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Genetic Reduction of Energy use and Emissions of Nitrogen through cereal production:

GREEN grain

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by

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1. ABSTRACT

This project initiated the development of a new wheat type for the UK – one with high energy grain, suited to alcohol production and livestock feeding, and with low nitrogen fertiliser requirements hence low environmental impacts. Primary beneficiaries of this 'GREEN grain' are predicted to be wheat growers through reduced costs of crop nutrition, but alcohol processors would also benefit, and emissions of nitrate, ammonia and nitrous oxide would reduce markedly. Wheat seed performance was also improved, but expected benefits for the livestock industry remain to be proven. Findings can be summarised as follows:

- Genetic variation amongst elite and 'global' germplasm indicated potential to breed varieties with the 'GREEN grain' ideotype – having both low canopy nitrogen and low grain protein. Varieties with lower grain protein tended to have higher grain yields when N was withheld.
- Examples of varieties demonstrating desirable traits were Defender (good N capture), Creativ (low leaf N), Solstice (low leaf sheath & stem N), Acropolis (low ear N), Exsept (low straw & chaff N), and Audi (low grain N).
- Canopy and N recovery traits showed genetic variation, but heritabilities were low, variation was often small, and few useful quantitative trait loci (QTL) were discovered, so a wider range of germplasm must now be explored.
- Grain protein had much greater heritability and, with its ease of measurement, breeding for low grain protein appears to offer the greatest scope to reduce N requirements. Genetic variation in grain protein can be as great as the fertiliser effect on grain protein, with fertilised low protein varieties having lower protein than unfertilised high protein varieties.
- Gliadins accounted for over 50% of grain protein and were much the most responsive fraction to nitrogen supply, but there was disappointingly little genetic variation in gliadin:total protein ratio.
- The inverse relationship between protein content and alcohol yield was confirmed, but alcohol yield also showed consistent genetic variation of up to 50 litres per dry tonne unrelated to protein content (probably related to non-starch polysaccharides).

- Varieties with the best alcohol yields (e.g. Glasgow, Denman, Zebedee, Istabraq) tended to have high grain yields and low grain protein, facilitating combined selection for alcohol yield and reduced N requirements. Maximum alcohol yields were 481 litres per dry tonne, and 4,638 litres per hectare.
- Near infrared spectrometer (NIR) calibrations for alcohol yield and gliadin content were developed that should prove useful for distilling and bioethanol processors, and also for plant breeders.
- Two commercial broiler feeding trials tested diets containing proto-type GREEN grain (6-8% protein instead of normal 10-11% protein). Manure N content and ammonia emissions were reduced, and effects on manure output, bird mortality, health and performance were not significant.
- Analysis of mapping populations returned several potentially useful QTL for protein composition and grain size. These QTL will be validated by Syngenta prior to possible use in their commercial wheat breeding programmes.
- If the industry and the wider environment are to benefit from this work, and from associated research, there is now an urgent need for variety tests by plant breeders, and for National and Recommended Lists, to include criteria determining N requirements.

2. TECHNICAL DETAIL

2.1 Objectives

The GREEN grain project was set up to improve the value of UK wheat for ethanol production and non-ruminant feeding, and to reduce costs of growing wheat for these and other markets by enabling development of high-energy varieties with reduced input requirements, especially of fertiliser N.

Specific objectives were:

1. To refine the specifications of wheat grain for distilling and for feeding to non-ruminants, and to devise protocols for its production.
2. To quantify the benefits of using wheat grain with high energy content and improved amino acid balance in commercial production of both ethanol and poultry meat.
3. To develop robust near infrared spectrometer (NIR) calibrations (or other rapid screening techniques) for energy content and alcohol yield from wheat grain, and for gliadin content of both hard and soft wheat types.
4. To identify germplasm expressing positive 'GREEN grain' characteristics and to establish any associations with other aspects of varietal performance.
5. To test the hypothesis that high energy content, high ethanol yield, and improved amino acid balance of wheat grain are consistent with reduced requirements for fertiliser N.
6. To identify gene-based markers that can be used to monitor the segregation of 'GREEN grain' and alcohol yield characters across a wide range of germplasm.

2.2 Background

The 21st century is seeing expanded markets in medium latitudes (e.g. NW Europe) for a grain type that delivers an energy-dense feed-stock for the bio-energy, livestock (especially non-ruminants), food and bio-materials industries; a market equivalent to the maize grain markets in lower latitudes. Wheat is the most suitable cereal for this use; indeed it is already the dominant cereal type used for grain whisky and potable white spirit production. But the problems are

that: (i) wheat has not been bred for these markets, being bred mainly for high protein content, (ii) wheat requires large applications of fertiliser N which have large greenhouse gas (GHG) costs, (iii) digestion or processing of many wheat varieties is affected by unacceptable levels of viscosity, and (iv) some of the information needed to overcome these deficiencies is lacking.

Wheat production in NW Europe is intensive, hence expensive, and is being challenged by impending EU legislation concerning CO_2 and N emissions. Wheat varieties in the UK have always been bred and tested with ample levels of fertiliser N; there has been no attempt hitherto to reduce N requirements of wheat by breeding, and indeed N requirements have increased substantially through recent breeding (Foulkes *et al.* 1998; Sylvester-Bradley *et al.* 2009).

The main breeding focus for wheat quality has been bread-making. Gliadins, the largest class of protein in wheat grain (Sylvester-Bradley & Folkes 1976), are storage proteins held in the endosperm which, together with glutenins, form the gluten needed for making bread. However, gliadins are less useful for feeding; they are very low in lysine and other amino acids essential for non-ruminant nutrition (Pomeranz 1988). They therefore constitute low-grade protein when fed to pigs, poultry, etc., (Yin *et al.* 1993; Wiseman 2001) and almost certainly contribute significantly to N excreted, and thus to N in non-ruminant manures (Lee & Kay 2003). Gliadins are also probably detrimental in distilling because of reduced processing efficiency, reduced lysine content of distiller's grains (Pomeranz 1988), and increased volume and N content of down-stream wastes (Smith *et al.* 2000). Wheat for non-bread uses may therefore be improved by reducing storage proteins, as has been attempted with other cereals (Mertz *et al.* 1964; Munck 1972).

With respect to *performance in distilling*, although the texture of grain and its starch content are likely to be important, analysis of variety data from Scotland demonstrate that there is no clear relationship between alcohol yield and hardness, specific weight or grain size; the strongest relationship (inverse) is with grain N content (Taylor & Roscrow 1990; Brosnan *et al.* 1998).

With respect to *non-ruminant feeding*, although wheat constitutes the major component of pig and poultry rations, ration formulation focuses primarily on ensuring least cost for a standard composition (Harris *et al.* 1968). Commercial formulations commonly treat all wheat as having a fixed composition, so there

can often be a significant oversupply in some constituents (e.g. protein), and hidden costs.

With respect to *N requirements and N use efficiency* of crops grown for energy yield, the main considerations are (i) recovery of soil-derived N (Sylvester-Bradley *et al.* 2001), (ii) recovery of fertiliser N (Bloom *et al.* 1988), (iii) effectiveness of canopy N in photosynthesis (DM formation; Sylvester-Bradley *et al.* 1997a) and (iv) harvest index (Sylvester-Bradley *et al.* 1997b). Recoveries of soil-derived N and fertiliser N are very variable, and unpredictable. Soil-N recovery is most likely related to subsoil rooting (King *et al.* 2003). Work on drought (Foulkes *et al.* 2007) supports the evidence that subsoil-N recovery has not improved through recent breeding (Foulkes *et al.* 1998). On the other hand, recovery of fertiliser N, which remains in the topsoil during growth, has apparently improved. Fertiliser N recovery relates inversely to soil immobilisation of N, hence to carbon deposition by roots in the topsoil (King *et al.* 2001). Thus there appear to be prospects for an improvement in N recovery through reduced topsoil rooting and greater subsoil rooting i.e. more uniform vertical distribution of roots. Difficulties in measuring roots dictate that selection for this will be best achieved through direct screening of N recovery.

