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THE INFLUENCE OF NITROGEN FERTILISERS ON THE EXPRESSION OF FUNCTIONAL PROTEINS IN WHEAT

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THE INFLUENCE OF NITROGEN FERTILISERS ON THE EXPRESSION OF FUNCTIONAL PROTEINS IN WHEAT

by

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Abstract

Wheat of the breadmaking variety Hereward was grown at a single site in Suffolk and a number (ten) of fertiliser regimes ranging from 40 to 280 kgN/ha (including a uniform basal level of 40 kgN/ha) were applied in a randomised block system involving four replicates of each treatment. The treatments included both soil-applied ammonium nitrate and foliar-applied urea.

Immature caryopses (ten spikes per plot) were harvested at weekly intervals from ten days after anthesis and transported frozen to Chorleywood. After freeze-drying, caryopses weight and protein contents were measured. A total of five immature sample sets were handled.

The expression of functional protein (glutenin and gliadin) was monitored using gel electrophoresis. Results showed that there were no qualitative differences in the proteins expressed in the mature grain, but there was evidence that both high levels of ammonium nitrate and the use of foliar urea resulted in accelerated formation of gliadins.

Of particular note was the finding that the amount of the technologically significant high-molecular weight glutenin subunits (HMW-G) found in samples 31 days after anthesis correlated ($r=0.84$) with the amount found in mature harvested grain. Subject to confirmation with other varieties, HMW-G could be used to monitor crop development allowing targeted application of foliar urea to improve the breadmaking potential of the crop.

The harvested grain was test baked by the Chorleywood Bread Process. Baking performance was highly correlated with protein content ($r=0.93$), gel-protein content ($r=0.94$) and SDS sedimentation volume ($r=0.94$).

The trial showed that foliar urea alone (80 kgN/ha) did not increase grain yield, but the baking performance was better than that achieved with an equivalent amount of ammonium nitrate. This trial showed that good baking performance could be achieved at relatively low levels of nitrogen fertiliser. If confirmed in tests with other varieties, this finding could lead to significant economic and environmental benefits.

CONTENTS

1. INTRODUCTION

The development of the Chorleywood Bread Process (CBP), which opened the way through its ability to tolerate lower protein content wheat, increased the use of United Kingdom-grown wheat in the breadmaking grist. Currently some 4 million tons of breadmaking quality wheat are required each year (NABIM, 1993) and up to 90% of this has been UK-grown in seasons when harvest conditions have been favourable, ie when high *alpha*-amylase levels have been avoided.

Recent developments in world trade, namely, the reform of the Common Agricultural Policy (CAP) and the Uruguay round of GATT have opened the way for increased imports of third-country wheat into the EU. Also nostalgic memories of North American wheat remain within the UK Milling and Baking Industries. The protection of this market for UK-grown wheat depends upon the consistent supply of high quality wheat.

This consistent supply depends upon a number of factors including choice of wheat variety and agronomic and seasonal variation. Increasing understanding of the biochemical basis of wheat protein quality in breadmaking has come about through the study of the high-molecular weight subunits of glutenin (HMW-G) (Payne *et al* 1987). Recent work at the Flour Milling and Baking Research Association (FMBRA) now part of the Campden and Chorleywood Food Research Association (CCFRA), much of it funded by the HGCA has resulted in the development of the gel-protein tests (Bhandari and Pritchard 1994, Oliver and Pritchard 1993, Pritchard and Brock 1994).

Increasingly, new breadmaking varieties have optimised complements of HMW-G. Consequently, other influences on quality variation in wheat are becoming more dominant. These include other protein fractions (low-molecular weight glutenin subunits and redox enzyme systems) and agronomic factors such as nitrogen fertilisation.

A recent study funded by the Home-Grown Cereals Authority (HGCA) entitled "Management of breadmaking wheat: Effects of extra nitrogen on yield, grain and flour quality" (Dampney *et al* 1995) investigated the efficacy of additional (to that required for yield) nitrogen fertiliser in raising the content of functional protein of breadmaking wheat. This work showed that both ammonium nitrate and foliar-applied urea spray could be beneficial for Mercia variety wheat.

Of particular interest, however, was the fact that while both nitrogen sources increased gel-protein weight by similar amounts, the elastic modulus of gel-protein increased linearly with foliar urea addition but was relatively unaffected by ammonium nitrate. A correlation ($r=0.94$) was shown to exist between elastic modulus and loaf volume in long fermentation process baking and a similar trend was observed in the Chorleywood Bread Process (CBP).

The elastic modulus of gel-protein is a measure of protein-protein interactions developed within the grain. The two nitrogen sources are applied at different growth stages (Tottman and Makepeace 1979): 32 for ammonium nitrate, and around 75 for foliar urea, and it is conceivable that the differences are related to timing.

This current project was, therefore, designed to study the effect of timing and quantity of

nitrogen addition on the expression of proteins which are functional in breadmaking, during the growth period between anthesis and maturity. The project involved collaboration with Levington Agriculture, Levington Park, Ipswich, Suffolk, IP10 0LU.

The objectives were:-

* To monitor the laying down of functional protein (gliadin and glutenin) in the developing wheat endosperm.

* To monitor the creation of technologically significant protein-protein interactions.

* To assess the influence of quantity and timing of nitrogen fertilizers in processes listed above.

* To develop guidance about how to improve protein quality for breadmaking while maintaining yield.

2. MATERIALS AND METHODS

2.1 Materials

Wheat samples were grown under contract by Levington Agriculture in a randomized plot system encompassing ten nitrogen treatments replicated four times. The treatments were:-

Trial No.	Main	Late kgN/ha	Total
1	0	0	0
2	80	0	80
3	120	0	120
4	160	0	160
5	200	0	200
6	0	80	80
7	40	80	120
8	80	80	160
9	120	80	200
10	160	80	240

Note: An early (March) dressing of 40 kg/ha solid ammonium nitrate was also applied. Main dressing was solid ammonium nitrate applied in April and late nitrogen was foliar-applied urea. Nitrogen rates were chosen to extend below and above normal farm practice with a range of 40 to 280 kgN/ha (including the uniform early application of 40 kgN/ha to all treatments). This range was intended to produce as wide a range of grain protein contents and quality characteristics as possible. For comparison, the average nitrogen fertiliser application to winter wheat was 185 kgN/ha in 1993/94 (HGCA 1995). This average would of course include non-breadmaking varieties.

Hereward variety wheat was grown at one site in Suffolk, selected to be of N index 0 on

"good" wheat land. Full details of the site, fertiliser application and trial diary are listed in Appendix 1. Growth environment details; weekly rainfall, evapotranspiration, soil moisture deficit and ambient temperatures are listed in Appendix 2.

Hereward, a breadmaking variety, was chosen because it is known to have a lower (than Mercia) off-take of soil nitrogen, and therefore, a greater need for fertiliser nitrogen (Scott *et al* 1994). This variety has been of variable quality on occasions and the milling industry has some reservations about its long-term future. This project, by focusing on the link between nitrogen fertilisation and quality expression could lead to guidelines as to how the quality of Hereward might be maximised and made more consistent through husbandry practice on the farm.

Sampling of immature wheat caryopses commenced 10 days after anthesis (July 1) and continued weekly up to maturity (see trial diary, Appendix 1). A total of five immature samples were received at Chorleywood prior to harvest which occurred on the 9 August. 10 ears per plot were packed into containers with dry-ice and sent by courier to Chorleywood. On arrival they were immediately stored at -18°C until being freeze-dried.

Grain yield and dry matter were determined for each plot at harvest and 10 kg grain from each plot was despatched for testing at Chorleywood.

2.2 Methods

2.2.1 Quantity of functional protein

Gliadins were analysed by 1-D acid-PAGE (one-dimensional acid-polyacrylamide gel electrophoresis) at pH 3.1 (Salmon and Burbridge 1985), using the sensitive Coomassie R 250 staining technique of Sapirstein and Bushuk (1985).

Total proteins including glutenins were analysed by 1-D SDS-PAGE (Laemmli and Favre 1973).

2-D PAGE (two-dimensional polyacrylamide gel electrophoresis) was performed with isoelectric focusing in the first dimension followed by SDS-PAGE in the second (Görg *et al* 1992; Payne *et al* 1984, 1985). Samples from selected treatments and time points were examined by 2-D PAGE using pre-cast gels and immobilised pH gradients (Immobilines), on the horizontal flat-bed Multiphor II system with the EPS 3500 XL power supply (Pharmacia).

Proteins on 1-D electrophoregrams, either directly from the gels or photographs, were quantified by scanning densitometry on a computerised Shimadzu CS-9001PC instrument.

Total protein content was measured by a modified Lowry method (Markwell *et al* 1978).

Functional protein and its interactions in immature caryopses and harvested grain were measured by gel-protein weight and rheology (Pritchard *et al* 1992) see Appendix 3. Harvested grain was also assessed by the SDS sedimentation volume test (Axford *et al* 1978).

2.2.2 Breadmaking performance

Breadmaking performance was assessed by test baking using the Chorleywood Bread Process (CBP), see Appendix 3. Loaf volumes were measured by seed displacement and crumb structure score was rated on a scale (1 - 10) where high points are awarded for a close uniform structure.

3. RESULTS AND DISCUSSION

3.1 Developing grain

3.1.1 Yield

After removal from the freeze-dried wheat spikes the immature caryopses were weighed to yield a crude estimate of grain quantity. Protein contents were determined on this dried material and the products of caryopsis weight and protein content are listed in table 1 as the means of the four replicate plots. Statistical analysis showed that there were significant differences between treatments at all time points.

Generally the data show that in these samples of ten spikes per plot, protein weight increased with applied nitrogen, and that treatment 1 (no added fertiliser) was significantly lower than all other treatments. There was no significant difference between 80 kg/ha ammonium nitrate (treatment 2) and 80 kg/ha foliar urea (treatment 6), but there was evidence that combination of the two nitrogen sources could be beneficial to the laying down of protein. However, it must be remembered that these data were generated from ten spikes and not from yield estimates of whole plots. It is likely that spike density would have increased at higher fertiliser-N levels.

