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ORIGINAL PAPER

The genetics of nitrogen use in hexaploid wheat: N utilisation, development and yield

Dimah Z. Habash · Stephanie Bernard · Jörg Schondelmaier · Jens Weyen · Steve A. Quarrie

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Abstract A genetic study is presented for traits relating to nitrogen use in wheat. Quantitative trait loci (QTLs) were established for 21 traits relating to growth, yield and leaf nitrogen (N) assimilation during grain fill in hexaploid wheat (*Triticum aestivum* L.) using a mapping population from the cross Chinese Spring × SQ1. Glutamine synthetase (GS) isozymes and estimated locations of 126 genes were placed on the genetic map. QTLs for flag leaf GS activity, soluble protein, extract colour and fresh weight were found in similar regions implying shared control of leaf metabolism and leaf size. Flag leaf traits were negatively associated with days to anthesis both phenotypically and genetically, demonstrating the complex interactions of metabolism with development. One QTL cluster for GS activity co-localised with a *GS2* gene mapped on chromosome 2A, and another with the mapped *GSr* gene on 4A. QTLs for GS activity were invariably co-localised with those for grain N, with increased activity associated with higher grain N, but with no or negative correlations with grain yield components. Peduncle N was positively correlated, and QTLs co-localised, with grain N and flag leaf N assimilatory traits, suggesting that stem N can be indicative of grain N status in wheat. A major QTL for ear number per plant was identified on chromosome 6B which was negatively co-localised with leaf fresh weight, peduncle N, grain N and grain yield. This locus is involved in processes defining the control of tiller number and consequently assimilate partitioning and deserves further examination.

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D. Z. Habash (✉) · S. Bernard
Crop Performance and Improvement Division,
Rothamsted Research, Harpenden, Hertfordshire,
AL5 2JQ, UK
e-mail: Dimah.Habash@bbsrc.ac.uk

S. A. Quarrie
Kraljice Natalije 39, 11000 Belgrade, Serbia

J. Schondelmaier · J. Weyen
Saaten-Union Resistenzlabor GmbH, Hovedisser Str. 92,
33818 Leopoldshoehe, Germany

S. A. Quarrie
Schools of Biology and Agriculture,
Food and Rural Development, Newcastle University,
Newcastle Upon Tyne, UK

Introduction

Genes, networks and pathways controlling the uptake, utilisation and partitioning of nitrogen and carbon contribute to the plant's final yield in a particular environment. The molecular components of a complex trait such as yield are polygenic, as demonstrated by genetic studies (Börner et al. 2002; Huang et al. 2003; Quarrie et al. 2005). Quantitative trait locus (QTL) analysis of wheat (*Triticum aestivum* L.) has shown that, as well as grain yield, grain protein content is also under the control of several genomic regions (Blanco et al. 2002; Groos et al. 2003; Prasad et al. 2003; Charmet et al. 2005). Very few studies have tackled the question of whether there is genetic diversity for N use in wheat, though Le Gouis and Pluchard (1996) have shown that differences exist in agronomic nitrogen use efficiency

(NUE; grain yield/nitrogen supplied) amongst 26 wheat varieties which may relate to the number of shoots and senescence profile. Studies on N budget in wheat hybrids showed reasonable heritability and differences at the post-anthesis stage in either N uptake or N utilisation (Cox et al. 1985). As yet, there are no reported studies specifically designed to establish QTLs for N use in wheat across several levels of organisation for that trait. Establishing QTLs for N use in wheat would enable us to identify and understand the genomic components of this trait and especially the possible interactions amongst loci. This would be a prerequisite to identify molecular markers to monitor introgression of loci for improved N use in future breeding programmes of a major crop. Quarrie et al. (2005) have recently described a new genetic map for hexaploid wheat (Chinese Spring × SQ1). Field experiments in 24 site × treatment × year combinations showed the mapping population to vary widely for yield and yield components. Seventeen yield QTL clusters were obtained in five or more trials and several of these yield QTL clusters corresponded to QTLs already reported in other studies using different wheat mapping populations. Observations also pointed to diversity in this population for responses to nitrogen supply, making it suitable to establish genomic regions associated with yield and nitrogen use in plants during grain filling.