Turning to photosynthetic N use efficiency, leaves have been found to need levels of N per unit green area of 1-2 g m^{-2}, depending on ambient light intensity (Hirose & Werger 1987). Analysis of UK wheat canopies (Critchley 2001) showed that leaf laminae tend to match these levels, but also found substantial additional N in the true stem and leaf sheaths. There has been very little research on the form and purpose of this true stem N, but merely by its location it appears surplus to photosynthetic requirements, and represents a ‘store’ that may be inessential in managed environments, especially if grain protein is inherently low.

Phenotypic analyses of variety differences in both canopy and grain characters are generally laborious (Bringhurst *et al.* 1996; Sylvester-Bradley *et al.* 1998); they are not therefore appropriate for screening the large numbers of lines required for comprehensive genetic analysis, so more rapid methods are needed. Near infrared reflectance (NIR) is a technique that has greatly improved the capacity to screen for grain protein (Millar 2003), but it has not been developed for alcohol yield or starch in wheat, and calibrations are not available for protein fractions in feed varieties.

The use of molecular markers linked to target traits in 'Marker Assisted Selection' (MAS) offers an efficient alternative to direct phenotypic selection. It can also be used to identify suitable parents, when breeding to meet specific targets. Mapping of quantitative trait loci (QTL) integrates phenotypic and genotypic data and identifies the number, chromosomal location, and significance of QTL affecting the characters under study; thus it provides potential markers for use in MAS. Prior to deploying markers in MAS, it is best to validate the marker-trait association in a wider gene pool. Previous QTL studies of wheat have focussed on wide crosses to maximise polymorphism and, particularly for quality characters, have not supported MAS in commercial germplasm (Thomas 2003). Few previous studies have addressed traits or germplasm relevant to the new wheat type envisaged here.

In conclusion, wheat has not been specifically bred for the biofuels, distilling and feeding markets, so it should hold considerable potential for improvement. Development of new wheat varieties tailored to these markets is hampered by incomplete understanding of biochemical processes and their underlying genes, lack of rapid screening techniques and poor parental characterisation. Necessary research could not be justified within commercial plant breeding programmes, yet the findings of a suitable research programme should improve substantially the effectiveness of the UK wheat industry, from breeder through to consumer, and into the future.

Thus, this project addressed the hypothesis that: Wheats with low stem N and low gliadin contents will have low N fertiliser requirements, high energy yields, and grain with increased value for ethanol production and non-ruminant feeding.

2.3 Predicted impacts of the GREEN grain ideotype

The GREEN grain ideotype was modelled, prototype grain was grown and it was then assessed for its impacts on nitrogen use efficiency, seed performance, value for non-ruminant feeding and for alcohol production, and greenhouse gas (GHG) emissions.

2.3.1 N use efficiency

The term 'nitrogen use efficiency' (NUE) has been widely defined (Van Sanford & MacKown 1986) and used to assess crop performance. However, the commercial value of this characteristic was unclear (e.g. Berendse & Aerts 1987) so a paper

was published during the project (Sylvester-Bradley & Kindred 2009), based on data from HGCA Report 438, to redefine NUE as it applies in a commercial UK farming environment.

Abstract: 'The efficient use of fertilizer nitrogen (N) is crucial to sustainable human nutrition. All crops receive significant amounts of additional N in temperate environments, through fixation or fertilizer use. This paper reviews progress towards the efficient use of fertilizer N by winter wheat (*Triticum aesitivum* L.) and spring barley (*Hordeum vulgare* L.) in the UK, acknowledging that on-farm this is governed by economics. Recent multi-site N response experiments on old and modern varieties show that yield improvements since the 1980s have been accompanied by increases in economic optimum N amounts for wheat but not for spring barley. On-farm N use efficiency (NUE) has increased for barley because increased yields with optimum N were associated with compensatory decreases in grain N concentration, whereas on-farm NUE has not increased for wheat because grain N concentration has not changed and improvements in N capture were insufficient to make up for the increased yield. Genetic effects on NUE are shown to differ markedly depending on whether they are determined at a single N rate, as in variety trials, or with optimum N amounts. It is suggested that, in order to elicit faster improvement in NUE on farms, breeding and variety testing should be conducted at some sites with more than one level of applied N, and that grain N%, N harvest index, and perhaps canopy N ratio (kg N per ha green area) should be measured more widely. It is also suggested that, instead of using empirical functions, N responses might be analysed more effectively using functions based on explanations of yield determination for which the parameters have some physiological meaning.'

2.3.2 Impacts on crop performance and grain value

Taking this new definition of NUE, a simple quantitative model was formulated to explain and estimate NUE and other aspects of wheat performance as they are affected by N capture, N distribution between stem, leaf and ear, and N redistribution to grain. Three cases were considered as follows:

(i) high-yielding wheat crops as are normally observed in the UK (Sylvester-Bradley *et al.* 1997b),

(ii) the GREEN grain ideotype as was proposed at the outset of the project, and

(iii) a 'composite cultivar' devised assuming that traits observed during the project for individual cultivars could be combined with minimal interaction to approach as far as possible the GREEN grain ideotype. (The feasibility of minimising trait interactions is explored in Sections 2.6-2.8.)

The changes from case (i) to (iii) were better capture of applied N, slightly less N per unit area of leaf, smaller leaf sheaths, significantly smaller stems with less N

content, and less N in chaff. At harvest, case (iii) was taken to have the same grain yield, but with less straw and smaller overall N contents than case (i).

Changes were incorporated into the model to compute impacts on target outcomes of the project in each case and these are reported in Table 1. Whilst the assumptions necessary to compute emissions and performance in alcohol processing are robust, feeding value and seed performance were better tested empirically, by comparing large grain lots for which the only difference was the fertiliser N used to grow them (see 2.3.3 below).

Table 1. Trait values and impacts estimated by modelling of normal high-yielding wheat, the GREEN grain ideotype, and a theoretical 'composite cultivar' with the best combination of individual canopy and grain traits, as demonstrated by individual cultivars tested in this project.

Trait / Impact	*units*	Normal wheat	GREEN grain ideotype	Composite cultivar[1]
Canopy N content at GS61	*kg/ha*	200	158	152
Grain N content at harvest	*kg/ha*	153	122	115
Protein in dry grain	*%*	11.4	9.1	8.6
Requirement for applied N	*kg/ha*	217	133	129
Fertiliser N cost at £0.50/kg	*/ha*	£108	£66	£65
N Use Efficiency[2]	*ratio*	31	45	46
N emissions from land[3]	*kg/ha*	54	30	29
GHG (CO_2 equiv.) emissions	*kg/t*	415	279	282
Value for poultry feeding[4]	*/t*	£100	no effect	no effect
Seed establishment[4]	*%*	42	50	52
Net value added to grain through alcohol processing[5]	*/t grain*	£46.16	£46.54	£46.64

[1] Tests were with sub-optimal N, so these results will tend to be slightly under-stated.

[2] Weight ratio (kg/kg) between grain DM formed and soil N supply plus fertiliser N.

[3] Includes nitrate to water, ammonia and nitrous oxide to air.

[4] Based on direct feeding or establishment trials with prototype GREEN grain (see Section 2.3.3 below).

[5] Ethanol value (at £0.4/litre) plus DDGS value (at £97/t), net of price paid for grain and processing costs.

In general the results of analysing UK and associated germplasm supported the theoretical predictions made for the GREEN grain ideotype at the start to the project: i.e. it should prove possible to breed a new GREEN wheat type needing 40% less fertiliser N. The most contentious element of this conclusion is the

assumption that grain protein concentration with optimal N supplies could be reduced by 2-3% i.e. from 11.5% to 9%. This is based on observations of several cultivars, particularly those from Denmark, which were tested at three sites (but only in 2008). It is supported by a recent analysis of grain N% with optimum N in the UK compared to Denmark (Sylvester-Bradley & Clarke, 2009). Accepting these and other observed traits as being repeatable, it appears possible to increase N capture by 10%, reduce canopy N by ~40 kg/ha and grain N by 30-40 kg/ha, with a net effect of reducing requirements for applied N by 80 kg/ha or 40%. This should have equivalent large effects on growing costs, on direct N emissions of nitrate, ammonia and nitrous oxide, and thus on GHG emissions. Advantages are also predicted from the increased grain starch and reduced gliadin in the grain when it is used for feed, for seed and for distilling, but these are relatively small compared to effects on growing costs. Examples of varieties demonstrating desirable traits were Defender (good N capture), Creativ (low leaf N), Solstice (low leaf sheath & stem N), Acropolis (low ear N), Exsept (low straw & chaff N), and Audi (low grain N).

