010 and 2011).

Yield and grain %N data

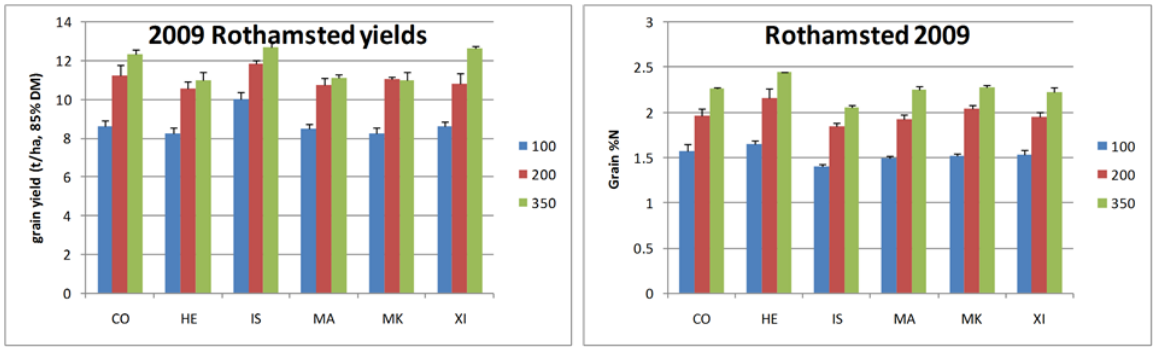


Figure 3.1 Yield and %N data in 2009 at Rothamsted

Grain yields and grain %N contents for the trials are shown in Figs. 3.1, 3.2 and 3.3. In 2009, at Rothamsted (Figure 3.1), with near average spring and summer rainfall (Table 3.3), yields varied between 8.2 and 12.7 t/ha (at 85% dry matter), with grain %N varying from 1.4 to 2.4 (equating to 8–13.7% protein using a conversion factor of 5.7). All varieties responded positively to applied N both in terms of yield and grain %N. Istabraq had the highest yields and lowest %N.

In 2010, all months had below average rainfall, with the exception of August which was very wet (Table 3.3; data only available for the Rothamsted site). Yield and grain %N for all sites are presented in Figure 3.2. Rothamsted grain yield varied from 7.3 to 10.2 t/ha, substantially lower than 2009. Grain %N varied from 1.4–2.8. Yield and grain %N both responded to applied N, with %N responding most strongly. Yield and grain %N also responded to N applications at Limagrain, RAGT and Syngenta but not at KWS. Yields were lowest at RAGT and highest at KWS and Syngenta.

In 2011, March to May was characterised by exceptionally low rainfall followed by a relatively wet summer (Table 3.3). Yield and grain %N for all sites are presented in Figure 3.3. Yields at Rothamsted ranged from 7.6 to 11.5, and grain %N from 1.6 to 3.2. Responses to applied N were generally poor for all sites except Rothamsted and Limagrain. The lowest yields were at RAGT, Syngenta and KWS, 3 sites which cluster closely geographically and all suffered from low rainfall.

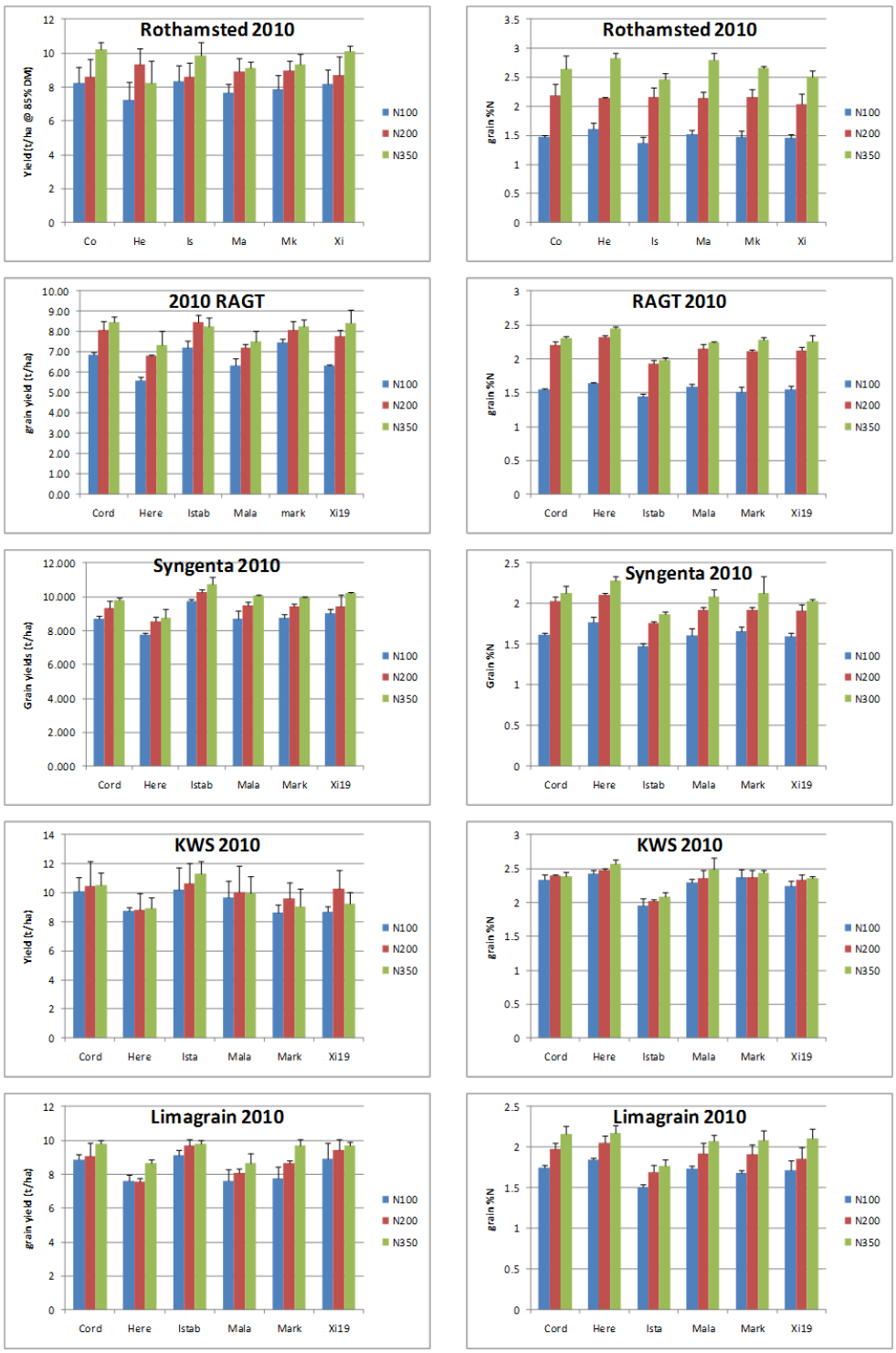


Figure 3.2 Yield and %N data in 2010 at all sites

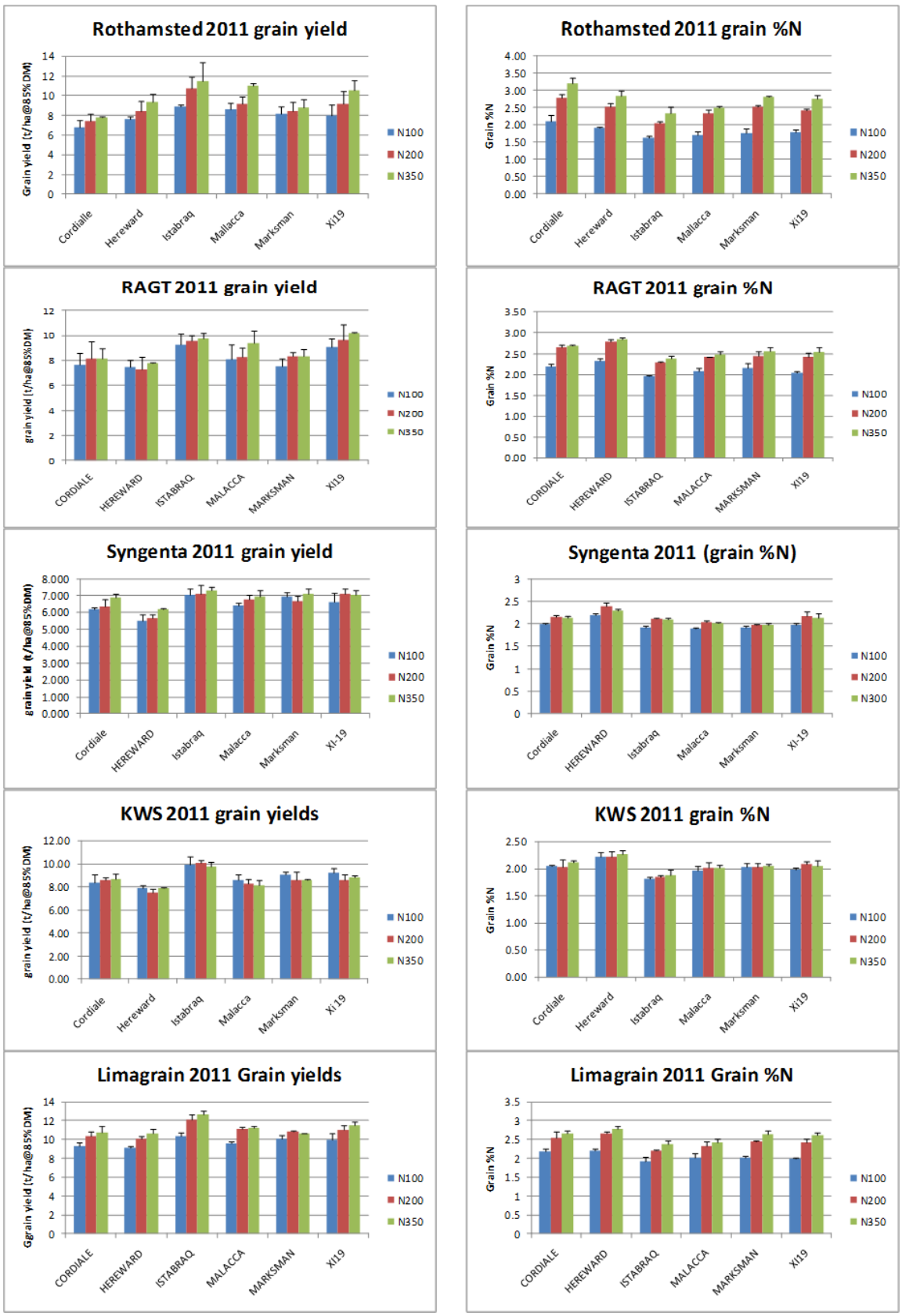


Figure 3.3 Yield and %N data in 2011 at all sites.

Grain protein deviation

Based on the grain yield and %N data (Figures 3.1–3.3), correlation plots examining the relationship between these parameters can be derived (Figures 3.4–3.7). The grain yield/protein

content relationships for the varieties grown at Rothamsted at 3 nitrogen levels (100, 200 and 350 kg/ha N) in 2009 are shown in Figure 3.4). A clear inverse relationship between grain yield and grain %N is clear for all three N input rates. The regressions are also approximately parallel and shifted, as both yield and % N are similarly responsive to applied N in this dataset. Grain protein deviation (GPD) is a deviation from this regression. For all N levels, Hereward shows a positive deviation, Cordiale at 100 and 350, Marksman at 200 and Xi19 at 350 kg/h, while Istabraq never showed a positive deviation.

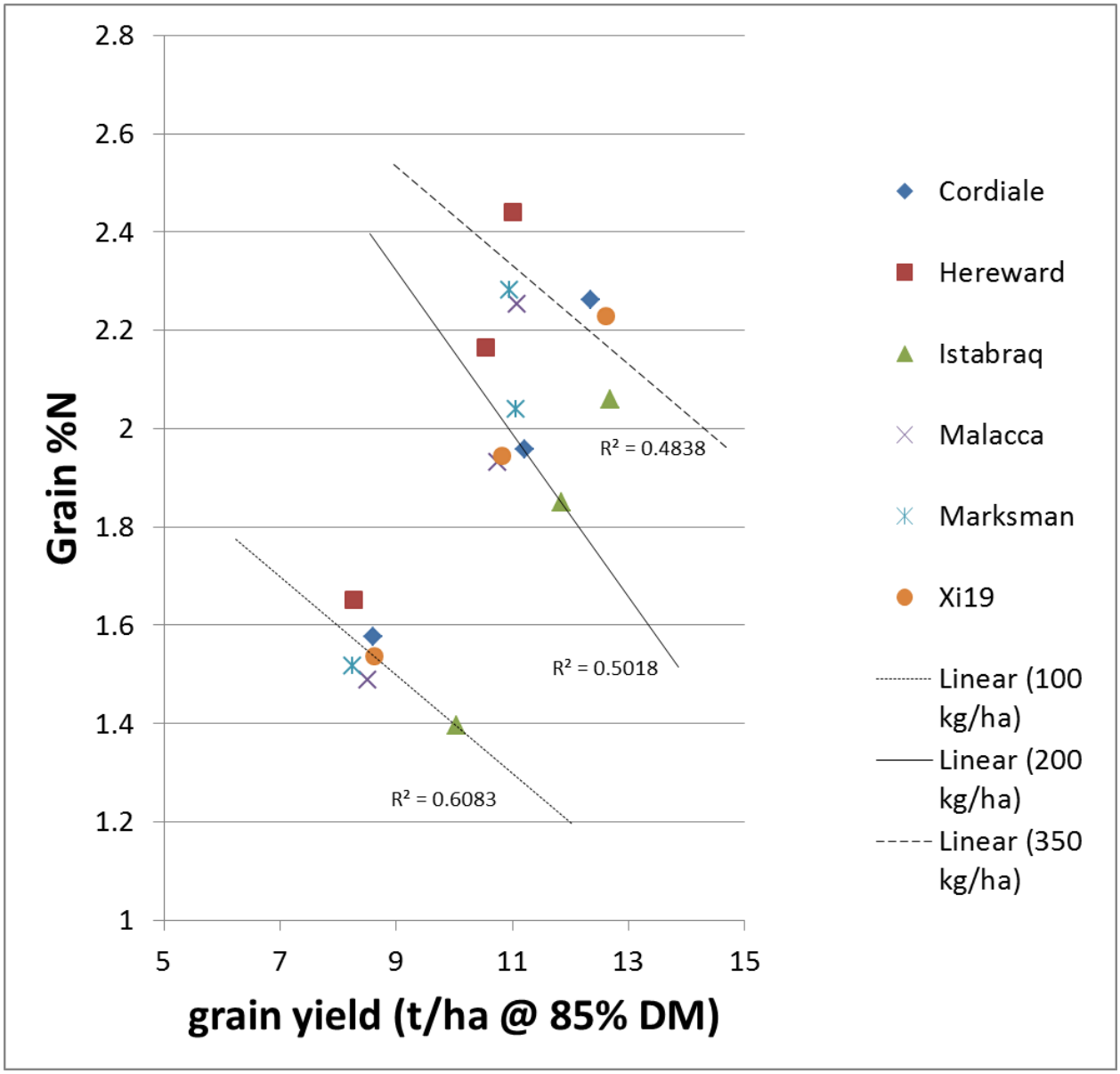


Figure 3.4 GPD in 2009 at 4 N levels at Rothamsted with linear regression, and R^2 values shown to indicate significance.

As the trial was part of a larger germplasm trial conducted as part of the WGIN project, it was possible to compare the GPD of the 6 varieties to a broader range of 25 varieties, as shown for the 200 kgN/ha treatment (Figure 3.5). Hereward and Marksman clearly show a positive GPD.

GPD for all sites for the 200 kgN/ha treatment in 2010 as shown in Figure 3.6. The inverse relationship between yield and grain %N was consistent in all of the trials with negative linear regressions for all sites; these are parallel and reflect the overall productivity of the individual sites. Cordiale and Marksman show positive GPD at the KWS site; Marksman and Hereward at Rothamsted; Hereward, Marksman and Cordiale at RAGT and Cordiale alone at Limagrain and Syngenta.

Little GPD was observed in 2011 with the exceptions of positive GPD for Hereward at Syngenta and KWS, Xi19 and Hereward at KWS, the two lowest yielding locations (Figure 3.7).

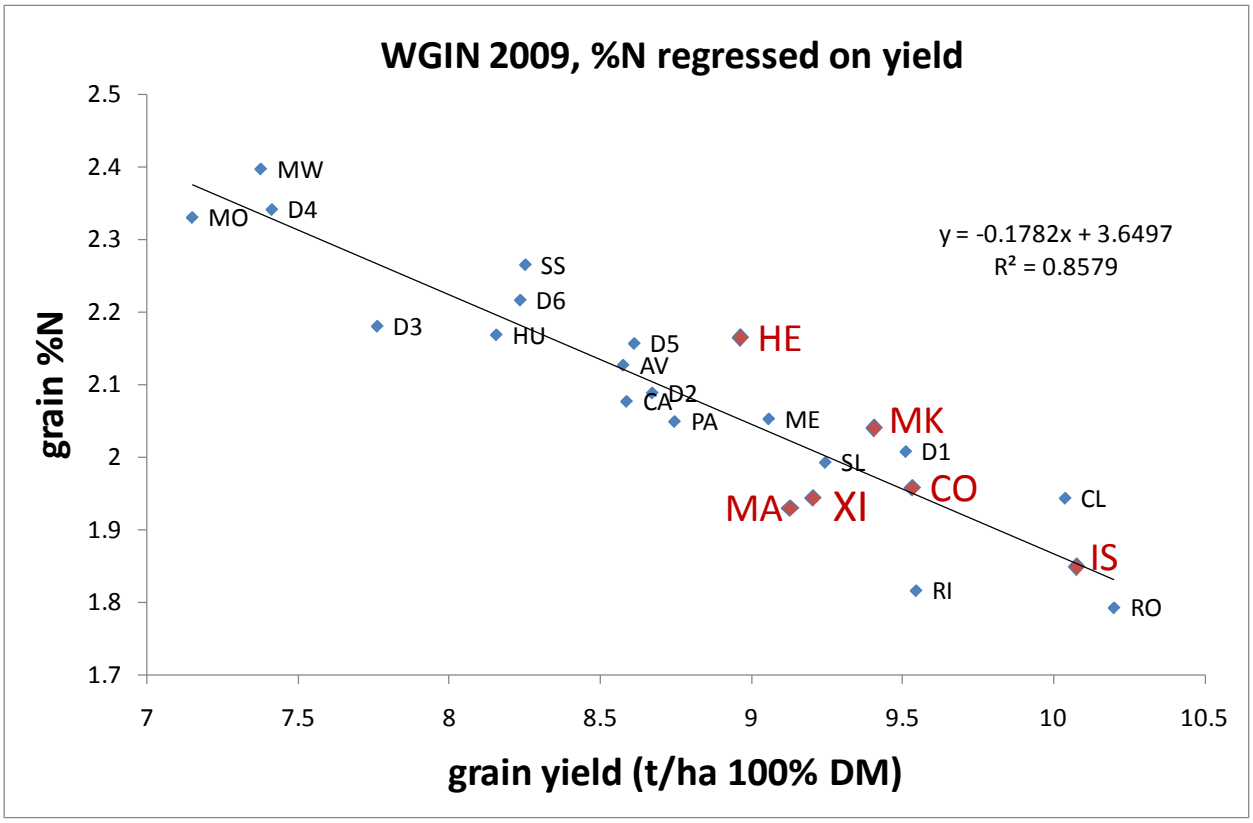


Figure 3.5 GPD at 200 kg N/ha at Rothamsted in 2009, comparing the 6 varieties in this study with 19 others in the same trial, as part of the WGIN project.

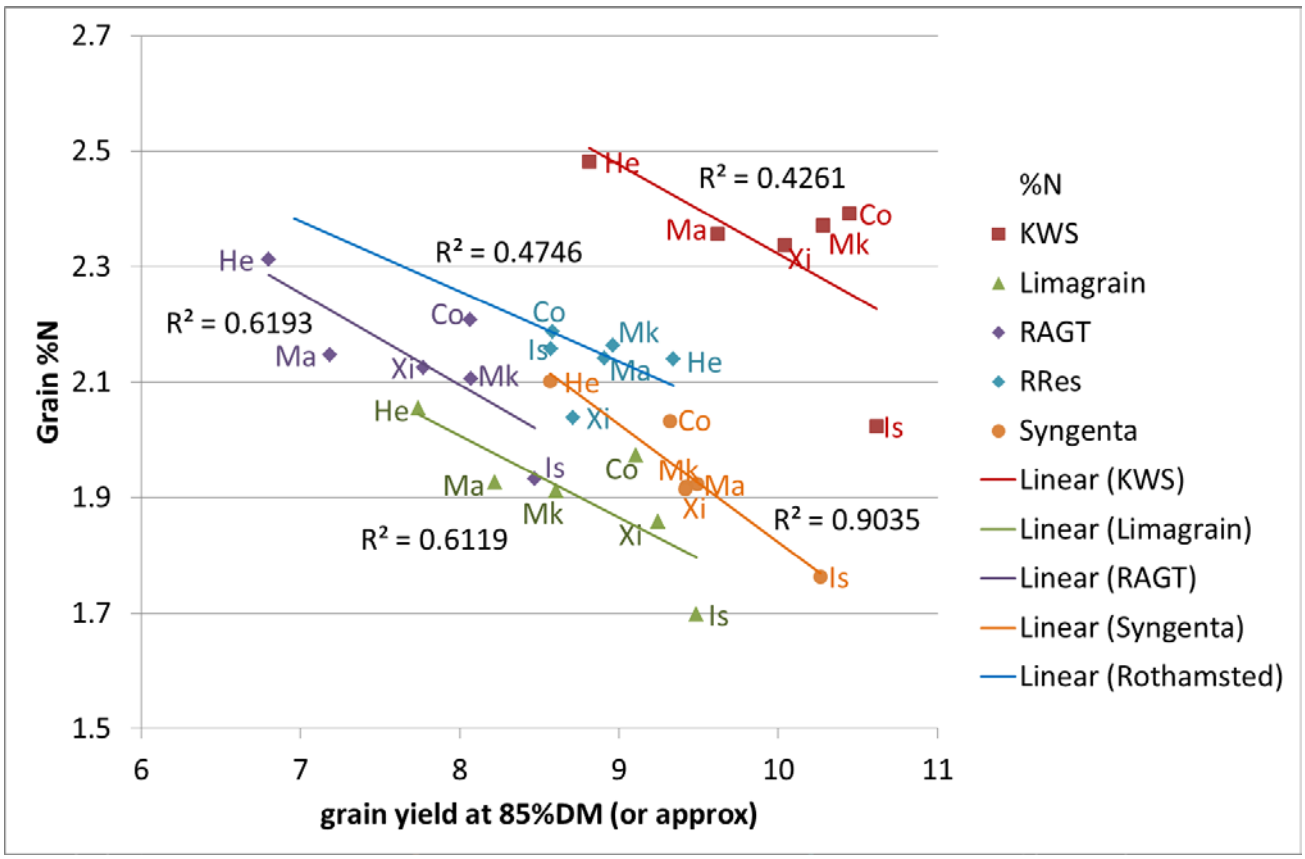


Figure 3.6 GPD at the 5 sites in 2010 at 200 kg N/ha.

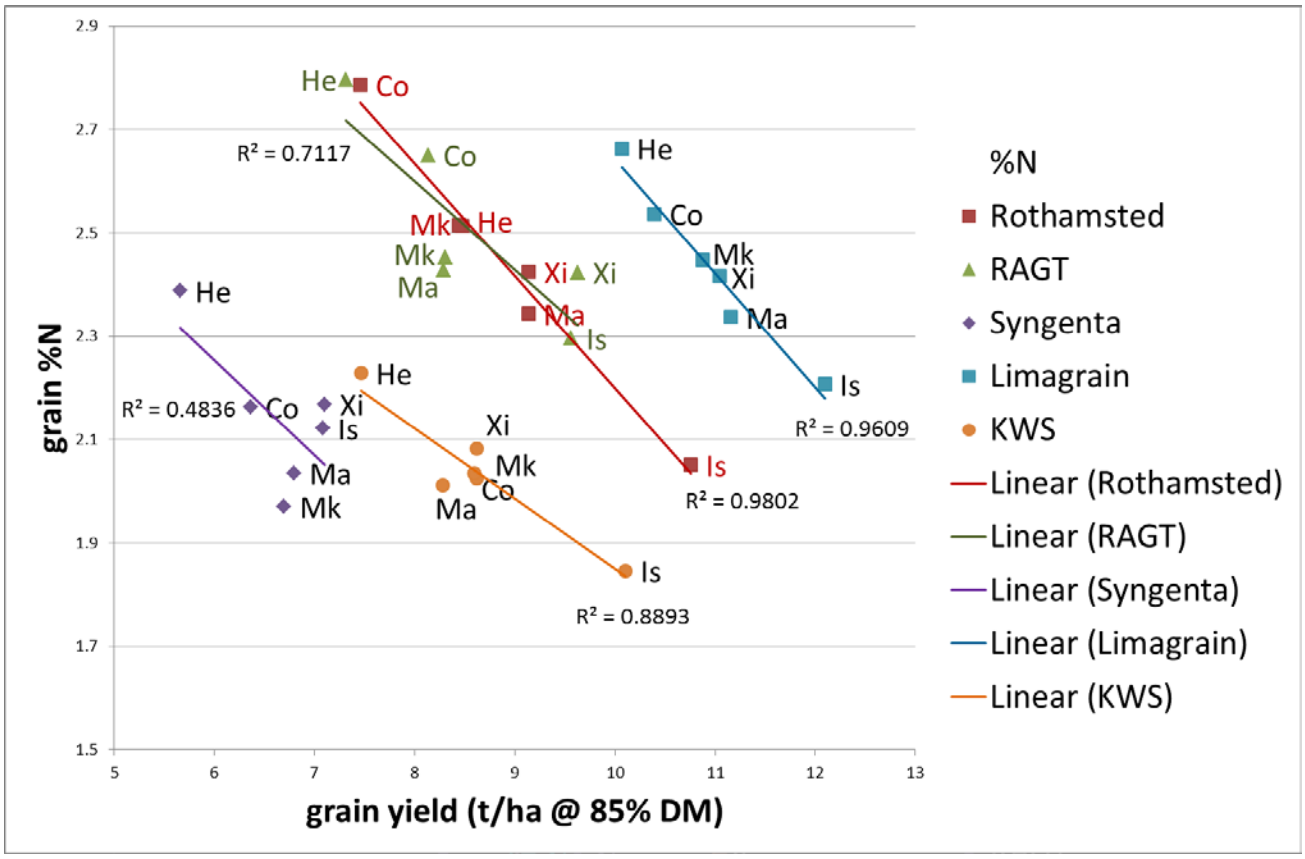


Figure 3.7 GPD at the 5 sites in 2011 at 200 kg N/ha.

3.3.2. **Transcriptomics**

Up- and down-regulated genes

Gene expression profiling of 21 dpa caryopses revealed multiple nitrogen-regulated genes (Figure 3.8). 107 genes were up-regulated significantly in both 2009 and 2010 in response to increasing N application, with a greater responsiveness being apparent in 2010. 52 genes were down-regulated in response to increasing N application in both 2009 and 2010. Of the 107 up-regulated genes, 13 encoded storage proteins and 6 encoded defence-related genes, 5 were photosynthetic genes and 81 were un-annotated 'unknown' genes (Figure 3.9).

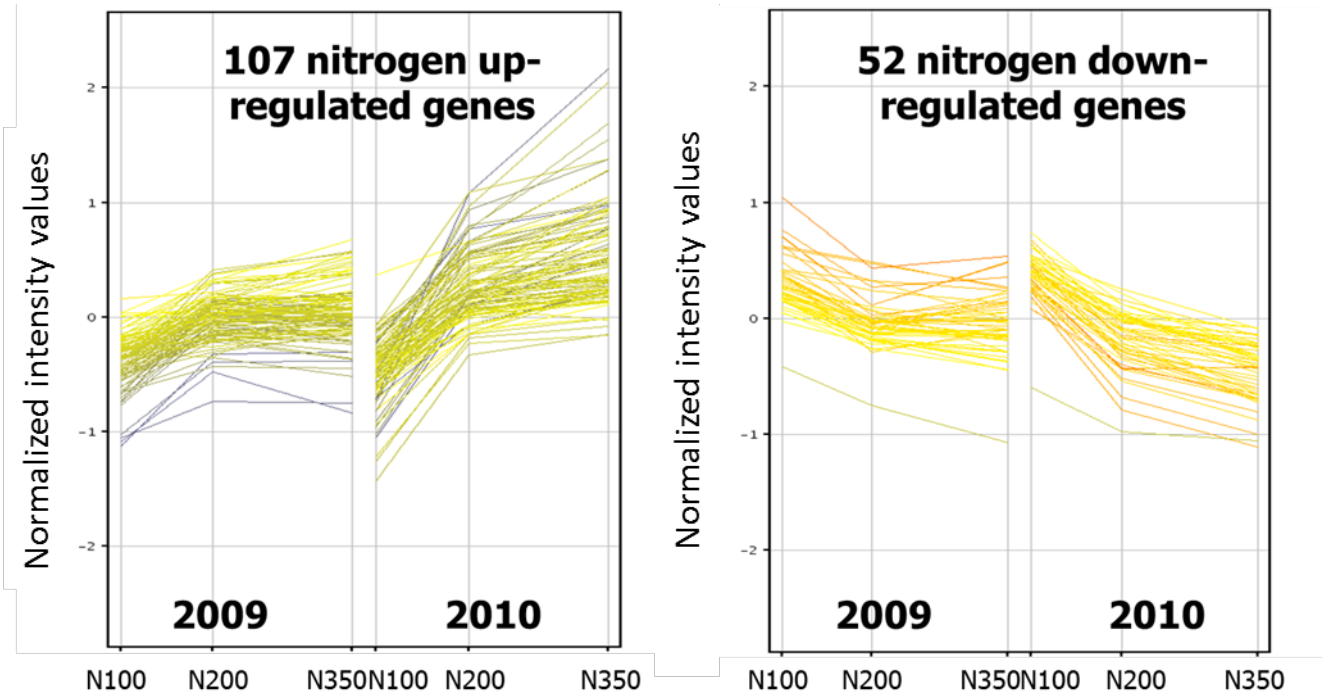


Figure 3.8 Significantly up- and down-regulated gene expression in 2009 and 2010 at Rothamsted.

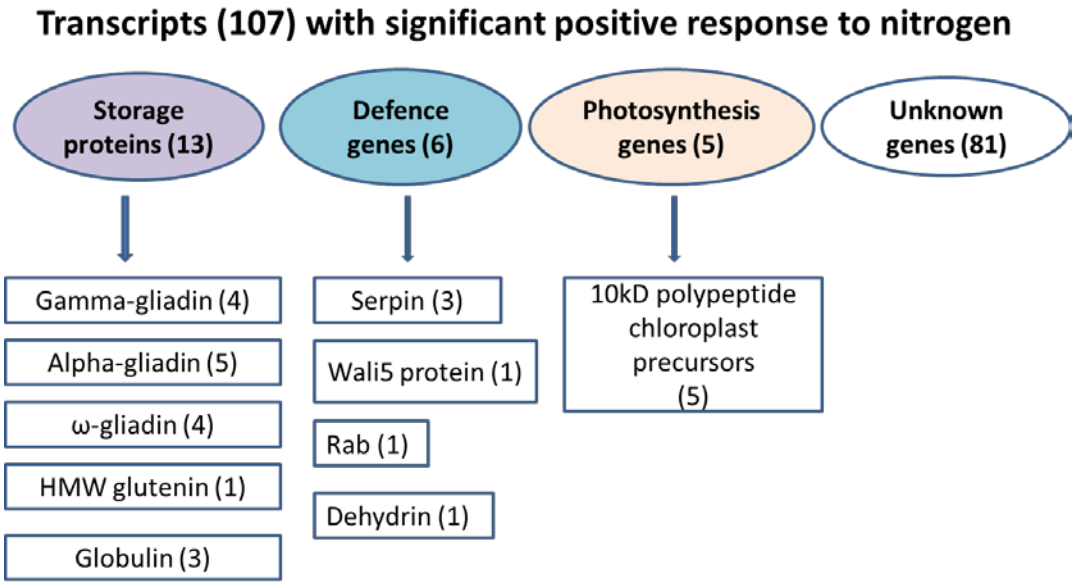


Figure 3.9 Classes of genes whose expression was significantly up-regulated by increasing N-application in both 2009 and 2010 at Rothamsted (107 in total).