The efficient use of N in wheat depends on inorganic N uptake and assimilation and on the successful remobilisation and partitioning of organic N. In wheat post-anthesis, 51–91% of the N in the spike comes from vegetative N remobilisation of organic N in the form of free amino acids and macromolecules such as Rubisco (Peoples et al. 1980; Van Sanford and MacKown 1987). The enzyme glutamine synthetase (GS) plays a central role in assimilating ammonia produced in the leaf from metabolic processes spanning assimilation (ammonia from photorespiration and nitrate reduction), transamination reactions and catabolic processes (Miflin and Lea 1980; Miflin and Habash 2002). Glutamine and glutamate, produced by the concerted action of GS and glutamate synthase, respectively, are then transported from the leaf to the developing sinks or grain in wheat. Recent genetic studies on maize and rice NUE have provided strong evidence that the activity or content of GS, in particular the cytosolic isozyme GS1, colocalises with QTLs for N remobilisation and grain size in both species (Galais and Hirel 2004; Obara et al. 2004). Studies of rice mutants lacking *OsGS1*;*1* also implicate cytosolic GS with growth and grain fill (Tabuchi et al. 2005).

Here we test the contribution of GS to the genetics of N use in wheat within the context of its central role in N remobilisation, establish genomic regions regulating leaf soluble protein, locate QTLs for the N storage capacity in the stem, determine the role of plant development and establish the level of genetic variation in grain yield and N content. In addition we mapped the recently cloned GS genes from wheat and located 126 loci of genes associated with aspects of nitrogen and carbohydrate metabolism, growth, yield and development to enable comparison with QTLs identified in this study.

Materials and methods

CS × SQ1 mapping population

The mapping population consisted of 95 doubled haploid lines (DHLs) generated from the cross between hexaploid wheat (*Triticum aestivum* L.) genotypes Chinese Spring (CS) and SQ1 (a high abscisic acid breeding line) according to Quarrie et al. (2005) and available from the John Innes Centre, Norwich (john.snape@bbsrc.ac.uk).

Experimental design and plant growth-GH

Ninety-one DHLs from the CS × SQ1 mapping population and their parental lines were studied. Four replicate plants per line were grown as one plant per 2 l pot. After vernalisation for 6 weeks at 5°C, plants were grown in two glasshouses (latitude 51° and 20 m^2 each) from March to July. Temperature was not allowed to fall below 16/14°C day/night and natural light was supplemented with 600 W SON-T sodium lamps to ensure the PFD did not fall below 350 μmol m^{-2} s^{-1} at plant level. Pots were grouped randomly within four blocks to take account of plant height differences. A peat-based compost was used with a nutrient system delivering a supply of N for six weeks from PG mix (Yara (UK) Ltd, Immingham, Lincs) and a sustained slow release fertiliser over four months from Osmocote (Scotts UK Professional, Ipswich, Suffolk). This resulted in 0.59 g N available per plant over the whole period of growth and near optimal for growth under these conditions.

Biochemical traits

Leaf and peduncle sampling

As the population varied for flowering time the strategy was to sample leaves when ears reached the same stage of grain development. Flag leaves and peduncles of the main shoot or first tiller were sampled at the ear

soft dough stage: Zadoks stage 85 (Zadoks et al. 1974). Whole leaf laminas and peduncles were weighed, frozen in liquid nitrogen and stored at −80°C. One leaf was harvested per plant and four replicate plants were sampled for each DHL. Biochemical and agronomic data are presented for three and four replicates, respectively.

Leaf extraction and biochemical measurements

Whole leaves were ground under liquid nitrogen to a fine powder and 0.1 g material was extracted in 1 ml of extraction buffer (pH 7.6) containing 100 mM triethanolamine, 1 mM EDTA, 10 mM $MgSO_4$, 5 mM glutamate, 10% v/v ethylene glycol at pH 7.6 and DTT to a final concentration of 6 mM. Crude extracts were centrifuged at 21,000*g* for 30 min at 4°C. Leaf soluble protein was determined using the Bradford (1976) assay. Leaf extract colour was scored on a scale of 1–8 with 8 being very green and 1 yellow. GS activity was measured using the synthetase assay based on the method of Lea et al. (1990).

Agronomic traits

Development

Vernalised plants were moved into glasshouses at the two-leaf stage (Zadoks 12). Time to anthesis was determined as the time taken from the two-leaf stage (Zadoks 12) to the time of anther appearance (Zadoks 61). Grain fill duration was measured as the time from anthesis (Zadoks 61) to grain dough stage (Zadoks 85). Plant height was measured at maturity to the tip of the ear (excluding awns).