3.1.2 Total protein

A typical SDS-PAGE gel of a total protein extract is shown in figure 1. At the top of the gel are the larger protein species that migrate slowest through the gel. The largest of these are the high-molecular weight glutenin subunits (HMW-G), known to be important markers of breadmaking quality. These proteins are also the principal components of gel-protein (Pritchard and Brock 1994).

For selected replicate plots an estimate of the weight of HMW-G was obtained from the protein content of the immature caryopsis and harvested grain and the area proportion of HMW-G peaks in the total area of the scan. The sample selection was aimed at determining: i) the influence of fertiliser treatment and ii) the predictability of final wheat quality from that of immature grain. The samples were all time points for treatments 1, 2, 5 and 6; and all treatments at time points 2, 4 and 6. Mean data for the four replicates are listed in table 2.

Statistical analysis of these data showed that at time points 1 and 2 there were no significant differences between treatments. Close examination of the gels and the scans suggested that material in this area of the gel was not clearly differentiated into HMW-G until time point 3, see figure 2. At this time point (24 days after anthesis) some significant differences were found; ammonium nitrate, but not foliar urea was boosting HMW-G formation.

At time point 4 significant differences between treatments were observed but generally not between those with similar levels of nitrogen, ie, treatments 2 and 6 (80 kg/ha), 3 and 7 (120 kg/ha) and 5 and 9 (200 kg/ha). The densitometer scans showed that at high levels of nitrogen addition, resolution of HMW-G improved. Figure 3 shows the influence of ammonium nitrate (treatments 1-5). The same was true at time point 6. Figure 4 shows the influence of ammonium nitrate, but in the harvested grain all treatments exhibited good resolution of the HMW-G.

This general similarity between time points 4 and 6 suggested that a correlation might exist. The plot is shown in figure 5, $r=0.84$. Thus, on this set of samples (Hereward variety, 1994 at one site) analysis of the quantity of HMW-G in immature caryopsis at 31 days after anthesis gave an indication of the quality of the harvested mature grain.

There is, therefore, a potential for the manipulation of grain quality by late application of nitrogen fertiliser. It has been demonstrated (Dampney *et al* 1995) that late application of foliar urea is most effective in increasing protein content when applied during growth-stage (GS) 70-79 (the milk development stage) (Tottman and Makepeace 1979). Dampney *et al (1995)* also showed that at some sites the GS at which foliar urea first failed to produce a positive response to late foliar urea was as late as GS 85.

In this study, sampling commenced ten days after anthesis at GS 75. We estimate that time point 4 would have been beyond GS 80. Also, 1994 was atypical in that warm dry weather resulted in an early harvest which for these samples took place on August the 9th. In other seasons it is conceivable that the time interval between the growth-stages represented by our time points 4 and 6 would be longer than the 18 days that occurred in this work. There would seem to be a good case for a more extensive study involving other varieties at multiple growing sites.

3.1.3 Gliadin proteins

Gliadin fraction analyses were limited to four selected fertiliser treatments. These were analysed by ACID-PAGE (Salmon and Burbridge, 1985) using gels which were run for a long time (1 hour 28 minutes) and a shorter time (1 hour). The long-run time produces gels which contain essentially ω-, γ-, β-, and α-gliadin, migrating in that order, see figure 6. The short-run time gels yield the globulins and albumins in addition to the gliadins (figure 7). All the earliest time point 1 samples displayed considerable streaking, with diffuse and faint gliadin bands in both gel types, a feature also reported by Ng *et al* 1991, and Johansson *et al* 1994. Although the globulins and albumins were present as major components, no significant differences were detected between the following treatments: 1 (no added fertiliser; 5 (200 kgN/ha ammonium nitrate); 2 (80 kgN/ha ammonium nitrate); and 6 (80 kgN/ha foliar urea) treatments, at time point 1. These results indicate that no useful electrophoretic information may be gleaned from the first time point (10 days after anthesis). At time point 2, majority of the gliadins could be detected in the samples of all four treatments.

Examination of later time points on the short-run gel showed that even at 24 days after anthesis (time point 3), treatment 5 (200 kgN/ha ammonium nitrate) produced the highest ω-gliadin content, with the lowest content found in treatment 1 (no added fertiliser), see table 3. These observations are consistent with other reports on the effects of nitrogen fertilisers

on the sulphur-poor ω-gliadin content of wheat flours (Wrigley *et al* 1984, Skerrit *et al* 1987, Mills *et al* 1994). The addition of equivalent amounts of ammonium nitrate and foliar urea resulted in boosting the proportion of ω-gliadin at an early stage, and maintained that proportion until maturity. Visual inspection of the short-run gels revealed similarity between the overall patterns for the treatments 5 and 6 (200 kgN/ha ammonium nitrate and 80 kg/ha foliar urea) which persisted with time.

Overall patterns for treatments 1 and 2 (no added fertiliser and 80 kgN/ha ammonium nitrate) at time point 5, closely resembled those of treatments 5 and 6 (200 kgN/ha ammonium nitrate and 80 kgN/ha foliar urea) at time point 4. This suggests that 80 kgN/ha foliar urea hastens the development of the gliadins to the same extent as the maximum levels of ammonium nitrate.

It was noted that at time point 5 (38 days after anthesis) treatments 5 and 6 (200 kgN/ha ammonium nitrate and 80 kgN/ha foliar urea) had near identical band patterns which were almost indistinguishable from the patterns of their mature, harvest counterparts. The time point 5 samples of treatments 1 and 2 (no added fertiliser and 80 kgN/ha ammonium nitrate) displayed near identical patterns, but differed from their mature counterparts (figure 7 short-run gel).

Quantitative densitometric analysis of the patterns obtained from the mature samples of the four treatments revealed that treatments 5 and 6 (200 kgN/ha ammonium nitrate and 80 kgN/ha foliar urea) yielded the highest content of total gliadin, and lowest content in treatment a)(no added fertiliser). The ω-gliadin content was the highest, ~25%, in treatment 6 (200 kgN/ha ammonium nitrate), while the other treatments all produced a lower common value of ~20%, on short- and long-run gels. Apart from the ω-gliadins, there was no evidence of any other significant variations in the remaining gliadin fractions, or in the globulins and albumins at maturity as a consequence of fertiliser treatment.

3.1.4 Two-dimensional gel-electrophoresis

Two-dimensional PAGE can achieve a better resolution of the endosperm proteins, than is generally possible with either ACID- or SDS-PAGE techniques. Several studies performed by Payne and co-workers (Jackson *et al* 1983, Payne *et al* 1984, 1985, 1986) have contributed to the identification of many of the major wheat components within two-dimensional PAGE maps. The extractions were performed with 9 M urea and 2 % NP-40 (a neutral detergent) to maximise the solubilisation of all classes of proteins (Dougherty *et al* 1990, Görg *et al* 1992). The two-dimensional PAGE maps contained several hundred components, and the variation in staining between individual gels made comparative quantitative detection impractical. Therefore, the gel maps were visually inspected.

Samples selected for analysis were limited to the four basic treatments: 1 (no added fertiliser); 5 (200 kgN/ha ammonium nitrate); 2 (80 kgN/ha ammonium nitrate) and 6 (80 kgN/ha foliar urea), at time points 1, 4, 5 and 6. Despite time point 1 two-dimensional maps having streaky spots and diffuse backgrounds, many lower molecular weight components corresponding to gliadins could be discerned (figures 8 & 9). Treatment 1 (no added fertiliser) map possessed the lowest proportion of α- and β- gliadins compared to the other treatments at the earliest stage. The consistency of the maps from time points 4, and 5

(figures 10 & 11) were unsatisfactory for an unequivocal interpretation, and no major differences were detected within the four treatments at time point 6 (figure 12 illustrates treatment 5 as an example).

Although two-dimensional PAGE is a very powerful technique for analysing proteins, the staining stage is very critical due to the highly sensitive nature of the silver stain and was one of the main causes of variation between gels. Many versions of the silver stain exist and these may detect different classes of proteins to varying degrees, making a comprehensive analysis of total wheat protein on a single gel somewhat difficult. The reasons for streaking and diffuse spots in some gels and not in others were not obvious. Future studies may achieve more satisfactory resolution on two-dimensional gels by applying the partly purified fractions separately, e.g. gliadins, glutenins or water-soluble, etc, and then staining with the most appropriate method for that fraction.

3.1.5 Protein-protein interactions

The weight and elastic modulus of gel-protein are now used as measures of the quantity and strength of the functional proteins existing in grain that are known to be influential in determining the breadmaking quality of wheat. It was of interest to determine whether such material can be detected in immature wheat caryopses.

Gel-protein levels are commonly measured on white flour and previous work (Pritchard *et al* 1992) has shown that both gel-protein weight and elastic modulus can be reduced in the presence of bran particles. Indeed, the extra-strong character of Fresco is not apparent in wholemeal flour. In this study only whole grain samples of the immature caryopses were available. Gel-protein weight and elastic modulus were determined on the bulked samples of the four replicate plots for selected treatments. For consistency the harvested grain was treated similarly.

For time points 1 to 3 there was no evidence of gel-protein. At time points 4 and 5 there was insufficient gel to allow meaningful rheological assessment. Gel-protein weights and elastic moduli data are listed in table 4. The elastic modulus data for harvested grain suggest that in the wholemeal ammonium nitrate was superior to foliar urea for developing protein-protein interactions. The weight data for 80 kg/ha showed that foliar urea was beneficial reflecting the results obtained on white flour.

In the absence of gel-protein, the viscosity (as a function of shear) of the SDS soluble protein material was measured. No consistent pattern with time or treatment was found. This work indicated that the protein-protein interactions detected by the gel-protein tests were not present in the immature caryopses. The test would have little value in predicting harvested grain quality.

3.2 Harvested grain

3.2.1 Yield

Mean yield data for the four replicate plots for each fertiliser treatment are listed in table 5. As ammonium nitrate levels were raised, significant ($p < 0.01$) increases in grain yield were

found. Comparison between ammonium nitrate and foliar urea used singly (treatments 2 and 6 respectively) showed that foliar urea had little influence on grain yield. When used in combination (treatments 7 to 10) it is clear that yield was determined by the amount of ammonium nitrate.