Relating GPD to the transcriptome

Firstly a value for GPD was determined for each variety and for each year (for all N-fertilisation rates), after normalisation for effects of N-fertiliser input and yield dilution effects (examples shown in Figure 3.10).

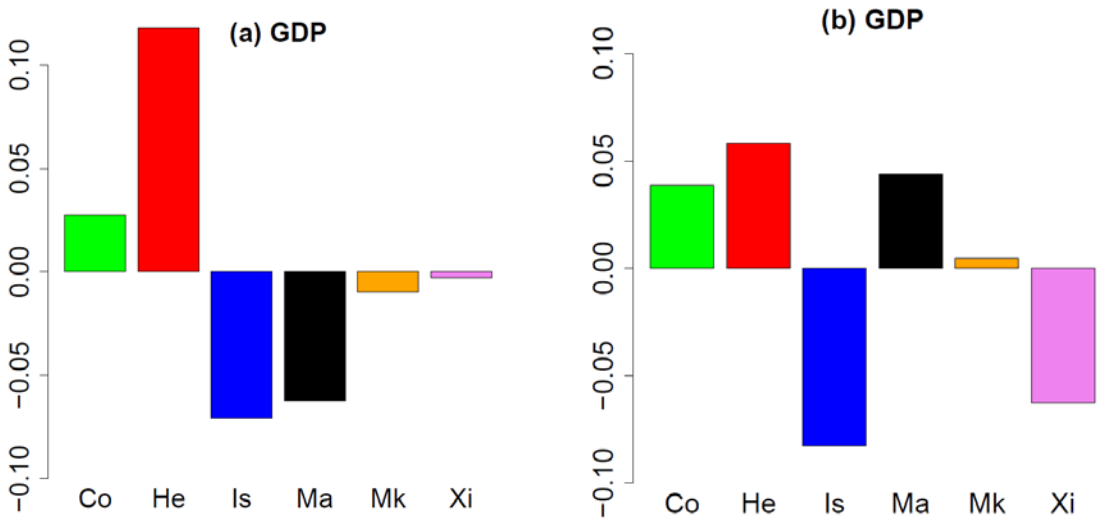


Figure 3.10 A measure of GDP for the 6 varieties along the x-axis; Cordiale (Co), Istabraq (Is), Hereward (He), Malacca (Ma), Marksman (Mk) and Xi19 (Xi)) grown at Rothamsted in 2009 (a) and 2010 (b) found by adjusting the protein content first by the direct effect of N-fertiliser level and secondly for the indirect effect of yield.

There was significant variation between the cultivars for uncorrected and corrected values for protein content and yield. However, whereas nitrogen level was significant for the uncorrected values it was not for the corrected values (Table 3.5), showing that the effect of N fertilisation had been successfully removed. There were no significant interactions between cultivars and nitrogen fertilisation for any of the parameters.

To identify genes related to the GPD, PCA was first performed on the gene expression data (the first 15 PCAs were evaluated), and PCs related to GPD but not to yield were identified (Table 3.6). This table indicates which principal components in the gene expression analysis correlate most significantly with the various parameters (yield, grain N and GPD). For 2009, PC2 and PC7 showed a significant relation to GPD but not to the yield or the N-fertilisation level. For 2010, PC2 and PC3 showed significant relation to GPD, but not to yield. The scores of these PCs are shown in Figure 3.11 as means of the cultivars where the arbitrary sign of the PCs are flipped for a comparison with the means of GPD for the cultivars in Figure 3.10. In both Figure 3.10 of GPD and in Figure 3.11 of the selected PCs, Hereward is positive and Istabraq is negative. The other cultivars vary in their position.

Table 3.5 p-values from ANOVA on the effect of the design parameters (CV, linear and quadratic effects of N, and the interaction between N and CV) on the phenotypic characteristics; Yield and Protein both corrected for the effect of N level, and the double correction of protein to give GPD, (a) 2009 and (b) 2010.

(a)

	pvalue_Y	pvalue_P	pvalue_Y_corr_N	pvalue_p_corr_N	pvalue_p_corr_N_and_Y
intercept	**0.000**	**0.000**	1.000	1.000	1.000
CV	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**
N_level	**0.000**	**0.000**	1.000	1.000	1.000
N_level^2	**0.000**	**0.000**	1.000	1.000	1.000
CV*N_level	0.233	0.766	0.233	0.766	0.766

(b)

	Yield	Protein	Yield corr N	Protein corr N	Protein_corr N&Y
intercept	**0.000**	**0.000**	1.000	1.000	1.000
CV	0.481	**0.027**	0.481	**0.027**	**0.063**
N_level	**0.001**	**0.000**	1.000	1.000	1.000
N_level^2	0.529	**0.000**	1.000	1.000	1.000
CV*N_level	0.636	0.636	0.636	0.636	0.878

Table 3.6 Results of ANOVA (FDR adjusted p-values) showing the effect of the scores of PCA of the gene expression data (input of the model) on the phenotypic characteristics (output of the model), (a) for 2009, (b) for 2010.

(a)

	Yield	Protein	Yield corr N	Protein corr N	Protein corr N and Y
mean PC1	0.078	**0.031**	0.740	0.527	0.591
mean PC2	0.065	0.998	0.053	**0.005**	**0.042**
mean PC3	0.152	0.453	**0.008**	**0.006**	0.126
mean PC4	0.736	0.682	0.139	0.911	0.412
mean PC5	0.362	**0.036**	**0.000**	**0.014**	0.653
mean PC6	0.357	0.195	0.823	0.572	0.412
mean PC7	0.853	0.062	0.199	**0.000**	**0.000**
mean PC8	0.071	**0.034**	0.431	0.642	0.285
mean PC9	0.314	0.444	0.080	0.067	0.299
maen PC10	**0.000**	**0.000**	0.447	0.089	**0.010**
maen PC11	**0.024**	0.379	**0.000**	**0.000**	0.099
mean PC12	0.088	**0.018**	0.414	0.641	0.276
mean PC13	0.525	0.716	0.445	0.437	0.152
mean PC14	**0.000**	**0.000**	0.427	0.252	0.398
mean PC15	0.191	0.179	0.263	0.600	0.174

(b)

	Yield	Protein	Yield corr N	Protein corr N	Protein corr N & Y
mean PC1	**0.001**	**0.000**	0.293	0.392	0.756
mean PC2	0.603	**0.000**	0.170	**0.012**	**0.038**
mean PC3	0.942	**0.021**	0.285	**0.030**	0.062
mean PC4	0.966	0.085	0.449	0.081	0.111
mean PC5	0.143	0.255	0.369	0.837	0.708
mean PC6	**0.013**	**0.000**	0.860	0.244	0.120
mean PC7	0.313	**0.005**	0.641	0.491	0.608
mean PC8	0.283	**0.295**	0.740	0.084	0.061
mean PC9	**0.026**	0.245	0.136	0.087	0.301
mean PC10	0.836	0.948	0.704	0.561	0.329
mean PC11	0.204	0.014	0.812	0.483	0.306
mean PC12	0.840	0.384	0.762	0.549	0.604
mean PC13	0.639	0.798	0.380	0.752	0.317
mean PC14	0.966	0.916	0.963	0.855	0.849
mean PC15	0.135	0.338	0.221	0.913	0.473

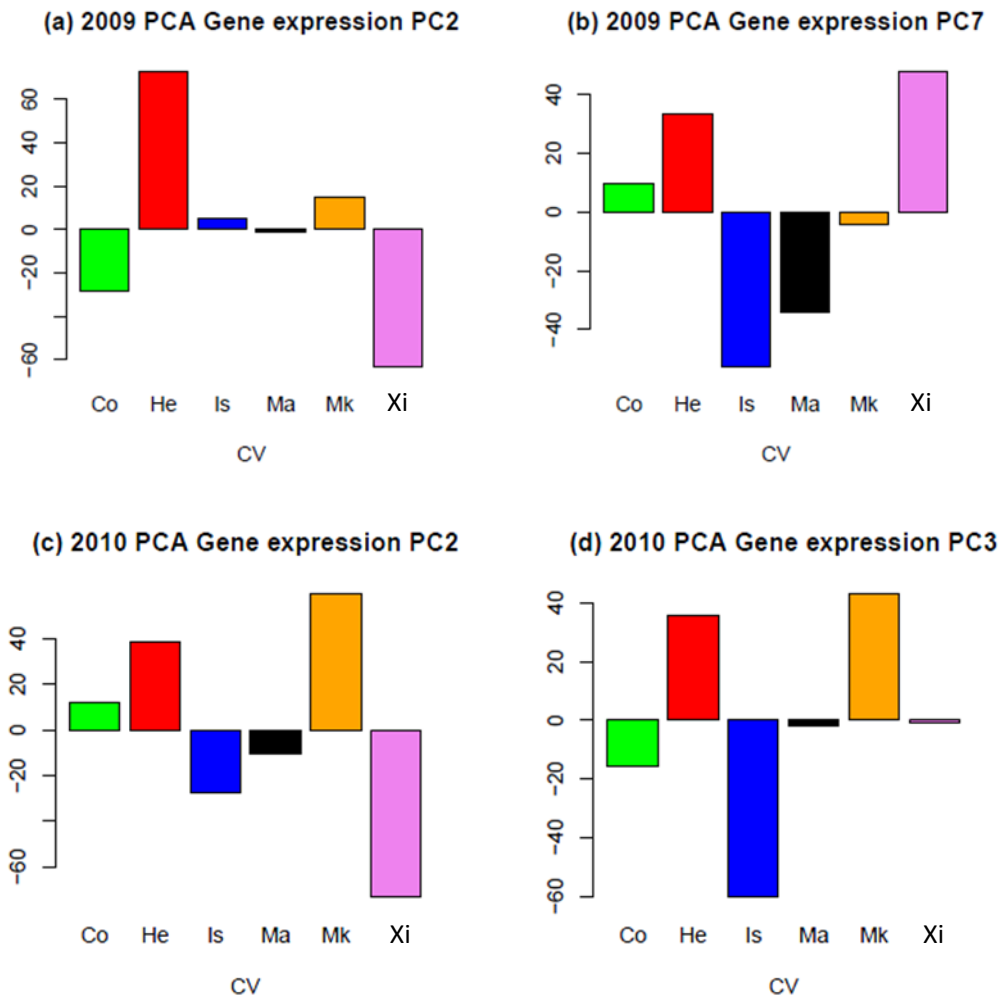


Figure 3.11 Cultivar means of scores of 4 selected principal components (PC2: a and c; PC3, b and d) PCA of the gene expression data obtained in 2009 (a and b) and 2010 (c and d) at Rothamsted. The PCs shown were those significant for GPD but not for grain yield.

Gene expression profiles of selected genes with the most positive and the most negative relation to GPD are shown in Figure 3.12 and 3.13. The selection of the genes was performed on the data set from Rothamsted in 2009 and 2010. Further analysis of the whole set of correlated genes will be required in the future.

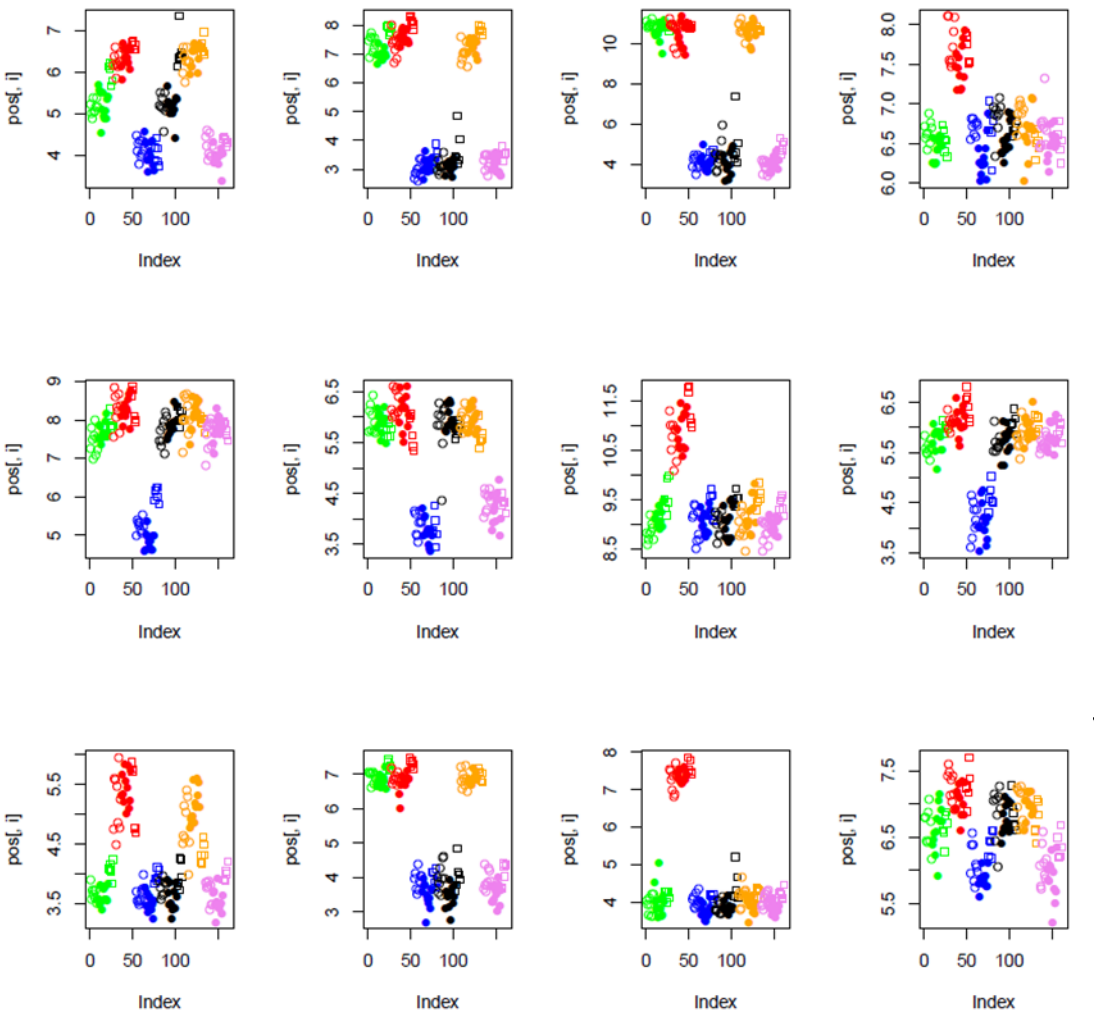


Figure 3.12 A gene expression profile of 12 of the genes with the most positive relation to GDP in 2009 (open circles), in 2010 (closed circles) and 2011 (open squares). The samples are sorted along the x-axis according to the cultivars for clarity. Green is Cordiale, red is Hereward, blue is Istabraq (Is), black is Malacca, yellow is Marksman and purple is Xi19)

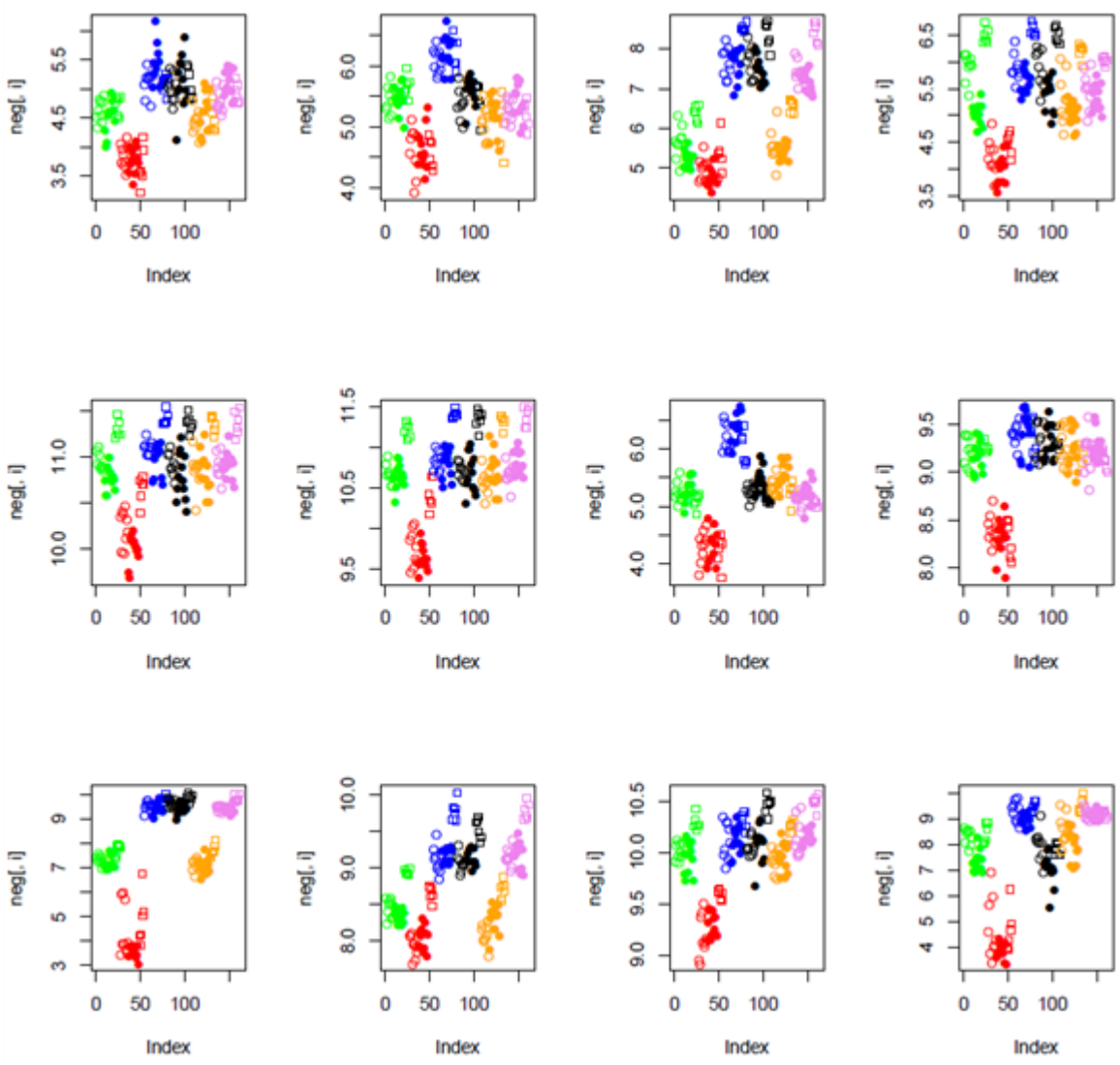


Figure 3.13 A gene expression profile of 12 of the genes with the most negative relation to GDP in 2009 (open circles), in 2010 (closed circles) and 2011 (open squares). The samples are sorted along the x-axis according to the cultivars for clarity. Green is Cordiale (Co), red is Istabraq (Is), blue is Hereward (He), black is Malacca (Ma), yellow is Marksman (Mk) and purple is Xi19 (Xi))

Gliadin gene expression

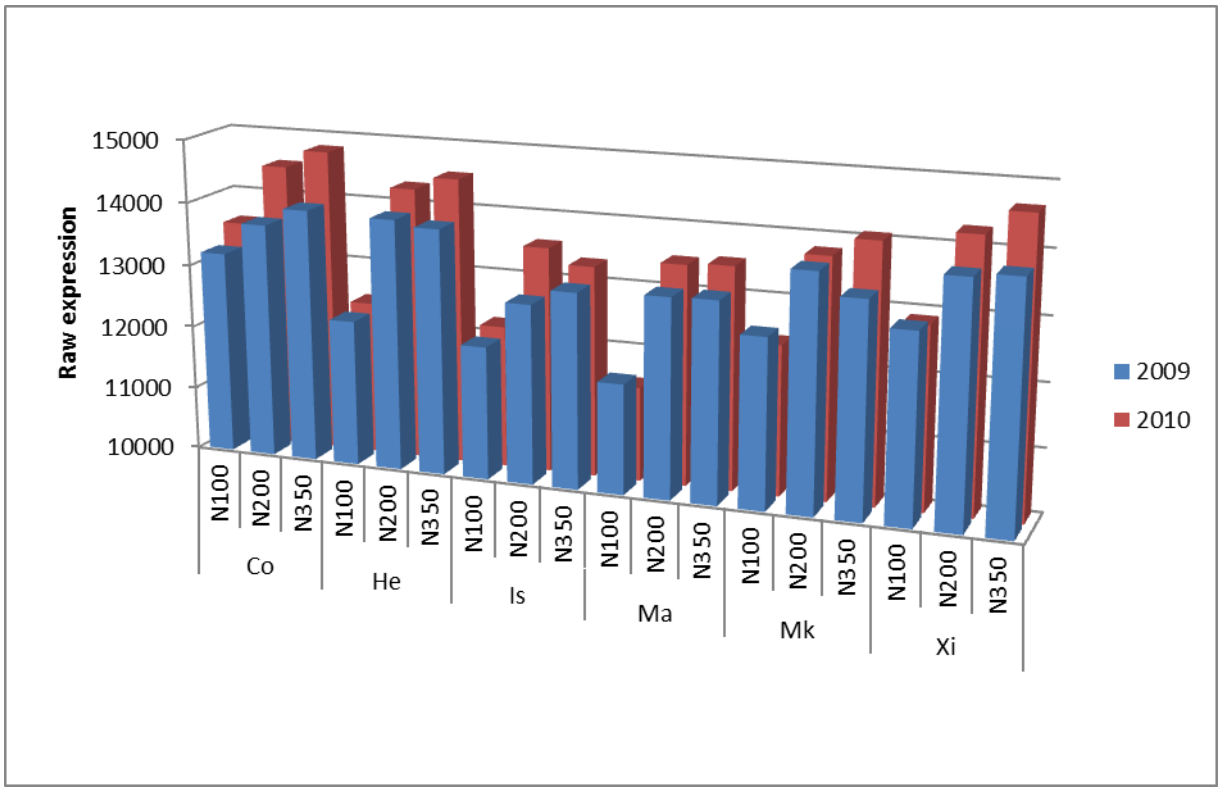


Figure 3.14 Gliadin gene expression in 2009 and 2010 at Rothamsted for the 6 varieties.

The expression profiles of all of the gliadin genes represented on the chip are shown in Figure 3.14. They show increased expression in response to applied N, particularly between the 100 and 200 kgN/ha treatments. The expression levels of gliadin genes were highest in Cordiale, Hereward, Marksman and Xi19. The gliadin genes represented on the array include a γ-gliadin which differs in its amino acid sequence from typical γ-gliadins. As only a partial sequence was available for this γ-gliadin, the complete sequence was determined. The expression of the gene is very responsive when N-fertilisation increases from 100 to 200 kgN/ha, particularly in Hereward, Istabraq and Malacca (Figure 3.15). The expression of this γ-gliadin was also determined during grain development, showing a peak at 21 dpa followed by a decrease (Figure 3.16). The full length sequence of the atypical γ-gliadin encoded by this gene is aligned with those of other γ-gliadins in Figure 3.17. The sequence of this gene and related γ-gliadin proteins are aligned with those of a 'typical' γ-gliadin from cv Chinese Spring (accession EF15018) and γ-3 hordein from *H. chilense* (accession AY338065) in Figure 3.17. The proteins all have a typical γ-gliadin structure with a signal peptide, a short *N*-terminal domain, a repetitive domain based on short motifs rich in proline and glutamine, and a *C*-terminal domain with eight cysteine residues which are known to form four intra-chain disulphide bonds (Shewry *et al*., 2009). However, the sequence of the novel γ-gliadin greatly differs from those of the typical γ-gliadins. The repeat sequence motif of the repetitive domains of typical γ-gliadins is $PFPQ_{1-2}$ $(PQQ)_{1-2}$, while the consensus repeat sequence motif in the novel γ-gliadin is $PLPQ_{3-4}$ with very few PQQ sequences. A complete report of this study has been published elsewhere (Wan *et al*., 2013).

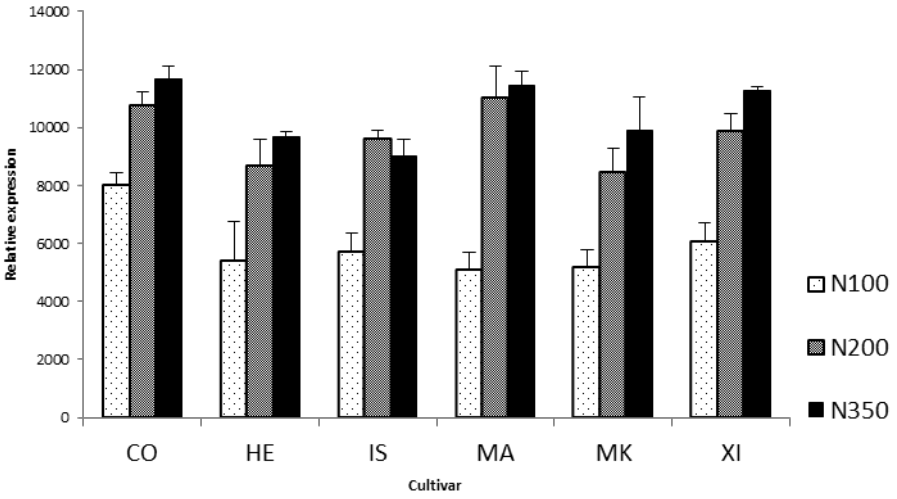


Figure 3.15 Expression as determined from the Affymetrix dataset of a novel N-regulated gamma gliadin (Rothamsted, 2010). Used with permission, Wan *et al*. 2013.

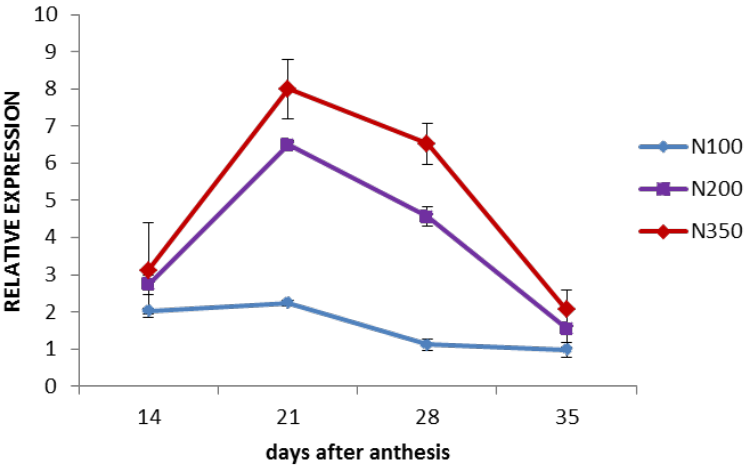


Figure 3.16 Abundance of transcripts related to the novel γ-gliadin in developing caryopses of wheat cv. Hereward grown in 2010, determined by RT-PCR, at 3 N inputs (100, 200 and 350 kg/ha). Used with permission, Wan *et al*. 2013.

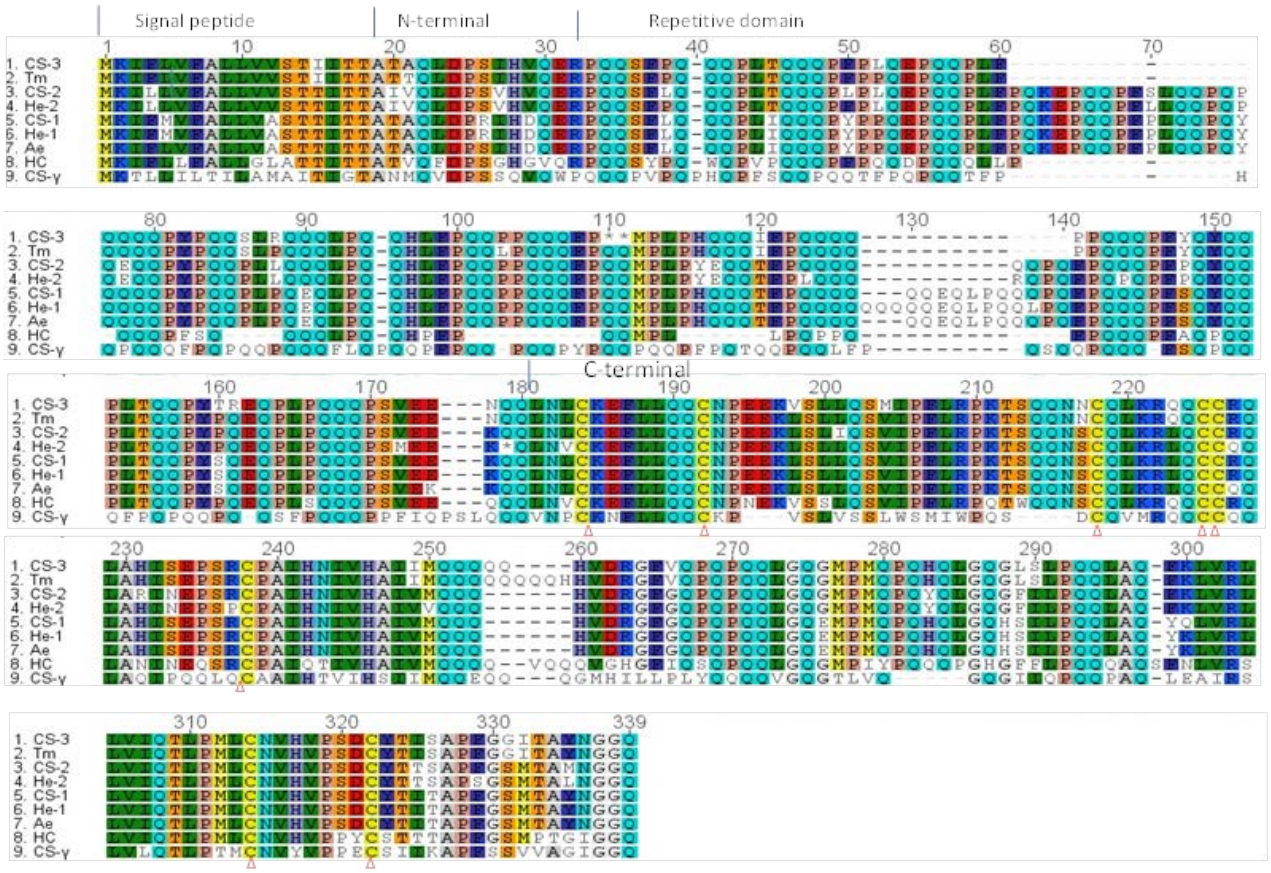


Figure 3.17 Alignment of novel γ-gliadin proteins. Alignment was performed with ClustalW using BLOSUM matrix of Geneious Pro5.5.6 version soft1. CS-1, CS-2, and CS-3 (pseudogene, predicted protein) novel γ-gliadin from Chinese Spring; CS-γ typical γ-gliadin from cv. Chinese Spring EF15018; He-1 and He-2 (pseudogene) novel γ-gliadin from cv. Hereward; Ae and Tm novel γ-gliadin from *Aegilop stauschii and Triticum monococcum*, respectively*;* HC from *Hordeumchilense* AY338065. Adapted from, Wan *et al*. 2013.