2.3.3 Value in non-ruminant diets and as seed

Two commercial feeding trials in 2006 and 2009 tested the effect of diets containing proto-type GREEN grain (with 6-8% protein) in place of normal wheat (with 10-11% protein) on the performance and excretion of broilers. In both trails effects on manure output, bird mortality, health and performance were too small to be considered significant, whilst manure N content and ammonia emissions were reduced. Replicated trials by AFBI (N. Ireland) further supported apparent prospects for reducing N excretion by both broilers and pigs through inclusion of GREEN grain in their diets, but more comprehensive confirmation is needed before feeding of low-protein grain can be recommended to the industry.

Seed tests at SCRI and ADAS Boxworth showed no effects of grain protein on germination and possible improved establishment with low protein grain. Thus it seems likely that the GREEN grain ideotype would retain good seed quality.

Publications with further detail: Sylvester-Bradley, R. (2007). “GREEN Grain”. Biofuels International 1, 37 39. Sylvester-Bradley, R. & Kindred, D.R. (2008). Developing and growing wheat for the biofuels market. In ‘Arable cropping in a changing climate’ HGCA conference 23-24 January 2008, pp. 110-125.

2.4 Rapid grain analysis for alcohol yield and gliadin content

Having devised a benchmark composition for wheat grain (Table 2: Smith *et al.*, 2006) yielding 435L/t alcohol, interrelationships between starch, protein and other grain constituents were studied, and best NIR calibrations were developed for rapid determination of alcohol yield and gliadin content.

Table 2. Benchmarks for composition (% dry matter) of wheat grain, grown with optimal N supplies and yielding 435 litres alcohol per dry tonne (Smith *et al.*, 2006).

Constituent	*% dry matter*
Starch	69.0
Sugars	3.0
Non Starch Polysaccharides	11.0
Crude protein (N x 5.7)	11.5
Lipid	2.5
Ash	2.0
Lignin	1.0

An initial study of interrelationships between grain constituents (Kindred *et al.*, 2008a) was undertaken on two varieties given a wide range of N nutrition:

Abstract: The effects of nitrogen (N) fertiliser on grain size and shape, starch and protein concentration, vitreosity, storage protein composition, and alcohol yield of two winter wheat varieties contrasting in endosperm texture were studied in a field trial in Herefordshire, UK in 2004. Averaged across varieties, the alcohol yield was 439 L/tonne for grain with a protein concentration of 11.5 g/100 g. The soft endosperm wheat variety Riband produced on average 7.7 L more alcohol per tonne of grain at a given protein concentration than the hard endosperm variety, Option. At the same time, N fertiliser was shown to have significant effects on alcohol production through its major influence on grain protein concentration. Averaged over both varieties, there was a reduction in alcohol yield of 5.7L for each 10 kg increase in protein content per tonne of grain. The starch concentration of Riband was 2.9 g/100 g higher than Option at a given grain protein concentration, supporting its higher observed alcohol yields. A low conversion of starch to alcohol in this study (6.30 L/ 10 kg starch) compared to the theoretical value (6.61 L/10 kg starch) indicated that there is potential for improvement of this character. The traits relating to grain size and shape were principally influenced by genotype, and were not influenced by N fertiliser. Conversely, there were only minor genotypic effects on grain protein concentration and vitreosity. An important finding was that there were no interactions between variety and N treatment for any of the variables considered, indicating that the response of the two varieties to changes in applied N was the same, resulting in consistent differences in starch concentration and alcohol yield

between genotypes at different levels of grain protein. An analysis of the composition of the wheat storage proteins by size-exclusion chromatography showed that the gliadins increased on average by 0.56 g per g increase in total grain protein and were quantitatively the major protein fraction, suggesting that selection for low gliadin content may be a desirable means by which to reduce grain protein, and thereby increase alcohol yield in wheat. The relationship between alcohol yield per unit area and applied N rate was described by a quadratic function and the maximum alcohol yield per unit area was ca. 3630 L/ha. Statistical analysis suggested that the economic optimum rate of N applied for grain yield was close to the optimum N rate for maximum alcohol productivity.

This study showed that available starch analysis methods were not accurate for samples with low protein and that although the Ewers method was preferable to the Megazyme method, neither was sufficiently precise for prediction of alcohol yield. Protein was a better predictor of alcohol yield but it could not account for important non-protein effects, largely genetic in origin. Thus a near infrared reflectance (NIR) calibration for alcohol yield was developed, referenced against the 'wheat cook' method (Agu *et al.*, 2006). This was improved successively through the project, eventually being based on analyses of 513 reference samples (specially selected from GREEN grain trials to represent the full range of protein and non-protein variation) plus 420 samples from RL trials (CEL & SWRI funded). The final direct calibration accounted for about 80% of variation in alcohol yield; this is adequate for quality control by distillers, and is now being commercialised. A further calibration which accounted separately for protein and non-protein effects accounted for 83% of variation in alcohol yield.

Alcohol: The relationship in available data from Scottish Whisky Research Institute (SWRI) between alcohol yield and protein appeared near to the theoretical (0.66L/kg starch), if increases in grain protein are taken to displace just starch, allowing for yeast growth. In addition there was a variety effect that appeared independent of the protein effect: at a fixed protein level there was a range in alcohol yield of 50L/kg. In general the varieties with low protein also showed high alcohol yield, relative to protein.

Protein fractions, especially gliadin: The initial study (Kindred *et al.*, 2008a) reconfirmed for modern varieties old findings (Dubetz *et al.*, 1979) that gliadin accounted for most of the extra protein due to fertiliser N. It also confirmed that a rapid gliadin assay would be valuable in developing the GREEN grain ideotype, for instance by enabling detection of low gliadin mutants or null gliadin lines.

Size-Exclusion HPLC was used to develop NIR calibrations based on the 513 reference samples; gliadin calibrations (R^2=0.92) and those for low molecular weight glutenins (R^2=0.81) had good precision but maybe mainly due to their correlation with total protein. Calibrations were poorer for high molecular weight glutenins (R^2=0.44) and albumins-plus-globulins (R^2=0.23). The gliadin calibration must now be validated using samples with genetic variation in gliadin:total protein ratio.

Grain characters and endosperm texture: Distillers currently use only soft wheat varieties such as Istabraq, Robigus, Alchemy and Zebedee. Analysis of the reference dataset showed that increases in protein (due to N nutrition) and vitreosity (due to the 1B1R translocation, and to grain weathering) augmented the hardness conferred by the *pin* loci. However alcohol yields indicated little fundamental effect of grain hardness. Bioethanol processors may show no preference for soft types, but more experience will be required to confirm this.

Alcohol yields were positively associated with large, plump grains, although this applied more for some varieties e.g. Riband, than others e.g. Glasgow. An affiliated studentship (HGCA-funded, Project 3261) investigated grain dimensions including pericarp thickness, crease depth and crease cavity width using image analysis of grain sections and crush tests (using the SKCS analyser) on individual grains. Pericarp represented about 2.4% of grain width and the proportion of endosperm in grains ranged from 78-83%. Averaged profiles from SKCS grain crush tests could be used to improve predictions of alcohol yield made from protein and grain width.

Summary: Starch analysis alone is too imprecise to support alcohol yield predictions. There were strong varietal and environmental (principally N) effects on alcohol yield and, promisingly, these seldom interacted. There was little variation in gliadin:total protein ratio. The role of this ratio as well as characters such as hardness and vitreosity will only become evident when lines are found with more variation. NIR calibrations developed here for alcohol yield and gliadin content have additional predictive power beyond that available from protein alone, and are suitable for use by breeders and distillers. However, the additional non-protein effect of variety on alcohol yield remains to be explained.