A crude measure of the amount of fertiliser nitrogen recovered from the grain was obtained from the dry matter basis yield and protein content data. This ranged from 49% (80 kg/ha foliar urea) to 74% (80 kg/ha ammonium nitrate) and clearly demonstrated the importance of early application of nitrogen fertiliser for yield purposes. All recoveries were high relative to those achieved in the earlier study (Dampney *et al* 1995) which ranged from 30% at 30 kg/ha down to 15% at 180 kg/ha applied nitrogen. This difference can probably be attributed to the lower levels (40 kg/ha) of base nitrogen applied to all plots in this study. In the earlier study this ranged from 50 to 225 kg/ha.

3.2.2 Quality

Measures of flour quality (protein content, SDS sedimentation volume, gel-protein weight and elastic modulus) and baking quality (CBP loaf volume and crumb score) are listed in table 6. The results show generally, that as the quantity of nitrogen fertiliser increased, the flour quality improved and with it the baking performance. An exception was the gel-protein elastic modulus which was relatively unaffected.

Of particular interest was the comparison between treatment 2 (80 kg/ha ammonium nitrate) and treatment 6 (80 kg/ha foliar urea). Foliar urea increased flour protein content by 2.95% and gel-protein weight by 4.33g relative to the untreated control (treatment 1), the equivalent figures for ammonium nitrate being 1.24% and 1.73g respectively. These differences were reflected in the baking performance where foliar urea brought about a loaf volume increase of 123ml whereas ammonium nitrate only resulted in a loaf volume increase of 33 ml.

An unexpected result was the fall in the elastic modulus of gel-protein in treatment 6. This result is at variance with those of Dampney *et al* (1995) which showed that foliar urea significantly increased elastic modulus in samples of Mercia wheat grown in 1990 and 1991. This study featured Hereward, known to respond differently to added fertiliser than does Mercia (Scott *et al* 1994). Also, the 1994 harvest was unusual in that the climatic conditions- warm and dry - resulted in an early harvest, and it was observed that in many other samples elastic moduli were higher than in recent years.

In this trial, correlation coefficients between measured quality parameters and loaf volume were:-

gel-protein elastic modulus	0.37
gel-protein weight	0.94
flour protein content	0.93
SDS sedimentation volume	0.94

The similarity between protein content and gel-protein weight was reflected in a correlation coefficient of 0.996 between them. These results clearly indicate that, in this trial the

quantity of functional protein was more important than its quality. Also, there is evidence of a direct link between applied nitrogen and functional protein as well as to protein content.

This trial differed from previous work (Dampney *et al* 1995) in that a very low level of basal nitrogen was applied. Thus the added fertiliser had a larger influence on yield, protein content and quality than seen previously. The economic down side of this study was that under these lean nitrogen conditions, foliar urea alone had no influence on yield. Thus early nitrogen is a prerequisite to trigger the plant to lay down the foundations for a good yield. If these conditions are not met, available nitrogen appears to be directed towards total and functional protein.

4. CONCLUSIONS

This study, limited though it was to one wheat variety, Hereward, grown at one site during one season, showed that monitoring of the expression of functional proteins during maturation, has potential for the prediction and manipulation of the quality of harvested grain.

Electrophoresis of both total protein extracts and of gliadin proteins suggested that the amount and timing of fertiliser nitrogen application had little influence on the protein species expressed, although there was evidence that some gliadin proteins were seen earlier when high levels of fertiliser N were applied. This was probably due to more vigorous growth allowing more rapid development when abundant nitrogen was available. Two-dimensional electro-phoresis confirmed that qualitatively the protein species were unaffected, reflecting a strong varietal influence.

The correlation between amounts of high-molecular weight glutenin subunits at around GS80 and those seen in the harvested grain appears the most promising outcome of this study. We believe that subject to the result being validated over a number of varieties, growing sites and seasons, HMW-G could be used to monitor the crop to determine whether additional nitrogen fertiliser would be beneficial for the attainment of desired quality traits. Such a programme would of course require the development of suitable assaying techniques, appropriate for use on the farm. A necessary first step would be validation.

The measured flour quality and baking performance of the harvested grain, showed that foliar applied urea did not result in increases in elastic modulus of gel-protein. These results, therefore, differed from those seen by Dampney *et al* (1995) in Mercia variety wheat. This difference could be due to variety or to the short ripening period which was a feature of this study. Such differences will only be resolved as more variety/seasonal data becomes available.

The grain yield data showed that foliar-applied urea is not sufficient for adequate yield potential in the absence of sufficient ammonium nitrate. However, at 80 kgN/ha of foliar urea, the baking performance was better than that achieved with an equivalent amount of ammonium nitrate. This trial showed that good baking performance could be achieved at lower levels of nitrogen fertilisers than commonly used. If this finding can be substantiated in other varieties, and under different growing conditions, there is potential for reaping significant economic and environmental rewards.

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Table 1

Yield of protein in immature grains from ten spikes
(mean of four plots)

(g x 100)

Treat-ment	Time 1	Time 2	Time 3	Time 4	Time 5
1	58.06	88.02	149.18	142.86	147.74
2	63.51	100.11	171.13	197.87	203.36
3	72.31	105.00	197.43	214.48	229.06
4	70.07	115.71	210.05	235.55	232.63
5	67.95	105.43	224.76	273.92	256.39
6	73.77	111.69	190.48	196.57	229.88
7	76.66	113.39	205.74	238.59	240.01
8	74.25	115.26	195.88	247.09	241.55
9	68.67	114.89	217.08	274.57	252.87
10	73.29	114.48	232.06	271.84	279.29
LSD 5%	9.86	14.77	-	-	-
LSD 10%	-	-	36.02	39.55	32.31

Table 2

Weight of high-molecular weight glutenin subunits
(mean of four plots)

(g/100g grain)

Treat-ment	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6
1	0.41	0.28	0.33	0.22	0.35	0.37
2	0.44	0.35	0.45	0.28	0.42	0.49
3	-	0.36	-	0.32	-	0.54
4	-	0.34	-	0.35	-	0.59
5	0.50	0.36	0.53	0.39	0.52	0.71
6	0.48	0.37	0.40	0.28	0.44	0.60
7	-	0.39	-	0.37	-	0.60
8	-	0.41	-	0.44	-	0.62
9	-	0.37	-	0.42	-	0.64
10	-	0.36	-	0.39	-	0.67
LSD 5%	NS	NS	0.086	0.092	NS	0.116

Note At time points 4 and 6 the LSD figures based upon treatments 1,2,5 and 6 were 0.070 and 0.116 respectively.

Table 3

Acid-PAGE of gliadin proteins
Densitometer scan data- total and ω-gliadins

SHORT GEL TIME POINT 3				SHORT GEL TIME POINT 4			
Treat-ment	ω-gliadins	Total gliadins	% ω-gliadins	Treat-ment	ω-gliadins	Total gliadins	% ω-gliadins
1	5759	39854	14.45	1	7282	45708	15.93
5	20737	77839	26.64	5	26461	101594	26.05
2	16592	79624	20.84	2	17880	96745	18.48
6	22864	124952	18.30	6	22673	111088	20.41
SHORT GEL TIME POINT 6				LONG GEL TIME POINT 6			
1	17076	87771	19.46	1	193415	990709	19.52
5	50125	193809	25.86	5	375935	1569139	23.96
2	33717	173649	19.42	2	282994	1262758	22.41
6	49469	256664	19.27	6	329613	1571972	20.97

Table 4

Gel-protein characteristics of immature and harvested grain
(bulked samples)

Treatment	Time 4 Weight g/5g	Time 5 Weight g/5g	Time 6 Weight g/5g	Time 6 Elastic modulus Pa
1	*	1.02	4.59	4.87
2	*	1.50	5.79	11.90
4	0.74	1.56	7.54	16.30
5	1.09	2.46	8.40	22.65
6	0.61	1.60	8.04	6.22
8	1.13	2.07	7.32	13.90

* Too little to measure

Table 5

Harvested grain yield and nitrogen recovery
(mean data)

Treat-ment	Grain yield T/ha as is	Grain yield T/ha dmb	Grain protein content % dmb	Protein yield kg/ha	Increase in N yield kg/ha	Added fertiliser N kg/ha	N re-covery %
1	5.10	4.33	10.58	458	-	-	-
2	7.55	6.41	12.42	796	59.3	80	74.1
3	8.14	6.92	13.43	929	82.7	120	68.9
4	8.54	7.26	14.53	1055	104.7	160	65.4
5	9.11	7.75	16.15	1252	139.2	200	69.6
6	5.50	4.67	14.61	682	39.3	80	49.2
7	6.86	5.83	15.20	886	75.1	120	62.6
8	7.65	6.50	15.87	1032	100.6	160	62.9
9	8.28	7.03	16.26	1143	120.2	200	60.1
10	8.61	7.32	17.17	1257	140.1	240	58.4
LSD 5%	0.364	0.310	-	-	-	-	-
1%	0.492	0.418	-	-	-	-	-

Table 6

Flour quality and baking performance of mature harvested grain
(mean of 4 plots)

Treat-ment	Protein content (Nx5.7) (14%mb) %	SDS sed. vol ml	Gel-protein weight g/5g	Gel-protein elastic modulus Pa	CBP loaf volume ml	CBP crumb score (1-10)
1	7.20	62	7.79	36.18	1669	5.25
2	8.44	74	9.52	37.28	1702	6.25
3	9.18	82	10.41	40.50	1770	6.75
4	9.92	86	11.51	39.15	1807	7.0
5	11.01	92	12.95	41.11	1812	7.0
6	10.15	81	12.12	32.50	1792	7.50
7	10.33	85	12.06	35.56	1793	7.25
8	10.76	90	12.63	39.94	1786	7.5
9	10.95	92	12.94	39.15	1814	7.5
10	11.51	91	13.44	46.19	1812	7.5
LSD 5%	-	-	-	-	-	1.21
LSD 1%	0.57	8.2	1.08	6.27	89	-

Figure 1

Typical SDS--PAGE of flour total protein extract

(Harvested mature grain)