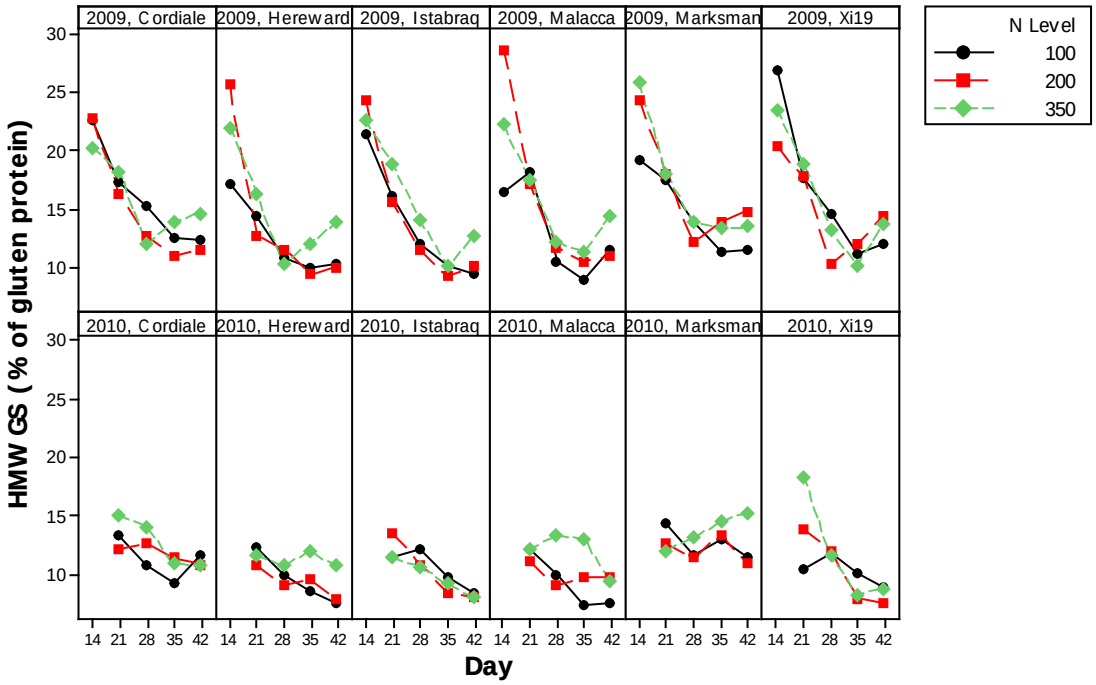
3.3.3. **Protein analysis**

SDS-PAGE analysis was carried out on individual replicates of ground wholegrain material for developing grain and mature grain. SE-HPLC analysis was carried out on pooled replicates of ground wholegrain material for developing grain, and white flour for whole grain. Developing grain samples were sampled from Rothamsted Research for 2009 and 2010, while mature grain was sourced from Rothamsted Research alone in 2009, and Rothamsted Research plus four additional sites in 2010 and 2011.

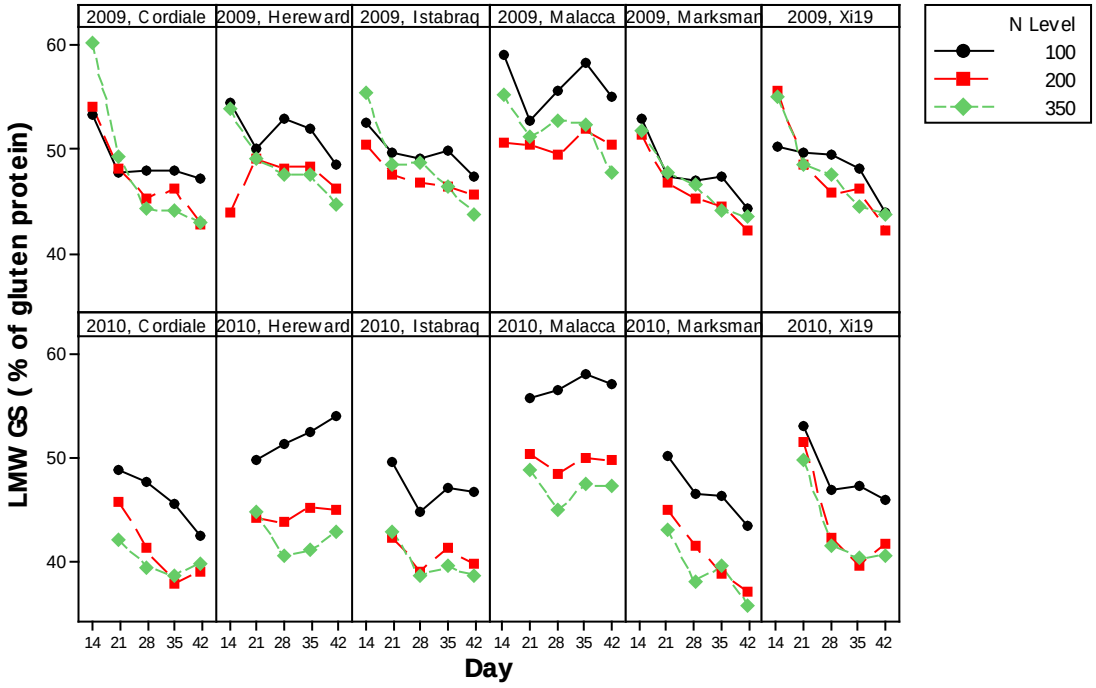
SDS-PAGE

In developing grain, the proportions of LMW glutenin subunits decreased significantly and the gliadins increased with increasing levels of N application (Figure 3.18). This effect was most apparent in 2010. With increasing days after anthesis, the proportion of HMW glutenin subunits decreased, with a corresponding increase in the proportion of gliadins.

A


2009, Cordiale
2009, Hereward
2009, Istabraq
2009, Malacca
2009, Marksman
2009, Xi19
2010, Cordiale
2010, Hereward
2010, Istabraq
2010, Malacca
2010, Marksman
2010, Xi19
HMW GS (% of gluten protein)
Day
N Level
100
200
350

B


2009, Cordiale
2009, Hereward
2009, Istabraq
2009, Malacca
2009, Marksman
2009, Xi19
2010, Cordiale
2010, Hereward
2010, Istabraq
2010, Malacca
2010, Marksman
2010, Xi19
LMW GS (% of gluten protein)
Day
N Level
100
200
350

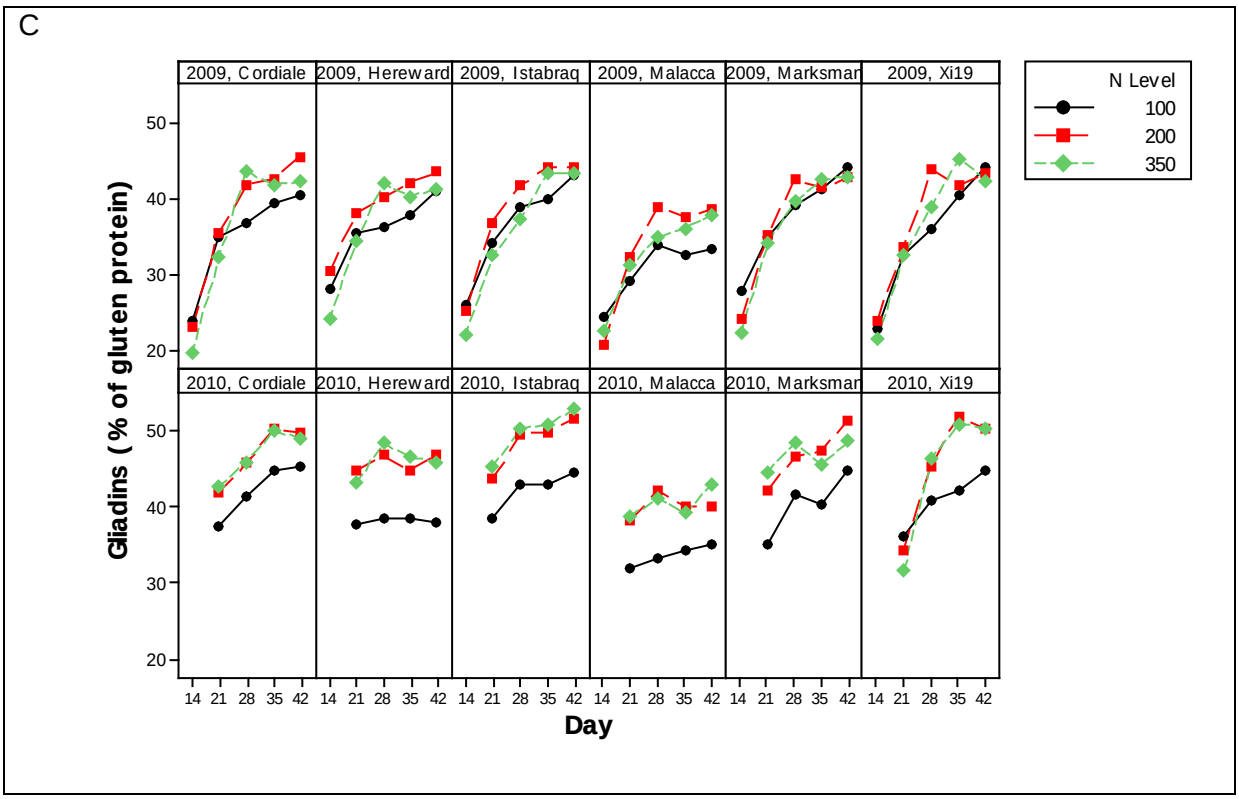
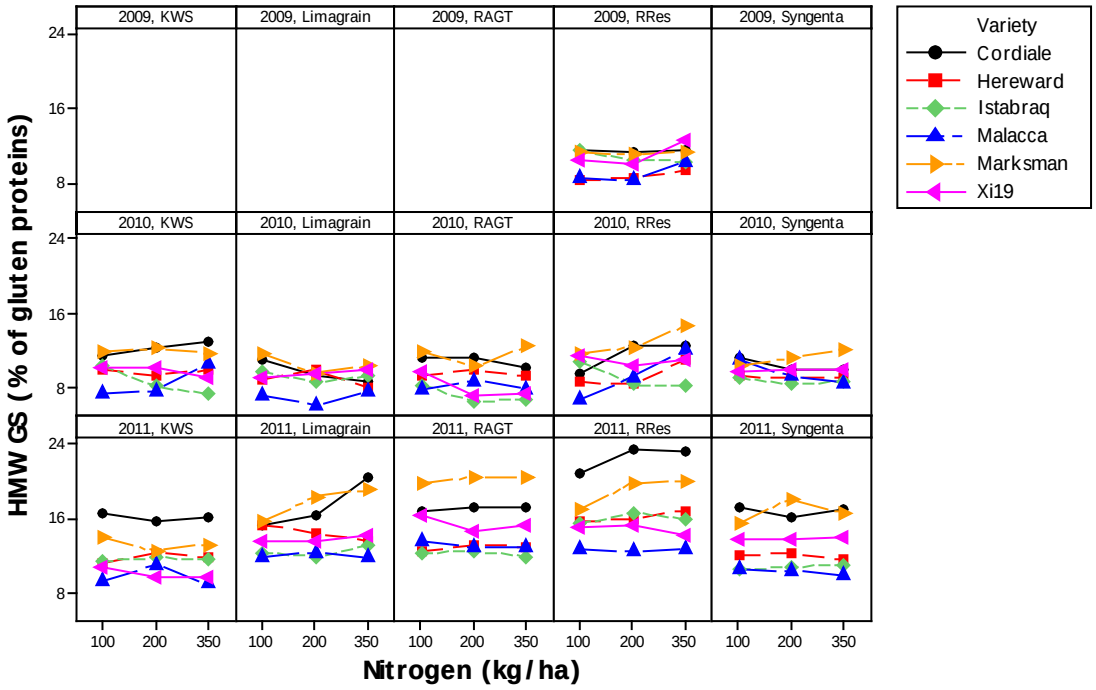


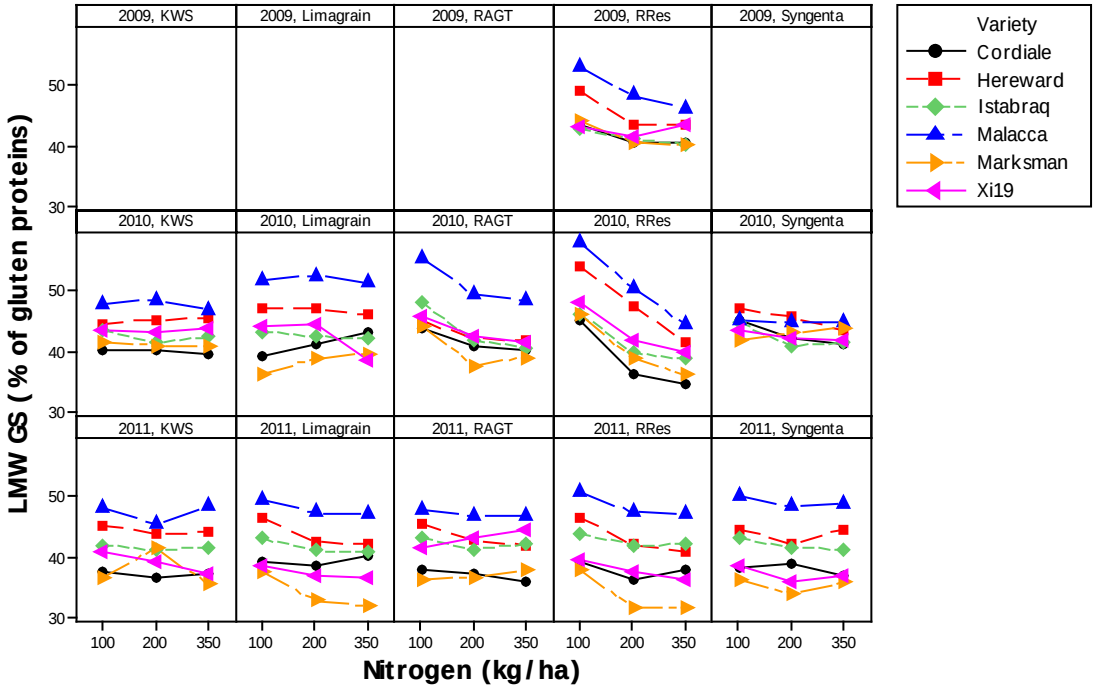
Figure 3.18 The proportion of HMW glutenin subunits (A), LMW glutenin subunits (B) and gliadins (C) as a proportion of total gluten proteins in developing grain grown at Rothamsted Research in 2009 and 2010. Values are a mean of three replicates. Each gel was run in duplicate.

The proportions of HMW glutenin subunits, LMW glutenin subunits and gliadins in mature grain were determined in mature grain over three years (Figure 3.19). In general, the proportion of LMW glutenin subunits decreased with increasing N fertilisation; that of gliadins increased, and that of HMW glutenin subunits increased slightly. These patterns were consistent across the three years of the trial. The same trends were not consistent between all five sites but this may be due to inconsistencies in the nitrogen application regime. However, the differences between varieties were consistent between years and sites where Cordiale and Marksman were characterised by higher levels of HMW glutenin subunits and lower levels of LMW glutenin subunits, while Malacca was characterised by high levels of LMW glutenin subunits and lower levels of gliadins.

A


2009, KWS
2009, Limagrain
2009, RAGT
2009, RRes
2009, Syngenta
2010, KWS
2010, Limagrain
2010, RAGT
2010, RRes
2010, Syngenta
2011, KWS
2011, Limagrain
2011, RAGT
2011, RRes
2011, Syngenta
HMW GS (% of gluten proteins)
Nitrogen (kg/ha)
Variety
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19

B


2009, KWS
2009, Limagrain
2009, RAGT
2009, RRes
2009, Syngenta
2010, KWS
2010, Limagrain
2010, RAGT
2010, RRes
2010, Syngenta
2011, KWS
2011, Limagrain
2011, RAGT
2011, RRes
2011, Syngenta
LMW GS (% of gluten proteins)
Nitrogen (kg/ha)
Variety
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19

C

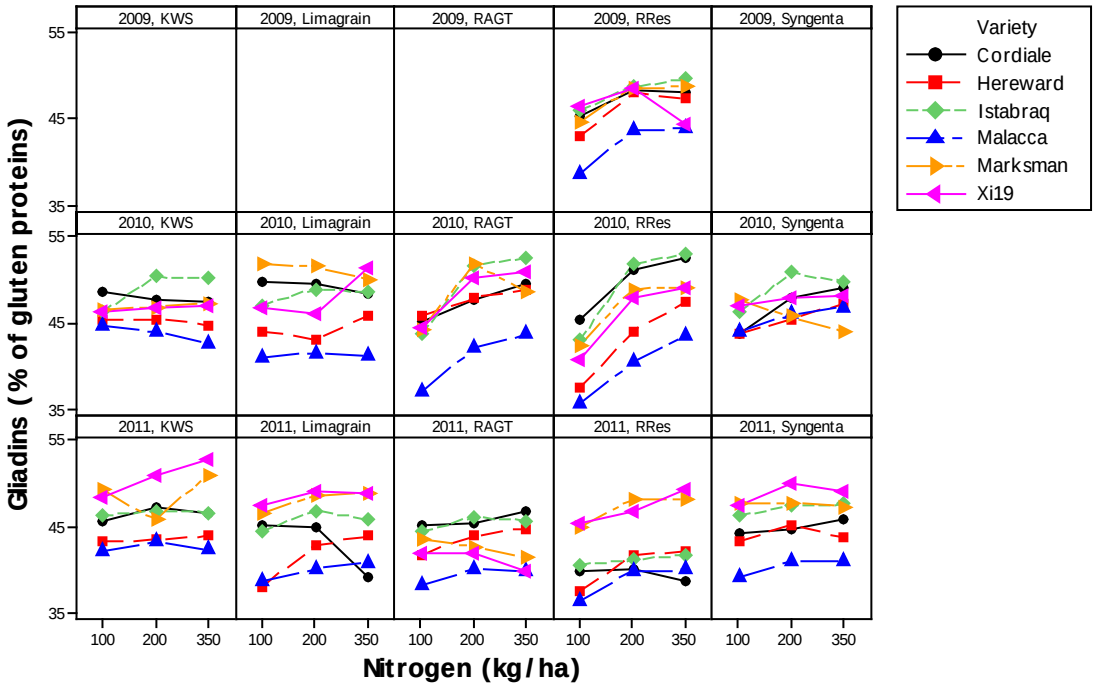
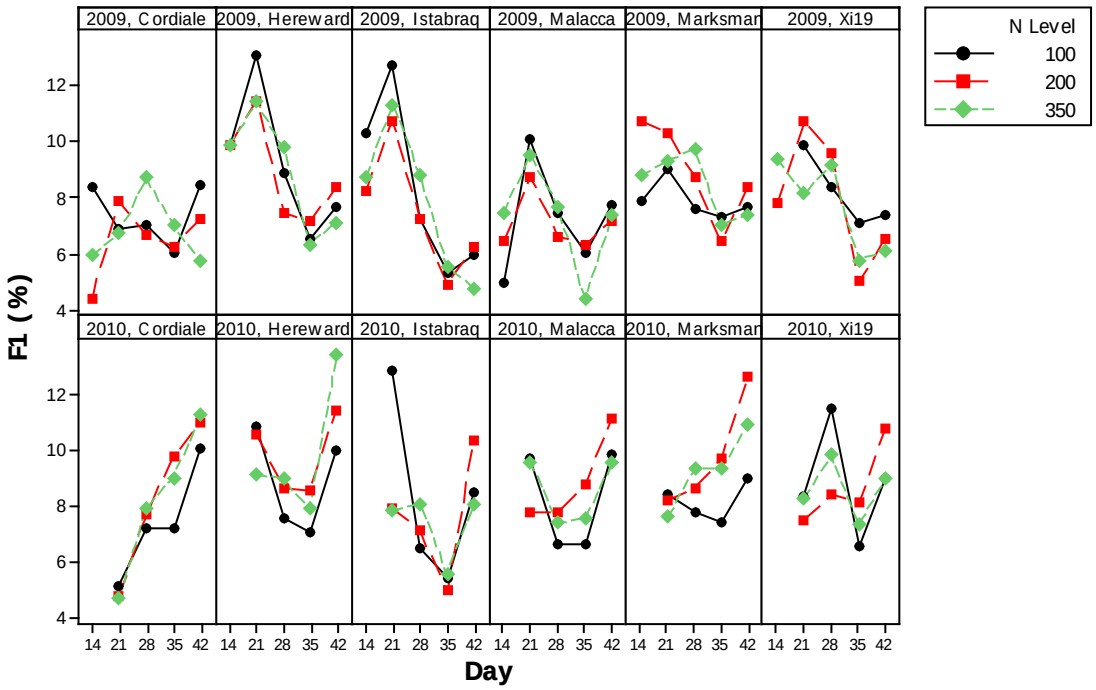


Figure 3.19 The proportion of HMW glutenin subunits (A), low molecular weight subunits (B) and gliadins (C) as a proportion of total gluten proteins in mature grain grown at a range of sites in 2009, 2010 and 2011. Values are a mean of three replicates. Each gel was run in duplicate.

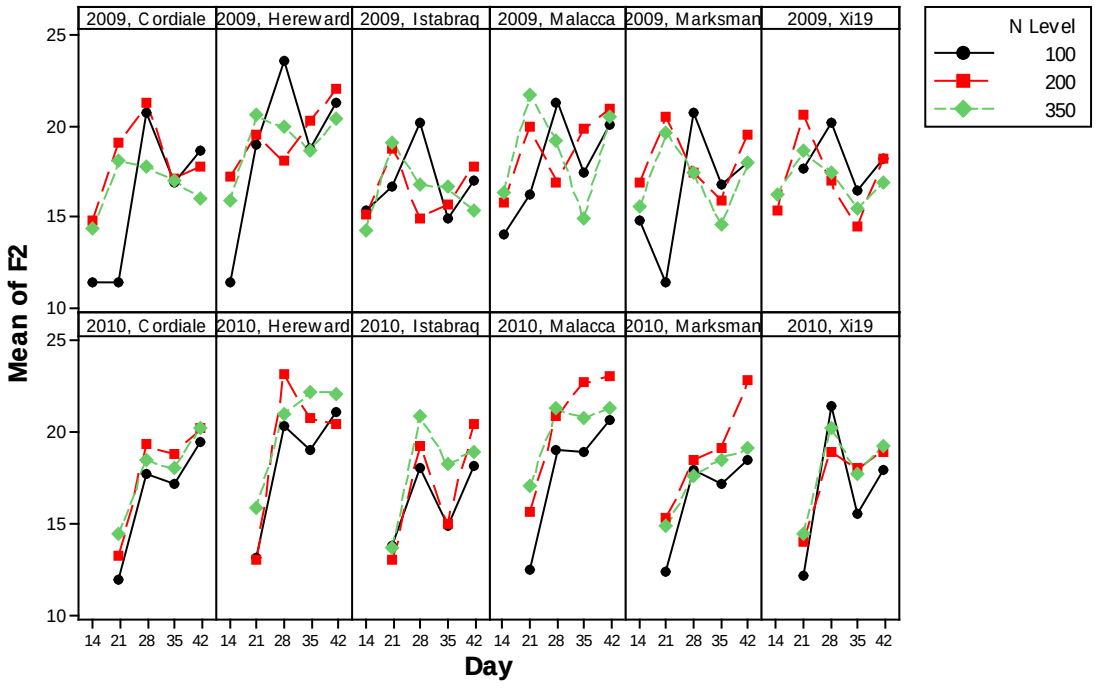
SE-HPLC

Analyses of developing grain showed clearer changes in protein composition in samples from 2010 compared with 2009 (Figure 3.20). In general, the proportion of polymeric proteins increased, while that of the monomeric proteins decreased. Only the proportions of the F4 and F5 fractions were affected by the level of nitrogen fertilisation, where the proportion of the F4 fraction increased with increasing nitrogen addition, while that of the F5 fraction decreased.

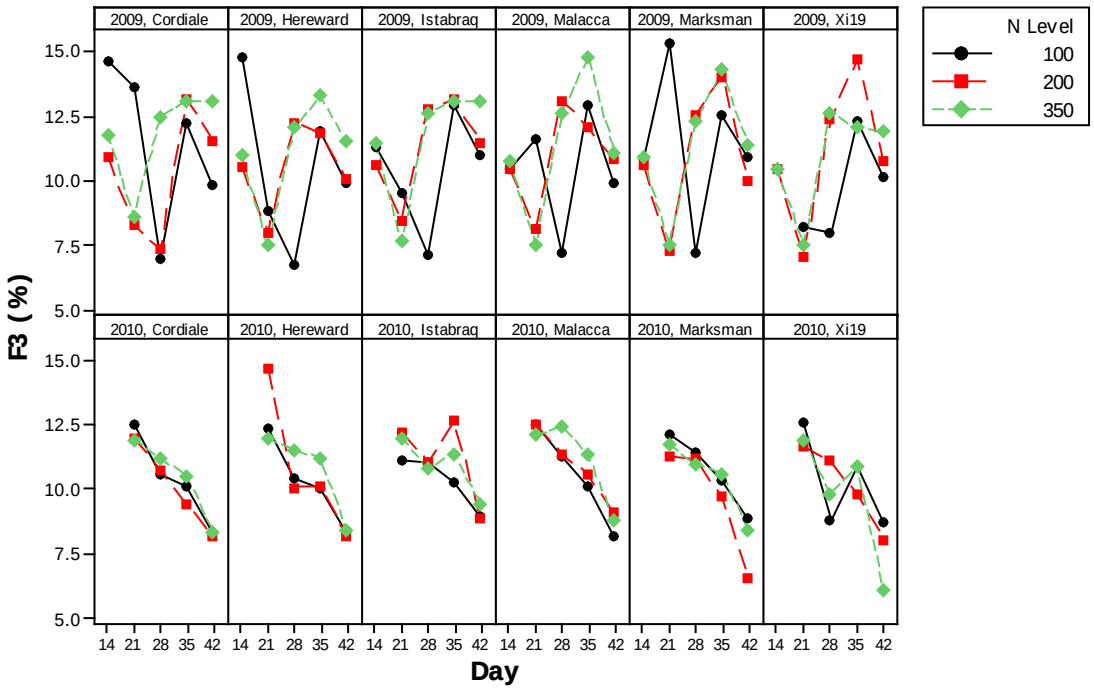
A


2009, Cordiale
2009, Hereward
2009, Istabraq
2009, Malacca
2009, Marksman
2009, Xi19
2010, Cordiale
2010, Hereward
2010, Istabraq
2010, Malacca
2010, Marksman
2010, Xi19
F1 (%)
Day
N Level
100
200
350

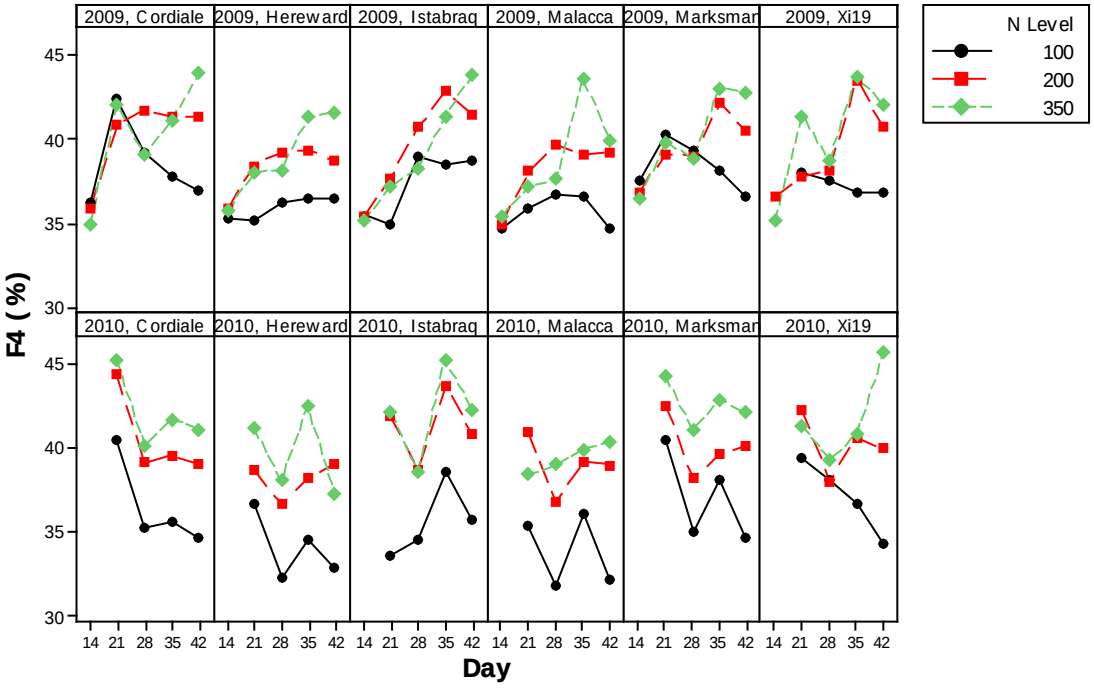
B


2009, Cordiale
2009, Hereward
2009, Istabraq
2009, Malacca
2009, Marksman
2009, Xi19
2010, Cordiale
2010, Hereward
2010, Istabraq
2010, Malacca
2010, Marksman
2010, Xi19
Mean of F2
Day
N Level
100
200
350

C


2009, Cordiale
2009, Hereward
2009, Istabraq
2009, Malacca
2009, Marksman
2009, Xi19
2010, Cordiale
2010, Hereward
2010, Istabraq
2010, Malacca
2010, Marksman
2010, Xi19
F3 (%)
Day
N Level
100
200
350

D


2009, Cordiale
2009, Hereward
2009, Istabraq
2009, Malacca
2009, Marksman
2009, Xi19
2010, Cordiale
2010, Hereward
2010, Istabraq
2010, Malacca
2010, Marksman
2010, Xi19
F4 (%)
Day
N Level
100
200
350