Publications with further detail: Smith *et al*., (2006), Swanston *et al*. (2007), Kindred *et al.* (2008a & b), Weightman & Kindred (2009), Davis-Knight & Weightman (2008), Misailidis (2010).

2.5 N storage in wheat canopies

Previous studies indicated significant quantities of inessential N in crop canopies at anthesis and also remaining in straw at harvest (Grindlay *et al.*, 1997; Justes *et al*. 1994; Critchley, 2001). To facilitate breeding of new N-efficient varieties, a new N partitioning model was developed, based on minimum concentrations of N in vegetative tissues, to estimate structural N (SN) and reserve N (RN) separately from photosynthetic N (PN). Data were obtained at anthesis and harvest in three field experiments established in October 2005 and October 2006 at ADAS Terrington, and in June 2006 at Lincoln, New Zealand. The experiments tested six fertiliser N treatments from nil to supra-optimal on cv. Istabraq (and at Terrington also on Atlanta, Claire, and Savannah).

Canopy N at anthesis: SN plus PN of leaf laminae was 2 g per m^2 green area, as estimated from the relatively consistent maximum response in photosynthesis (g DM per MJ intercepted solar radiation) to leaf N. Optimal canopies at anthesis contained 259kg/ha N; 41% RN, 39% PN and 20% SN. RN was 26, 106 and 117kg/ha with nil, optimum and maximum N treatments respectively. RN was located primarily in true stem (42%) and ear (27%), with less in leaf lamina (18%) and sheath (13%). RN allocation was consistent across N treatments, except with nil where negligible RN was allocated to lamina or sheath. Variety differences in RN of true stem with optimum N supply were only 47-54kg/ha. Presence of RN even with deficient N supplies for grain yield indicates a significant 'functional' role. RN accumulation may increase the crop 'sink' for N, hence increasing recovery of soil mineral N in competition with soil biomass. RN may also provide a dynamic N store to support leaf expansion.

Canopy N at harvest: Grain N was derived from 163kg/ha redistributed canopy N and 64kg/ha post-anthesis N uptake. SN accumulated by anthesis was assumed to remain in the straw at harvest. RN in leaf lamina, leaf sheath and true stem appeared to be redistributed to the grain before PN. Remobilised RN was 24, 92 and 96kg/ha with nil, optimum and maximum fertiliser N, but RN remaining in the straw at harvest ('accumulation N') was 15kg/ha with optimum N and

22kg/ha with maximum N. With optimum fertiliser N, all of the RN located in leaf lamina and leaf sheath was remobilised (19 and 14kg/ha, respectively), whilst 90% of chaff N (25kg/ha) and only 73% of true stem N (33kg/ha) was remobilised. The true stem was the predominant location for 'accumulation N'; amounts increased with N supply from 12 kg/ha with optimum N to 17kg/ha with maximum N. Of the N in canopy components <50% of true stem N was remobilised compared to >75% from leaf lamina and >60% from leaf sheath. There was relatively little variation between the four varieties in RN remobilisation from true stem (42-49%).

RN accumulation and subsequent remobilisation may be important during yield formation by delaying mobilisation of photosynthetic and metabolic proteins, thus maintaining post-anthesis green canopy area and photosynthesis. Thus it appears that RN could only be reduced with impunity if grain N demand was also reduced. On the other hand, 'accumulation N' increases crop N demand without increasing yield so its reduction may represent an opportunity for crop improvement. Breeding for optimised RN would seem to depend on reducing true stem traits such as wall thickness, height and N content.

Publication with further detail: Pask (2009).

2.6 Genetic variation in canopy N storage of UK wheat

Variety trials: Genetic variation in grain yield, NUE and traits associated with the GREEN grain ideotype was more fully assessed in experiments at two or three sites (ADAS Terrington, ADAS High Mowthorpe and SCRI Invergowrie) in each of four harvest years (2005-8) when 30-40 varieties were tested with both nil and just-sub-optimal fertiliser N (120-180 kg/ha). For harvest 2005 & 2006 elite UK varieties were tested; for harvests 2007 & 2008 a broader 'global' range of germplasm was tested including 14 core elite varieties, N efficient lines identified in parallel projects e.g. WGIN[1], selections from the Syngenta global collection, eight interesting lines from a Canterbury x Eclipse mapping population (see Section 2.8), and some new candidate varieties; in 2008 only, ten additional varieties included cultivars from Denmark.

[1] the Defra-funded Wheat Genetic Improvement Network: results from the diversity trial conducted by Rothamsted Research provided by P Barraclough.

Table 3. Maxima (max) and minima (min) of REML* over-trial means for traits of 74 varieties (with N applied) determining canopy N content, grain yield and NUE. Standard errors of means (SEM) indicate measurement error, and heritability (H) indicates the portion of total variation across trials due to the main effect of variety. The *r* coefficients indicate correlations with grain yield at nil N (Y_0), above-ground N uptake at harvest (TN), and N use efficiency (NUE) with N applied. Positive or negative effects are shown by the sign of r; r values outside the range ±0.23, ±0.30 and ±0.38 indicate probabilities of 95%, 99% and 99.9% respectively that effects were real.

Trait	*units*	Min	Max	SEM	H	*r values*		
						Y_0	*TN*	NUE
At anthesis (GS61)								
Fertile Shoots (= ears)	*#/m²*	286	463	10.6	10%	-0.29	0.56	0.25
Stem & sheath DM	*g/shoot*	1.2	2.3	0.05	16%	0.10	-0.32	-0.26
Stem & sheath N	*%DM*	0.84	1.07	0.03	2%	0.23	-0.32	-0.30
Stem & sheath N	*kg/ha*	47	67	2.0	1%	-0.20	0.34	-0.11
Leaf lamina N	*mg/shoot*	9.1	16.8	0.46	15%	-0.41	0.15	0.07
Ear N	*mg/shoot*	8.4	13.2	0.49	29%	-0.25	-0.35	-0.23
Total canopy N	*kg/ha*	115	174	4.27	0%	-0.53	0.48	0.16
Proportion N in stem		0.36	0.47	0.01	11%	0.46	-0.29	-0.26
N:green area ratio (CNR)	*kg/ha*	21.0	33.9	1.51	3%	0.11	-0.30	-0.19
After harvest								
50% senescence date	*in July*	7	20	0.8	13%	0.34	0.65	0.28
Grains	*#/ear*	38.3	55.3	1.17	13%	0.55	-0.43	0.28
Grain weight	*g/1000*	35.1	52.0	0.55	32%	0.25	-0.35	-0.10
Grain yield at 85% DM	*t/ha*	7.74	10.1	0.20	22%	0.65	0.11	1.00
Grain N	*kg/ha*	119	138	2.7	2%	0.38	0.30	-0.04
Straw N	*kg/ha*	36.7	57.3	1.68	2%	-0.54	0.72	0.13
N harvest index	*%*	68.6	77.8	0.02	1%	0.55	-0.56	-0.13
Protein in dry grain	*%*	8.50	11.2	0.17	31%	-0.35	-0.05	-0.86
N uptake efficiency – soil	*ratio*	0.96	1.04	0.21	1%	0.65	0.05	0.15
N uptake efficiency – fert.	*ratio*	0.65	0.74	0.22	2%	-0.41	0.75	-0.23
Total N uptake	*kg/ha*	161	184	4.01	3%	-0.28	1.00	0.11
N conversion efficiency	*DM:N*	37.3	51.8	1.04	18%	0.74	-0.42	0.85
N Use Efficiency (NUE)	*DN:N*	31.3	41.0	1.11	16%	0.65	0.11	1.00

*restricted maximum likelihood

At ADAS sites, canopies were measured at anthesis for DM and N traits and canopy senescence; at all sites harvest measurements included grain yield, grain protein, DM and N harvest indices and yield components. Data from each of

>100 measured or calculated traits from all site-seasons were analysed together using REML (restricted maximum likelihood) with variety as a random term to extract the main effects of all 74 genotypes tested. Best Linear Unbiased Predictors (BLUPs) were derived for the varieties because the variety x site combinations were incomplete over all four years; BLUPs are conservative predictions of variety means.