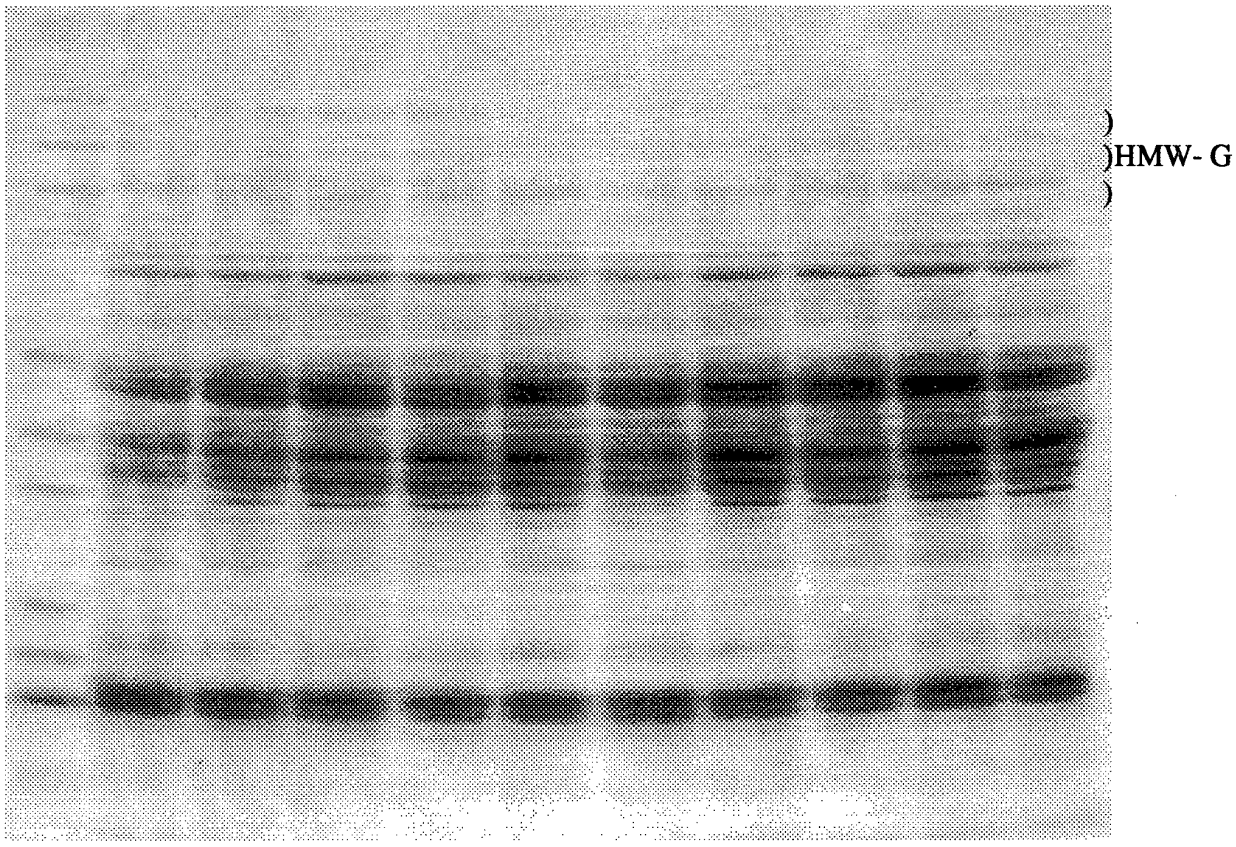


Figure 2

Densitometer scans of total protein extracts: effect of grain maturity. (treatment 5 - 200KgN/ha)

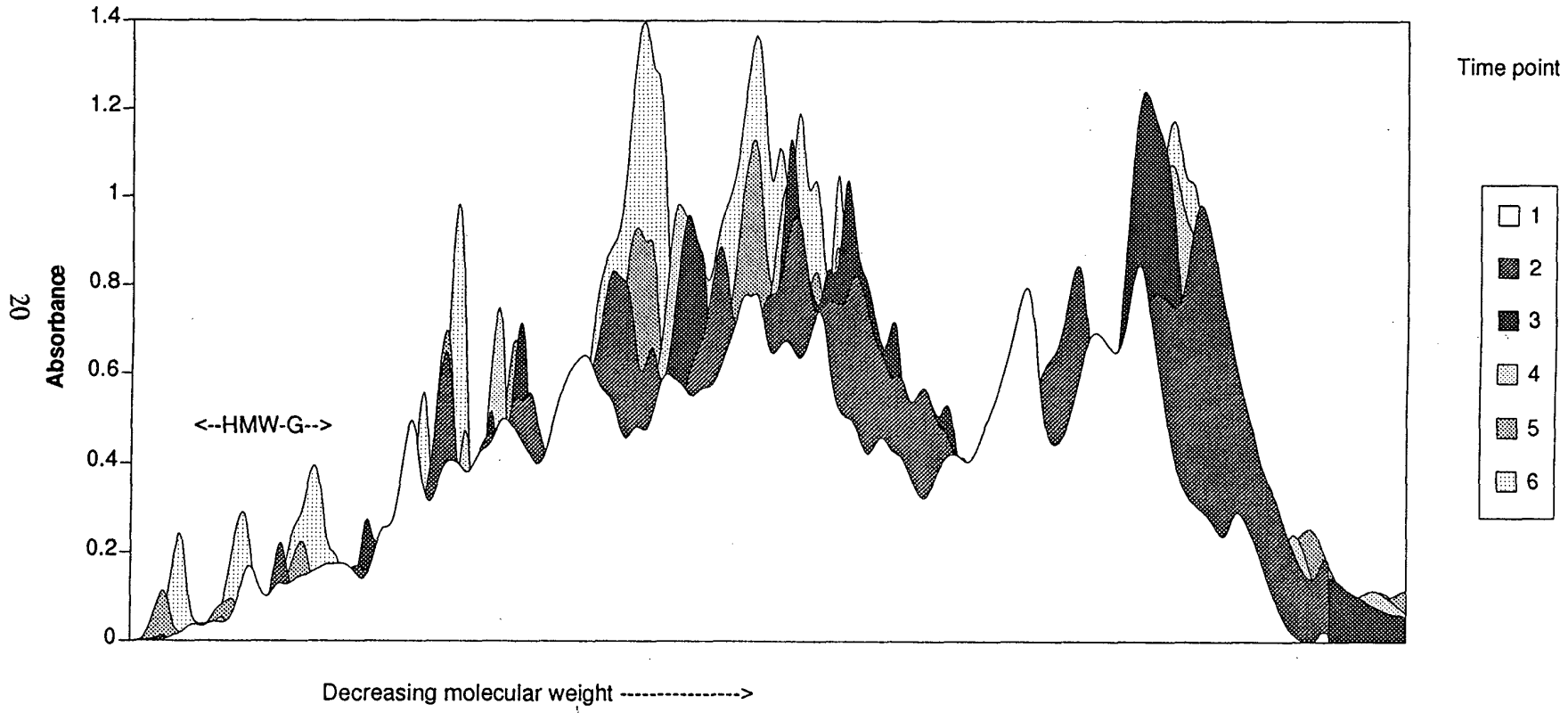


Figure 3

Densitometer scans of total protein extracts: effect of ammonium nitrate (time point 4 - 31 days after anthesis)

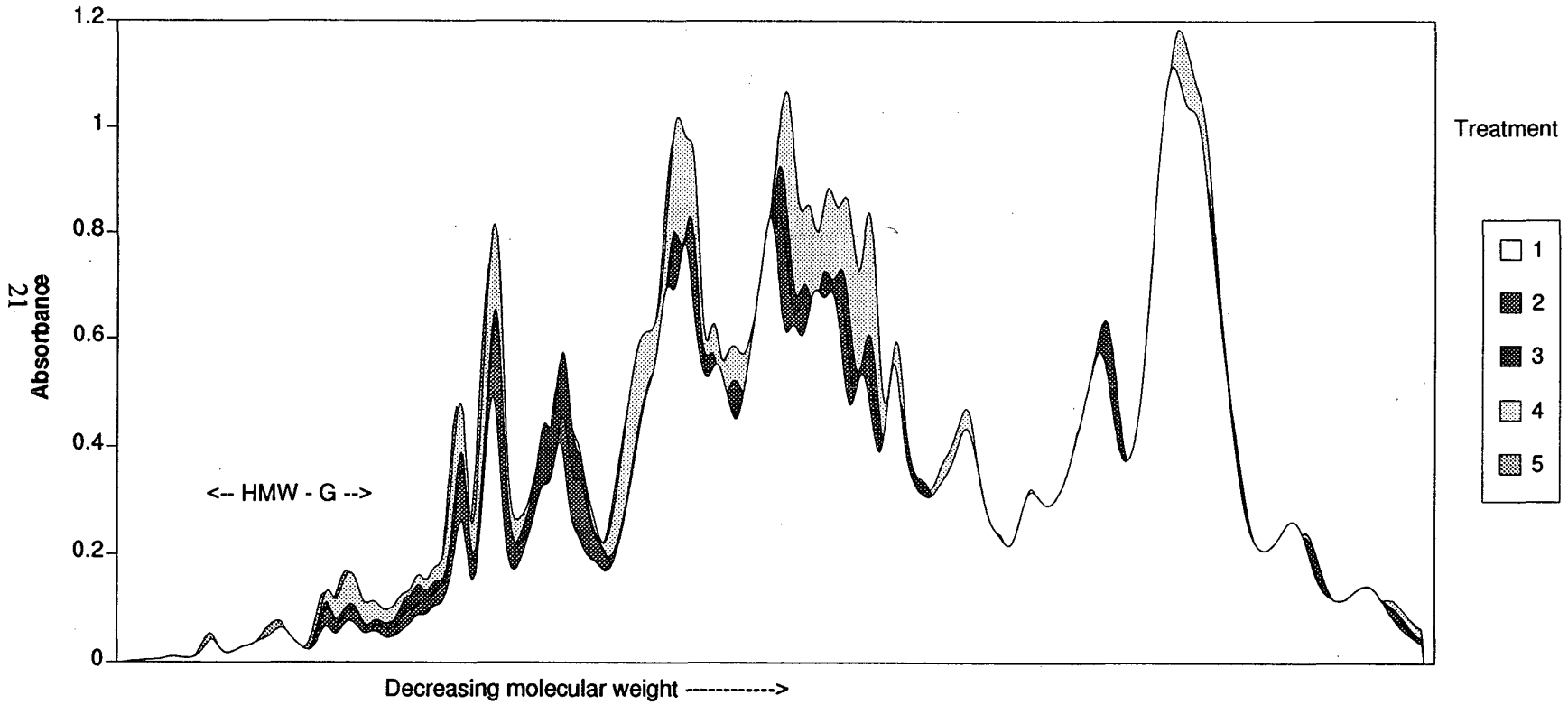


Figure 4

Densitometer scans of total protein extracts: effect of ammonium nitrate. (time point 6 - harvested grain)