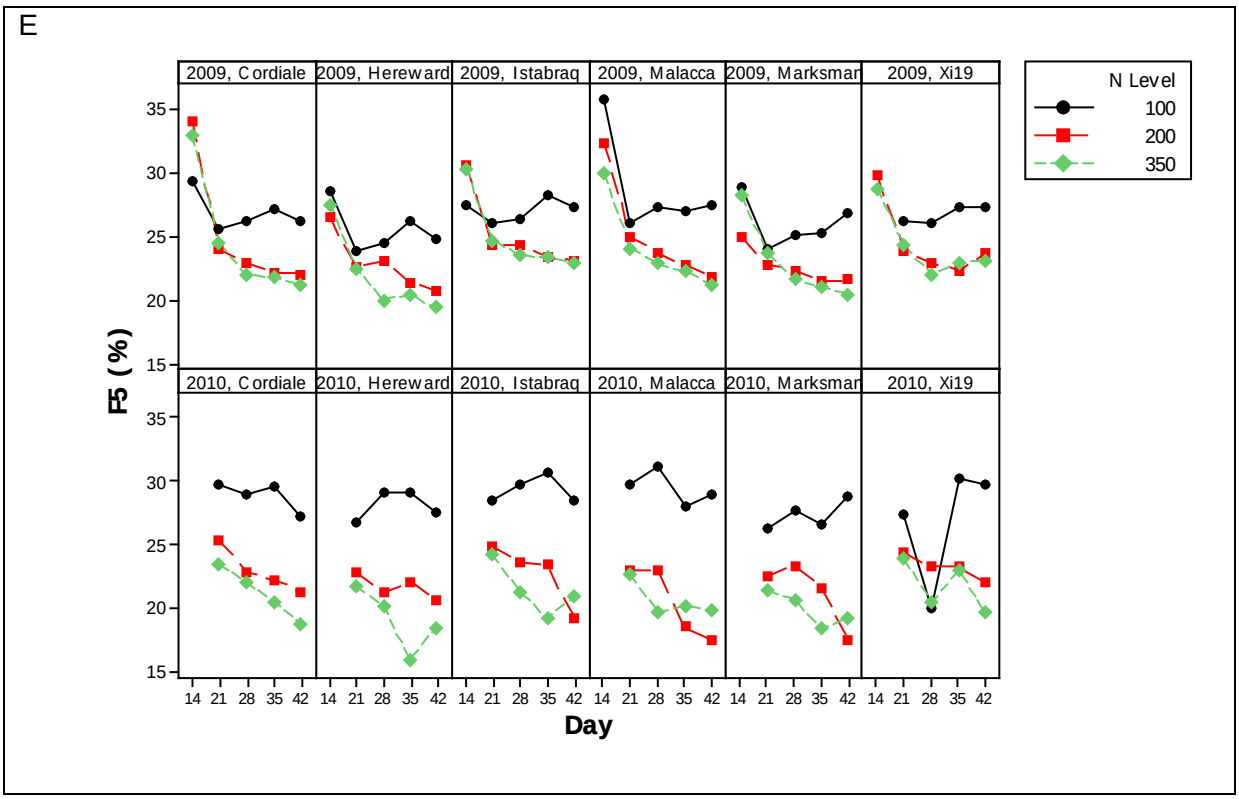
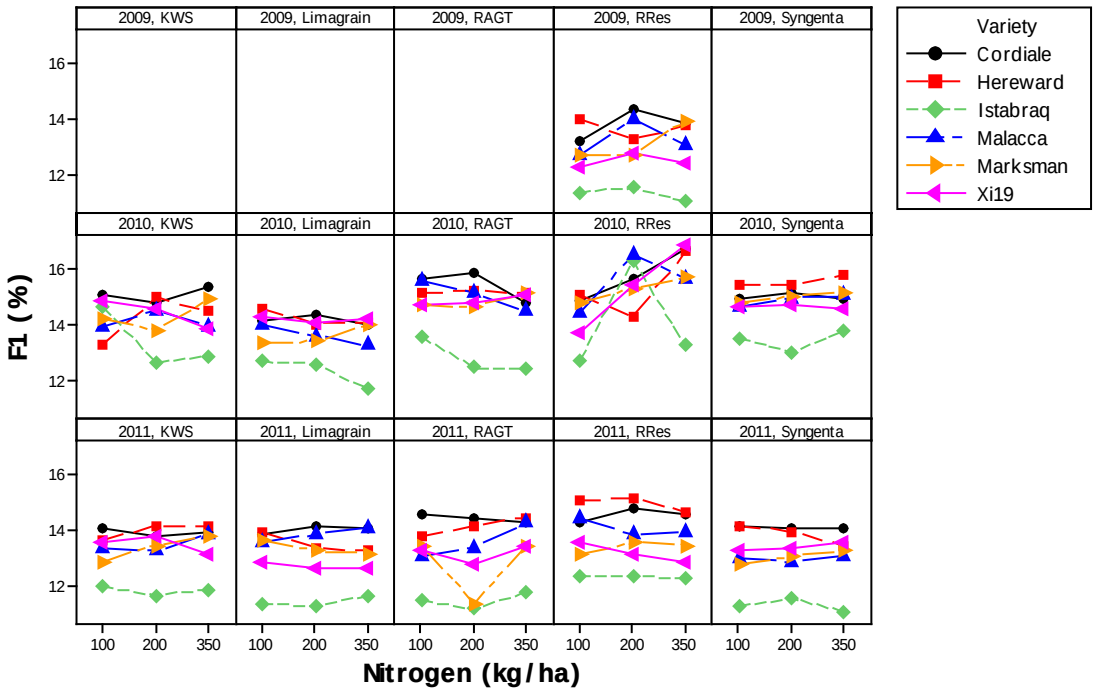


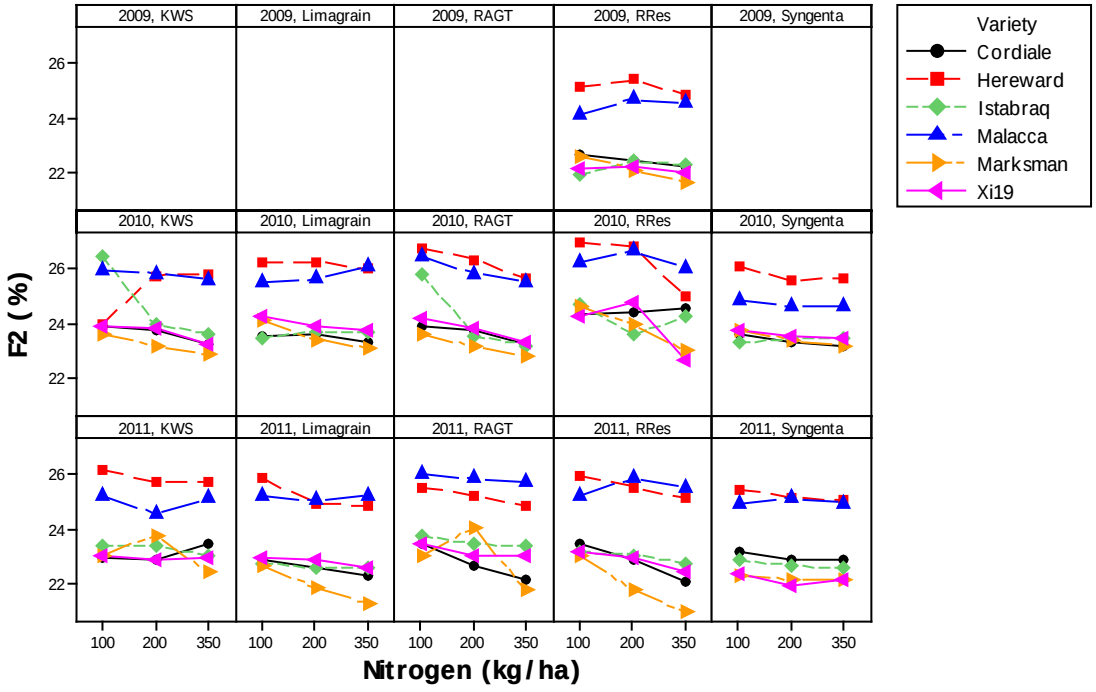
Figure 3.20 The proportion of HMW glutenin polymers (F1, A), LMW glutenin polymers (F2, B), gliadins (F3, C and F4, D) and albumins and globulins (F5, E) in mature grain grown at a range of sites in 2009, 2010 and 2011.

In mature grain, only the proportions of fractions F2 to F5 were affected by the level of nitrogen application; with the proportions of the F3 and F4 fractions increasing with increasing nitrogen application, the proportion of the F5 fraction decreasing and the F2 fraction showing a small decrease (Figure 3.21, Table 3.7b). The protein composition differed between varieties and these differences were consistent across sites and years. Thus, no significant three-way interactions were observed (Table 3.7b). Istabraq contained the lowest proportion of the F1 fraction. The highest proportion of the F2 fraction and the lowest proportion of the F5 fraction were in Malacca and Hereward. The lowest proportions of the F3 fraction were in Cordiale and Xi19.

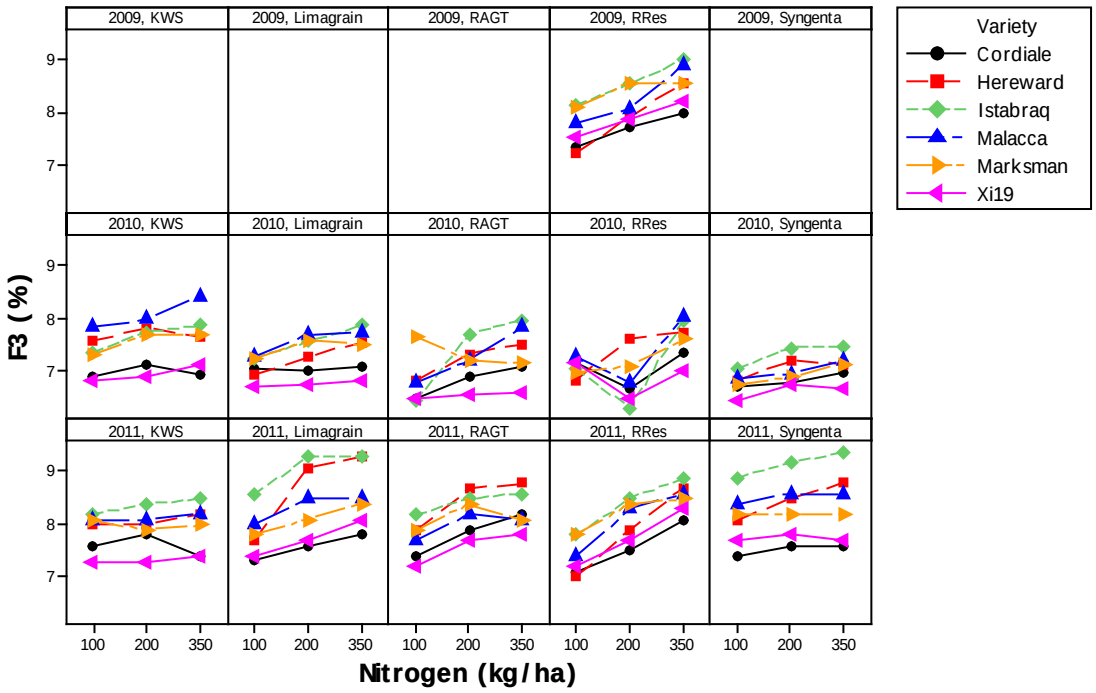
A


2009, KWS
2009, Limagrain
2009, RAGT
2009, RRes
2009, Syngenta
2010, KWS
2010, Limagrain
2010, RAGT
2010, RRes
2010, Syngenta
2011, KWS
2011, Limagrain
2011, RAGT
2011, RRes
2011, Syngenta
F1 (%)
16
14
12
100
200
350
Nitrogen (kg/ha)
Variety
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19

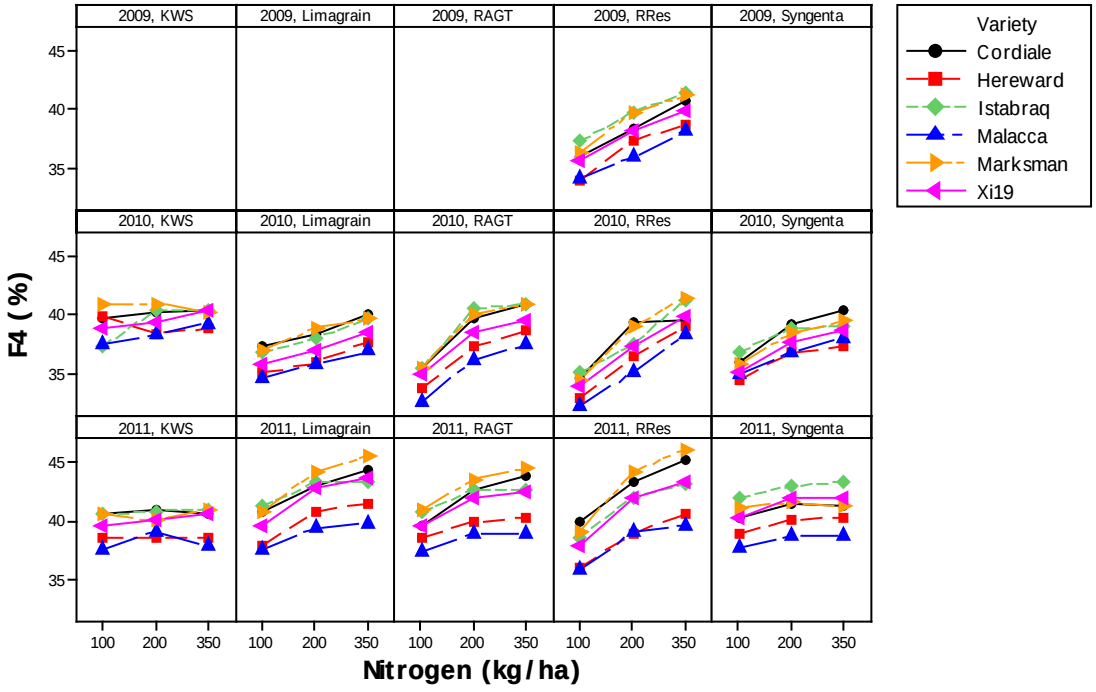
B


2009, KWS
2009, Limagrain
2009, RAGT
2009, RRes
2009, Syngenta
2010, KWS
2010, Limagrain
2010, RAGT
2010, RRes
2010, Syngenta
2011, KWS
2011, Limagrain
2011, RAGT
2011, RRes
2011, Syngenta
F2 (%)
26
24
22
100
200
350
Nitrogen (kg/ha)
Variety
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19

C


2009, KWS
2009, Limagrain
2009, RAGT
2009, RRes
2009, Syngenta
2010, KWS
2010, Limagrain
2010, RAGT
2010, RRes
2010, Syngenta
2011, KWS
2011, Limagrain
2011, RAGT
2011, RRes
2011, Syngenta
F3 (%)
Nitrogen (kg/ha)
Variety
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19

D


2009, KWS
2009, Limagrain
2009, RAGT
2009, RRes
2009, Syngenta
2010, KWS
2010, Limagrain
2010, RAGT
2010, RRes
2010, Syngenta
2011, KWS
2011, Limagrain
2011, RAGT
2011, RRes
2011, Syngenta
F4 (%)
Nitrogen (kg/ha)
Variety
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19

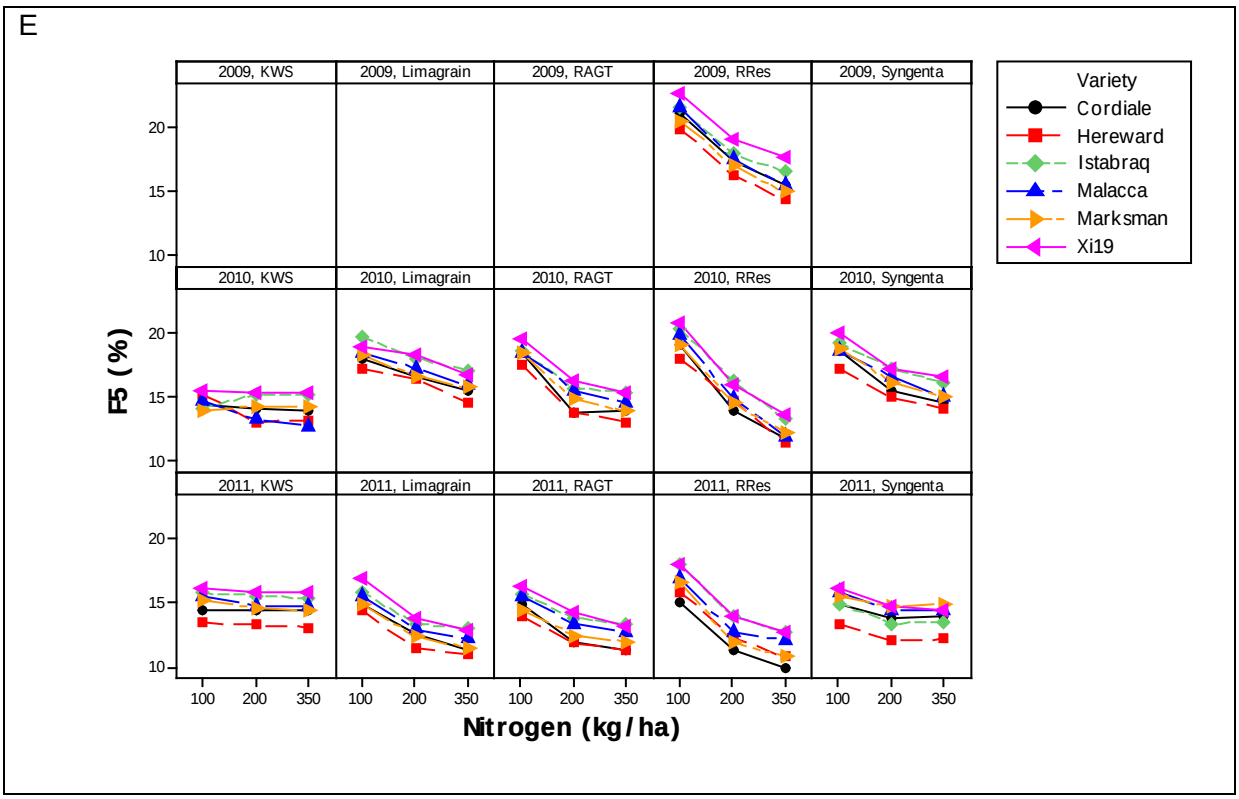


Figure 3.21 The proportion of HMW glutenin polymers (F1, A), LMW glutenin polymers (F2, B), gliadins (F3, C and F4, D) and albumins and globulins (F5, E) in developing grain grown at Rothamsted Research in 2009 and 2010. Analyses were performed on pooled replicates.

The proportions of the F2 and F3 fractions responded differently to N level in the different cultivars, with a significant interaction between cultivar and N level (Table 3.7 and Figure 3.21).

Taken together, the protein analyses show that there is an effect of nitrogen on protein composition and that this effect varies in extent with variety.

3.3.4. **Functionality**

Sufficient samples for milling were not obtained for samples grown at 100 and 350N from the RAGT site in 2010. Functionality and baking tests were therefore only undertaken on four sites in 2010 (Rothamsted Research, KWS, Limagrain and Syngenta).

NIR analysis of grain and flour

Determination of total grain N (protein) showed that the response to the level of nitrogen fertilisation was dependent on site (Figure 3.22). For samples grown at Rothamsted Research the amount of grain N increased with increasing levels of N fertilisation. The degree of response was lower at the Syngenta site, and lower still at the Limagrain site, while for samples grown at KWS there was no observable response to N fertilisation in terms of grain N content. Results for flour protein (as is) showed similar trends (data not shown).

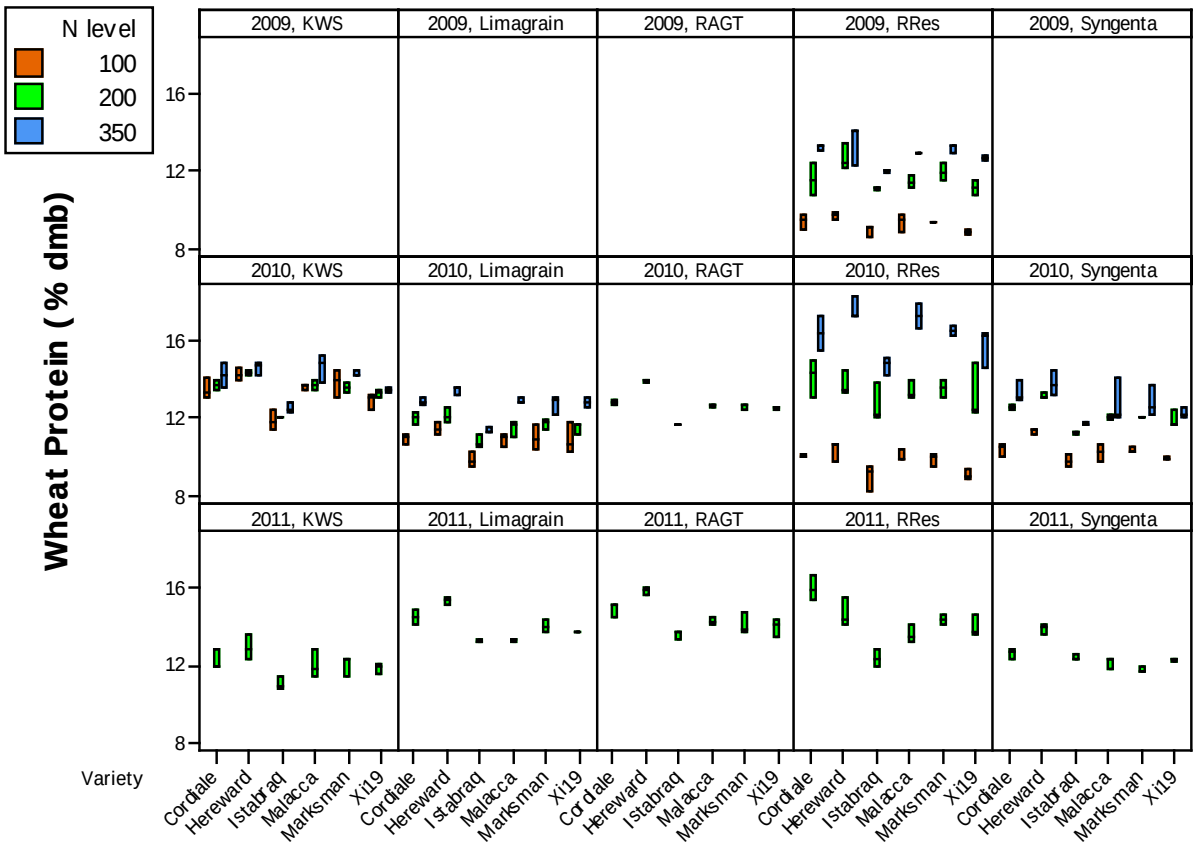
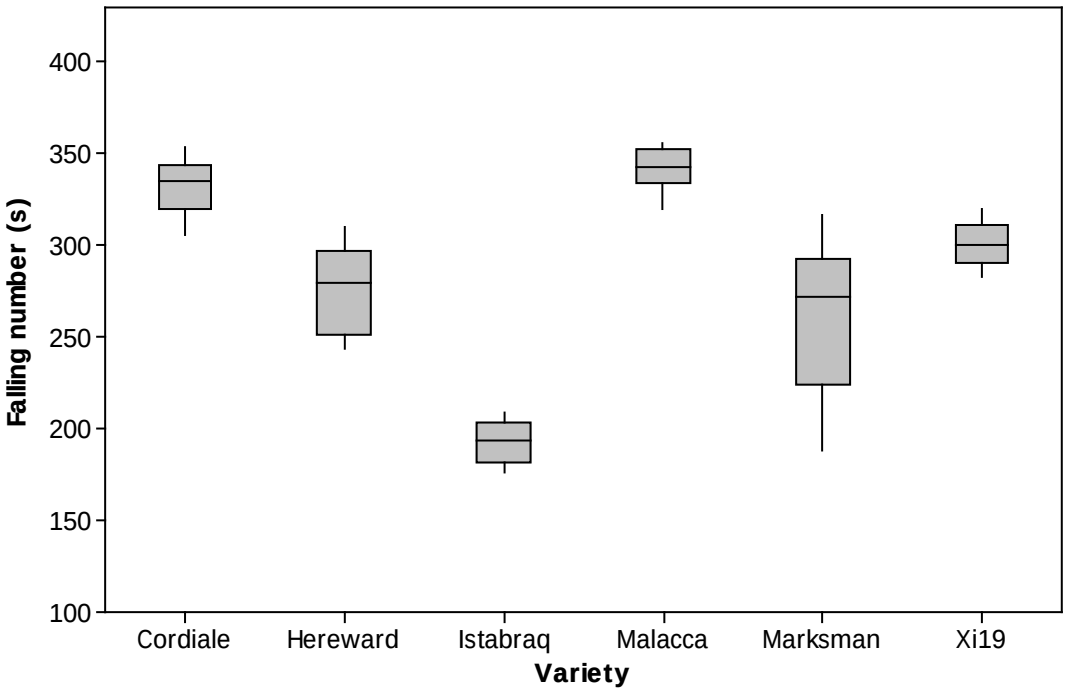


Figure 3.22 Wheat protein content (% dmb) in samples grown at different sites and nitrogen fertilisation regimes over three years. Data are means of three biological replicates.

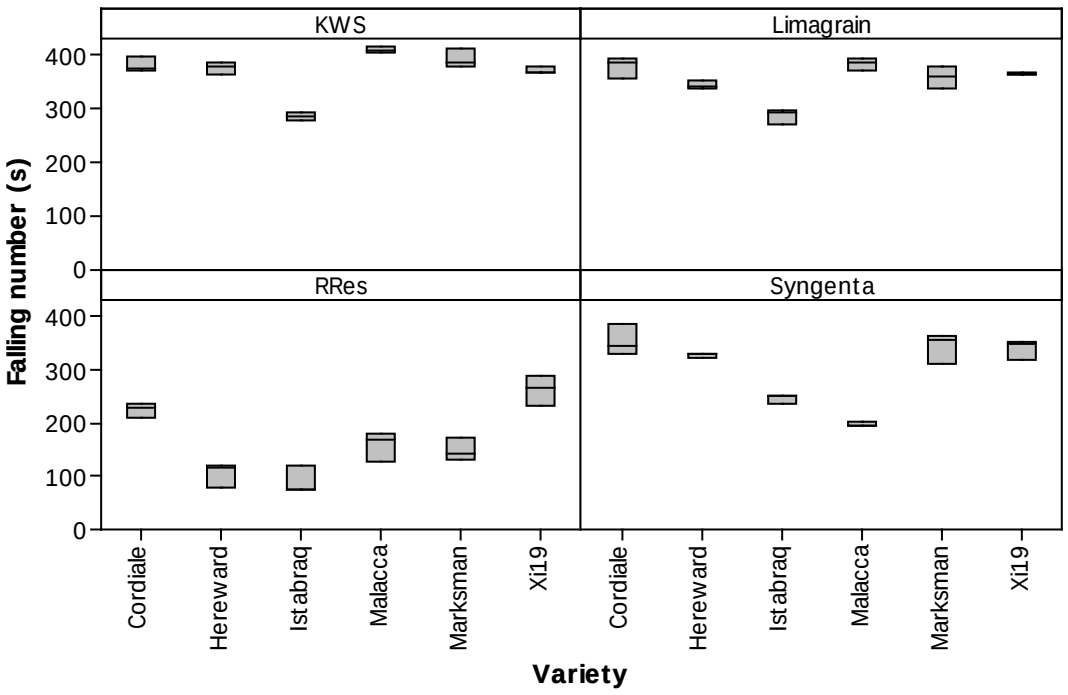
Hagberg Falling Number

There was no effect of the level of nitrogen fertilisation on the Hagberg Falling Number. The Falling Number was generally lowest for Istabraq at each site (Figure 3.23), and was generally low (indicating high α-amylase activity) for all varieties grown at Rothamsted Research in 2010.

A


Falling number (s)
400
350
300
250
200
150
100
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19
Variety

B


KWS
Limagrain
RRes
Syngenta
Falling number (s)
400
300
200
100
0
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19
Variety

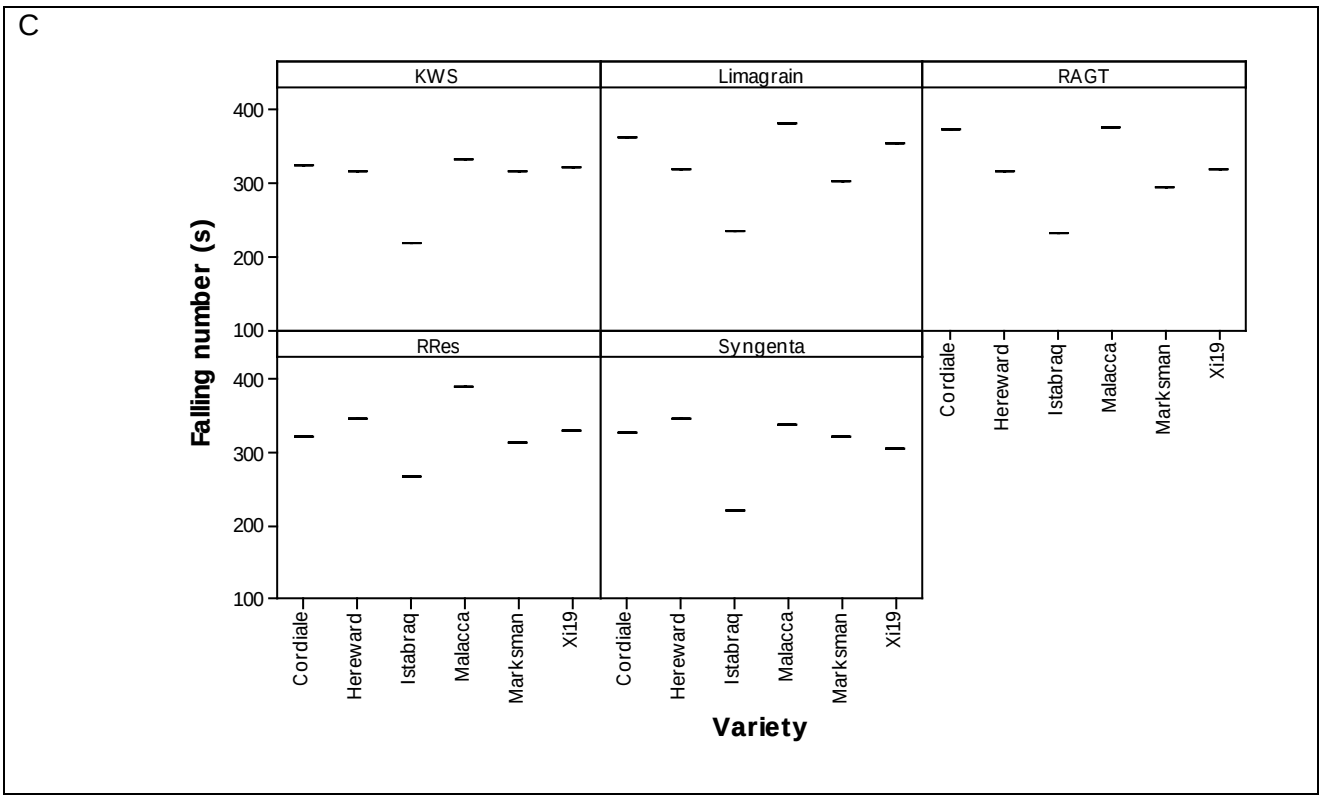
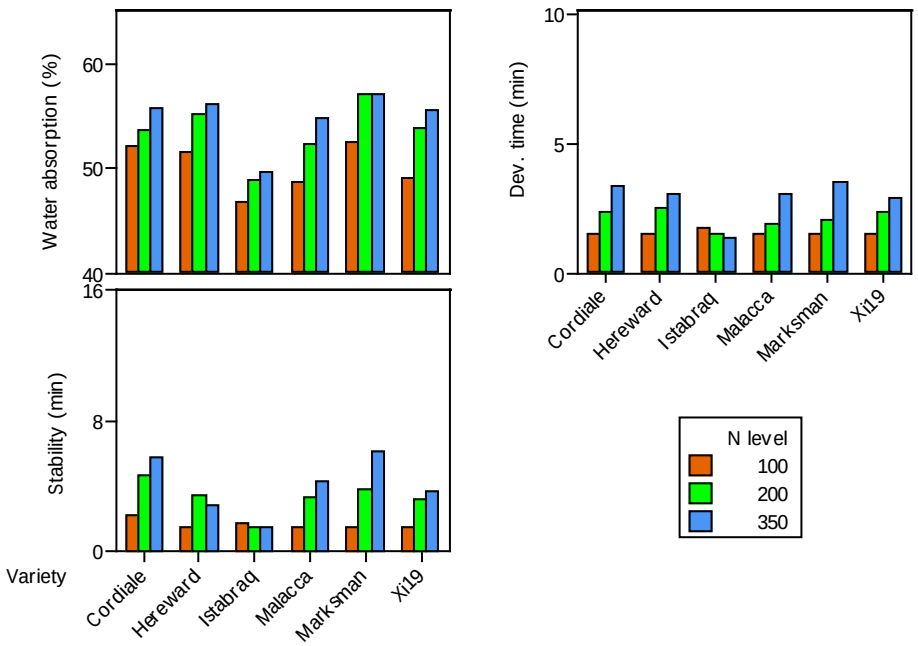


Figure 3.23 Hagberg Falling Number(s) in samples grown at different sites in 2009 (A), 2010 (B) and 2011 (C). For 2009, data are means of three levels of nitrogen fertilisation grown at Rothamsted Research only, for 2010 data are means of three levels of nitrogen fertilisation grown at four different sites, and 2011 data are from one level of nitrogen fertilisation (200 kg/ha) grown at five different sites. Samples are made up of three replicates pooled prior to milling.