Ranges of genetic variation in target canopy traits are summarised in Table 3 showing confidence limits, heritabilities[2] and correlations with NUE. A sub-study of 6 experiments at the ADAS sites with 6 N-levels and 4 varieties showed that NUE-opt (see Section 2.3.1) was best indicated in a 2 N-level experiment (such as those reported here) by grain yield with nil N applied (Y_0). Hence correlations are shown here with both NUE (at the fixed N level) and Y_0. Similarly correlations with total crop N at harvest show the best indicators of N capture.

Confidence limits for all traits (except N uptake efficiencies) were satisfactorily small and the genetic ranges in Table 3 suggest that in principle there was sufficient variation within the germplasm tested to create the GREEN grain ideotype described in 2.3.1. Results suggested that a reduced requirement for canopy N at flowering might best be achieved by selecting for low stem & sheath DM rather than low stem & sheath N%, and for low lamina and ear N, because canopy N (kg/ha) itself showed near-nil heritability. Similarly a reduced requirement for N at harvest would probably be achieved most effectively by selecting for low grain protein. There seems some limited scope to improve N capture from the soil and from fertiliser, and heritabilities were low. The most desirable combinations of traits were not however represented by any one cultivar, and several challenges were apparent in targeting these combinations:

- Most heritabilities were low, reflecting the large environment and genotype-by-environment effects on these traits.
- Some traits showed counteracting relationships that could hinder their combination in a single genotype. For example, traits that showed reasonable genetic variation on a per shoot basis were often inversely related to shoot population (per m^2) so that differences were not necessarily expressed on a

[2] Strictly, because we are not examining the properties of a segregating population, these are ‘repeatabilities’ rather than ‘heritabilities’.

per ha basis; genetic variation and heritabilities on a per ha basis were generally much less than on a per shoot basis.

- Some advantageous traits also had potential disadvantages. For instance, low canopy N storage might be achieved through reducing stem DM per ha and stem N% (as well as ear N), but this might also compromise lodging, storage of redistributable assimilates, or N uptake.

In the event, reduced stem DM and N per ha were associated with higher grain yields (r^2=0.10 and r^2=0.25 respectively) but also with worse total N recovery (r^2=0.34). On the other hand, there was a strong relationship (r^2=0.52) between stem N per ha and canopy N ratio (CNR), and negative relationships between CNR, stem N and ear N per ha with nil-N yields, supporting the GREEN grain hypothesis that reduced canopy N can benefit NUE_{opt}. Perhaps, in adopting low canopy N as a route to efficient N use, it will be necessary to find independent means of improving N recovery.

Canopy senescence is generally accompanied by redistribution of N from photosynthetic tissues to support protein formation in the maturing grain, and here late senescence was related to high grain yields (r^2=0.26) and low grain protein (r^2=0.28). Low grain protein was clearly consistent with high yields and high NUE: the varieties with the lowest protein contents (Acropolis and Denman) gave the highest nil-N yields. However, some of these were only tested in 2008, a season which generally gave high yields with low protein, and need further validation.

To conclude, canopy and N recovery traits showed genetic variation, but their heritabilities were low and variation was sometimes small. Grain protein was the trait with the greatest heritability and, with its ease of measurement, this appeared to offer the greatest scope to improve NUE. However, varieties with low grain protein must be tested in full N response experiments, to prove that their N optima are indeed low. To reduce canopy N, it seems that a wider range of germplasm will be required, if useful genetic variation is to be found.

2.7 Genetic variation in alcohol yield and protein composition of UK wheat

Trait data: Grain quality traits were measured on all plots from the 10 variety experiments described in Section 2.6, including physical measurements of grain weight, length & width using the Marvin grain analyser, standard measures of protein, starch and hardness (using an Infratec 1241 NIR spectrometer with standard FOSS calibrations) and measures of alcohol yield, residue viscosity and protein composition (using the NIR calibrations described in Section 2.4). Data were analysed using REML as described in Section 2.6 to generate cross-site varietal means (BLUPs) with and without N applied.

Alcohol yield: Genetic variation in the main traits of interest and relationships with grain protein and alcohol yield are shown in Table 4. Genetic variation and heritability of grain traits were generally greater than for canopy traits. Varieties with the lowest protein and highest alcohol yields were Audi and Denman, though some caution is needed as these were only present in 2008. Alcohol production per ha was driven more by grain yield than differences in alcohol yield, being greatest in Denman.

As shown by previous work (e.g. Riffkin, 1990; Smith *et al*., 2006; Swanston *et al*., 2007; Kindred *et al*., 2008a; Agu *et al.*, 2009) the strong negative relationship with protein explained much of the variation in alcohol yield per tonne (r^2=0.64). Figure 1 shows this relationship to be similar both with and without N applied, and close to the theoretical relationship of 6.6 litres per tonne per % difference in protein (Smith *et al.*, 2006). It also shows that the genetic variation can be as great as the fertiliser effect, with the fertilised lowest protein varieties having lower protein than the unfertilised high protein varieties (though this result is influenced by 2 experiments in Scotland where fertiliser N caused nil or negative responses in % protein).

Alcohol yield and grain protein were related to some whole crop or canopy traits; as predicted by the GREEN grain ideotype, alcohol yield related positively to GAI and delayed senescence, and negatively to N in the stem. There was a tendency for earlier maturing varieties to show higher grain protein and lower grain yields; some of these were ‘global’ lines, not necessarily adapted to the UK.

Table 4. Maxima (max) and minima (min) of REML* over-trial means for traits of 74 varieties (with N applied) determining alcohol yield and gliadin content of grain. Standard error of the mean (SEM) indicates measurement error, and heritability (H) indicates the portion of total variation across trials due to the main effect of variety. The *r* coefficients indicate correlations with crude protein (% of grain DM; CP) and alcohol processing yield (litres per dry tonne; AY) with N applied. Positive or negative effects are shown by the sign of r; r values outside the range ±0.23, ±0.30 and ±0.38 indicate probabilities of 95%, 99% and 99.9% respectively that effects were real.

Trait	*units*	Min	Max	SEM	H	*r* value	
						CP	AY
Whole crop traits							
Green Area Index	*ratio*	3.4	5.1	0.16	1%	-0.43	0.46
Specific leaf weight	*g/m²*	42	71	2.33	2%	-0.34	0.08
Leaf DM	*g/shoot*	0.30	0.54	0.01	22%	-0.33	0.04
Heading date (GS59)	*in June*	-4	12	0.55	16%	-0.64	0.39
Senescence date	*in July*	7	20	0.75	13%	-0.61	0.37
N per stem	*mg*	11	22	0.62	8%	0.37	-0.50
Grain yield	*t/ha*	7.7	10.1	0.20	22%	-0.86	0.65
Grain traits							
Protein (Nx5.7) in grain DM	*%*	8.5	11.2	0.17	31%	1.00	-0.80
Alcohol Yield (AY)	*l/ dry t*	433	455	1.5	20%	-0.80	1.00
Deviations from expected AY	*l/ dry t*	93	105	0.5	45%	0.15	0.43
Alcohol production	*l/ha*	2,894	3,996	30.6	21%	-0.89	0.73
Starch (by NIR) in grain DM	*%*	68.9	72.0	0.24	9%	-0.68	0.80
Grain hardness (by NIR)	*SKCS units*	10.6	47.3	1.40	8%	0.43	-0.70
Residual viscosity	*mPa.s*	1.47	1.61	0.02	7%	-0.01	-0.33
Weight per grain (=TGW)	*mg*	40.5	55.6	0.51	25%	0.16	-0.20
Grain length:width	*ratio*	1.67	1.97	0.01	41%	-0.19	0.03
Specific weight	*kg/hl*	70.7	77.5	0.44	37%	0.40	-0.28
Gliadin in grain DM	*%*	4.3	6.1	0.13	29%	0.96	-0.76
HMW glutenin in grain DM	*%*	0.7	1.7	0.03	51%	0.39	0.10