Ear and grain yield

Plants were harvested at maturity. Ears were removed, counted, dried at 80°C for 24 h and threshed. Grain was weighed and counted for each DHL.

Nitrogen content

The total nitrogen content of grain and peduncle was determined on dried and ground material by the Dumas method using a LECO FP Combustion Analyser (St Joseph, Michigan, USA).

Molecular markers, genetic map and genetic analysis

The genetic map for CS × SQ1 of 567 marker loci, presented in Quarrie et al. (2005), was updated with a further 38 informative markers, mainly SSRs, using methods described in Quarrie et al. (2005). QTL analysis was carried out using the new genetic map containing 449 loci (redundant loci and other loci of low information content having been excluded). QTLs were located using the QTLCartographer suite (Basten et al. 1996), using both marker-by-marker linear regression (LRmapqtl) and composite interval mapping (CIM) (Zmapqtl). Composite interval mapping was carried out using the 20 most significant markers, genome-wide, as background loci identified with the program SRmapqtl. All likelihood ratios (LRs) greater than 20 (equivalent to LOD scores greater than 4.34) were regarded as significant for the purposes of comparison with those QTLs significant ($P < 0.05$) using marker-by-marker linear regression. The percentage of phenotypic variation accounted for by each QTL was determined using Zmapqtl. A co-location of QTLs was assumed when linear regression or CIM LR maxima were within a 10 cM interval of the most significant marker or CIM LR maximum, representing a minimum precision typical for QTL detection (Mangin and Goffinet 1997). Microsoft Excel was used to determine correlations amongst traits, ANOVA and to calculate heritabilities on a genotype mean basis, using the formula $h^2 = s^2g/(s^2g + s^2e)$, where s^2g is the genotype variance and s^2e is the error variance obtained using two-factor ANOVA without replication.

Mapping GS and candidate genes

Recently cloned GS genes from wheat (AY491971 GSe, AY491969 GSr, DQ124214 GS2, DQ124211 GS1) were aligned with ESTs from public databases (http://www.wheat.pw.usda.gov/GG2/blast.shtml) and primers designed using primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www_slow.cgi) (Table 1 ESM). Primer pairs included InDels (mainly in the 3′ UTR regions) visible in the alignment were checked for polymorphism between CS and SQ1 as described in Steiner et al. (2004) and Quarrie et al. (2005). Polymorphic fragments were scored in the CS × SQ1 DH population and monomorphic fragments were assigned to chromosomes using nullisomic–tetrasomic lines of Chinese Spring. Locations on the genetic map of several genes for N uptake, assimilation, carbohydrate metabolism, growth, agronomy and development were determined either directly (by mapping with RFLP probes or PCR-amplified primers) or estimated according to chromosome bin locations of wheat ESTs or comparative mapping. Procedures used to assign chromosomal locations to these genes are summarised in Table 2 ESM.

Results

Mapped genes

A wheat GS2 gene was mapped to chromosome 2AL, and two other monomorphic bands were identified and assumed to be 2B and 2D homoeologues (Fig. 1). Cytosolic GSr and GSe genes were mapped to chromosomes 4AS and 4BS, respectively, with nullisomic–tetrasomic analysis showing each to form homoeologous series on the group 4s (Fig. 1). Cytosolic GS1 gene was mapped to chromosome 6BL (Table 1 ESM) with a monomorphic homoeologue located to 6A by nullisomic–tetrasomic analysis. Predicted locations of 126 genes likely to be associated with either N utilisation or grain yield, both directly and indirectly, were identified in the CS × SQ1 genetic map (Fig. 1) using database information and publications (Table 2 ESM).

Phenotypic characterisations

ANOVA showed significant (at $P < 0.5$) phenotypic variation amongst the population for all traits measured and many traits were highly correlated (Table 1). Frequency distributions of traits showed their quantitative nature with values outside those for the parents demonstrating transgressive segregation for every trait except thousand grain weight (TGW) (data not shown). The population showed a high degree of heritability for all 21 traits, with values on a mean basis calculated to be from 0.62 for GS activity per mg protein to 0.98 for plant height.