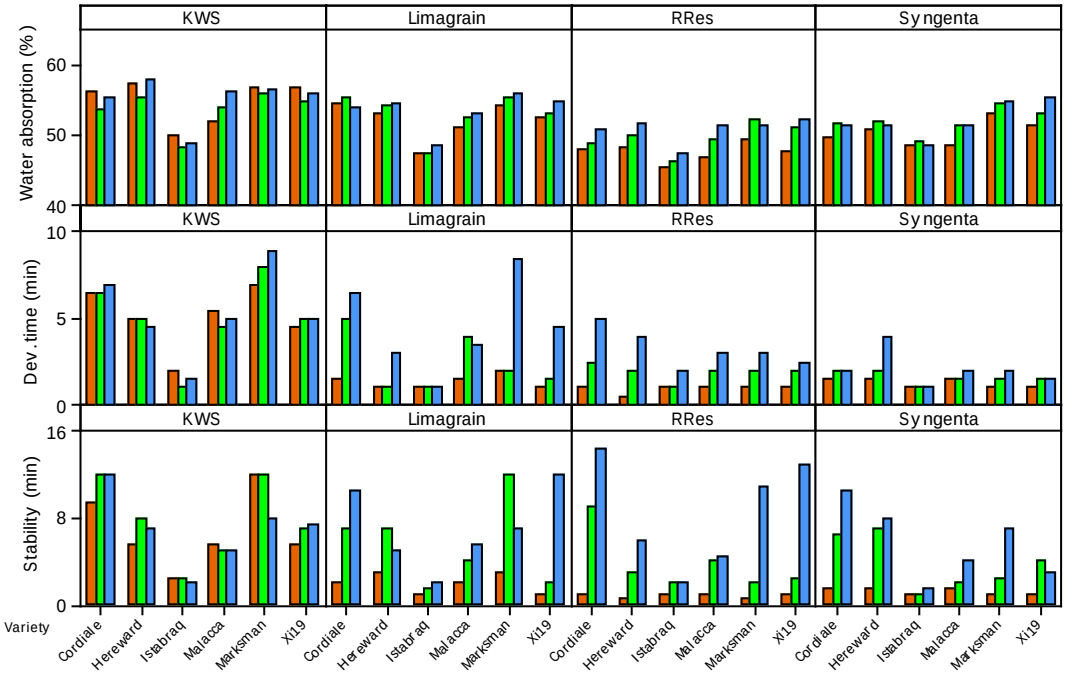
Dough rheology

Dough rheology was determined on white flour samples made from pooled replicates. In general, Istabraq showed the lowest values for water absorption, development time, stability, resistance and extensibility (Figures 3.24 and 3.25). Samples grown at Rothamsted Research showed an effect of nitrogen fertilisation where an increase in the level of nitrogen fertilisation resulted in increases in water absorption, development time, stability, resistance and extensibility. Difference also occurred between varieties in their response to nitrogen fertilisation. A similar trend was seen for samples grown at the Limagrain and Syngenta sites, but not for samples grown at KWS.

A


Water absorption (%)
60
50
40
Dev. time (min)
10
5
0
Stability (min)
16
8
0
Variety
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19
N level
100
200
350

B


KWS
Limagrain
RRes
Syngenta
Water absorption (%)
60
50
40
Dev.time (min)
10
5
0
Stability (min)
16
8
0
Variety
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19

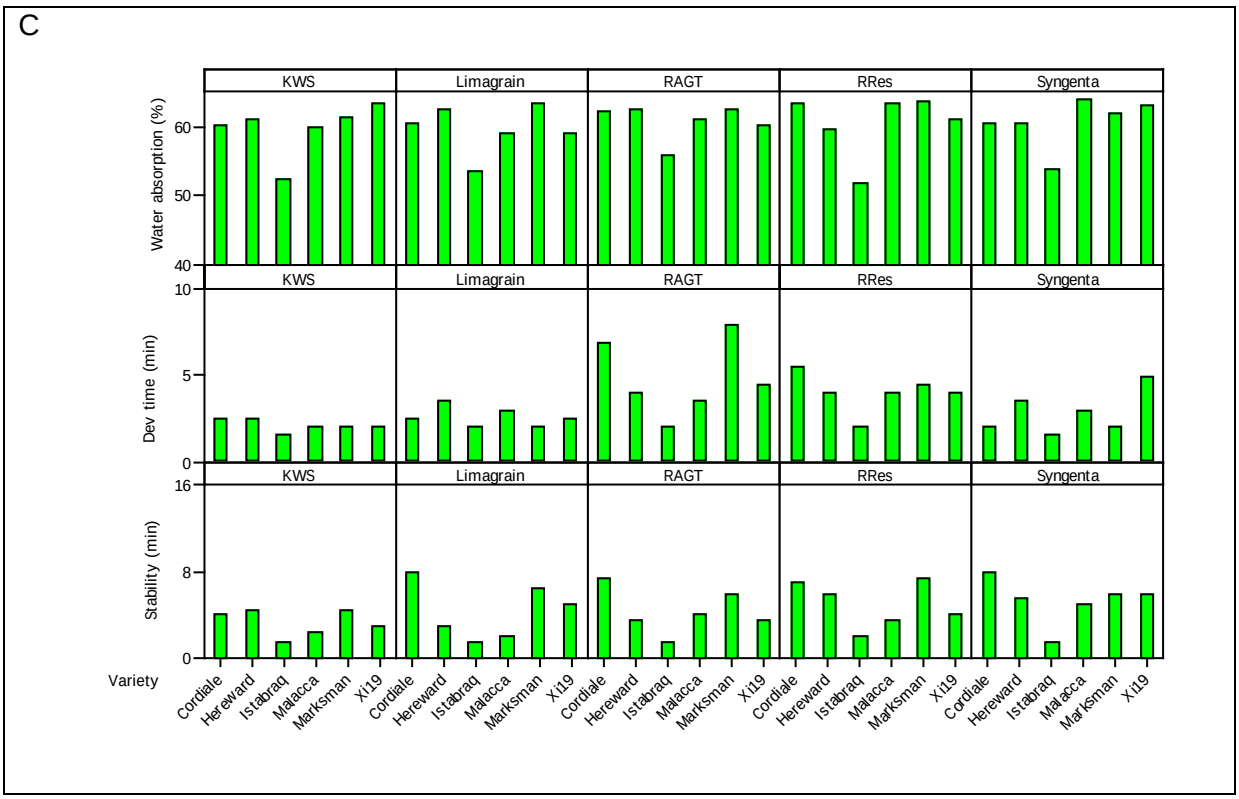
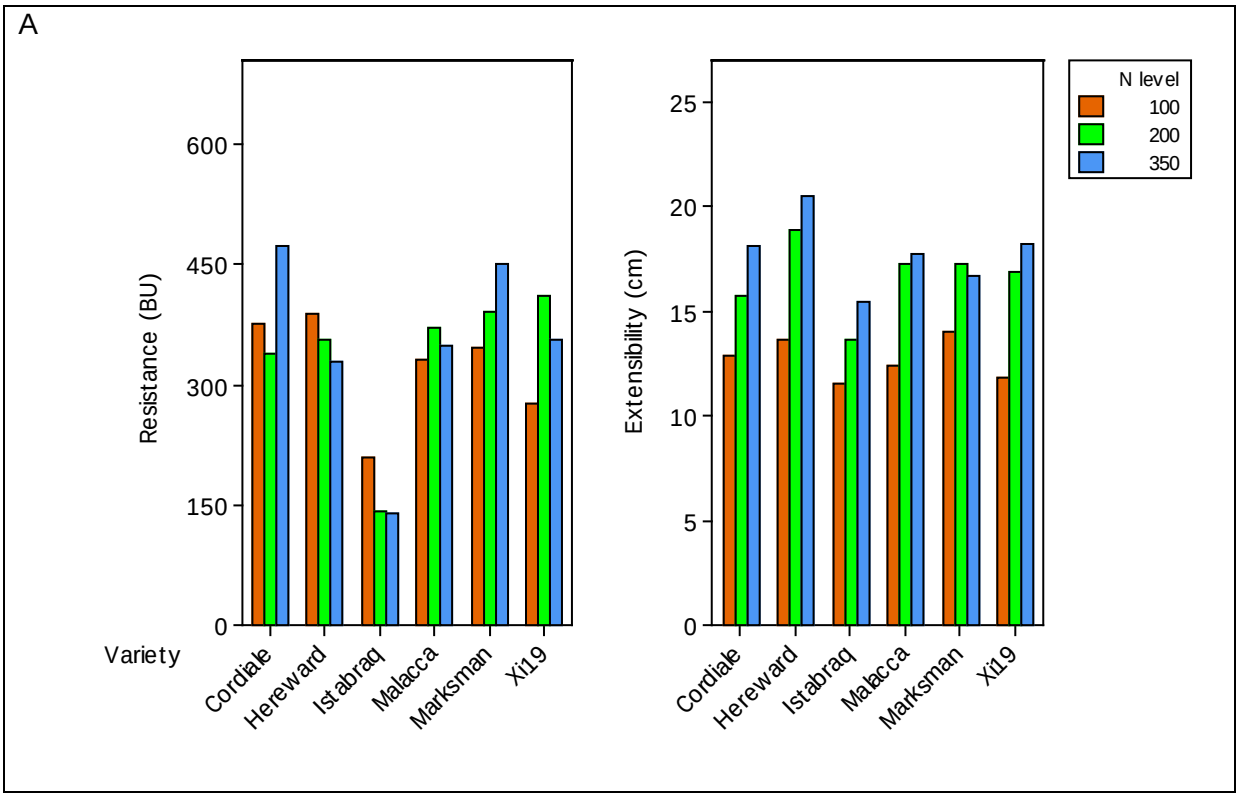


Figure 3.24 Farinograph water absorption, development time and stability for samples grown at different sites over three years. For 2009 (A), data are means of three levels of nitrogen fertilisation grown at Rothamsted Research only, for 2010 (B) data are means of three levels of nitrogen fertilisation grown at four different sites, and 2011 (C) data are from one level of nitrogen fertilisation (200 kg/ha) grown at five different sites. Samples are made up of three replicates pooled prior to milling.



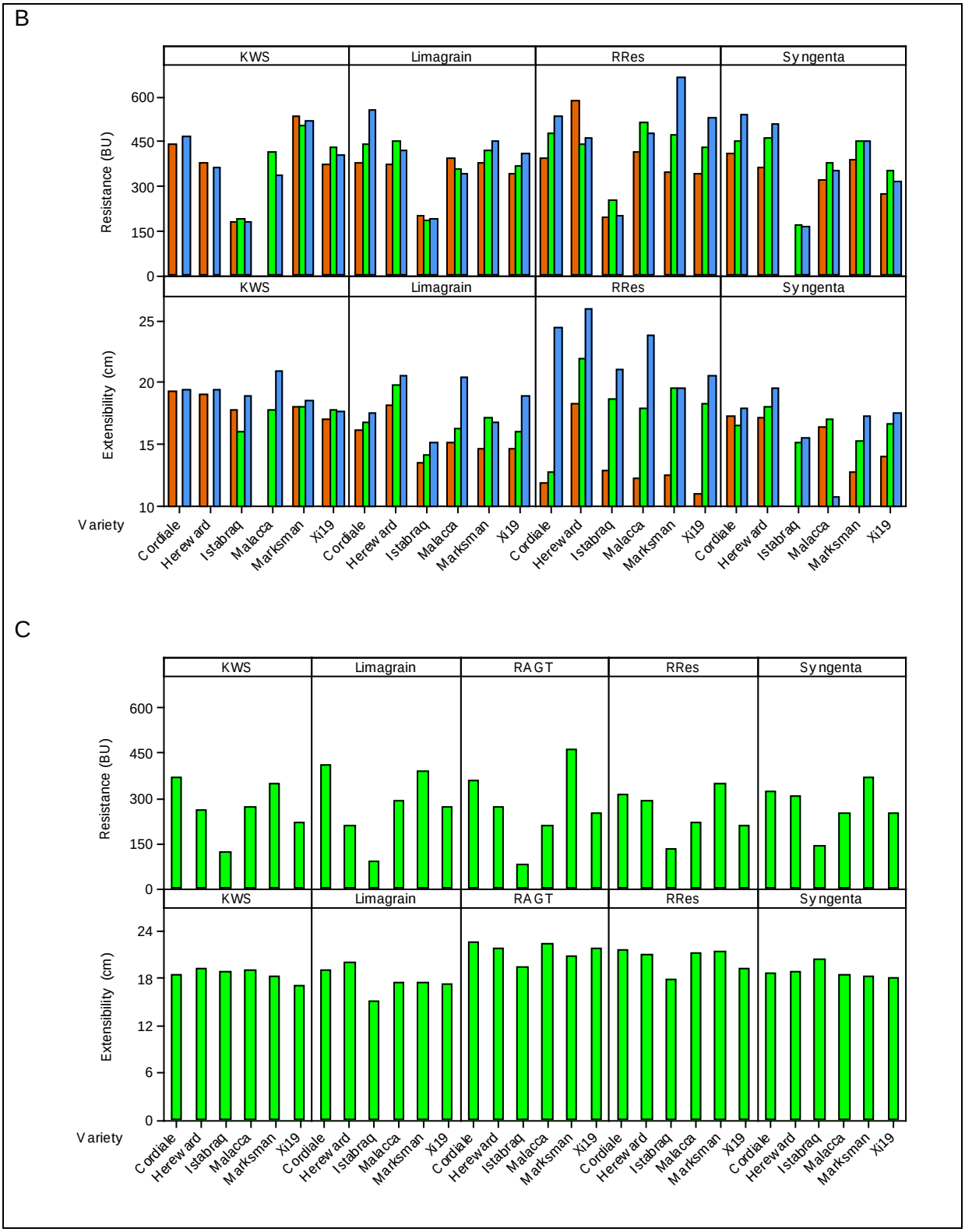


Figure 3.25 Extensibility and resistance for samples grown at different sites over three years, 2009 (A), 2010 (B) and 2011 (C) For 2009, data are means of three levels of nitrogen fertilisation grown at Rothamsted Research only, for 2010 data are means of three levels of nitrogen fertilisation grown at four different sites, and 2011 data are from one level of nitrogen fertilisation (200 kg/ha) grown at five different sites. Samples are made up of three replicates pooled prior to milling.

Reomixer traces were determined in duplicate for each biological replicate. The traces collected were reduced to two principal components from which a quality map was plotted. When all the data for all samples were plotted together, the samples on the quality map were separated by both the level of nitrogen fertilisation (Figure 3.26A) and variety (Figure 3.26B).

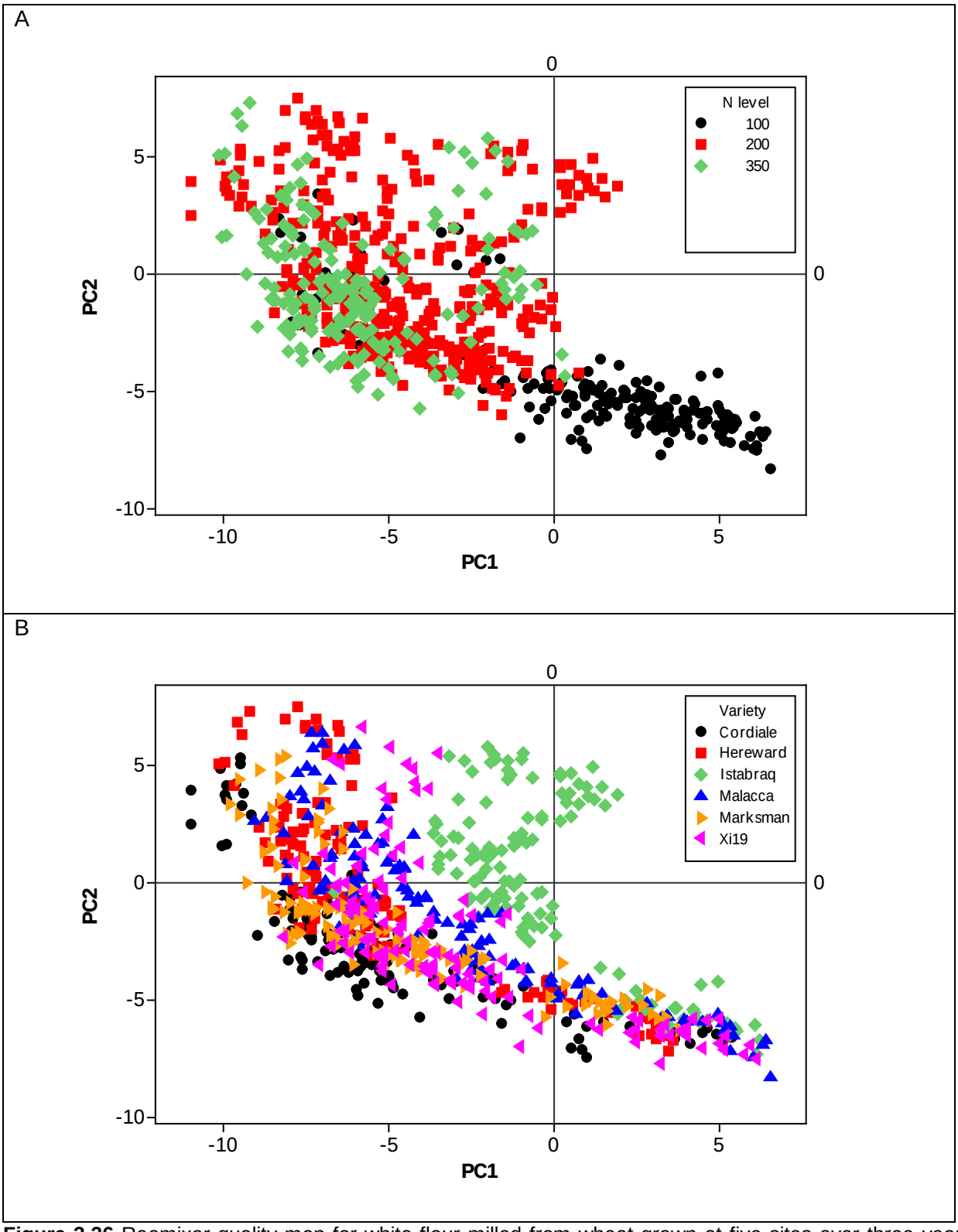


Figure 3.26 Reomixer quality map for white flour milled from wheat grown at five sites over three years at different levels of nitrogen fertilisation showing the effect of nitrogen fertilisation (A) and variety (B).

Figure 3.26 shows some overlap between the N levels which can be explained when the data for 2010 are examined in detail (Figure 3.27). The best separation for nitrogen fertilisation was seen in samples grown at Rothamsted Research, with all samples overlapping for samples grown at KWS.

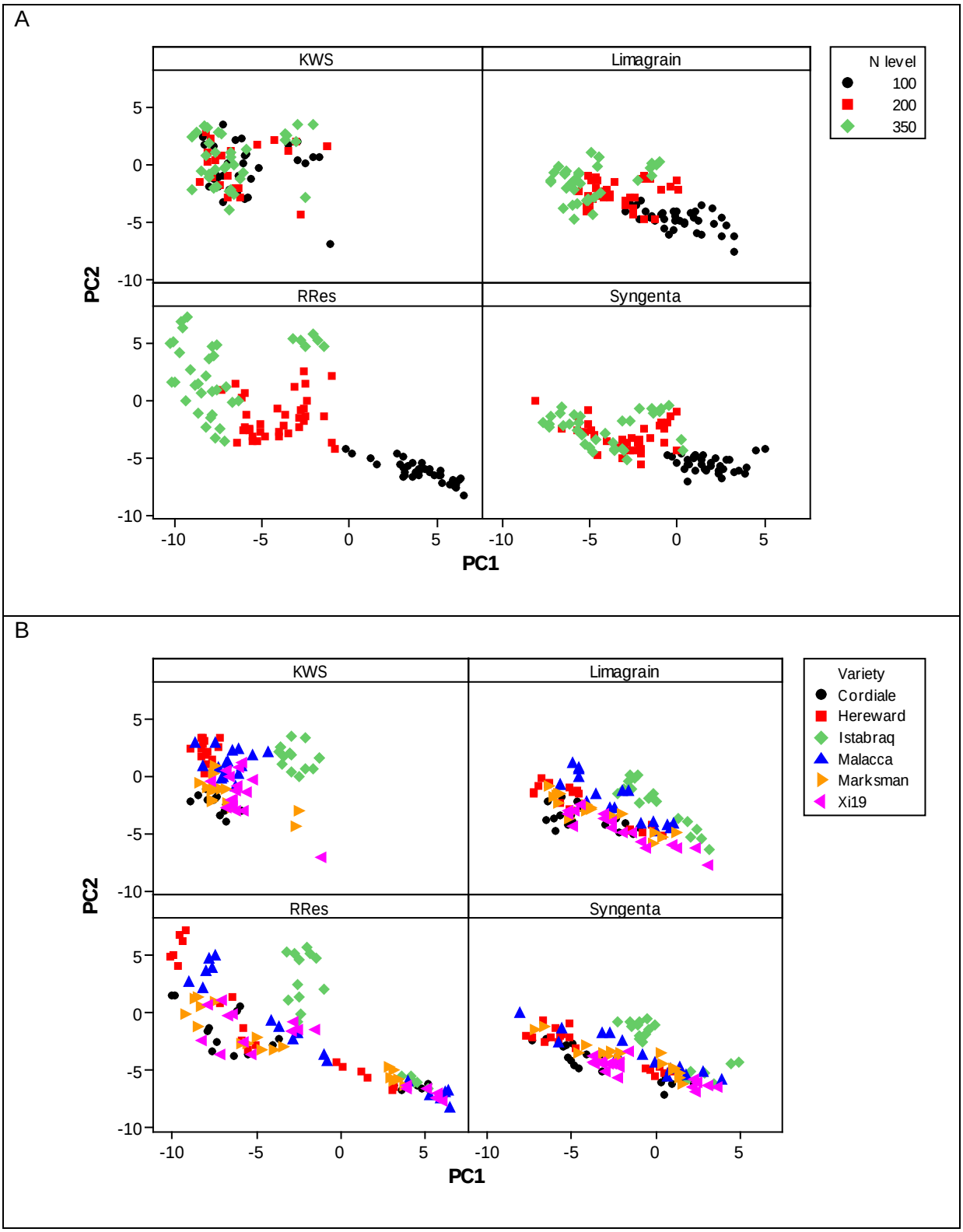


Figure 3.27 Reomixer quality map for white flour milled from wheat grown at four sites in 2010 at different levels of nitrogen fertilisation showing the effect of nitrogen fertilisation (A) and variety (B).

Taken together, the results for total protein and flour rheology indicate that there may have been a problem with the application of the nitrogen fertilisation treatment at the KWS site. There are differences between the varieties in their response to nitrogen fertilisation, with PC1 of the reomixer (reflecting the dough strength) showing a significant interaction between cultivar and nitrogen fertilisation, see Table 3.8.

3.3.5. **Bread baking quality tests**

Campden BRI

The loaf volume of bread baked at Campden BRI generally increasing nitrogen fertilisation (Figures 3.28, 3.29 and 3.30). Bread made from Istabraq consistently had the lowest loaf volume and poorest crumb structure compared with bread made from the other varieties which was expected as Istabraq is not a bread making variety.

For samples grown at Rothamsted Research, there was an increase in loaf volume with an increase in nitrogen fertilisation. This trend was seen for both 2009 and 2010. There was also an increase in crumb score. There was some evidence of a similar effect on bread baked from samples grown at Syngenta and Limagrain in 2010, but not for bread baked from samples grown at KWS.

There was little evidence of an effect of nitrogen fertilisation on the brightness or the number of cells. Brightness varied between bread baked from samples grown at different sites, being lowest in bread baked from samples grown at Rothamsted Research. Crumb scores (subjective assessment) for the bread baked from samples grown in 2011 were lower compared with those for bread baked from samples grown in 2009 and 2010.

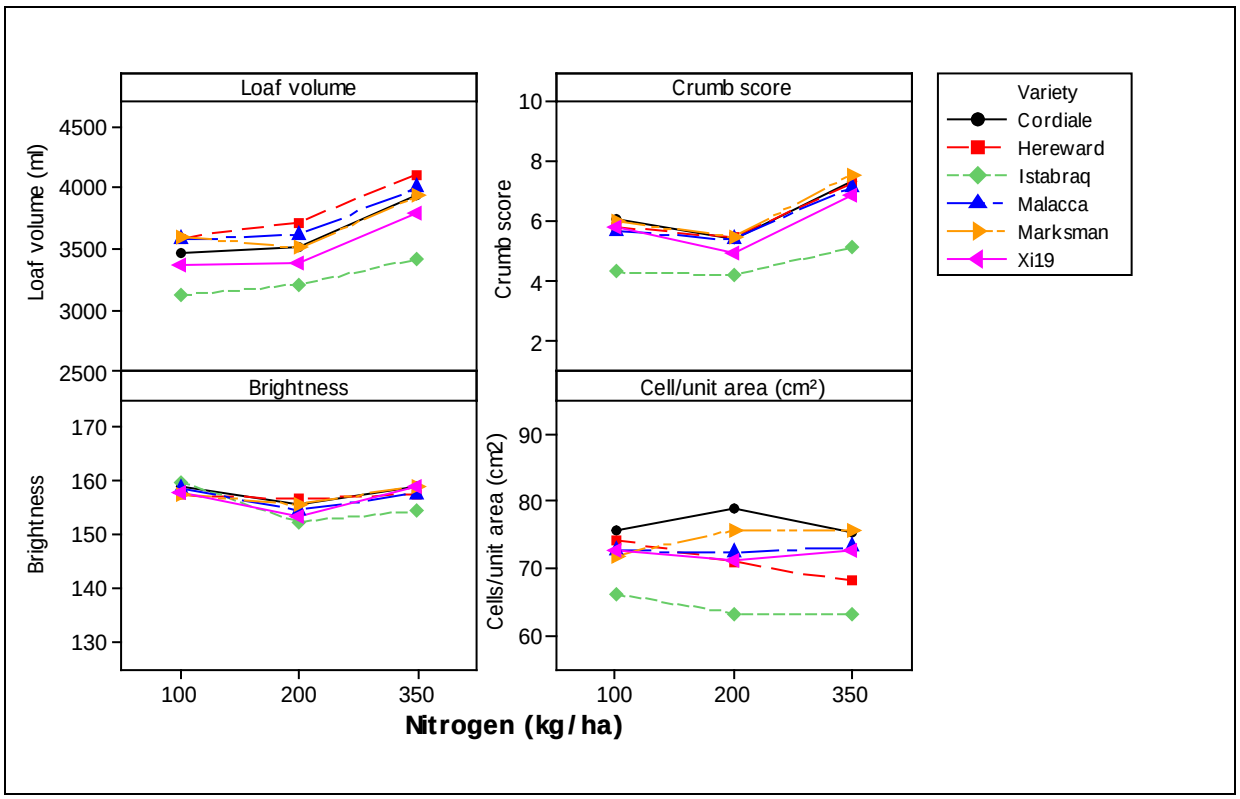
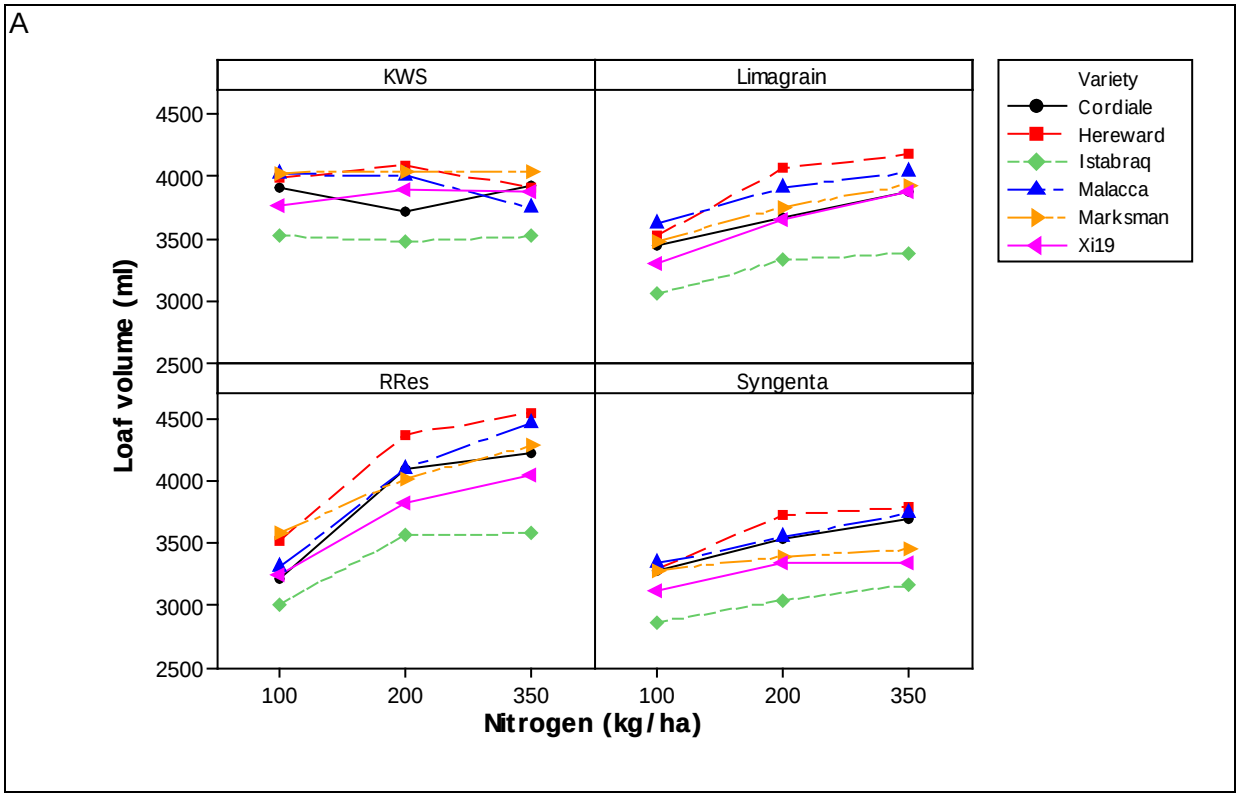
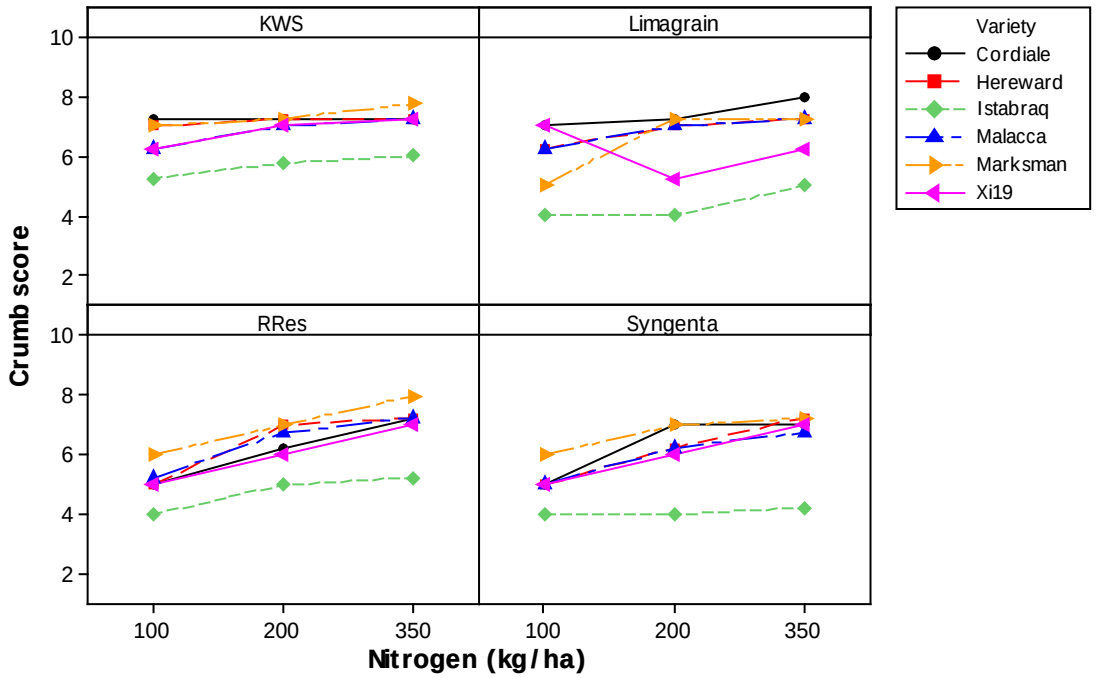