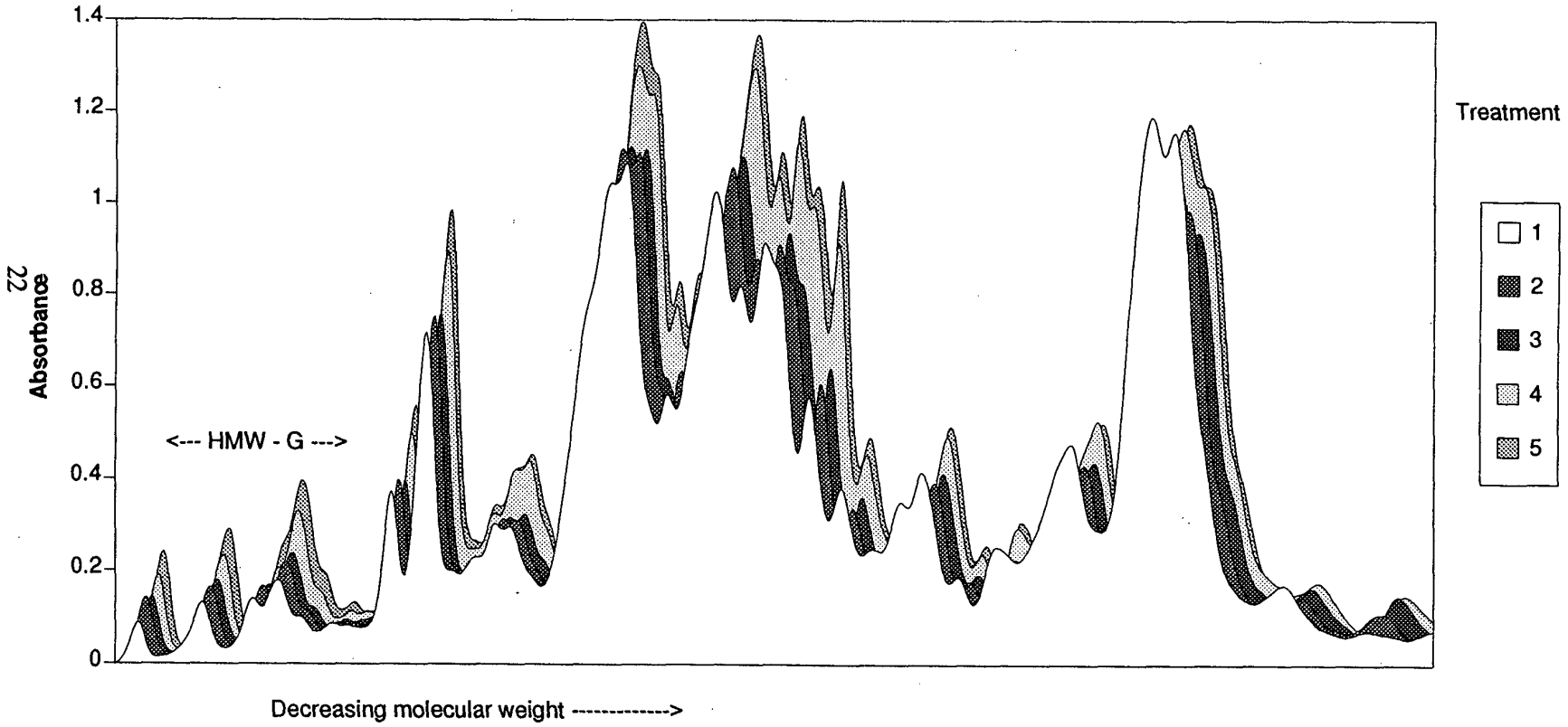


Figure 5. Weight of HMW-G

Correlation between time point 4 (31 days after anthesis) and time point 6 (harvested grain)

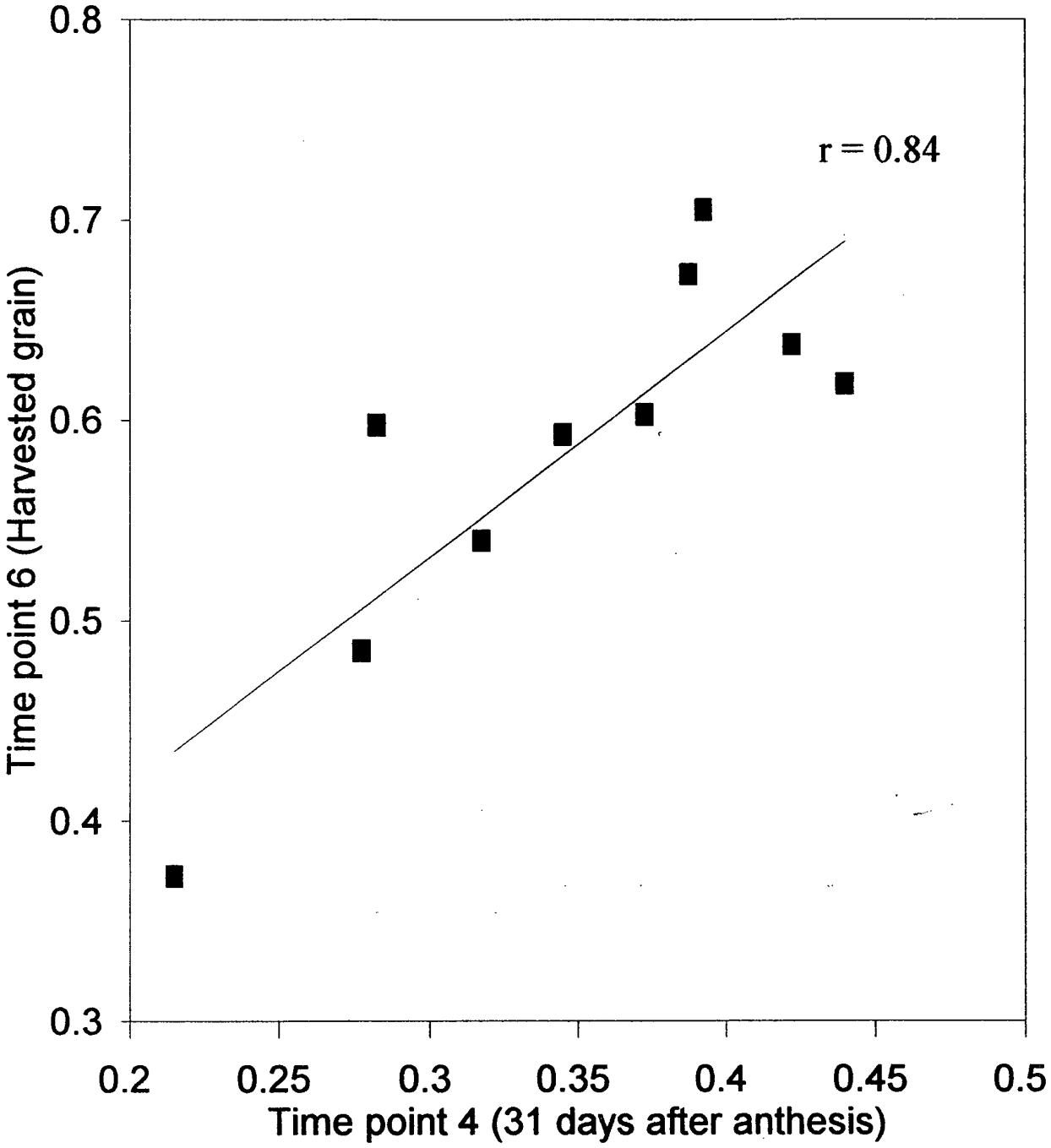
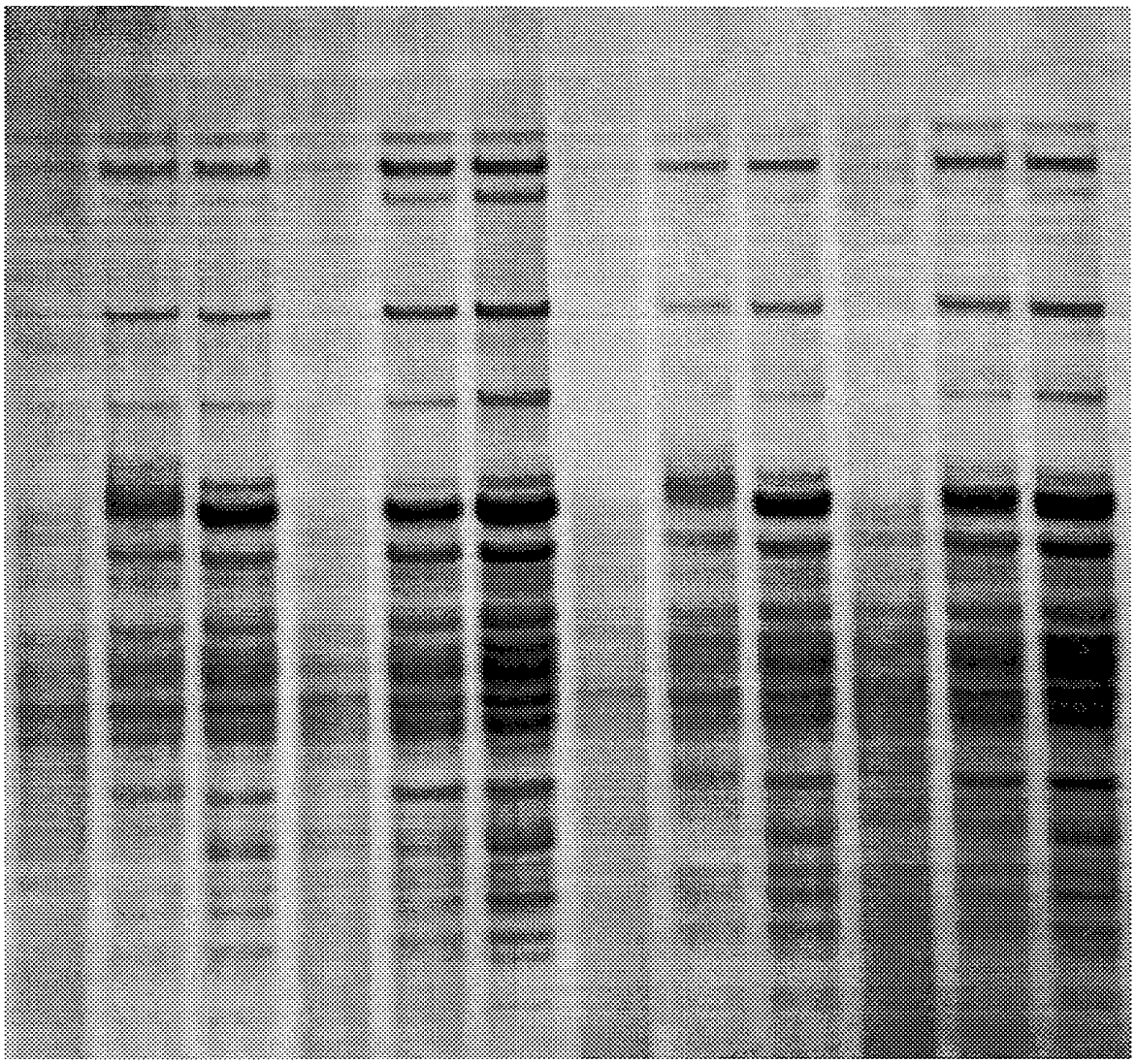