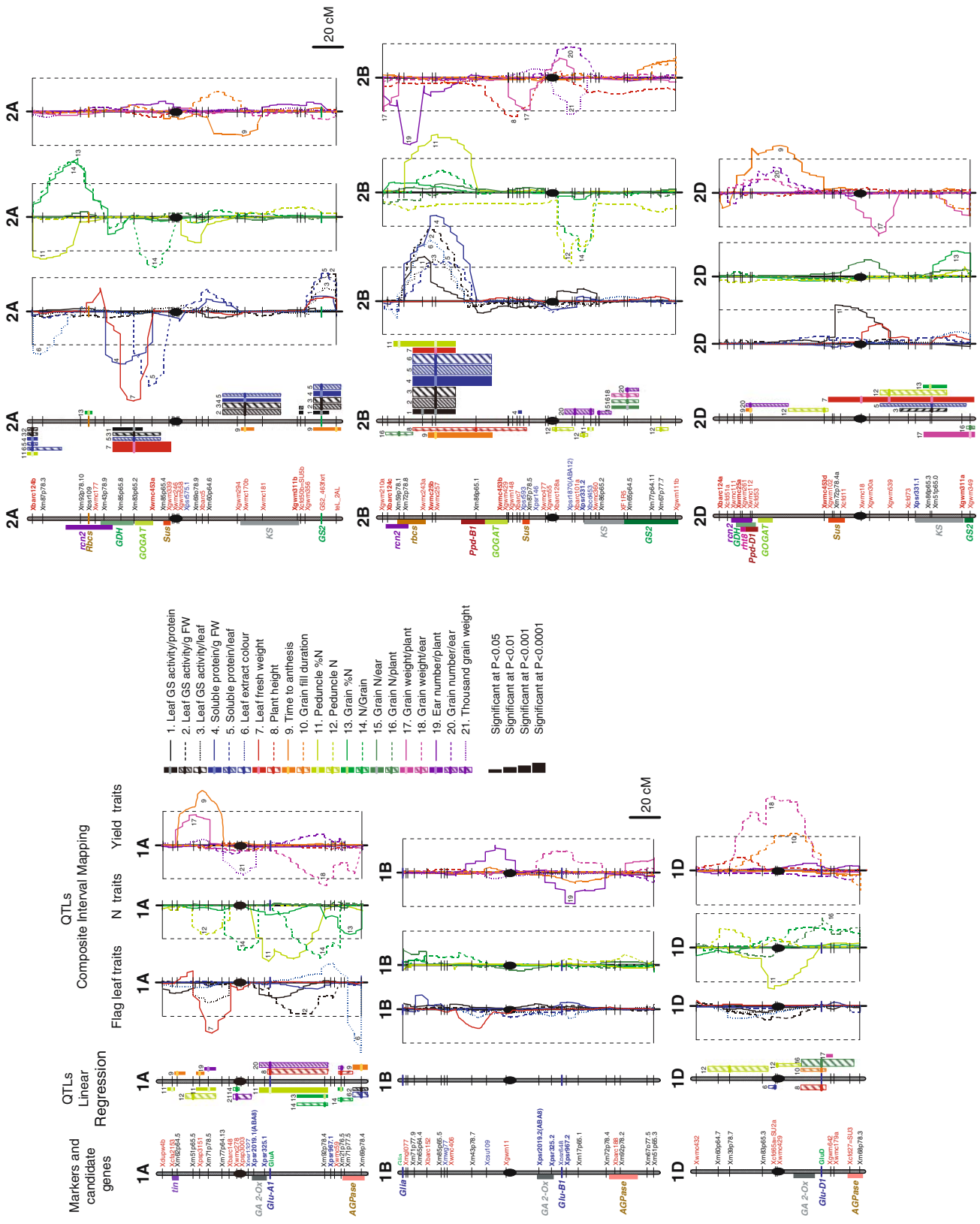
QTL identification

The locations of QTLs were identified using two methods of analysis: single-marker linear regression and CIM (Fig. 1; Table 2). Comparison of QTLs identified by both single-marker analysis and CIM (data in bold in Table 2) showed that 60% of the QTLs were common to both methods of analysis (according to the criteria used to declare the presence of a QTL—see Materials and methods). However, in reality, this underestimates the similarity between the two methods, as CIM frequently gave LR maxima below 20 which were coincident with significant single-marker regressions, and vice versa. In the subsequent text, code numbers for each trait are given in parenthesis after the trait name to facilitate trait identification in Fig. 1. Major QTLs will be discussed and minor ones only when associated with other major QTLs or candidate genes. For simplicity, QTLs at the same locus with increasing additive effects coming from the same parental alleles will be referred to as QTLs with positive co-localisation, and QTLs with increasing additive effects from opposite parental alleles will be referred to as QTLs with negative co-localisation.