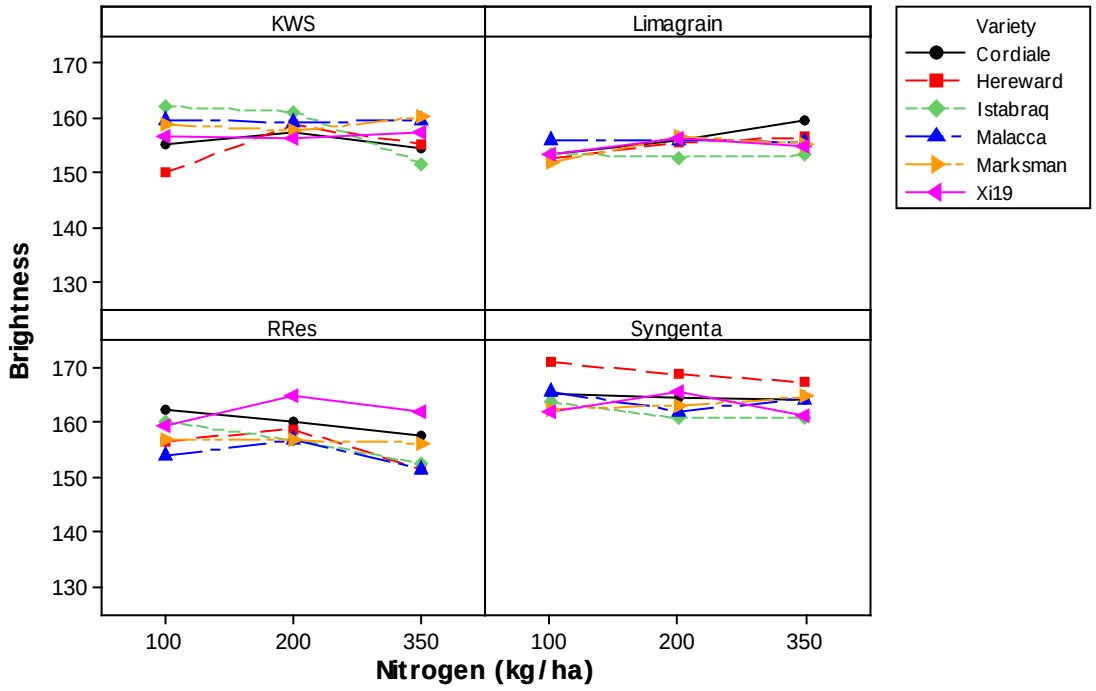
Figure 3.28 Loaf volume, crumb score, brightness and cells per unit area for bread baked from wheat grown at Rothamsted Research at three levels of nitrogen fertilisation in 2009. Data represents the mean of three replicates.



B


KWS
Limagrain
RRes
Syngenta
Crumb score
Nitrogen (kg/ha)
Variety
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19

C


KWS
Limagrain
RRes
Syngenta
Brightness
Nitrogen (kg/ha)
Variety
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19

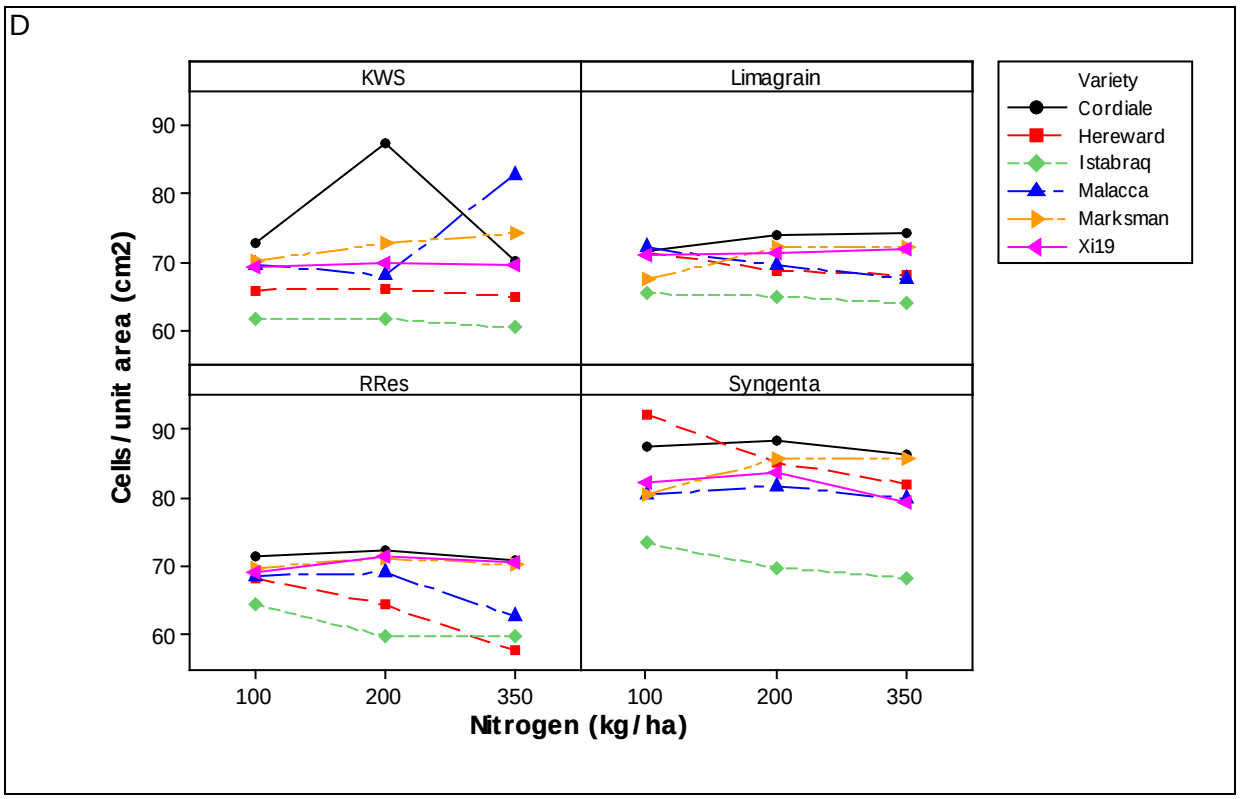
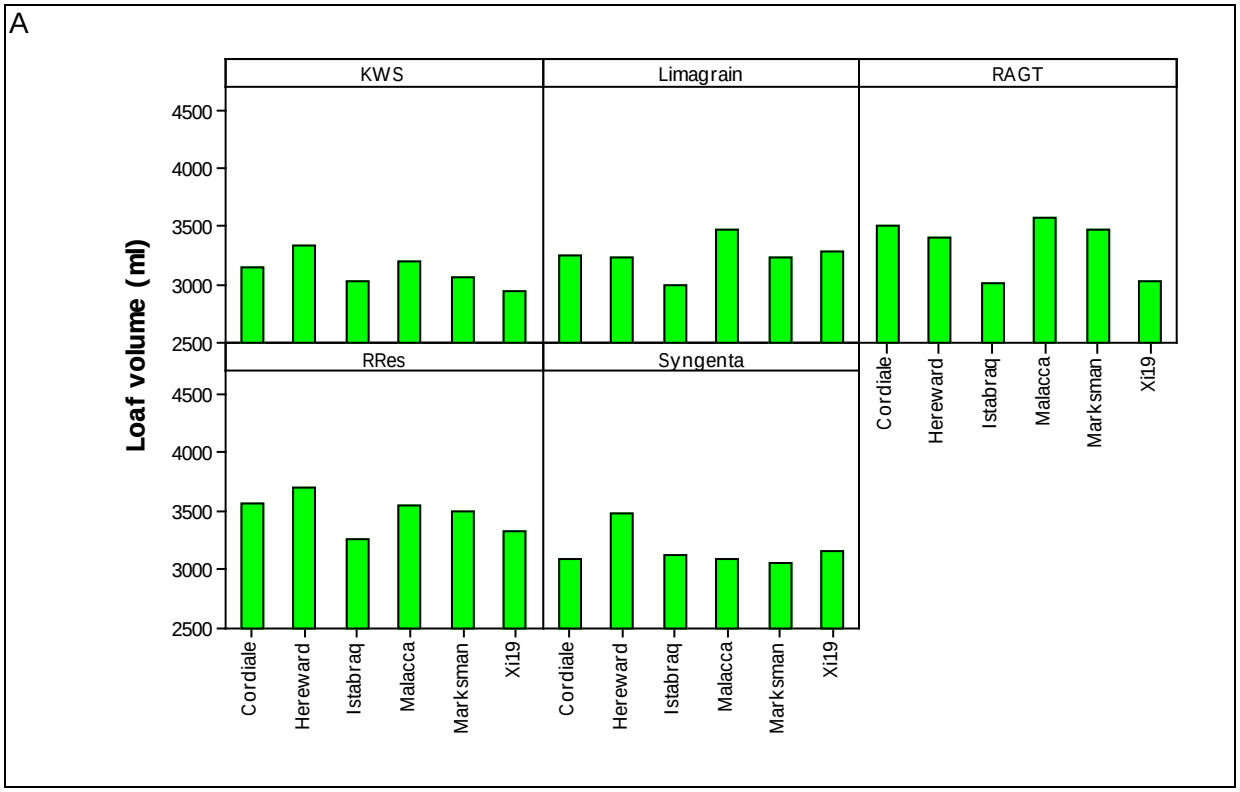
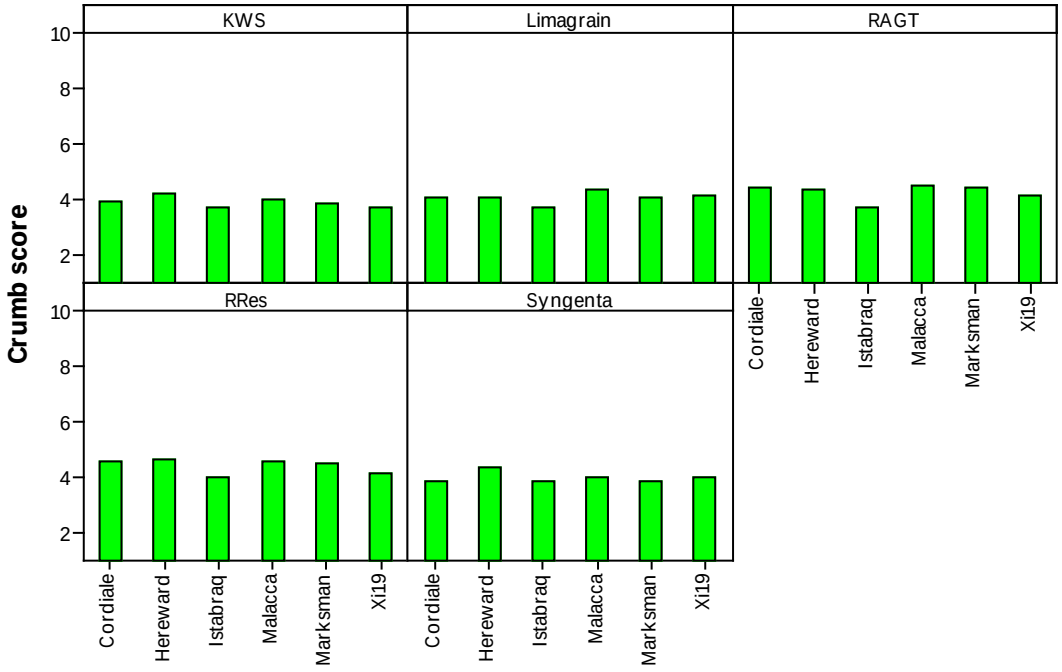


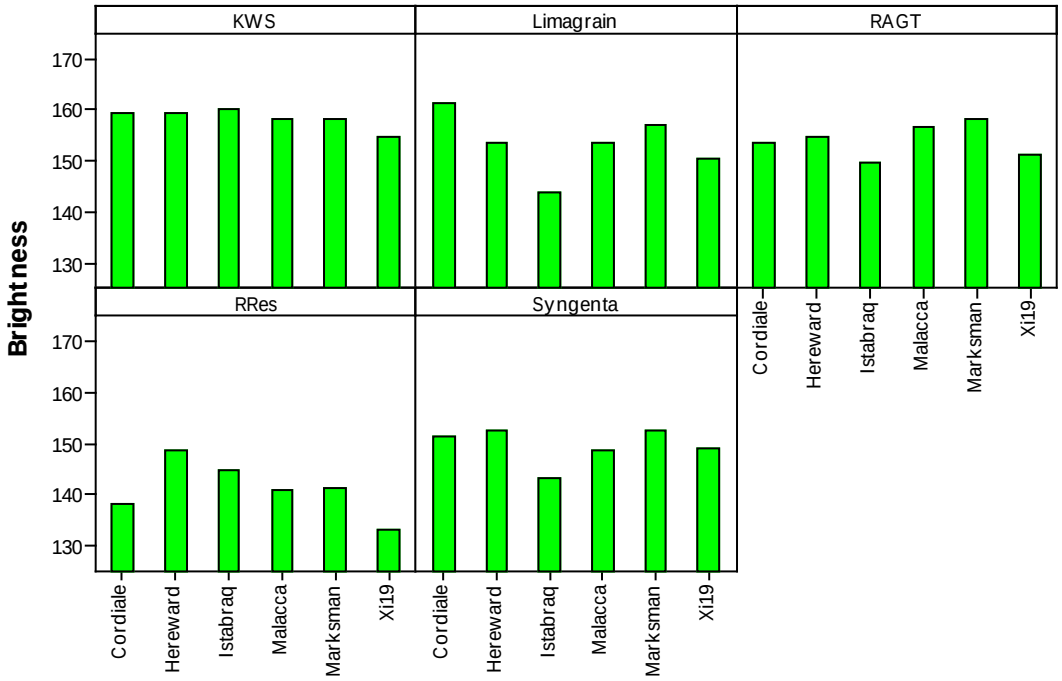
Figure 3.29 Loaf volume (A), crumb score (B), brightness (C) and cells per unit area (D) for bread baked from wheat grown at four different sites and at different levels of nitrogen fertilisation in 2010. Bread was baked from flour milled from three pooled biological replicates.



B


KWS
Limagrain
RAGT
RRes
Syngenta
Crumb score
10
8
6
4
2
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19

C


KWS
Limagrain
RAGT
RRes
Syngenta
Brightness
170
160
150
140
130
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19

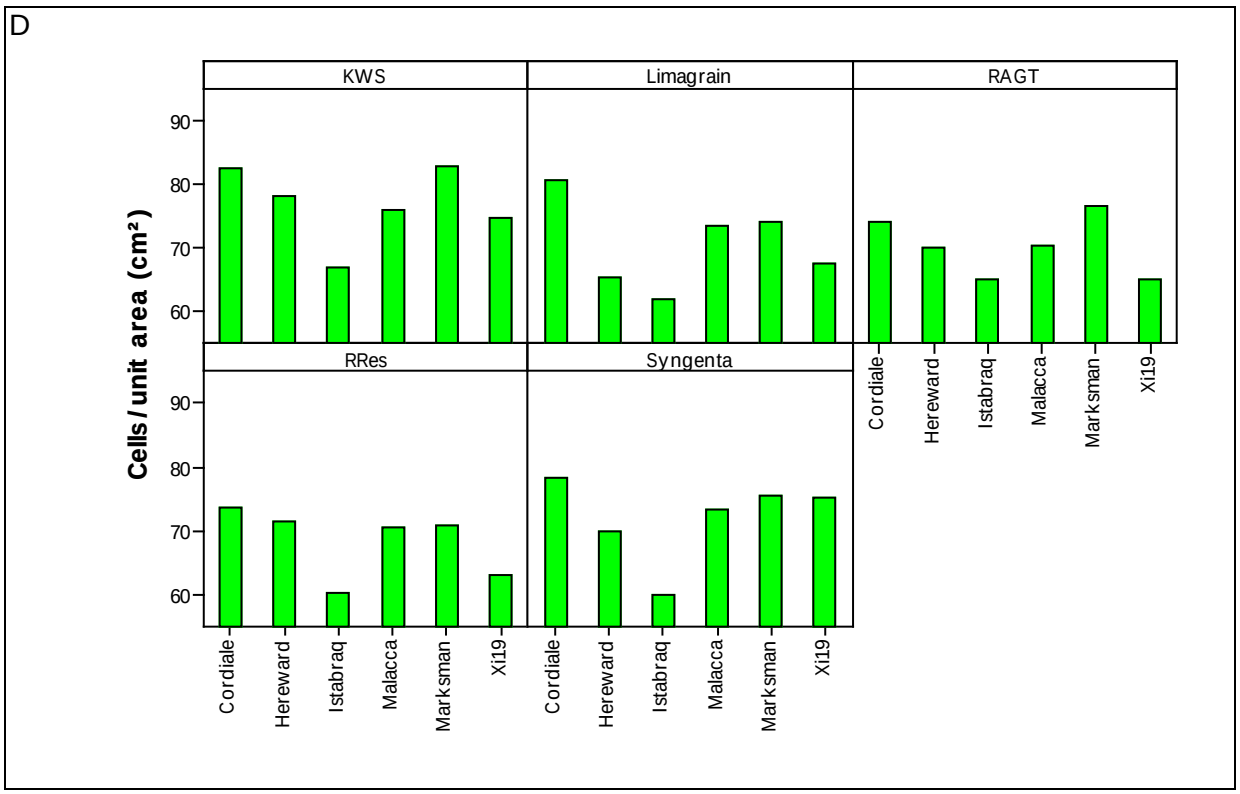
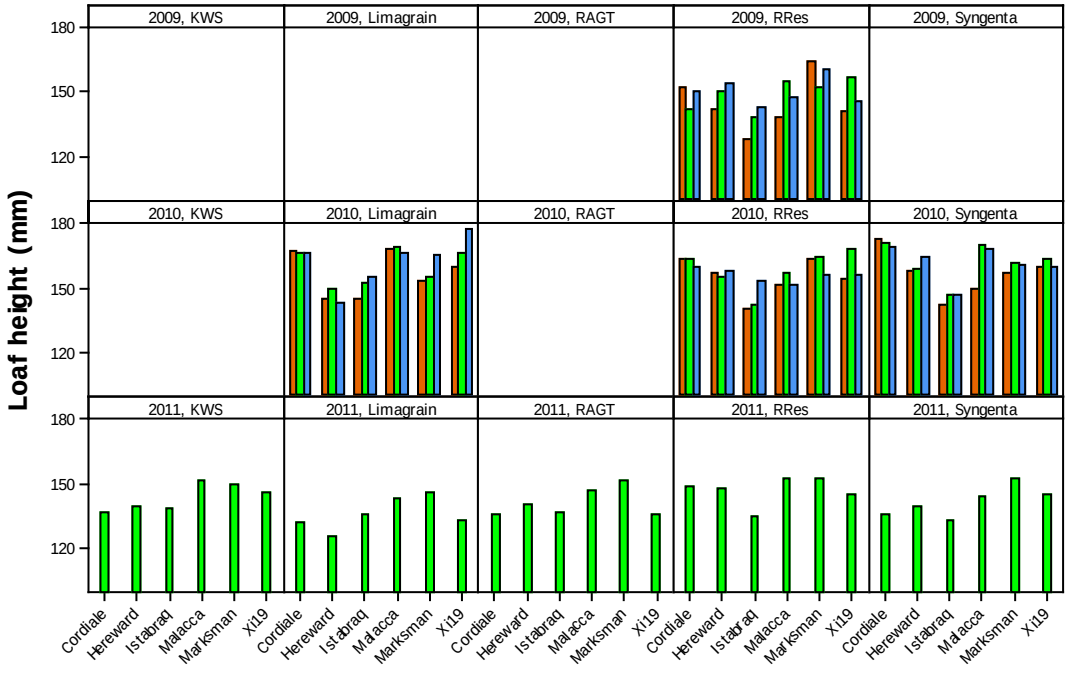


Figure 3.30 Loaf volume (A), crumb score (B), brightness (C) and cells per unit area (D) for bread baked from wheat grown at five different sites and at one level of nitrogen fertilisation (200 kg/ha) in 2011. Bread was baked from flour milled from three pooled biological replicates.

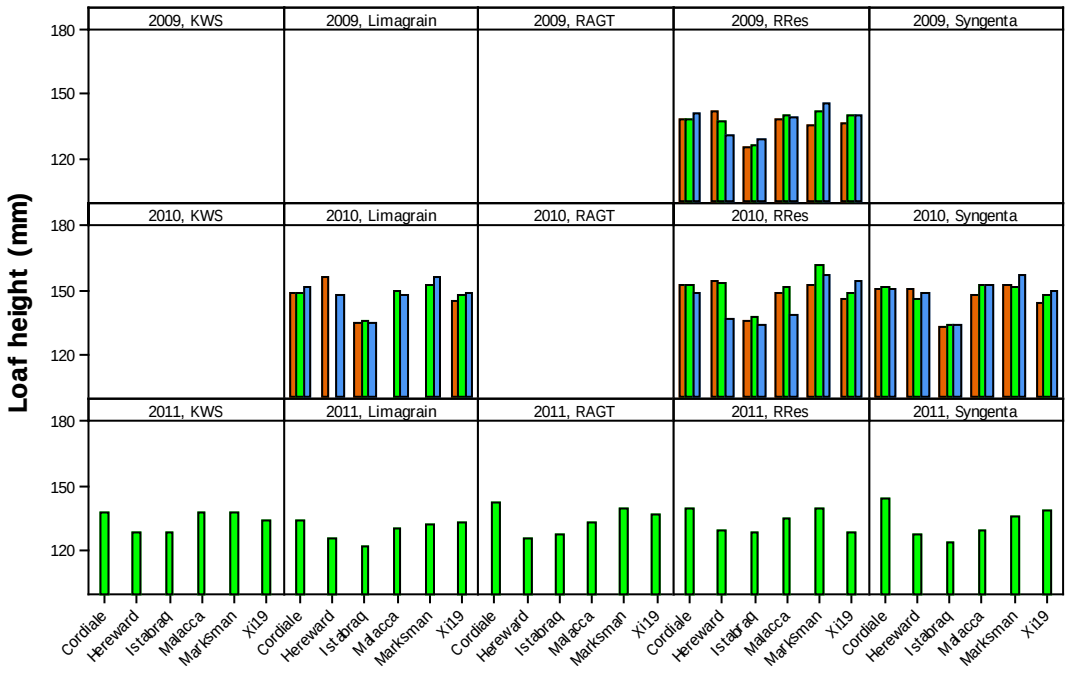
Industry partner data

Bread was baked by industry partners in 2009, 2010 and 2011. In 2010, the samples received from RAGT were not sufficient to allow for baking by the industry partners. The baking assessment was carried out by the industry partners according to their standard protocols, and therefore, the data recorded varied between partners. Data for loaf height was collected by all of the industry partners (Figure 3.31), and this can be used to compare the results. Overall, Warburtons reported lower loaf volumes. There was no consistent effect of nitrogen fertilisation on the height of loaves baked at Warburtons (Table 3.7). Premier and Warburtons reported lower loaf heights for bread baked in 2011.

A


2009, KWS
2009, Limagrain
2009, RAGT
2009, RRes
2009, Syngenta
2010, KWS
2010, Limagrain
2010, RAGT
2010, RRes
2010, Syngenta
2011, KWS
2011, Limagrain
2011, RAGT
2011, RRes
2011, Syngenta
Loaf height (mm)
180
150
120
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19

B


2009, KWS
2009, Limagrain
2009, RAGT
2009, RRes
2009, Syngenta
2010, KWS
2010, Limagrain
2010, RAGT
2010, RRes
2010, Syngenta
2011, KWS
2011, Limagrain
2011, RAGT
2011, RRes
2011, Syngenta
Loaf height (mm)
180
150
120
Cordiale
Hereward
Istabraq
Malacca
Marksman
Xi19

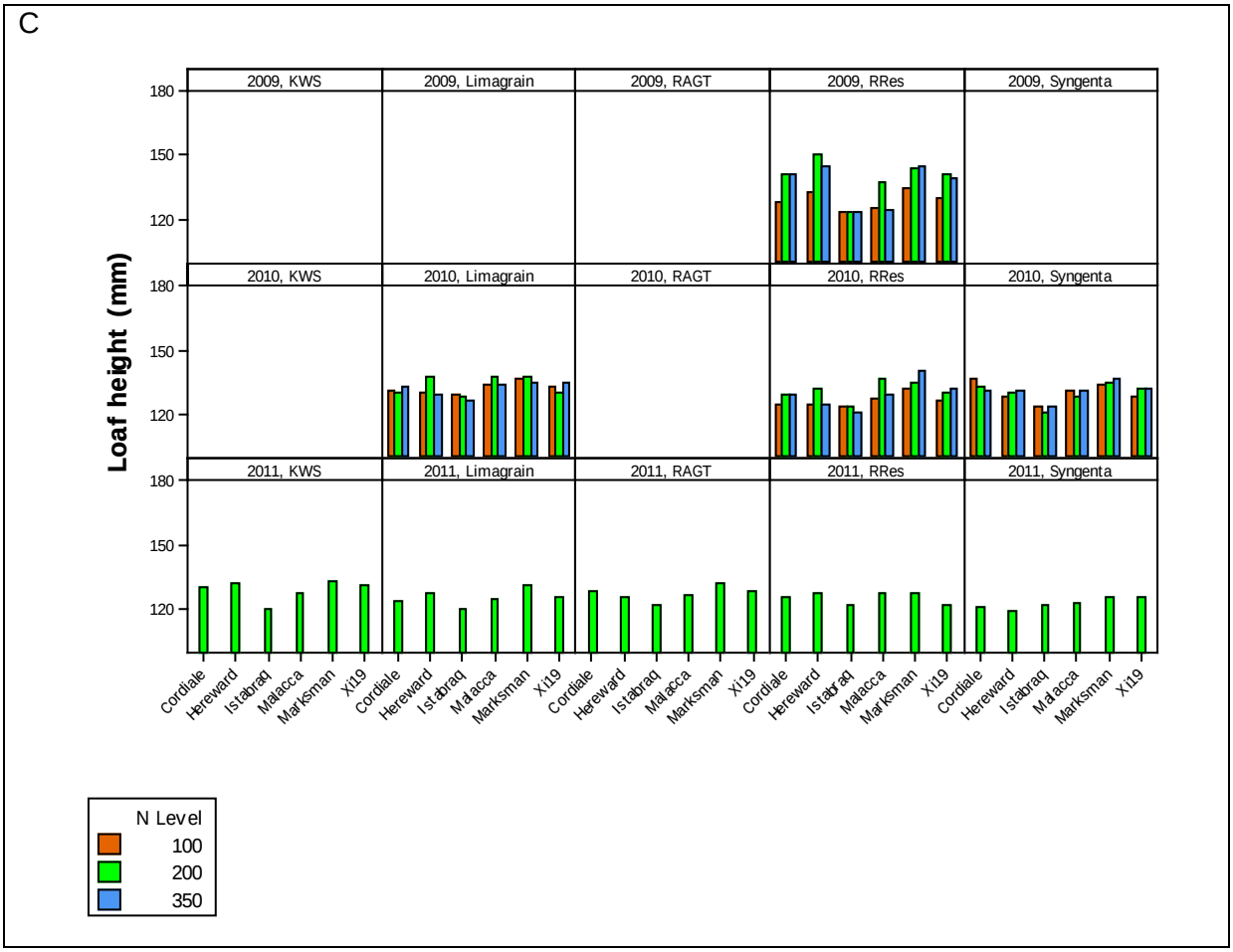


Figure 3.31 Loaf volume of bread baked at Premier (A), Warburtons (B) or ATC (C) from samples grown at five different sites over three years at three levels of nitrogen fertilisation.

Loaf volume was recorded by Premier and ATC (Figure 3.32) and these data can be compared with the loaf volumes recorded by Campden BRI. There was no consistent effect of nitrogen fertilisation on loaf volume.