*restricted maximum likelihood

There was clearly some variation in alcohol yield that was not protein-related (this is referred to here as 'deviations'); NIR analyses showed varieties differed by up to 16 l/t at a constant protein level. Starch content by NIR was inversely related to grain protein (slope = -0.5 litres per dry tonne per % protein); starch correlated well with alcohol yield as well as protein content (r^2=0.64) and

explained some of the deviations in alchol yield (r^2=0.14). Grain hardness also correlated negatively with alcohol yield and deviations in alcohol yield (r^2=0.32), as well as positively with grain protein. Physical measures such as grain weight, specific weight, size and shape did not relate well to alcohol yield. Although protein, starch and hardness rely on very different reference tests and calibration sets, correlations between different NIR measures of these traits might arise through their common derivation from the same spectral data. Protein, starch content and grain hardness were previously identified as factors contributing to alcohol yield (e.g. Agu *et al.*, 2009). Whilst Swanston *et al.* (2007) showed that grain size and shape related to alcohol yields in a subset of these samples, they were not a major explanatory factor in this fuller analysis of varietal differences.

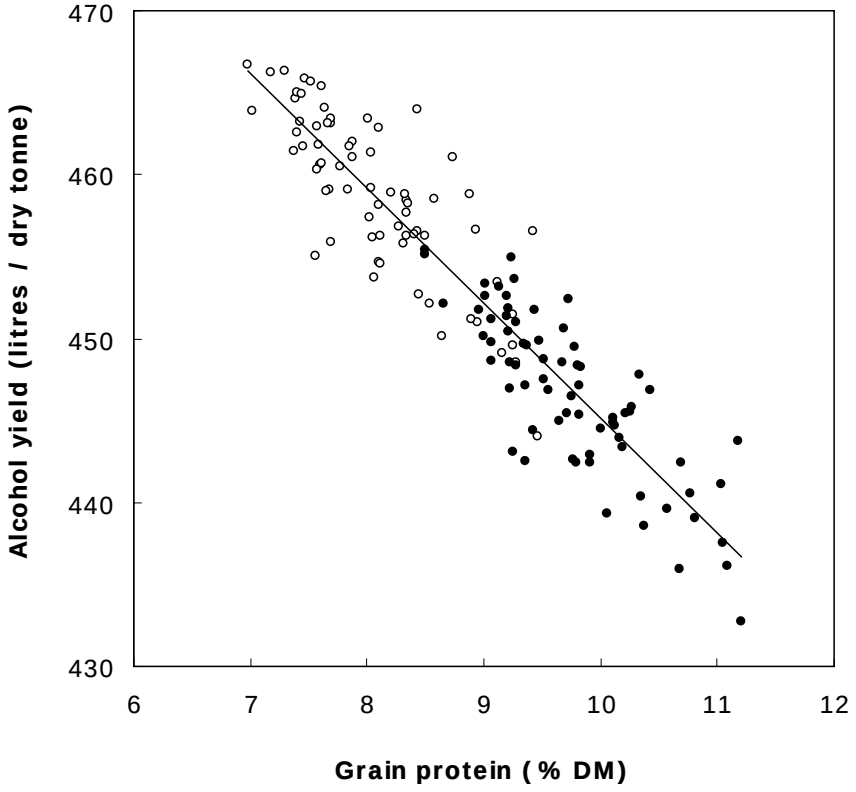

Figure 1. Relationship between protein content and alcohol yield for all 74 varieties tested, grown with (closed symbols) and without (open) applied N. Values are best linear unbiased predictions (BLUPs) from REML (restricted maximum likelihood) analysis of all 10 experiments (intercept 515 L/t; slope 7.0 L/t per %protein; R^2 0.86).

Grain protein composition: Varieties showed very distinctive grain protein composition, with high heritabilities. Denman and Audi again gave the lowest gliadin content, gliadin being very strongly correlated with total protein content. HMW glutenins were less well related to protein; Savannah gave the least and Mercia the most. Whilst the NIR predictions showed clear differences in protein composition between varieties, these results should be interpreted with caution because the NIR calibrations for the individual protein fractions were not perfect.

Conclusions: Alcohol yield could be increased, and gliadin content reduced, by breeding for low grain protein. But a significant part of the variation in alcohol yield was not explained by protein, starch or hardness, and probably not by grain physical characters; it may be better explained by starch quality or non-starch polysaccharide (NSP) content. NIR calibrations for alcohol yield, deviations from expected alcohol yield, starch and hardness could all be useful in breeding programmes to maximise alcohol yields.

NIR predictions of gliadin content and protein composition were mainly related to differences in total protein. NIR calibrations for these traits need to be tested on genetic material with more variable protein composition before they can be properly validated. It remains to be seen whether the better route to high alcohol yield and high feeding value could come through selecting for low gliadin content, rather than for low total protein content.

Varieties with the best alcohol yields (e.g. Glasgow, Denman, Zebedee, Istabraq) tended to have high grain yields and low grain protein also, facilitating combined selection for alcohol yield and reduced N requirements.

2.8 Genetic control of, and markers for, N Use Efficiency, protein composition and alcohol yield.

Whilst the variety trials provided information on the extent of genetic variation in UK-related wheat gene-pools, populations of random inbred lines (RILs) were developed, grown and analysed for more detailed information about genetic control of traits and the relationships between them. One population was derived from a cross between Canterbury x Eclipse (CxE) and a second was derived from crosses between Atlanta x Istabraq (AxI) and Istabraq x Wizard (IxW).

Mapping population trials: Ten experiments were conducted at ADAS Terrington, ADAS High Mowthorpe and SCRI Invergowrie over four harvest years. Five

experiments harvested in 2005 or 2006 tested 178 RILs of CxE with both nil and just-sub-optimal fertiliser N (120-180 kg/ha). Then five experiments harvested in 2007 & 2008 tested 87 RILs from AxI and 85 RILs from IxW with both nil and just-sub-optimal fertiliser N. A common set of 10 control varieties was included in each experiment. Six of the ten experiments (i.e. at ADAS sites) measured canopies for DM and N traits at anthesis and for canopy senescence; grain yield, grain protein, grain DM, N harvest index and yield components were measured in all experiments. Variance component analysis was used to estimate the relative importance of the genotype, genotype x environment (individual site and year combinations) and error variation for each trait. Data for 83 measured or calculated traits were analysed to search for QTL.

Genetic variation in the mapping populations: Grain yields of the mapping populations were 6.7-9.3 t/ha (Table 5), less than the 7.7-10.1 t/ha found in the variety experiments (Tables 3 & 4). Otherwise it was notable that the maxima of the populations were generally greater than those of the varieties for anthesis traits, alcohol yield, grains per ear and TGW but less than those of the varieties for most other quality traits. The minima did not show such an obvious trend, which is surprising as one would have expected unselected populations to have a generally lower level of performance. Provided there are no adverse associations of these traits with other characters, our results do suggest that improvement in some of the canopy characters is feasible. Genetic variation in mapping populations should provide a truer reflection of the heritability of each trait than was apparent from the varieties because the RILs were a random sample of segregating populations. The heritabilities detected in the mapping populations were frequently higher than those detected in the variety trials; the most obvious differences were in canopy partitioning and residual viscosity (Tables 3, 4 & 5). Together with the increased maxima, this indicates that selection for increased expression of the canopy traits is perfectly feasible.

Table 5. Maxima and minima of over-trial means and heritabilities (H) for traits of lines (with N applied) from the CxE and AxI & IxW (AIW)* mapping populations.