Fig. 1 Molecular markers, mapped genes and QTLs. Data are presented on five chromosome plots for each of the 21 chromosomes. The first chromosome plot shows the position of the mapped molecular markers as *horizontal bars* and the likely locations of genes relevant to nitrogen use and yield (described, with abbreviations, in Table 2 ESM) in wheat as *coloured horizontal* (if mapped in the population) or *vertical bars*. The second chromosome plot shows the location of QTLs for the 21 traits identified using linear regression marker-by-marker. Here, QTLs shown to the left of chromosomes indicate that the increasing allele comes from SQ1, and QTLs to the right of chromosomes have the increasing allele from CS. In this figure, the length of QTL bars is not a measure of the confidence interval, but is determined as the distance from the last significant ($P < 0.05$) marker either side of a QTL cluster to the mid-point between those markers and the first flanking non-significant markers. The third, fourth and fifth plots for each chromosome show likelihood ratio (LR) traces for QTLs identified using CIM; traits are grouped according to flag leaf traits (GS, protein and size, traits 1–7), N content traits (peduncle and grain, traits 11–16), and agronomy (yield, development and height, traits 8–10 and 17–21), respectively. For these figures, LR traces on the left indicate an increasing effect from SQ1 alleles and traces on the right indicate an increasing effect from CS alleles. *Dashed lines* show a LR of 20

QTLs for the rate of plant development, leaf N metabolism and leaf size

A major result, on the basis of both the phenotypic correlations (Table 1) and co-localisations of QTLs (Fig. 1), was the consistent negative association between flag leaf biochemical traits (1–6) and anthesis date (9). The parents of the mapping population differed in their anthesis dates (CS flowers later than SQ1) and this resulted in major QTLs for anthesis on chromosome 5A and 5D due to the vernalisation requirement genes *Vrn-A1* and *Vrn-D1* (Snape et al. 2001). These regions influenced other biochemical, physiological and agronomical traits in this population, as shown by co-localisation of QTLs for traits on 5A and 5D in Fig. 1. Thus individuals which reached anthesis faster, and in consequence also reached grain soft dough stage earlier, had greener leaves, higher leaf GS activity and more leaf soluble protein at the grain soft dough stage and thus showed delayed senescence during grain filling. The time from anthesis to grain dough stage, or approximately the grain fill duration (10), did not seem to be strongly associated with any leaf metabolic trait or any yield or N parameters (Table 1). Nevertheless, major QTLs for grain fill duration (10) were established on 4B (near Rht-B1) and 5D (near Vrn-D1) (Table 2; Fig. 1) that co-localised negatively with plant height (8) and days to



anthesis (9), respectively. Another major finding from our study is the positive co-localisations between flag leaf weight (7), a measure of leaf size or leaf area (data not shown), and QTLs for all flag leaf metabolic traits measured at grain dough stage, namely GS activity (1, 2, 3), soluble protein (4, 5) and colour (6), at loci on 2A,