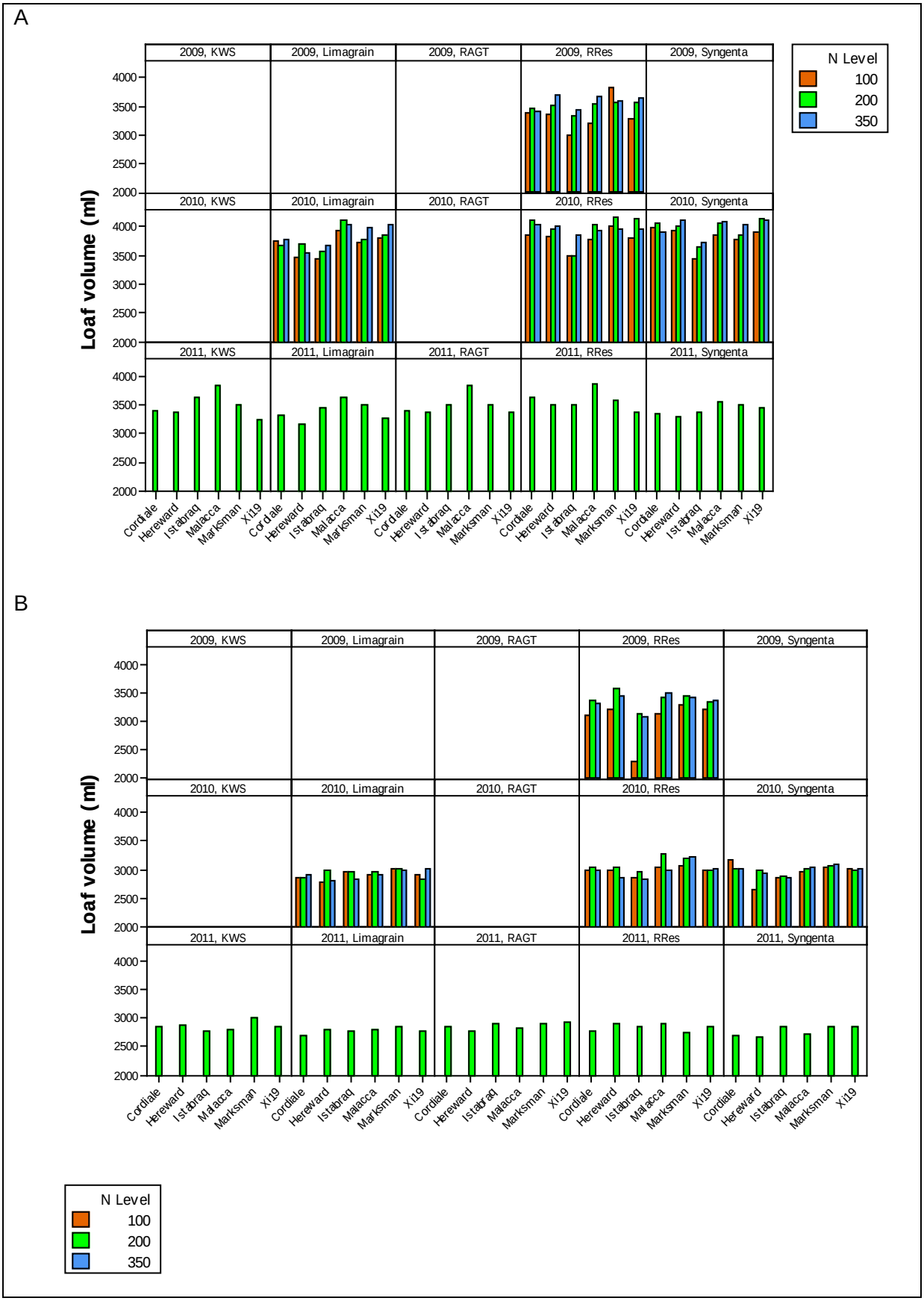


Figure 3.32 Loaf volume of bread baked at Premier (A) and ATC (B) from samples grown at five different sites over three years at three levels of nitrogen fertilisation.

The differences between the results shown by Campden BRI and the industry partners are likely to be due to differences between the processes. The spiral bake performed by Campden BRI is a lean recipe and is baked in an unlidded tin, while ATC and Warburtons loaves were baked in lidded tins. The quality criteria demanded by industry will include parameters other than simply a high loaf volume, such as handling properties, slicing etc.

Data correlations - statistical validation by univariate test

The statistical analysis of all the parameters showed highly significant effect of the genotypic and environmental factors and their interactions for most of the characteristics analysed (Table 3.7). For reference, correlations between parameters are summarised in Figures 3.33 and 3.34 and Tables 3.7–3.11. Where relevant these tables are referred to in the preceding text. The statistical analyses highlight the expected importance of cultivar, N-fertiliser regime and site on protein content and composition, and subsequently dough functionality and baking performance.

The 3-way interaction plots (Figures 3.33 and 3.34) visually illustrate the interactions of a number of variables. Dough strength (as illustrated by PC1 from the reomixer analysis, section 3.3.4) is most strongly related to cultivar and nitrogen (Figure 3.33). Site and year to year variation were generally consistent; however this analysis illustrated a breakdown in the nitrogen effect for the 2010 KWS data.

Table 3.4 Statistical validation of univariate tests of the individual parameters are given in the following tables (a) Three Baking tests : Premium Food, W and ATC, (b) HPLC and SDS-PAGE, (c) HMW subunits x E. The design factors are YS which is a combination of year and site, CV = cultivar, N = N-level and interactions between these factors.

(a) Baking tests

	YS	CV	N	YSxCV	YSxN	CVxN	YS*CV*N
PF_DoughCon10.mins	0,00	0,39	0,00	0,98	0,19	0,67	1,00
PF_Lheight	0,00	0,00	0,04	0,27	0,70	0,75	1,00
PF_OvenSpring	0,00	0,00	0,02	0,03	0,27	0,78	1,00
PF_Volume	0,00	0,00	0,00	0,03	0,76	0,27	0,91
PF_BTex	0,00	0,00	0,22	0,00	0,36	0,90	1,00
PF_Yi	0,00	0,00	0,00	0,37	0,06	0,90	1,00
PF_Wi	0,00	0,00	0,00	0,42	0,04	0,94	1,00
PF_a	0,00	0,00	0,00	0,00	0,00	0,01	0,02
PF_b	0,00	0,00	0,00	0,59	0,01	0,27	0,40
PF_L	0,00	0,00	0,07	0,42	0,04	0,99	1,00
W_height	0,00	0,00	0,85	0,02	0,04	0,00	0,80
W_CColour	0,00	0,80	0,00	0,16	0,00	0,14	0,86
W_Texture	0,00	0,03	0,00	0,17	0,00	0,07	0,94
W_Softness	0,12	0,01	0,83	0,20	0,70	0,04	0,55
W_Resilience	0,00	0,28	0,00	0,16	0,00	0,06	0,09
W_CStrength	0,00	0,00	0,00	0,00	0,00	0,00	0,00
ACT_MixTime	0,00	0,00	0,19	0,98	0,03	0,90	1,00
ATC_DoughTemp	0,00	0,04	0,82	0,02	0,40	0,88	0,40
ATC_ProofHeight	0,00	0,00	0,00	0,00	0,30	0,46	0,50
ATC_Bheight	0,00	0,00	0,09	0,81	0,40	0,90	1,00
ATC_OvenSpring	0,00	0,00	0,83	0,96	0,37	0,92	1,00
ATC_LoafVol	0,00	0,01	0,08	0,71	0,08	0,99	1,00
ATC_BreadMoist	0,00	0,00	0,06	0,37	0,01	0,46	0,55
ATC_Ccolour_L	0,00	0,02	0,42	0,00	0,40	0,99	1,00
ATC_CColourLb	0,00	0,00	0,00	0,00	0,00	0,32	0,40
ATC_Ccompres	0,00	0,00	0,00	0,37	0,00	0,92	0,99
ATC_Height	0,00	0,00	0,02	0,76	0,02	0,94	1,00
ATC_TotConcavity	0,00	0,01	0,00	0,46	0,52	0,92	1,00
ATC_Left.Concavity	0,04	0,47	0,04	0,99	0,54	0,99	1,00
ATC_RightConcavity	0,00	0,06	0,01	0,67	0,46	0,55	1,00
ATC_Side.wallConcavity	0,01	0,27	0,02	0,98	0,46	0,94	1,00
ATC_TopConcavity	0,00	0,00	0,03	0,04	0,86	0,97	0,64
ATC_SliceBrightness	0,00	0,01	0,00	0,00	0,00	0,14	0,40
ATC_NumberOfCells	0,00	0,58	0,04	0,42	0,00	0,90	0,86
ATC_NormalisedCellCount	0,00	0,00	0,00	0,37	0,01	0,90	0,86
ATC_WallThickness	0,00	0,02	0,01	0,46	0,04	0,94	0,81
ATC_CellDiameter	0,00	0,00	0,01	0,93	0,18	0,90	1,00
ATC_CellVolume	0,00	0,00	0,01	0,81	0,05	0,90	1,00

(b) **Statistics of the SE-HPLC data**

	F1	F2	F3	F4	F5
YS	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**
CV	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**
N	0,14	**0,00**	**0,00**	**0,00**	**0,00**
YSxCV	0,65	**0,00**	**0,00**	0,65	1,00
YSxN	**0,01**	**0,01**	**0,00**	**0,00**	**0,00**
CVxN	0,43	**0,01**	**0,01**	0,91	0,96
YS*CV*N	1,00	0,17	1,00	1,00	1,00

SDS-PAGE groups of proteins analysed at Rothamsted

	HMW GS_%	Omega GLIADIN_%	LMW_GS + GLIADINS %
YS	**0**	**0**	**0**
CV	**0**	**0**	**0**
N	**0**	**0**	**0**
YS x CV	**0**	**0**	**0**
YS x N	**0**	**0**	**0**
CV x N	**0.05**	**0**	0.8
YS*CV*N	0.96	**0**	0.96

SDS-PAGE groups of proteins analysed at Campden

	HMW GS %	Omega GLIADIN %	LMW GS + GLIADINS %
YS	**0.00**	**0.00**	**0.00**
CV	**0.00**	**0.00**	**0.00**
N	**0.00**	**0.00**	**0.00**
YSxCV	**0.00**	**0.00**	**0.00**
YSxN	**0.00**	**0.00**	**0.00**
CVxN	**0.00**	0.21	0.12
YS*CV*N	**0.00**	**0.00**	**0.00**

(c) HMW subunits and ω-gliadins x E

Model Term	1Ax1	1Dx	1Bx	1By	1Dy	ω5a	ω5b	ω5c	ω(1+2)a	ω(1+2)b	LMS
Site	0.169	0.538	<0.001	<0.001	0.001	<0.001	<0.001	0.010	0.119	<0.001	<0.001
Year	<0.001	<0.001	0.037	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cultivar	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.040	<0.001	<0.001	<0.001
N	<0.001	0.002	<0.001	<0.001	0.551	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Site.Year	0.147	<0.001	0.013	0.101	<0.001	0.324	<0.001	0.646	0.099	0.739	<0.001
Site.Cultivar	**0.035**	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	0.007	<0.001	<0.001	<0.001
Year.Cultivar	0.389	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Site.N	0.268	0.549	0.112	0.005	0.111	<0.001	<0.001	0.068	<0.001	0.345	**<0.001**
Year.N	<0.001	<0.001	0.234	0.035	0.747	<0.001	<0.001	**0.017**	<0.001	<0.001	**<0.001**
Cultivar.N	0.505	**<0.001**	**0.001**	**<0.001**	**<0.001**	<0.001	0.018	0.192	0.006	<0.001	0.984
Site.Year.Cultivar	0.999	**<0.001**	**<0.001**	**<0.001**	**0.006**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	<0.001	**<0.001**
Site.Year.N	0.638	**<0.001**	**0.007**	**<0.001**	0.515	**0.056**	**0.003**	0.162	**0.021**	0.277	0.119
Site.Cultivar.N	0.168	0.300	0.910	0.875	0.485	0.950	0.750	0.134	0.407	0.007	0.992
Year.Cultivar.N	**0.036**	0.282	0.179	0.265	0.170	**0.015**	**0.028**	0.121	**0.005**	<0.001	0.954
Site.Year.Cultivar.N	0.416	0.162	0.890	0.991	0.811	0.320	0.239	0.402	0.114	**<0.001**	0.980

Table 3.5 Statistical validation (p-values, significant if p<0.05) of the effect of the design parameters (Year*Site, CV=cultivar, N-level, and their interaction) on results from Reomixer data given as Principal Component scores from Principal Component Analysis (PCA). The data analysed comprises all data from 2009 and 2010, and samples as 200 N level in 2011.

	Reo_strength_pc1	Reo_stability_pc2
Year_Site	0,000	0,000
CV	0,001	0,000
N-level	0,003	0,001
Year_Site*CV	0,286	0,000
Year_Site*N-level	0,030	0,000
CV*N-level	0,013	0,651
Year_Site*CV*N-level	0,030	0,016

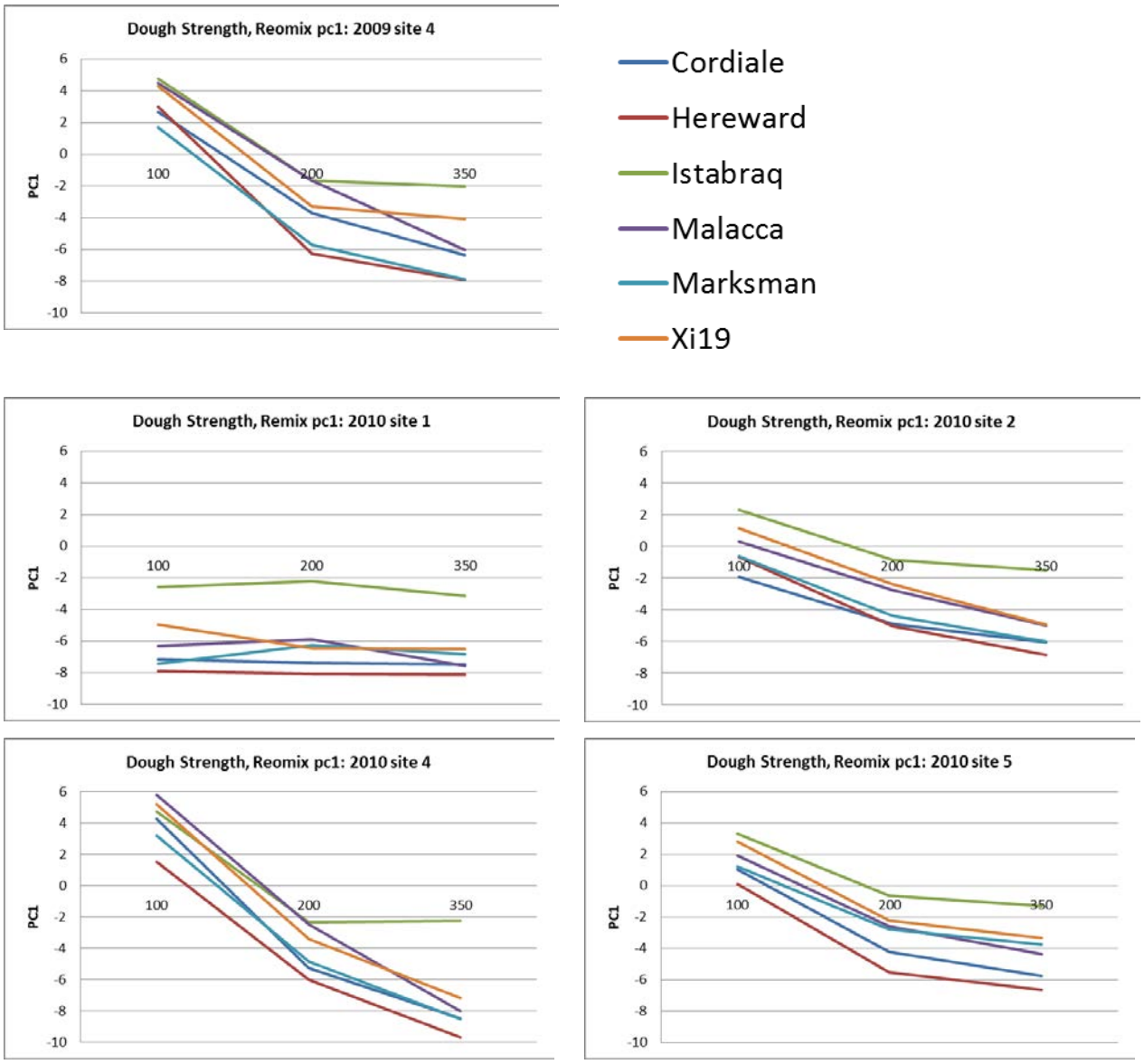


Figure 3.29 Plots of the three-way interaction Cultivar * N at different location for Dough Strength (x-axis represents the 3 N-inputs in kg/ha). The sites are as follows: 1, KWS; 2, Limagrain; 4, Rothamsted (data for 2009 and 2010); 5, Syngenta.

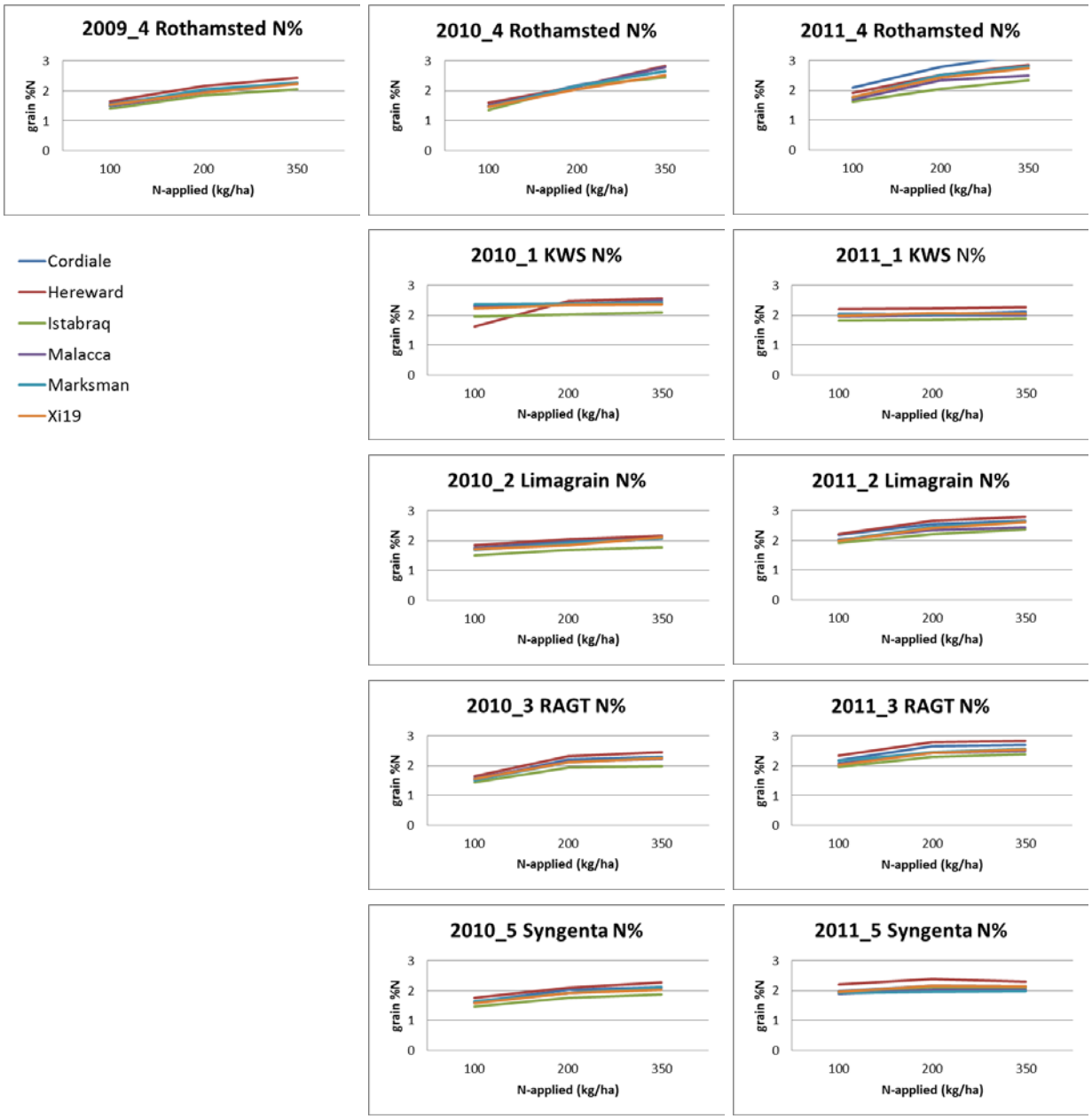


Figure 3.30 Plots of the three-way interaction Cultivar * N at different location for N% in the grain analysed by Rothamsted.

Table 3.6 Statistical validation (p-values, significant if p<0.05) of the effect of the design parameters (Year*Site, CV=cultivar, N-level, and their interaction) on the yield and protein content obtained on all samples of the experiment.

	R_Yield	R_N
Year_Site	0.000	0.000
CV	0.000	0.000
Nlevel	0.000	0.000
Year_Site*CV	0.001	0.000
Year_Site*N-level	0.000	0.000
CV*Nlevel	0.369	0.369
Year_Site*CV*N-level	0.999	0.999

Table 3.7 Simple correlation among yield, quality and baking characteristics for 2009 and 2010 at Rothamsted. Correlation coefficients r = 0.70 and above are marked yellow.

	R_yield	R_n	Dx	Bx	By	Dy	ω-gliadin 5	ω-gliadin 1_2	R_%_HWGS	R_%_ω-gliadins	R_%_LWGS	Specific weight	Protein AsIs	SKCS	F_Water_Abs	ReoPC1	ReoPC2	F1	F2	F3	F4	F5	F1.F2	F3F4.F1	AT	F1F2	F3F4	F1F2.F3F4	Gluten Protein
R_yield	1	0.35	0.19	-0.24	0.01	-0.08	0.14	-0.14	0.60	0.31	-0.54	0.72	0.07	0.41	0.50	-0.46	0.28	-0.44	-0.55	0.72	0.68	-0.22	-0.19	0.72	0.52	-0.57	0.75	-0.80	0.47
R_n	0.35	1	0.03	-0.17	0.14	0.03	0.40	-0.40	0.73	0.58	-0.77	0.05	0.90	0.89	0.55	-0.94	0.87	0.50	0.06	0.19	0.74	-0.95	0.44	-0.06	0.94	0.32	0.69	-0.20	0.97
Dx	0.19	0.03	1	0.50	-0.03	0.74	0.57	-0.57	-0.02	0.35	-0.20	0.08	-0.06	-0.16	-0.26	0.08	0.24	-0.33	0.12	0.21	-0.06	0.11	-0.43	0.30	-0.02	-0.13	0.00	-0.12	-0.03
Bx	-0.24	-0.17	0.50	1	-0.54	0.48	0.68	-0.68	-0.38	0.33	0.05	-0.15	-0.15	-0.43	-0.43	0.25	0.03	-0.06	0.62	-0.04	-0.45	0.10	-0.39	-0.11	-0.26	0.32	-0.40	0.42	-0.24
By	0.01	0.14	-0.03	-0.54	1	-0.24	-0.29	0.29	0.15	0.04	-0.11	-0.31	0.13	0.32	0.16	-0.07	0.06	0.13	-0.13	0.01	0.07	-0.06	0.18	-0.09	0.10	0.00	0.06	-0.07	0.10
Dy	-0.08	0.03	0.74	0.48	-0.24	1	0.41	-0.41	-0.19	0.11	0.03	-0.16	0.02	-0.18	-0.28	0.07	0.15	0.04	0.36	-0.22	-0.17	-0.01	-0.18	-0.08	-0.08	0.22	-0.19	0.19	-0.06
ω-gliadin 5	0.14	0.40	0.57	0.68	-0.29	0.41	1	-1.00	0.23	0.88	-0.62	0.02	0.31	0.10	-0.13	-0.30	0.64	0.00	0.40	0.29	0.17	-0.41	-0.26	0.14	0.30	0.23	0.22	0.02	0.32
ω-gliadin 1_2	-0.14	-0.40	-0.57	-0.68	0.29	-0.41	-1.00	1	-0.22	-0.88	0.62	-0.01	-0.31	-0.10	0.14	0.30	-0.64	0.00	-0.40	-0.29	-0.17	0.41	0.27	-0.14	-0.29	-0.23	-0.22	-0.02	-0.32
R_%_HWGS	0.60	0.73	-0.02	-0.38	0.15	-0.19	0.23	-0.22	1	0.47	-0.88	0.37	0.57	0.81	0.69	-0.80	0.64	0.05	-0.44	0.48	0.85	-0.61	0.25	0.36	0.81	-0.22	0.85	-0.61	0.79
R_%_ω-gliadins	0.31	0.58	0.35	0.33	0.04	0.11	0.88	-0.88	0.47	1	-0.83	0.02	0.44	0.36	0.07	-0.49	0.75	0.06	0.22	0.41	0.42	-0.58	-0.10	0.20	0.49	0.16	0.46	-0.14	0.51
R_%_LWGS	-0.54	-0.77	-0.20	0.05	-0.11	0.03	-0.62	0.62	-0.88	-0.83	1	-0.25	-0.59	-0.70	-0.47	0.77	-0.81	-0.06	0.15	-0.52	-0.75	0.69	-0.10	-0.33	-0.77	0.06	-0.78	0.45	-0.77
Specific weight	0.72	0.05	0.08	-0.15	-0.31	-0.16	0.02	-0.01	0.37	0.02	-0.25	1	-0.24	0.16	0.51	-0.23	0.02	-0.57	-0.49	0.66	0.36	0.09	-0.29	0.65	0.30	-0.61	0.46	-0.62	0.24
ProteinAsIs	0.07	0.90	-0.06	-0.15	0.13	0.02	0.31	-0.31	0.57	0.44	-0.59	-0.24	1	0.75	0.32	-0.80	0.80	0.63	0.14	-0.04	0.61	-0.89	0.52	-0.24	0.78	0.45	0.52	-0.02	0.81
SKCS	0.41	0.89	-0.16	-0.43	0.32	-0.18	0.10	-0.10	0.81	0.36	-0.70	0.16	0.75	1	0.77	-0.91	0.66	0.43	-0.15	0.20	0.72	-0.79	0.48	-0.03	0.92	0.16	0.68	-0.31	0.92
F_Water_Abs	0.50	0.55	-0.26	-0.43	0.16	-0.28	-0.13	0.14	0.69	0.07	-0.47	0.51	0.32	0.77	1	-0.72	0.27	0.11	-0.24	0.40	0.51	-0.45	0.26	0.12	0.74	-0.07	0.54	-0.36	0.71
ReoPC1	-0.46	-0.94	0.08	0.25	-0.07	0.07	-0.30	0.30	-0.80	-0.49	0.77	-0.23	-0.80	-0.91	-0.72	1	-0.77	-0.41	0.03	-0.27	-0.77	0.90	-0.40	-0.02	-0.96	-0.22	-0.73	0.28	-0.97
ReoPC2	0.28	0.87	0.24	0.03	0.06	0.15	0.64	-0.64	0.64	0.75	-0.81	0.02	0.80	0.66	0.27	-0.77	1	0.29	0.07	0.26	0.71	-0.84	0.19	0.12	0.76	0.21	0.68	-0.24	0.79
F1	-0.44	0.50	-0.33	-0.06	0.13	0.04	0.00	0.00	0.05	0.06	-0.06	-0.57	0.63	0.43	0.11	-0.41	0.29	1	0.48	-0.65	-0.01	-0.62	0.79	-0.87	0.36	0.87	-0.15	0.60	0.41
F2	-0.55	0.06	0.12	0.62	-0.13	0.36	0.40	-0.40	-0.44	0.22	0.15	-0.49	0.14	-0.15	-0.24	0.03	0.07	0.48	1	-0.42	-0.53	-0.23	-0.05	-0.66	-0.12	0.86	-0.55	0.85	-0.06
F3	0.72	0.19	0.21	-0.04	0.01	-0.22	0.29	-0.29	0.48	0.41	-0.52	0.66	-0.04	0.20	0.40	-0.27	0.26	-0.65	-0.42	1	0.46	-0.06	-0.42	0.82	0.34	-0.62	0.63	-0.73	0.30
F4	0.68	0.74	-0.06	-0.45	0.07	-0.17	0.17	-0.17	0.85	0.42	-0.75	0.36	0.61	0.72	0.51	-0.77	0.71	-0.01	-0.53	0.46	1	-0.67	0.19	0.49	0.78	-0.31	0.98	-0.72	0.77
F5	-0.22	-0.95	0.11	0.10	-0.06	-0.01	-0.41	0.41	-0.61	-0.58	0.69	0.09	-0.89	-0.79	-0.45	0.90	-0.84	-0.62	-0.23	-0.06	-0.67	1	-0.45	0.20	-0.85	-0.49	-0.59	0.02	-0.89
F1.F2	-0.19	0.44	-0.43	-0.39	0.18	-0.18	-0.26	0.27	0.25	-0.10	-0.10	-0.29	0.52	0.48	0.26	-0.40	0.19	0.79	-0.05	-0.42	0.19	-0.45	1	-0.60	0.42	0.44	0.07	0.21	0.44
F3F4.F1	0.72	-0.06	0.30	-0.11	-0.09	-0.08	0.14	-0.14	0.36	0.20	-0.33	0.65	-0.24	-0.03	0.12	-0.02	0.12	-0.87	-0.66	0.82	0.49	0.20	-0.60	1	0.07	-0.89	0.61	-0.87	0.02
AT	0.52	0.94	-0.02	-0.26	0.10	-0.08	0.30	-0.29	0.81	0.49	-0.77	0.30	0.78	0.92	0.74	-0.96	0.76	0.36	-0.12	0.34	0.78	-0.85	0.42	0.07	1	0.15	0.76	-0.36	1.00
F1F2	-0.57	0.32	-0.13	0.32	0.00	0.22	0.23	-0.23	-0.22	0.16	0.06	-0.61	0.45	0.16	-0.07	-0.22	0.21	0.87	0.86	-0.62	-0.31	-0.49	0.44	-0.89	0.15	1	-0.41	0.84	0.21
F3F4	0.75	0.69	0.00	-0.40	0.06	-0.19	0.22	-0.22	0.85	0.46	-0.78	0.46	0.52	0.68	0.54	-0.73	0.68	-0.15	-0.55	0.63	0.98	-0.59	0.07	0.61	0.76	-0.41	1	-0.79	0.74
F1F2.F3F4	-0.80	-0.20	-0.12	0.42	-0.07	0.19	0.02	-0.02	-0.61	-0.14	0.45	-0.62	-0.02	-0.31	-0.36	0.28	-0.24	0.60	0.85	-0.73	-0.72	0.02	0.21	-0.87	-0.36	0.84	-0.79	1	-0.31
GlutenProtein	0.47	0.97	-0.03	-0.24	0.10	-0.06	0.32	-0.32	0.79	0.51	-0.77	0.24	0.81	0.92	0.71	-0.97	0.79	0.41	-0.06	0.30	0.77	-0.89	0.44	0.02	1.00	0.21	0.74	-0.31	1
PF_DoughCon10.ins	0.69	0.40	0.17	-0.28	0.10	-0.20	0.08	-0.08	0.69	0.22	-0.55	0.57	0.22	0.58	0.54	-0.50	0.26	-0.20	-0.58	0.51	0.57	-0.19	0.10	0.46	0.58	-0.45	0.61	-0.68	0.53
PF_Height	-0.28	0.28	-0.54	-0.40	0.31	-0.26	-0.31	0.31	0.08	-0.15	0.03	-0.26	0.33	0.40	0.38	-0.33	0.14	0.57	0.28	-0.30	0.06	-0.42	0.41	-0.50	0.25	0.50	-0.01	0.34	0.28
PF_OvenSpring	-0.56	-0.16	-0.41	-0.02	0.09	-0.14	-0.30	0.30	-0.34	-0.32	0.38	-0.27	-0.11	-0.06	0.04	0.14	-0.30	0.42	0.52	-0.50	-0.50	0.04	0.19	-0.65	-0.21	0.54	-0.55	0.68	-0.19
PF_Volue	-0.35	0.45	-0.35	-0.20	0.31	-0.04	-0.08	0.08	0.04	0.05	-0.05	-0.54	0.56	0.41	0.18	-0.41	0.35	0.74	0.49	-0.42	0.10	-0.62	0.43	-0.60	0.29	0.71	-0.01	0.47	0.35
PF_BTex	-0.57	0.03	-0.16	0.08	0.22	0.09	-0.04	0.04	-0.35	-0.08	0.26	-0.56	0.18	-0.03	-0.26	0.07	-0.02	0.56	0.62	-0.60	-0.37	-0.16	0.20	-0.69	-0.15	0.68	-0.46	0.68	-0.10
PF_Yi	0.58	0.30	0.13	0.00	-0.25	-0.21	0.33	-0.33	0.57	0.43	-0.59	0.58	0.15	0.33	0.31	-0.35	0.28	-0.30	-0.47	0.56	0.49	-0.14	-0.04	0.51	0.44	-0.44	0.55	-0.58	0.40
PF_Wi	-0.55	-0.31	-0.18	-0.03	0.25	0.18	-0.38	0.37	-0.58	-0.45	0.61	-0.56	-0.18	-0.32	-0.28	0.35	-0.33	0.30	0.46	-0.58	-0.48	0.14	0.05	-0.52	-0.43	0.44	-0.55	0.58	-0.40
PF_a	0.17	-0.32	0.20	0.21	-0.31	-0.06	-0.04	0.04	0.00	-0.21	0.10	0.56	-0.38	-0.17	0.18	0.20	-0.35	-0.48	-0.27	0.35	-0.22	0.50	-0.29	0.30	-0.12	-0.44	-0.12	-0.23	-0.18
PF_b	0.35	0.37	0.17	0.07	-0.04	-0.10	0.41	-0.41	0.45	0.51	-0.55	0.29	0.28	0.35	0.26	-0.35	0.37	-0.16	-0.20	0.50	0.34	-0.21	-0.06	0.34	0.42	-0.21	0.41	-0.37	0.40
PF_L	-0.30	-0.40	-0.02	0.01	0.35	0.12	-0.33	0.33	-0.48	-0.39	0.50	-0.29	-0.40	-0.27	-0.13	0.39	-0.50	0.08	0.37	-0.36	-0.57	0.33	-0.13	-0.38	-0.42	0.25	-0.58	0.45	-0.42
W_height	-0.39	0.12	-0.52	-0.26	0.20	-0.12	-0.43	0.43	-0.13	-0.35	0.27	-0.48	0.22	0.21	0.16	-0.15	-0.16	0.68	0.36	-0.57	-0.15	-0.28	0.50	-0.69	0.05	0.60	-0.26	0.53	0.08
W_CColour	0.08	-0.78	-0.12	-0.05	-0.12	-0.12	-0.49	0.49	-0.36	-0.57	0.53	0.27	-0.81	-0.53	-0.07	0.57	-0.82	-0.52	-0.24	0.04	-0.43	0.75	-0.38	0.22	-0.60	-0.44	-0.37	-0.08	-0.64
W_Texture	0.18	-0.69	-0.09	-0.12	-0.04	-0.08	-0.49	0.49	-0.26	-0.53	0.45	0.26	-0.74	-0.42	-0.01	0.48	-0.74	-0.48	-0.28	0.03	-0.32	0.66	-0.36	0.23	-0.51	-0.44	-0.28	-0.14	-0.56
W_Softness	0.20	-0.10	-0.29	-0.38	0.12	-0.15	-0.41	0.41	0.10	-0.29	0.10	-0.06	-0.07	0.07	0.10	0.00	-0.28	0.00	-0.24	-0.14	0.16	0.03	0.02	0.05	-0.07	-0.14	0.11	-0.15	-0.07
W_Resilience	0.16	-0.67	-0.01	-0.04	-0.08	-0.03	-0.37	0.37	-0.39	-0.40	0.46	0.21	-0.74	-0.49	-0.17	0.56	-0.68	-0.49	-0.21	-0.01	-0.32	0.64	-0.46	0.25	-0.54	-0.41	-0.28	-0.10	-0.58
W_CStrength	0.14	-0.50	-0.11	-0.20	0.02	-0.04	-0.42	0.42	-0.12	-0.38	0.28	0.10	-0.55	-0.26	-0.04	0.39	-0.56	-0.34	-0.29	-0.08	-0.15	0.47	-0.26	0.18	-0.40	-0.36	-0.15	-0.15	-0.43
ACT_ixTie	0.54	-0.04	0.08	-0.24	0.04	-0.30	-0.15	0.15	0.40	-0.09	-0.21	0.69	-0.22	0.21	0.53	-0.10	-0.12	-0.58	-0.64	0.66	0.25	0.27	-0.22	0.62	0.21	-0.71	0.37	-0.67	0.14
ATC_DoughTep	-0.05	-0.01	0.01	0.11	0.18	0.04	-0.06	0.07	0.09	-0.04	-0.03	-0.10	-0.03	0.08	0.16	0.03	-0.03	0.08	0.07	-0.03	-0.13	0.04	0.10	-0.14	-0.01	0.09	-0.12	0.10	-0.02
ATC_ProofHeight	0.43	0.02	0.20	-0.04	0.18	-0.23	0.16	-0.15	0.36	0.27	-0.37	0.46	-0.19	0.19	0.38	-0.10	-0.07	-0.38	-0.24	0.61	0.07	0.13	-0.22	0.37	0.20	-0.36	0.20	-0.36	0.15
ATC_Bheight	0.41	0.27	-0.27	-0.36	0.12	-0.27	-0.23	0.23	0.44	-0.10	-0.22	0.52	0.08	0.49	0.79	-0.46	0.00	0.01	-0.21	0.26	0.27	-0.18	0.15	0.08	0.47	-0.11	0.29	-0.25	0.43
ATC_OvenSpring	-0.06	0.27	-0.33	-0.16	0.23	-0.07	-0.10	0.10	0.10	0.00	-0.06	-0.12	0.21	0.38	0.39	-0.38	0.02	0.51	0.43	-0.32	-0.03	-0.38	0.23	-0.50	0.26	0.55	-0.10	0.40	0.28
ATC_LoafVol	0.42	0.19	-0.14	-0.29	0.22	-0.29	-0.11	0.12	0.35	0.06	-0.25	0.56	-0.06	0.39	0.70	-0.36	-0.01	-0.10	-0.11	0.36	0.14	-0.09	-0.02	0.13	0.38	-0.12	0.21	-0.20	0.33
ATC_Breadoist	0.12	0.49	-0.30	-0.30	0.19	-0.13	-0.14	0.15	0.50	-0.02	-0.30	0.09	0.41	0.63	0.76	-0.60	0.26	0.38	0.04	0.07	0.31	-0.48	0.36	-0.21	0.56	0.25	0.28	-0.01	0.55
ATC_Ccolour_L	-0.04	-0.29	-0.14	-0.09	-0.03	-0.19	-0.31	0.31	-0.19	-0.35	0.30	0.22	-0.41	-0.18	0.09	0.17	-0.30	-0.28	-0.15	0.08	-0.14	0.32	-0.22	0.17	-0.21	-0.25	-0.10	-0.09	-0.23
ATC_CColourLb	0.01	-0.54	0.04	-0.01	0.04	-0.16	-0.29	0.29	-0.25	-0.38	0.35	0.41	-0.67	-0.30	0.15	0.34	-0.58	-0.42	-0.06	0.16	-0.44	0.58	-0.39	0.14	-0.35	-0.28	-0.35	0.04	-0.40