Figure 6

Acid-PAGE of gliadin proteins showing effect of grain development

(long-run gel)



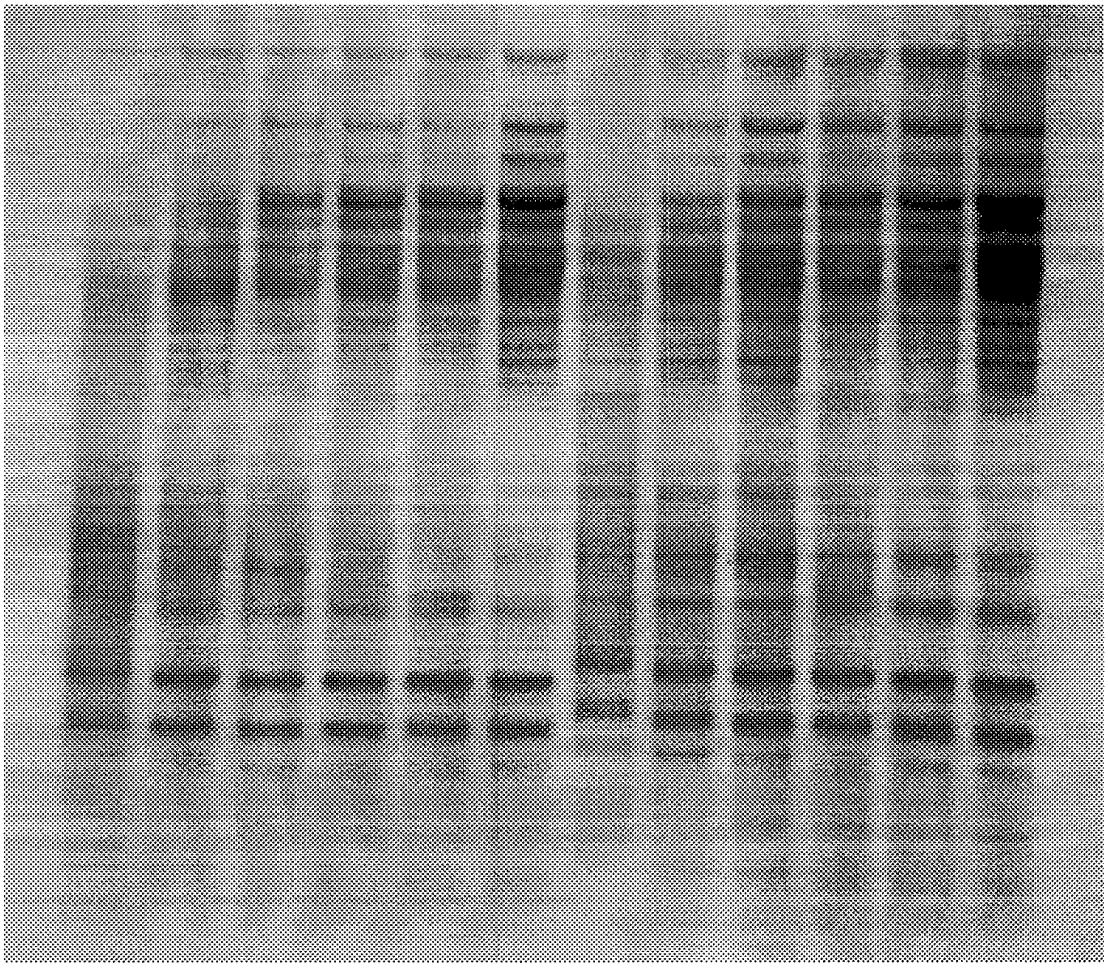
Time point

1	5	6	1	5	6	1	5	6	1	5	6
Treatment 1 (nil added) (nitrogen)			Treatment 5 (200 kgN/ha) (ammonium nitrate)			Treatment 2 (80 kgN/ha) (ammonium nitrate)			Treatment 6 (80 kgN/ha) (foliar urea)		

Figure 7

Acid-PAGE of gliadin proteins showing effect of grain development

(Short-run gel)



Time point

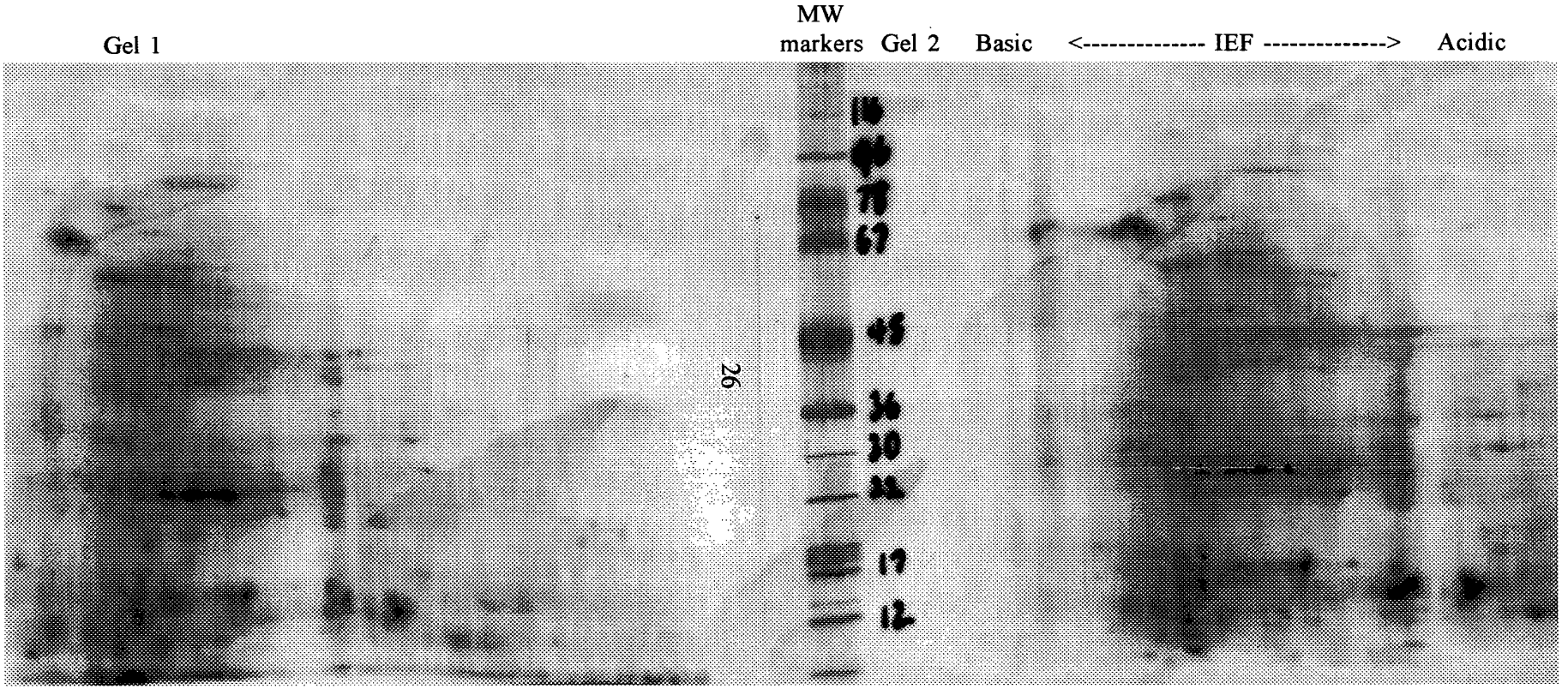
1 2 3 4 5 6 1 2 3 4 5 6

Treatment 2
(80 kgN/ha ammonium nitrate)

Treatment 6
(80 kgN/ha foliar urea)