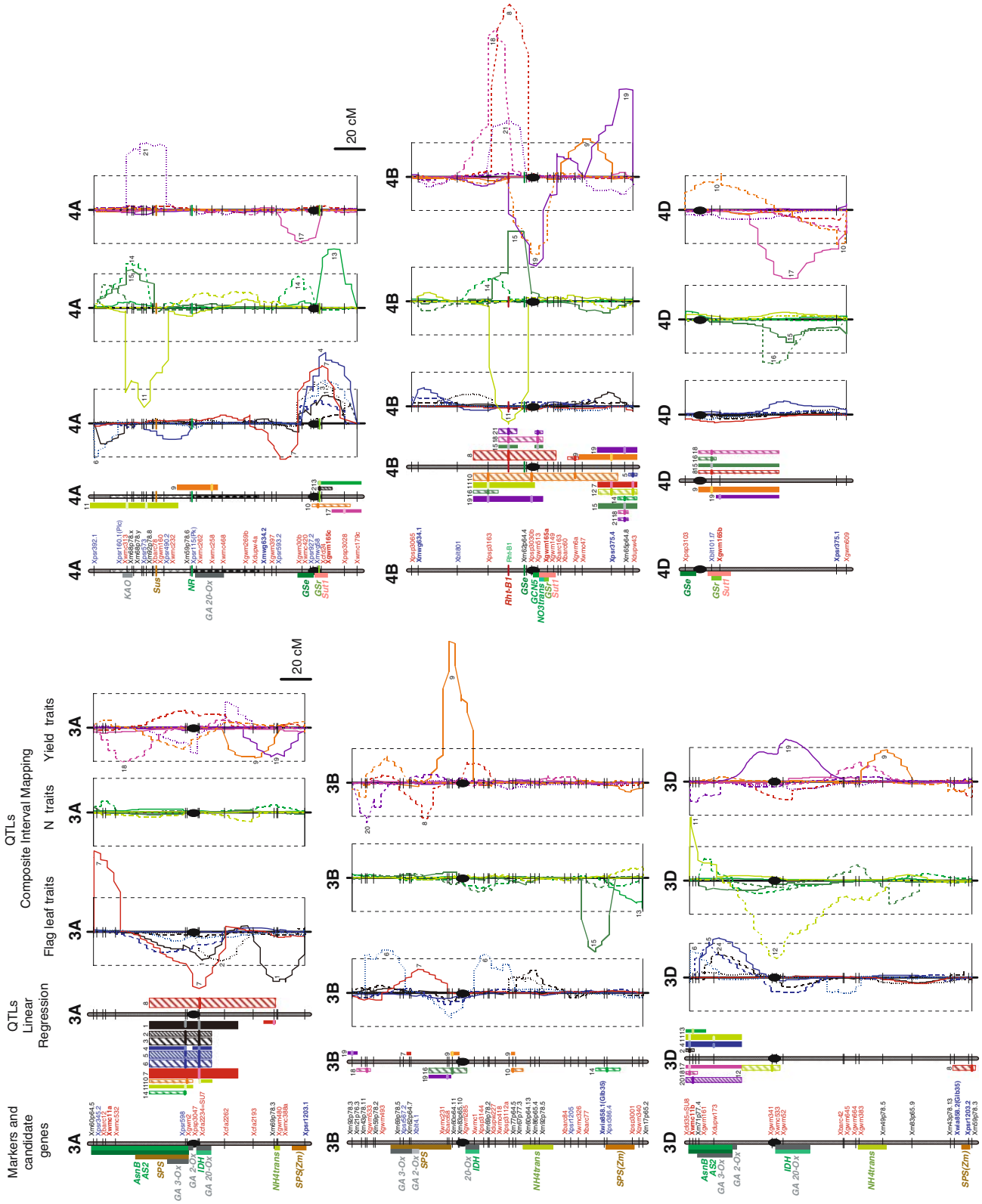


Fig. 1 continued

2B, 3A, 5A, 5D and 7A (Fig. 1). These strong and positive QTL co-localisations were also supported by the large positive phenotypic correlations in Table 1, such that leaves with larger fresh weight had higher soluble protein content/fwt and higher GS activity/protein compared with smaller leaves.