	R_yield	R_n	Dx	Bx	By	Dy	ω-gliadin 5	ω-gliadin 1_2	R_%_HW GS	R_%_ω-gliadins	R_%_LWG S	Specific weight	Protein AsIs	SKCS	F_Water_Abs	ReoPC1	ReoPC2	F1	F2	F3	F4	F5	F1.F2	F3F4.F1	AT	F1F2	F3F4	F1F2.F3F4	Gluten Protein
ATC_Ccopres	-0.49	-0.32	0.20	0.45	-0.19	0.40	0.24	-0.24	-0.58	0.06	0.33	-0.63	-0.12	-0.58	-0.88	0.49	-0.07	0.09	0.39	-0.47	-0.37	0.17	-0.14	-0.23	-0.56	0.28	-0.43	0.43	-0.51
ATC_Height	0.49	0.32	-0.14	-0.31	0.25	-0.25	-0.05	0.05	0.46	0.14	-0.36	0.53	0.04	0.54	0.76	-0.48	0.07	-0.05	-0.11	0.39	0.28	-0.23	0.02	0.14	0.50	-0.09	0.33	-0.26	0.46
ATC_TotConcavity	0.29	0.44	0.21	-0.12	0.11	0.11	0.12	-0.12	0.46	0.12	-0.37	0.31	0.37	0.47	0.47	-0.53	0.43	0.01	-0.14	0.37	0.36	-0.32	0.14	0.18	0.50	-0.07	0.40	-0.28	0.48
ATC_Left.Concavity	0.31	0.40	0.11	-0.24	0.18	0.05	-0.01	0.01	0.45	0.06	-0.33	0.26	0.34	0.48	0.49	-0.50	0.34	0.02	-0.25	0.32	0.40	-0.28	0.19	0.18	0.48	-0.13	0.42	-0.33	0.46
ATC_RightConcavity	0.34	0.39	0.32	-0.08	0.15	0.21	0.18	-0.18	0.37	0.17	-0.34	0.30	0.32	0.38	0.37	-0.46	0.44	-0.09	-0.12	0.44	0.33	-0.27	-0.01	0.26	0.43	-0.12	0.39	-0.32	0.42
ATC_Side.wallConcavity	0.33	0.43	0.21	-0.18	0.18	0.14	0.08	-0.08	0.44	0.11	-0.35	0.28	0.36	0.47	0.47	-0.52	0.41	-0.02	-0.20	0.38	0.39	-0.30	0.11	0.22	0.49	-0.12	0.43	-0.34	0.47
ATC_TopConcavity	0.10	0.25	0.16	0.03	-0.04	0.07	0.13	-0.13	0.26	0.06	-0.21	0.22	0.23	0.23	0.26	-0.30	0.31	0.02	0.01	0.18	0.16	-0.19	0.12	0.06	0.27	0.02	0.17	-0.07	0.27
ATC_SliceBrightness	-0.57	-0.37	-0.21	0.18	0.04	0.13	-0.11	0.11	-0.59	-0.19	0.47	-0.57	-0.25	-0.41	-0.45	0.37	-0.35	0.33	0.59	-0.61	-0.56	0.13	-0.01	-0.57	-0.51	0.53	-0.62	0.71	-0.46
ATC_NuberOfCells	0.09	-0.28	-0.03	0.01	0.11	0.11	-0.13	0.13	-0.32	-0.09	0.25	0.05	-0.45	-0.15	-0.01	0.19	-0.36	-0.01	0.19	-0.21	-0.27	0.19	-0.17	-0.15	-0.22	0.10	-0.29	0.22	-0.23
ATC_NoralisedCellCount	-0.37	-0.48	0.09	0.26	-0.12	0.38	-0.07	0.06	-0.70	-0.21	0.55	-0.43	-0.39	-0.57	-0.67	0.55	-0.34	0.09	0.31	-0.58	-0.47	0.31	-0.15	-0.30	-0.60	0.23	-0.54	0.45	-0.57
ATC_WallThickness	0.19	0.46	-0.15	-0.26	0.20	-0.31	0.03	-0.03	0.60	0.17	-0.46	0.14	0.44	0.53	0.53	-0.49	0.30	0.03	-0.21	0.42	0.42	-0.35	0.21	0.16	0.51	-0.10	0.46	-0.34	0.50
ATC_CellDiaeter	0.48	0.18	-0.06	-0.26	0.06	-0.41	-0.07	0.07	0.55	0.03	-0.36	0.67	0.01	0.38	0.68	-0.34	0.03	-0.36	-0.42	0.65	0.30	0.00	-0.09	0.44	0.40	-0.45	0.41	-0.52	0.35
ATC_CellVolue	0.52	0.24	-0.02	-0.24	0.04	-0.39	-0.01	0.01	0.61	0.09	-0.43	0.67	0.06	0.40	0.66	-0.38	0.11	-0.38	-0.46	0.70	0.38	-0.05	-0.09	0.50	0.44	-0.48	0.49	-0.59	0.39

Table 3.8 Simple correlation among baking characteristics for 2009 and 2010 at Rothamsted. Correlation coefficients r = 0.70 and above are marked yellow.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
PF_DoughCon10.ins	1	-0.27	-0.46	-0.39	-0.56	0.77	-0.76	0.43	0.60	-0.43	-0.37	-0.04	0.01	0.12	-0.06	0.00	0.69	0.01	0.56	0.43	-0.06	0.38	0.14	-0.03	0.07	-0.65	0.47	0.37	0.41	0.32	0.39	0.14	-0.72	-0.15	-0.59	0.44	0.68	0.71
PF_Height	-0.27	1	0.59	0.84	0.42	-0.44	0.45	-0.47	-0.38	0.20	0.68	-0.17	-0.10	0.16	-0.23	-0.06	-0.30	0.10	-0.24	0.25	0.57	0.22	0.58	-0.06	-0.01	-0.21	0.19	0.14	0.18	0.09	0.16	0.09	0.30	0.05	-0.08	0.15	-0.07	-0.11
PF_OvenSpring	-0.46	0.59	1	0.42	0.64	-0.47	0.50	-0.15	-0.34	0.52	0.62	0.10	0.06	0.03	-0.03	0.09	-0.32	0.16	-0.18	0.20	0.55	0.11	0.32	0.02	0.23	0.04	0.12	-0.11	-0.10	-0.17	-0.13	-0.07	0.55	0.19	0.12	-0.09	-0.12	-0.20
PF_Volue	-0.39	0.84	0.42	1	0.56	-0.56	0.54	-0.70	-0.37	0.20	0.71	-0.44	-0.36	0.09	-0.38	-0.24	-0.60	0.10	-0.43	0.01	0.56	-0.01	0.47	-0.12	-0.32	0.07	0.02	0.08	0.09	0.06	0.09	0.07	0.41	0.05	0.08	0.06	-0.34	-0.36
PF_BTex	-0.56	0.42	0.64	0.56	1	-0.64	0.67	-0.39	-0.45	0.62	0.52	-0.33	-0.32	-0.13	-0.25	-0.22	-0.66	-0.03	-0.38	-0.18	0.51	-0.23	0.03	-0.20	-0.09	0.41	-0.15	-0.07	-0.14	-0.04	-0.10	0.03	0.64	0.13	0.32	-0.21	-0.46	-0.50
PF_Yi	0.77	-0.44	-0.47	-0.56	-0.64	1	-0.98	0.49	0.80	-0.74	-0.56	-0.08	-0.10	-0.08	-0.06	-0.05	0.59	-0.06	0.51	0.18	-0.33	0.20	-0.10	-0.12	-0.04	-0.40	0.20	0.16	0.17	0.10	0.14	0.07	-0.71	-0.26	-0.47	0.27	0.51	0.56
PF_Wi	-0.76	0.45	0.50	0.54	0.67	-0.98	1	-0.50	-0.82	0.78	0.59	0.10	0.12	0.09	0.09	0.07	-0.61	0.10	-0.54	-0.14	0.39	-0.18	0.06	0.13	0.03	0.40	-0.18	-0.17	-0.18	-0.15	-0.17	-0.08	0.72	0.31	0.49	-0.28	-0.52	-0.57
PF_a	0.43	-0.47	-0.15	-0.70	-0.39	0.49	-0.50	1	0.35	-0.13	-0.45	0.38	0.32	-0.18	0.25	0.12	0.72	0.10	0.52	0.26	-0.29	0.24	-0.07	0.20	0.57	-0.41	0.19	0.16	0.14	0.18	0.16	0.08	-0.44	-0.08	-0.29	0.07	0.56	0.53
PF_b	0.60	-0.38	-0.34	-0.37	-0.45	0.80	-0.82	0.35	1	-0.61	-0.48	-0.24	-0.31	-0.15	-0.19	-0.21	0.49	-0.04	0.43	0.11	-0.26	0.13	-0.02	-0.17	-0.12	-0.29	0.17	0.14	0.15	0.17	0.17	-0.01	-0.58	-0.29	-0.43	0.33	0.40	0.45
PF_L	-0.43	0.20	0.52	0.20	0.62	-0.74	0.78	-0.13	-0.61	1	0.43	0.30	0.34	0.15	0.29	0.24	-0.25	0.10	-0.05	0.08	0.47	0.12	0.06	0.14	0.34	0.13	0.17	-0.15	-0.14	-0.11	-0.13	-0.14	0.58	0.51	0.34	-0.24	-0.18	-0.27
W_height	-0.37	0.68	0.62	0.71	0.52	-0.56	0.59	-0.45	-0.48	0.43	1	0.02	0.09	0.42	-0.04	0.18	-0.46	0.12	-0.29	0.17	0.62	0.11	0.46	0.03	-0.04	0.01	0.12	-0.11	-0.02	-0.18	-0.09	-0.15	0.59	0.26	0.17	0.00	-0.24	-0.30
W_CColour	-0.04	-0.17	0.10	-0.44	-0.33	-0.08	0.10	0.38	-0.24	0.30	0.02	1	0.96	0.45	0.79	0.76	0.31	0.04	0.23	0.16	-0.05	0.23	-0.12	0.34	0.65	-0.10	0.10	-0.30	-0.23	-0.26	-0.26	-0.23	0.20	0.41	0.22	-0.23	0.16	0.10
W_Texture	0.01	-0.10	0.06	-0.36	-0.32	-0.10	0.12	0.32	-0.31	0.34	0.09	0.96	1	0.58	0.82	0.83	0.31	0.07	0.24	0.22	0.03	0.28	-0.04	0.29	0.60	-0.15	0.19	-0.28	-0.18	-0.22	-0.21	-0.28	0.19	0.49	0.22	-0.23	0.16	0.09
W_Softness	0.12	0.16	0.03	0.09	-0.13	-0.08	0.09	-0.18	-0.15	0.15	0.42	0.45	0.58	1	0.56	0.76	0.04	-0.02	0.02	0.17	0.19	0.14	0.14	0.06	0.04	-0.10	0.18	-0.36	-0.14	-0.30	-0.22	-0.52	0.18	0.31	0.12	0.03	0.04	0.01
W_Resilience	-0.06	-0.23	-0.03	-0.38	-0.25	-0.06	0.09	0.25	-0.19	0.29	-0.04	0.79	0.82	0.56	1	0.86	0.19	0.01	0.16	0.00	-0.10	0.14	-0.32	0.35	0.53	0.04	0.08	-0.58	-0.41	-0.44	-0.45	-0.60	0.23	0.62	0.45	-0.48	-0.05	-0.11
W_CStrength	0.00	-0.06	0.09	-0.24	-0.22	-0.05	0.07	0.12	-0.21	0.24	0.18	0.76	0.83	0.76	0.86	1	0.16	0.11	0.16	0.11	0.01	0.15	-0.07	0.21	0.38	-0.01	0.15	-0.50	-0.32	-0.40	-0.37	-0.56	0.26	0.50	0.30	-0.25	0.01	-0.03
ACT_ixTie	0.69	-0.30	-0.32	-0.60	-0.66	0.59	-0.61	0.72	0.49	-0.25	-0.46	0.31	0.31	0.04	0.19	0.16	1	0.04	0.66	0.49	-0.30	0.50	0.17	0.21	0.42	-0.74	0.46	0.30	0.36	0.28	0.34	0.10	-0.74	-0.19	-0.63	0.41	0.82	0.82
ATC_DoughTep	0.01	0.10	0.16	0.10	-0.03	-0.06	0.10	0.10	-0.04	0.10	0.12	0.04	0.07	-0.02	0.01	0.11	0.04	1	-0.01	0.28	0.07	0.08	0.31	-0.02	-0.01	-0.15	0.13	0.05	0.12	0.06	0.11	-0.12	-0.10	0.05	-0.11	0.15	0.09	0.09
ATC_ProofHeight	0.56	-0.24	-0.18	-0.43	-0.38	0.51	-0.54	0.52	0.43	-0.05	-0.29	0.23	0.24	0.02	0.16	0.16	0.66	-0.01	1	0.26	-0.10	0.55	0.11	-0.09	0.42	-0.58	0.54	0.16	0.17	0.13	0.15	0.04	-0.38	0.04	-0.50	0.36	0.68	0.65
ATC_Bheight	0.43	0.25	0.20	0.01	-0.18	0.18	-0.14	0.26	0.11	0.08	0.17	0.16	0.22	0.17	0.00	0.11	0.49	0.28	0.26	1	0.52	0.74	0.66	0.10	0.25	-0.79	0.83	0.29	0.36	0.23	0.31	0.02	-0.28	0.17	-0.56	0.45	0.67	0.64
ATC_OvenSpring	-0.06	0.57	0.55	0.56	0.51	-0.33	0.39	-0.29	-0.26	0.47	0.62	-0.05	0.03	0.19	-0.10	0.01	-0.30	0.07	-0.10	0.52	1	0.40	0.47	-0.04	0.10	-0.22	0.55	0.05	0.04	0.00	0.03	0.00	0.47	0.46	-0.01	0.07	0.03	-0.04
ATC_LoafVol	0.38	0.22	0.11	-0.01	-0.23	0.20	-0.18	0.24	0.13	0.12	0.11	0.23	0.28	0.14	0.14	0.15	0.50	0.08	0.55	0.74	0.40	1	0.45	0.20	0.43	-0.80	0.85	0.23	0.27	0.15	0.22	0.09	-0.23	0.30	-0.50	0.34	0.69	0.63
ATC_Breadoist	0.14	0.58	0.32	0.47	0.03	-0.10	0.06	-0.07	-0.02	0.06	0.46	-0.12	-0.04	0.14	-0.32	-0.07	0.17	0.31	0.11	0.66	0.47	0.45	1	-0.06	0.00	-0.61	0.50	0.42	0.44	0.33	0.42	0.20	-0.15	-0.07	-0.47	0.43	0.35	0.33
ATC_Ccolour_L	-0.03	-0.06	0.02	-0.12	-0.20	-0.12	0.13	0.20	-0.17	0.14	0.03	0.34	0.29	0.06	0.35	0.21	0.21	-0.02	-0.09	0.10	-0.04	0.20	-0.06	1	0.48	-0.14	0.13	-0.05	0.01	-0.04	-0.02	-0.18	0.08	0.30	0.16	-0.23	0.10	0.07
ATC_CColourLb	0.07	-0.01	0.23	-0.32	-0.09	-0.04	0.03	0.57	-0.12	0.34	-0.04	0.65	0.60	0.04	0.53	0.38	0.42	-0.01	0.42	0.25	0.10	0.43	0.00	0.48	1	-0.33	0.31	-0.04	-0.07	0.03	-0.04	0.00	0.12	0.38	0.03	-0.19	0.36	0.27
ATC_Ccopres	-0.65	-0.21	0.04	0.07	0.41	-0.40	0.40	-0.41	-0.29	0.13	0.01	-0.10	-0.15	-0.10	0.04	-0.01	-0.74	-0.15	-0.58	-0.79	-0.22	-0.80	-0.61	-0.14	-0.33	1	-0.76	-0.41	-0.46	-0.29	-0.40	-0.21	0.59	0.05	0.74	-0.56	-0.85	-0.82
ATC_Height	0.47	0.19	0.12	0.02	-0.15	0.20	-0.18	0.19	0.17	0.17	0.12	0.10	0.19	0.18	0.08	0.15	0.46	0.13	0.54	0.83	0.55	0.85	0.50	0.13	0.31	-0.76	1	0.19	0.24	0.16	0.21	-0.05	-0.18	0.39	-0.50	0.43	0.68	0.62
ATC_TotConcavity	0.37	0.14	-0.11	0.08	-0.07	0.16	-0.17	0.16	0.14	-0.15	-0.11	-0.30	-0.28	-0.36	-0.58	-0.50	0.30	0.05	0.16	0.29	0.05	0.23	0.42	-0.05	-0.04	-0.41	0.19	1	0.91	0.90	0.95	0.74	-0.45	-0.41	-0.52	0.39	0.38	0.41
ATC_Left.Concavity	0.41	0.18	-0.10	0.09	-0.14	0.17	-0.18	0.14	0.15	-0.14	-0.02	-0.23	-0.18	-0.14	-0.41	-0.32	0.36	0.12	0.17	0.36	0.04	0.27	0.44	0.01	-0.07	-0.46	0.24	0.91	1	0.81	0.96	0.45	-0.45	-0.30	-0.47	0.37	0.37	0.40
ATC_RightConcavity	0.32	0.09	-0.17	0.06	-0.04	0.10	-0.15	0.18	0.17	-0.11	-0.18	-0.26	-0.22	-0.30	-0.44	-0.40	0.28	0.06	0.13	0.23	0.00	0.15	0.33	-0.04	0.03	-0.29	0.16	0.90	0.81	1	0.94	0.55	-0.37	-0.28	-0.37	0.24	0.29	0.32
ATC_Side.wallConcavity	0.39	0.16	-0.13	0.09	-0.10	0.14	-0.17	0.16	0.17	-0.13	-0.09	-0.26	-0.21	-0.22	-0.45	-0.37	0.34	0.11	0.15	0.31	0.03	0.22	0.42	-0.02	-0.04	-0.40	0.21	0.95	0.96	0.94	1	0.52	-0.44	-0.31	-0.44	0.33	0.35	0.38
ATC_TopConcavity	0.14	0.09	-0.07	0.07	0.03	0.07	-0.08	0.08	-0.01	-0.14	-0.15	-0.23	-0.28	-0.52	-0.60	-0.56	0.10	-0.12	0.04	0.02	0.00	0.09	0.20	-0.18	0.00	-0.21	-0.05	0.74	0.45	0.55	0.52	1	-0.30	-0.49	-0.40	0.27	0.23	0.25
ATC_SliceBrightness	-0.72	0.30	0.55	0.41	0.64	-0.71	0.72	-0.44	-0.58	0.58	0.59	0.20	0.19	0.18	0.23	0.26	-0.74	-0.10	-0.38	-0.28	0.47	-0.23	-0.15	0.08	0.12	0.59	-0.18	-0.45	-0.45	-0.37	-0.44	-0.30	1	0.53	0.68	-0.48	-0.61	-0.69
ATC_NuberOfCells	-0.15	0.05	0.19	0.05	0.13	-0.26	0.31	-0.08	-0.29	0.51	0.26	0.41	0.49	0.31	0.62	0.50	-0.19	0.05	0.04	0.17	0.46	0.30	-0.07	0.30	0.38	0.05	0.39	-0.41	-0.30	-0.28	-0.31	-0.49	0.53	1	0.58	-0.54	-0.25	-0.33
ATC_NoralisedCellCount	-0.59	-0.08	0.12	0.08	0.32	-0.47	0.49	-0.29	-0.43	0.34	0.17	0.22	0.22	0.12	0.45	0.30	-0.63	-0.11	-0.50	-0.56	-0.01	-0.50	-0.47	0.16	0.03	0.74	-0.50	-0.52	-0.47	-0.37	-0.44	-0.40	0.68	0.58	1	-0.88	-0.87	-0.90
ATC_WallThickness	0.44	0.15	-0.09	0.06	-0.21	0.27	-0.28	0.07	0.33	-0.24	0.00	-0.23	-0.23	0.03	-0.48	-0.25	0.41	0.15	0.36	0.45	0.07	0.34	0.43	-0.23	-0.19	-0.56	0.43	0.39	0.37	0.24	0.33	0.27	-0.48	-0.54	-0.88	1	0.72	0.76
ATC_CellDiaeter	0.68	-0.07	-0.12	-0.34	-0.46	0.51	-0.52	0.56	0.40	-0.18	-0.24	0.16	0.16	0.04	-0.05	0.01	0.82	0.09	0.68	0.67	0.03	0.69	0.35	0.10	0.36	-0.85	0.68	0.38	0.37	0.29	0.35	0.23	-0.61	-0.25	-0.87	0.72	1	0.99
ATC_CellVolue	0.71	-0.11	-0.20	-0.36	-0.50	0.56	-0.57	0.53	0.45	-0.27	-0.30	0.10	0.09	0.01	-0.11	-0.03	0.82	0.09	0.65	0.64	-0.04	0.63	0.33	0.07	0.27	-0.82	0.62	0.41	0.40	0.32	0.38	0.25	-0.69	-0.33	-0.90	0.76	0.99	1

3.4. Discussion

The principal aims of the breeder when developing new wheat lines are intrinsic yield and disease resistance (which determine farmers' yields). There is no doubt that breeding companies have been quite successful in achieving both targets. While end-use quality is regarded as being important, it has often been of a lower priority. There is some concern within the milling and baking industries that some potentially good breadmaking varieties may be screened out at the early stages, based on yield, growth and other agronomic performance criteria. Also, there is a need for a greater diversity, as at present there is a reliance on relatively few varieties that are classified in **nabim** Groups 1 and 2. Increased yield is generally associated with lower protein concentration in wheat crops (Kettlewell, 1996). At present, the standard breadmaking processes in the UK demand high protein (usually a minimum of 13% for grain) which can only be achieved with substantial and costly N fertiliser inputs. The sustainability of such farming practices are being questioned, in terms of economic returns, NVZ, diffuse pollution and water framework compliance. Increased awareness of the carbon footprint of fertiliser use will also increase the pressure on growers striving for both optimal yield and the higher protein targets.

There are growing concerns that the availability of UK-grown wheat for breadmaking with such a protein specification may be limited, as seen with the 2007 harvest. Just 11% of the 2007 milling wheat crop met the high quality bread wheat specification compared to over 40% in 2006. Given the on-going global *fuel v food* debate, allied to the advent of biofuel production in the UK, there is a real possibility that an increasing number of farmers will switch to growing high starch varieties rather than Group 1 quality wheats. There is already growing concern over the declining area devoted to growing breadmaking wheat. Therefore, there is a need for new varieties that are efficient in N utilisation, tolerant to seasonal variations and which could also be adaptable in terms of usage to give UK farmers the required flexibility to fulfil market demands.

Although the relationships between genetically determined differences in gluten protein composition and grain processing properties are well established (Payne *et al*., 1987; Shewry *et al*., 1995), little is known about the impacts of nutrition and other environmental factors on these relationships (Triboi *et al*., 2000). However, it is clear that complex genotype *x* environment interactions exist, as in other biological systems. Unravelling these interactions is essential to facilitate the development of new wheat varieties which have high stability to environmental fluctuations and, in particular, maintain high quality despite reduced application of nitrogen fertilisers. The combination in this study of new transcriptome technologies (Wan *et al*., 2008) with classical protein and functional studies has provided both new information on mechanisms and key links between genes, proteins, functionality and baking characteristics. Furthermore, the

identification of transcripts which are associated with the stability of quality and the maintenance of quality under reduced N inputs are potential new markers which may be developed for use in plant breeding programmes. This project has built on the discovery of some of the new QTLs for grain characteristics and end-use quality, as reported in a recent CCFRA-led LINK Project (FQS 23, Millar *et al*., 2008).

Key messages to emerge from the project

- The inverse relationship between wheat grain yield and grain N is very robust and consistent
- Grain protein deviation (GPD) is a much more variable trait. An analysis where all N-levels and years and sites are considered, but effects of N-fertiliser input and dilution effects of yield are all normalised, indicates a GPD principally for Hereward and to a lesser extent for Cordiale and Malacca.
- The year-to-year yield and quality stability of these varieties is poor, as is the GPD trait
- 2010 and 2011 were very dry with negative impacts on N-responses (either yield and/or grain N)
- Variety and nitrogen inputs have defined but complex impacts on dough quality
- 159 key genes have been identified which are N-responsive including important storage proteins
- More than 3000 genes correlating with GPD were identified; these have been ranked in order of significance
- Some GPD genes also correlate with baking quality

Implications for breeders/producers

- Methodologies used in this study and the extensive correlations with quality, compositional and baking traits will provide leads for high throughput selection protocols
- Further screening will identify varieties which make more efficient use of applied N, in terms of translocation to the grain and incorporation into quality-related grain proteins.
- The project has demonstrated the potential for determining the genes and mechanisms responsible for GPD and quality traits, which will facilitate the development of new wheat lines with improved N utilisation

Implications for millers/bakers

- Reducing the requirement for high flour protein in breadmaking wheats is important in managing costs and reducing environmental/energy footprints
- There is the possibility to identify varieties with stable high processing quality when grown at low N inputs using the approaches described

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