Figure 8

2-D Maps of samples of treatments 1 and 5 at timepoint 1

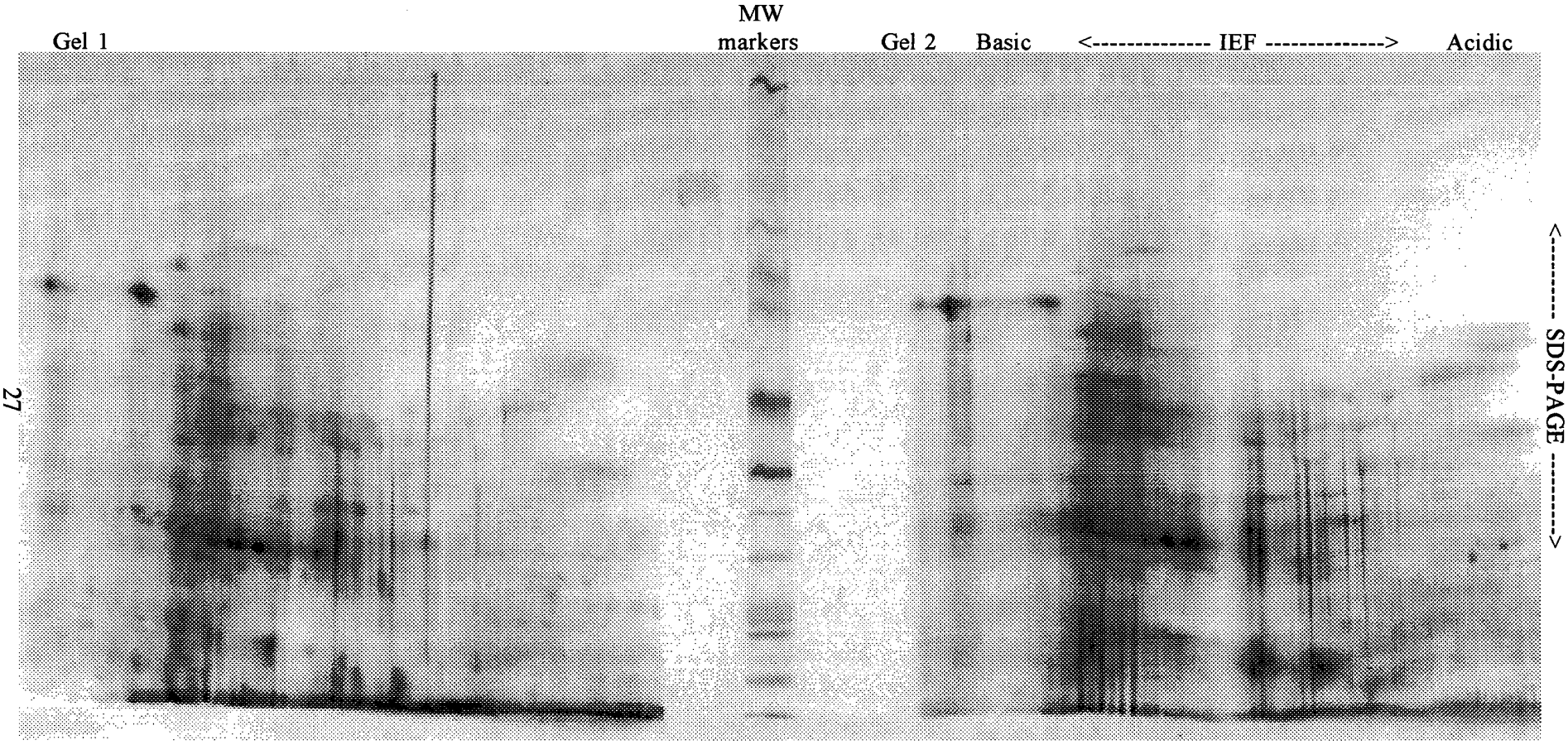


Treatment 1 (no added fertiliser)

Treatment 5 (200 kg N/ha ammonium nitrate)

Figure 9

2-D Maps of samples of treatments 2 and 6 at timepoint 1



Treatment 2 (80 kg N/ha ammonium nitrate)

Treatment 6 (80 kg N/ha foliar urea) nitrate)

Figure 10

2-D Maps of samples of treatments 1 and 5 at timepoint 4

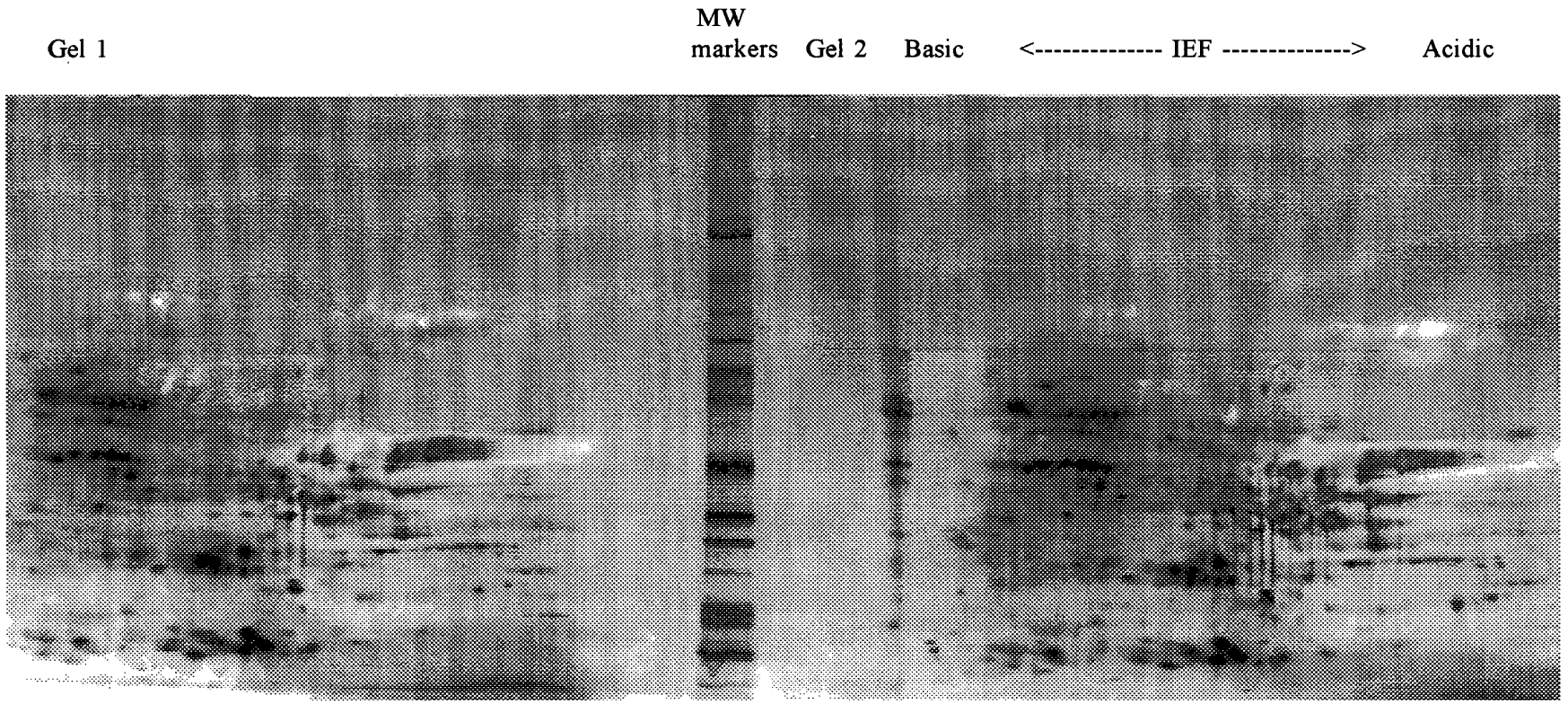
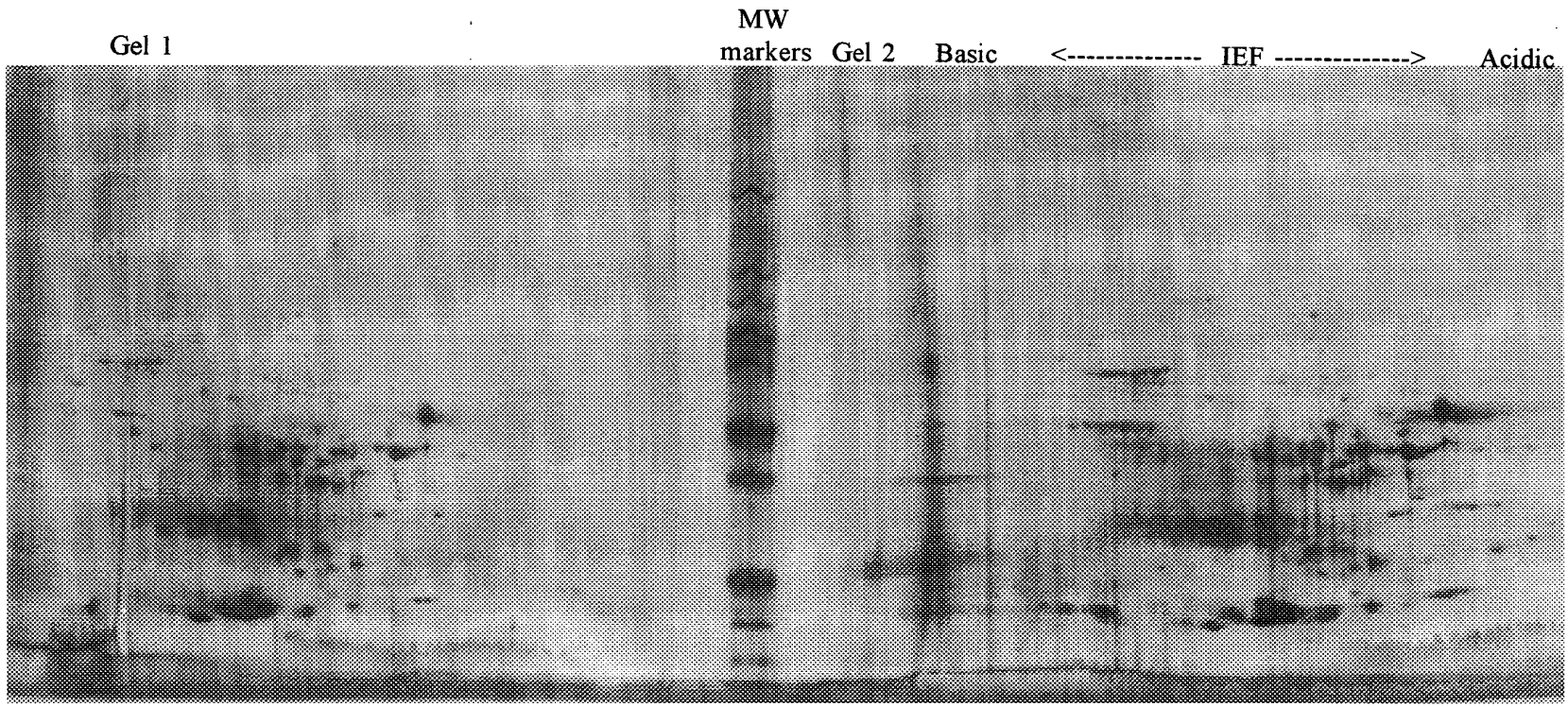