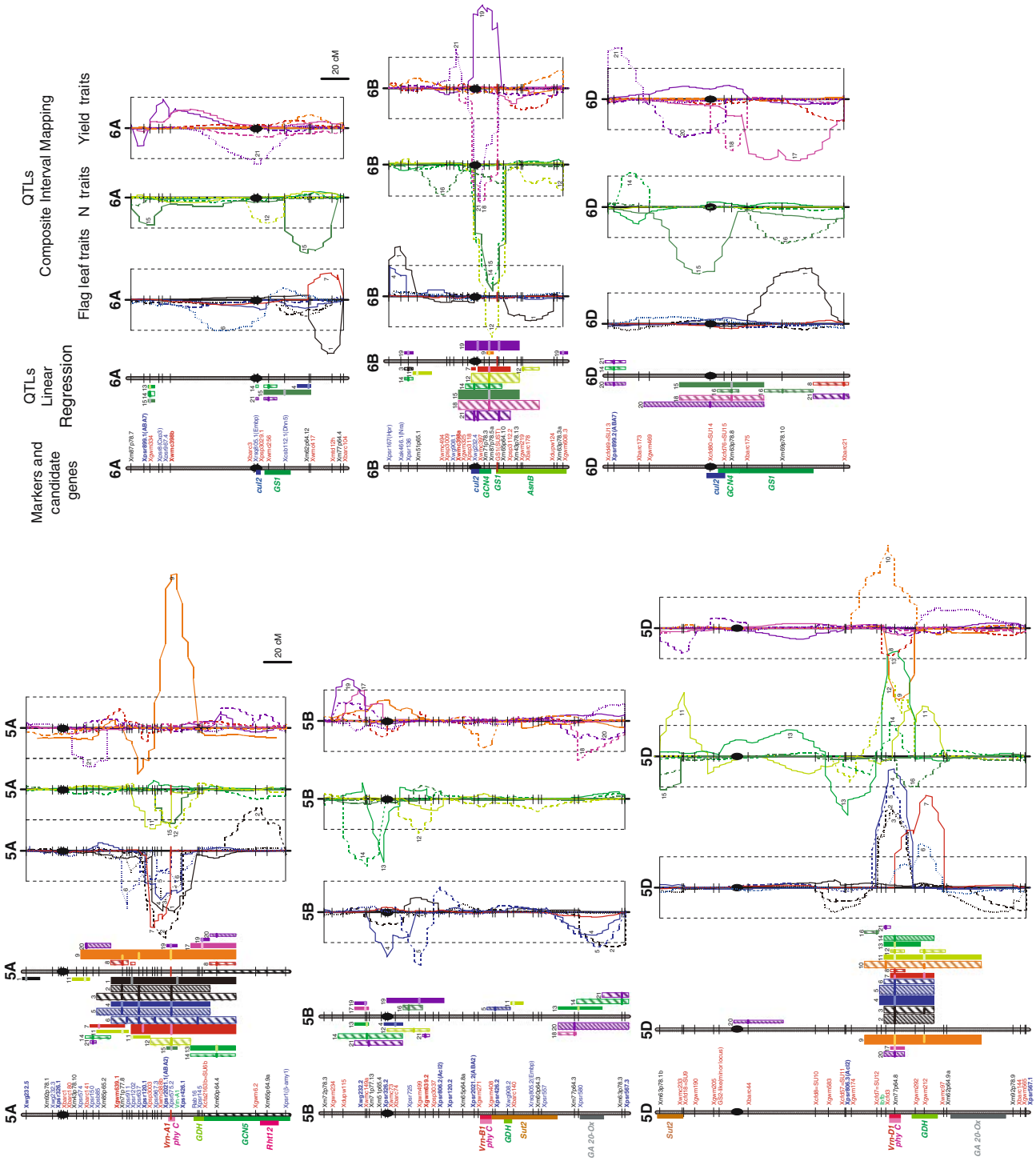
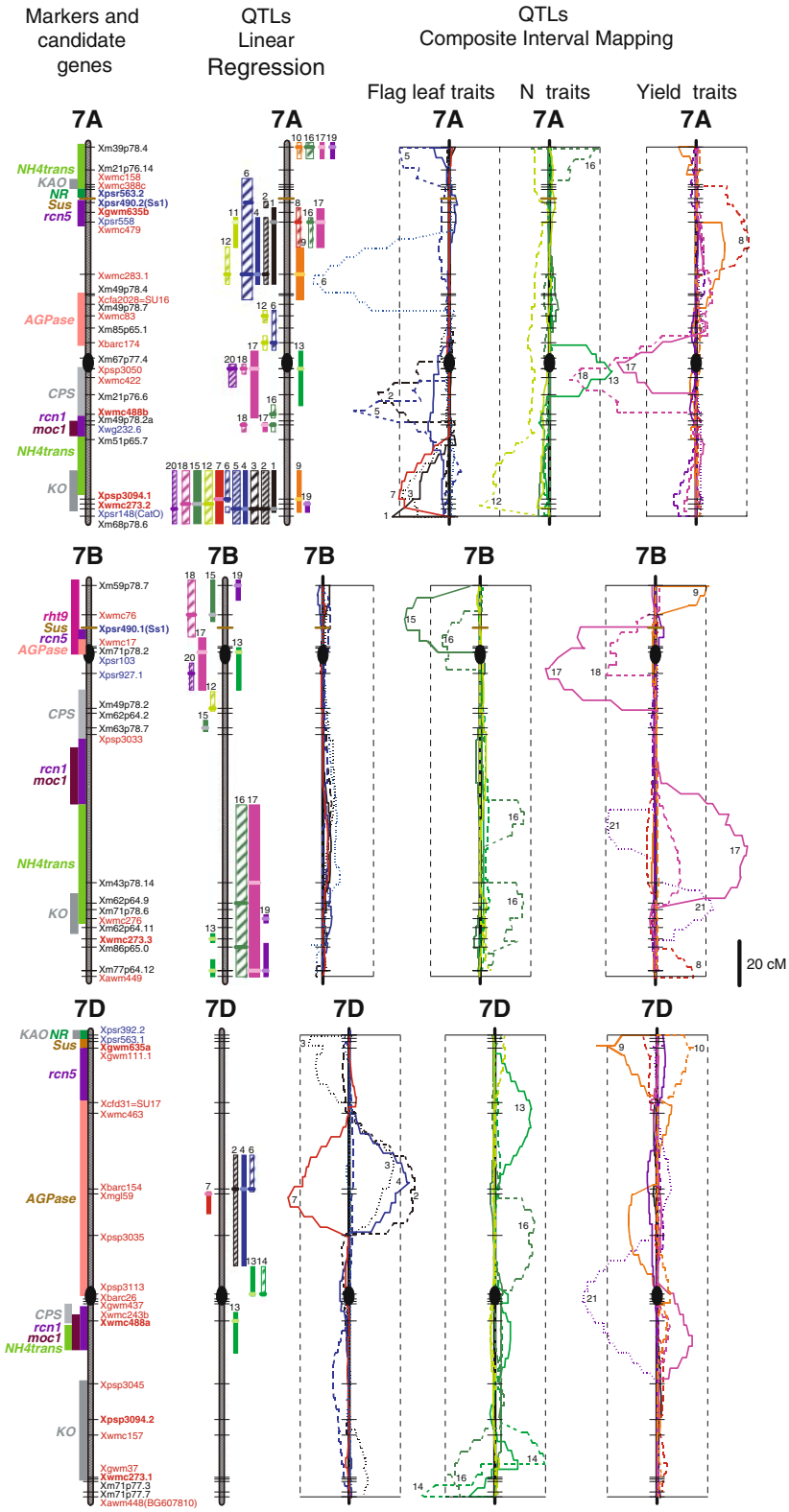