Trait	*units*	Min	Max	H CxE	H AIW
At anthesis					
Fertile Shoots (= ears)	*#/m²*	322	487	5%	5%
Stem & sheath N	*% DM*	0.69	1.46	24%	18%
Stem & sheath N	*kg/ha*	11	35	9%	35%
Stem & sheath N of canopy N	*%*	28	54	13%	37%
Leaf lamina N	*mg/shoot*	8.7	17.3	15%	41%
Ear N	*mg/shoot*	8.5	17.7	25%	31%
At harvest					
Grains	*#/ear*	38.8	66.5	20%	27%
Weight per grain (=TGW)	*mg*	42.7	54.8	41%	52%
Grain length:width	*ratio*	1.67	1.92	58%	44%
Grain yield at 85% DM	*t/ha*	6.87	9.26	17%	10%
Grain N	*kg/ha*	107	138	11%	12%
Straw N	*kg/ha*	28	58	18%	10%
N harvest index	*%*	68	80	18%	10%
Total N uptake	*kg/ha*	144	203	13%	11%
N conversion efficiency	*DM:N*	38.5	50.3	18%	13%
Grain quality traits					
Protein (Nx5.7) in grain DM	*%*	9.0	10.9	32%	29%
Alcohol Yield (AY)	*l/dry t*	438	461	19%	22%
Deviations from expected AY	*l/dry t*	93	107	26%	29%
Alcohol production	*l/ha*	2,589	3,618	17%	12%
Starch (by NIR) in grain DM	*%*	67.7	70.7	13%	15%
Grain hardness (by NIR)	*SKCS units*	16.3	38.2	1%	0%
Residual viscosity	*mPa.s*	1.46	1.63	33%	29%
Gliadin in grain DM	*%*	4.7	6.2	32%	28%
HMW glutenin in grain DM	*%*	0.9	1.4	23%	24%

*(CxE) = Canterburyt x Eclipse; (AxI)= Atlanta x Istabraq and (IxW)= Istabraq x Wizard

Genetic Maps: 250 RILs from the CxE population were genotyped with 201 molecular markers polymorphic between the parents; 199 were Simple Sequence Repeats (SSRs) and two were markers for the Glutenin (Glu-1A) and hardness (PinB) loci on chromosomes 1A and 5D. Similarly, the AxI and IxW populations were genotyped with 165 and 187 SSRs respectively. As the chromosomal locations of most of the markers were known, groups of markers could be assigned to specific chromosomes and then the loci could be ordered on each

chromosome to produce individual genetic maps for each population. Markers representing all 21 chromosomes were found for CxE and IxW but no polymorphisms were detected on chromosome 4A for AxI. Most of the chromosomes had relatively fragmentary coverage, which is not surprising as all crosses were between closely related UK cultivars. In general, coverage of Group 4, 5 and 6 chromosomes was limited, either in terms of numbers of markers or map distances. There were also some notable differences between the populations with AxI and IxW having very limited polymorphism on chromosomes 1A and 1B compared to CxE. Similarly, IxW was relatively monomorphic on chromosomes 3B and 3D compared to CxE and AxI.

QTL detection – Canterbury x Eclipse: Phenotypic data from CxE trials were combined with the appropriate subset of genotypic data for the 178 lines to search for QTL. The Biometris QTL x Environment procedures (Boer *et al*., 2007) implemented in Genstat 12 (Payne *et al.*, 2009) were used as they take account of the appropriate variance / covariance structure between environments and genotypes for each trait and thus represent a considerable advance over other QTL detection methods. QTL were detected for 53 of the 83 traits with a maximum of 8 QTL per trait (for starch % in grain DM). Overall, 148 QTL were detected in this population, an average of 1.8 for each trait. Traits with no QTL were all derived from grab samples; these comprised few plants per plot, hence were more variable than whole plot measures or grain samples, and provided less scope for QTL detection. More than 10 QTL were located on each of chromosomes 1B, 1D, 2B, 3A, 6D and 7D; these can be considered 'hot-spots'. No QTL were detected on chromosomes 4D and 7A. The A genome contained the fewest QTL (32) and the B genome the most (63). Just 39 of the 148 QTL showed a consistent directional effect (main effect) over all environments. Most of the 109 QTL-by-environment interactions were due to scaling but 30 showed clear evidence of cross-over, with significant alleles derived from Canterbury in one or more environments and significant alleles from Eclipse in others. These cross-over interactions were more frequently due to sites than to fertiliser levels.

QTL Detection – Atlanta x Istabraq: Using the same QTL analysis as for CxE, phenotypic data from 87 lines showed 90 QTL amongst 86 traits studied; 33 traits had no QTL. Most QTL (5) were detected for two protein fraction traits. Four QTL were detected for grain length to width ratio and three each for NUE, fraction of N in the ear at GS61 and gliadin:total protein ratio. Ten or more QTL

were located on chromosomes 3A, 4B, 6A and 6D. The A genome had the most QTL (40) and the B genome the least (22). The 33 traits with no QTL included whole plot and grain sample determinations (e.g. grain yield, grain protein) as well as those from grab samples. Less than 20% of the QTL (16) were main effects, and whilst many of the 74 QTL-by-environment interactions resulted from scaling, 13 were cross-over interactions.

QTL Detection – Istabraq x Wizard: Because of low seed availability in 2007, only data for 83 lines in 2008 were analysed. Overall, 89 QTL were detected, with none for 37 of the 86 traits. Four QTL were found for each of five traits: albumin and globulin (%DM), the fraction of low molecular weight glutenins derived from NIR predictions of total protein and % low molecular weight glutenins, N% in the ear at GS61, thousand grain weight and total plant height. Three QTL were detected for another 6 traits, mainly related to protein composition but also for stem length and one related to nitrogen partitioning in the ear. As with AxI, most of the QTL (54) were located on the A genome, with chromosomes 2A, 3A and 6A all having over 10 QTL. The D genome had very few QTL (8). Traits with no QTL represented a similar spectrum to those in the AxI population. The proportion of main effects QTL (33) was highest in this cross compared to the other two but the proportion of cross-over interactions was also higher at 32% compared to 27% for CxE and 18% for AxI.

QTL Comparisons: Whilst a number of QTL (40) were located on group 1 chromosomes in CxE, the polymorphism levels in the other two crosses were low and just 3 QTL from AxI and IxW were located here. There was much more homology on chromosome 2A with QTL for albumin and globulin content and alcohol yield from two crosses located here. There was a considerable hot spot on chromosome 2B in CxE but there were fewer QTL detected in the other two crosses. Nevertheless, QTL for grain length to width ratio, thousand grain weight, alcohol yield (at 10% protein content) and height measures from two crosses showed approximate co-location on this chromosome. Many QTL from all three crosses were detected on chromosome 3A and many of the markers were polymorphic between two or more of the populations, rendering comparisons of locations easier. QTL for albumin and globulin fraction, ear length, gliadin fraction and different measures of high and low molecular weight glutenins from at least two crosses were apparently co-located here (Figure 2).

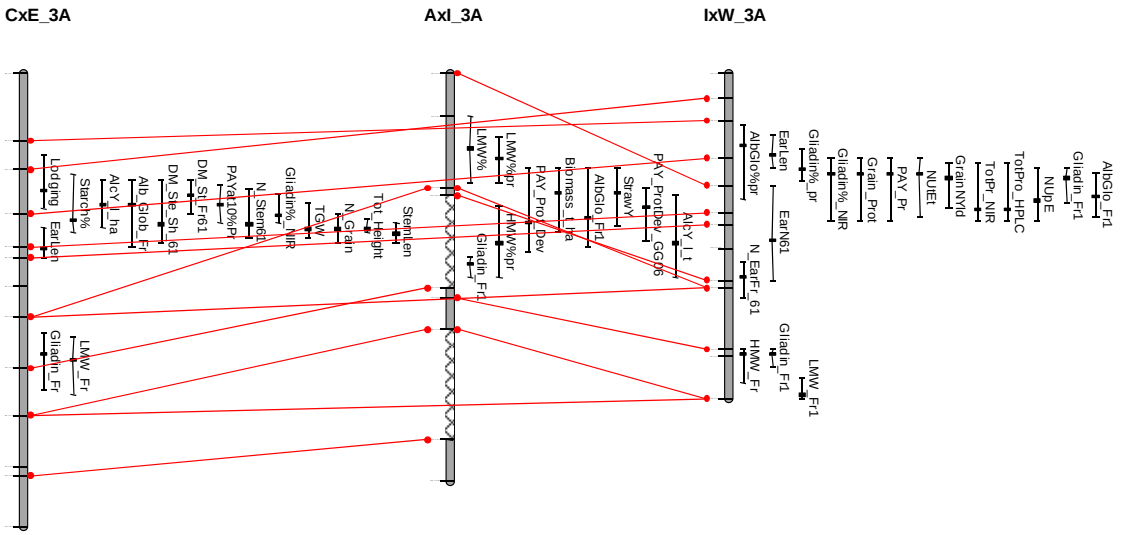

Figure 2. Genetic maps of chromosome 3A for Canterbury x Eclipse (CxE), Atlanta x Istabraq (AxI) and Istabraq x Wizard (IxW). Red dots joined by red connecting lines indicate markers segregating in more than one population. Black dots indicate the most probable location of a QTL for a character and whiskers denote where the QTL has at least a 10% chance of being located). The abbreviated trait names will be defined in the full chapter on phenotyping the mapping populations.