Figure 11

2-D Maps of samples of treatments 2 and 6 at timepoint 5

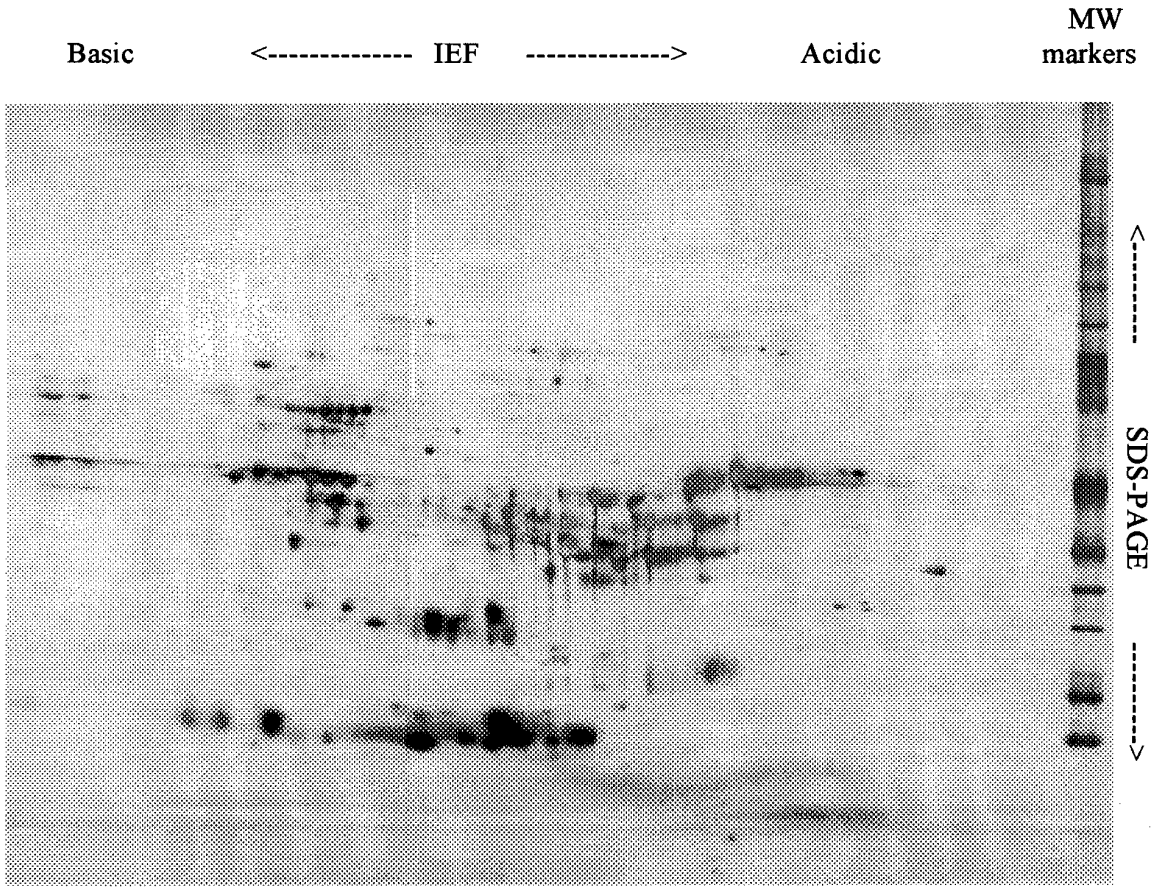


Treatment 2 (80 kgN/ha ammonium nitrate)

Treatment 6 (80 kg N/ha foliar urea)

Figure 12

2-D Maps of treatment 5 (200 kg N/ha ammonium nitrate) at timepoint 6



Appendix 1

Growing trial details

Levington Agriculture code	Project 495

Site details

OS Reference		TL 703 895
County		Suffolk
Soil:	Type	Sandy Clay Loam
	Clay %	28.0
	Silt %	18.0
	Sand %	54.0
	$CaCO_3$%	6.7
	Organic carbon %	1.1
	pH	8.1
	N Index	0
	Phosphorus (resin extract) mg/l	14
	Potassium (ammonium nitrate extract) mg/l	146
	Magnesium (ammonium nitrate extract) mg/l	51
	Available water capacity (0.2-15.0 bar) %	19.4
Crop:	Variety	Hereward
	Sowing date	22 Sept. 1993
	Seed-bed preparation	Sub-soiled and power harrow
	Seed rate kg/ha	200
	Row width mm	115
	sowing depth mm	30-50
	Seed treatment	Cerevax

Previous Cropping:	Previous year	Sugar beet
	Two years before	Winter wheat
	Three years before	Winter wheat
	Four years before	Winter wheat

Agrochemical sprays:	Dicurane	5 l/ha	20 Oct. 1993
	Flexidor	0.15 l/ha	20 Oct. 1993
	Toppel	250 ml/ha	6 Nov. 1993

Appendix 1 continued

Fertiliser application

	1st uniform ammonium nitrate application	2nd treatment ammonium nitrate application	3rd treatment foliar-urea application
Date	10 March 1994	21 April 1994	6 June 1994
Method	By hand	By hand	CO_2 sprayer
Soil temp. °C	9	11	16
Air temp °C	11	13	20
Relative humidity %	68	65	78
Soil surface	moist/dry	dry	moist
At 50 mm depth	wet	moist/wet	moist/wet
Crop growth stage (Zadoks)	22-24	31-32	52
Surface leaf moisture (Y/N)	N	N	N

Trial diary

31 Jan. 1994	Site marked out, growth stage 23.
10 March 1994	50 kgN/ha applied to all plots as ammonium nitrate.
21 April 1994	Soil sample taken for available water capacity.
	Main nitrogen application as ammonium nitrate.
6 June 1994	Foliar urea applied.
1 July 1994	10 ears per plot sampled and despatched to Chorleywood.
8 July 1994	10 ears per plot sampled and despatched to Chorleywood.
15 July 1994	10 ears per plot sampled and despatched to Chorleywood.
22 July 1994	10 ears per plot sampled and despatched to Chorleywood.
29 July 1994	10 ears per plot sampled and despatched to Chorleywood.
9 Aug. 1994	Site harvested, 10 kg grain per plot despatched to Chorleywood.
	No lodging of crop.

Appendix 2

Growing site moisture and temperature data

Table 1
Soil moisture data

Date	Soil moisture deficit mm	Rainfall preceding 7 days mm	Potential losses preceding 7 days evapo-transpiration mm	Drainage mm
11/4	0.0	22.5	11.9	8.2
18/4	0.0	14.0	12.9	3.0
25/4	13.5	3.0	17.0	0.0
2/5	38.9	0.0	25.4	0.0
9/5	33.7	23.6	18.4	0.0
16/5	50.4	5.5	22.2	0.0
23/5	54.0	11.0	14.6	0.0
30/5	71.6	0.0	17.6	0.0
6/6	92.6	12.0	17.6	0.0
13/6	115.4	4.0	26.8	0.0
20/6	144.6	0.0	29,2	0.0
27/6	161.0	14.0	30.4	0.0
4/7	192.1	0.0	31.1	0.0
11/7	208.6	4.5	21.0	0.0
18/7	212.3	1.5	5.2	0.0
25/7	198 .9	15.0	1.6	0.0
1/8	197.2	2.5	0.8	0.0
8/8	208.3	4.5	15.6	0.0
15/8	196.7	20.4	8.8	0.0

Ambient temperature

Between April 13 and July 19 ambient temperatures were recorded at two hourly intervals at a weather station at Norton in Suffolk within 5 miles of the growing site. Calculated daily averages showed that average temperatures greater than 15°C occurred on 3, 16 and 19 days in April, June and July respectively. Temperatures greater than 20° occurred on 1 day in June and 4 in July.

The total number of hours above 20°C were 5 in April, 89 in June and 145 in July. Temperatures above 25°C were experienced for 6 hours in June and 38 hours in July. On July 12 the temperature exceeded 30°C for 6.5 hours. These data are included for archival purposes as a record of the environmental conditions applying to this trial. They will be of value when comparing this trial with others in the future.

Appendix 3

CBP recipe and dough processing methods for white bread

Test baking procedure No. 1AA

Breadmaking process: CBP

Bread type: 400g white

Mixing machine: Morton

Recipe:

	% of flour weight	g/mix
Flour	100	1400
Yeast (compressed)	2.5	35
Salt	2.0	28
Water	As determined by Simon Extrusion Meter 10 min method	
Fat (Ambrex, slip point c. 45°C)	1.0	14
Ascorbic acid (100 ppm)	0.01	0.14

The *alpha*-amylase activity of the flour is adjusted to 80 FU by the addition of fungal *alpha*-amylase.

Dough processing:

Mixing machine	:	Morton
Beater speed	:	300 rev per min.
Work input	:	11Wh/kg
Pressure	:	Atmospheric
Dough temperature	:	30.5 +/- 1°C
Scaling	:	By hand to 454g
First moulding	:	Cylinder using Mono moulder
First proof	:	10 min. at ambient temperature
Final moulding	:	Single-piece cylinder, (R7, W5.5, P1.25)
Pan size	:	Top 160mm x 98mm, 83mm deep
Shape	;	Unlidded
Proving conditions	:	43°C, humidity to prevent skinning
Proving height	:	10cm
Baking temperature	:	244°C
Oven type	:	Direct gas-fired Reel
Baking time	:	25 min.
Baking humidity	:	No steam injected
Cooling	:	Open rack at room temperature
Storage	:	Closed cupboard overnight at 21°C