Whilst relatively few QTL were located on chromosome 3B, significant QTL for gliadin fraction were detected here in two crosses. There was more polymorphism on chromosome 4B for AxI and IxW than for CxE, and QTL for different measures of N accumulation in the ear (at GS61) were detected on this chromosome in both crosses. A heading date QTL was also detected in CxE and IxW on this chromosome, suggesting that these effects may be related. There was, however, little evidence of any heading date QTL from AxI on chromosome 4B, so the N accumulation effects appear to be genuine and not the result of pleiotropy. Just 6 QTL were located on chromosome 4D and none of these was detected in CxE. Nevertheless, QTL for albumin and globulin fraction from AxI and IxW were co-located here. The general level of polymorphism on the group 5 chromosomes was low and there was no great evidence of co-location of QTL for the same character from different crosses.

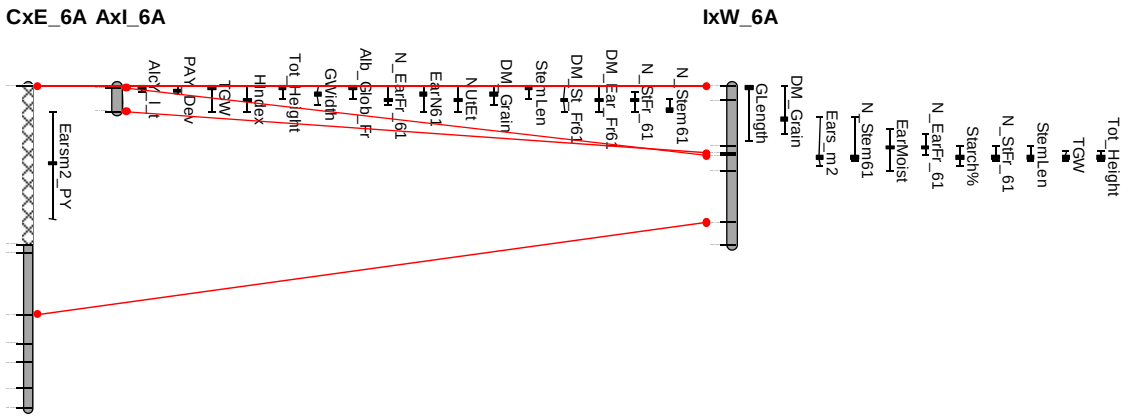

Figure 3. Genetic maps of chromosome 6A for Canterbury x Eclipse (CxE), Atlanta x Istabraq (AxI) and Istabraq x Wizard (IxW). Red and black lines indicate markers and QTL respectively, as described in Figure 2.

There was considerable evidence of QTL co-location on chromosome 6A in AxI and IxW with significant effects for various measures of N accumulation in the ear and stem at GS61, thousand grain weight and two measures of height (Figure 3). In contrast, only one QTL from CxE was located on chromosome 6A – this was co-located with one from IxW for ear number. Tight co-location of all these effects questions whether they might result from pleiotropy of, for instance, the height QTL where Istabraq alleles increase the character by approximately 3 cm. However, such a difference seems too small to account for effects of approximately 1g in TGW, and including height as a fixed effect in the QTL scans does not make the TGW QTL non-significant, so we can conclude that there are either a number of linked QTL or a general growth factor is located in this region. Whilst it is a relatively small region genetically, it is centromeric and could therefore be a large physical portion of the chromosome with the possibility that many QTL are linked here.

Whilst a number of QTL were detected on chromosome 6D in CxE and AxI, there was no evidence of any co-location for the same traits, although there was some co-location of similar trait types. There was considerable homology between all three crosses on chromosome 7D but no evidence of any QTL co-location. In CxE, QTL alleles from Canterbury increased alcohol yield and reduced residual viscosity, probably through reduced gliadin content. Thus these represent beneficial alleles for distilling quality derived from a hard wheat.

Conclusions: Previous QTL detection methods have assumed no variance / covariance structure between the same traits measured on the same population in different environments. Our analyses show this assumption to be adequate in just 3 out of 254 cases; QTL detection in this study has therefore been much more efficient than in previous studies and has identified a large number of QTL for a wide range of agronomic, grain quality and physiological characters. Many of these characters showed consistent effects over a range of environments, so may be used with some confidence in wheat breeding programmes to improve crop performance. Ideally, such QTL need to be validated before deployment and Syngenta are currently genotyping parental material for markers located in the hot-spots noted above, e.g. on chromosomes 3A and 6A. Once this information is available, phenotypes predicted from genotypic scores here can be compared with those observed in the parental trials described in Sections 2.6 & 2.7.

2.9 Conclusions and Research Suggestions

The new wheat ideotype envisaged here should suit feed and distilling markets giving high energy content grain, but also requiring less N fertiliser to achieve the same grain yield. The main beneficiaries should be farmers and the environment, predominantly through reduced use of N fertiliser, but also distillers and bioethanol producers. Genetic variation amongst the elite and 'global' germplasm examined here indicates that it should be possible to breed varieties with reduced N requirements if low protein grain can be combined with reduced canopy N. Genotypes with lower grain protein levels were found in the project, and these tended to have higher grain yields when N was withheld. Validation of these varieties in full N response experiments is required before such varieties can be confirmed as actually having lower N requirements. Whilst substantial reserve N was held in canopies, and there was genetic variation in components of canopy N (stem & ear dry matter & N%, shoots m^{-2}), these are not combined in any of the genotypes studied to give substantially reduced canopy N per hectare. Insufficient variation in canopy traits was found for much progress to be made with genetic analysis; few useful QTL for canopy N were discovered. It therefore seems that wider germplasm will be needed to find reduced canopy N, and that full exploitation of the GREEN grain ideotype in the UK will be a long term challenge.

The negative relationship between protein content and alcohol yield has been strongly confirmed by the project, showing that varieties with low protein content should give higher alcohol yields. However, the work also showed substantial variation in alcohol yield which is not related to protein content. This variation is reasonably consistent between varieties, with some consistently giving higher than expected alcohol yields (e.g. Glasgow, Zebedee), and others lower than expected (e.g. Kipling). These differences did not relate obviously to other grain measures such as size, shape, specific weight or starch content. The differences must ultimately be explained by starch contents or starch conversion, but starch analysis methods were insufficiently precise to identify which. It seems likely that differences in the non-starch non-protein component (predominantly non-starch polysaccharide) will be implicated in this; further work is required to understand better the drivers and to characterise the genetically controlled component of non-starch non-protein.

Successful NIR calibrations for alcohol yield have been developed in the project that should prove useful for distilling and bioethanol processors, and also for plant breeders. The calibrations for protein fractions also show promise as a screen for breeding material, though this needs validating on germplasm with more widely varying protein fractions. It is hoped that this will be enabled by spin-off work to screen the population of Paragon mutants created in WGIN.

There is an urgent need for variety testing (as conducted by plant breeders, as well as for the National and Recommended Lists) to address criteria determining N requirements, if the industry and the wider environment are to benefit from this and associated research.

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