Fig. 1 continued

QTLs for leaf ammonia assimilation at GS loci

The mapping of GS genes enabled the study of the genetic role of GS in N use. Results identified GS activity QTLs at the *GS2* locus on chromosome 2A with the increasing allele coming from CS and which co-localised with soluble protein content/leaf (4, 5) but with no other plant developmental or yield traits (Fig. 1). Other QTLs for total GS activity were established at the *GSr* locus on chromosome 4AS (Fig. 1) with a small effect (4.8% of variance for GS/leaf at LR 24.2 and 6.9% variance for GS/protein at LR max 16.9) but which co-localised positively with a grain %N (trait 13) QTL contributing 10.4% of the variance for that trait

Fig. 1 continued



(Table 2). No GS activity QTLs were identified in our study at the wheat *GS1* (*Gln1–1* in rice, *Gln4/3* in maize) locus on chromosome 6BL, but two large effect QTLs for GS/protein (1) were established using CIM about 20–30 cM from the predicted locations of *GS1* genes on 6A and 6D (Fig. 1). Nevertheless, large-effect QTLs for grain N (14, 15) and TGW (21) were associated, in our study, with the *GS1* locus on 6B (Figs. 1, 1 ESM).

Table 1 Correlation matrix using phenotypic mean values for the 21 traits studied

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	Leaf GS activity/ protein	1.00																				
2	Leaf GS activity/leaf fwt	0.61	1.00																			
3	Leaf GS activity/leaf	0.60	0.91	1.00																		
4	Leaf soluble protein/fwt	0.46	0.97	0.87	1.00																	
5	Soluble protein/leaf	0.50	0.90	0.96	0.92	1.00																
6	Leaf extract colour	0.65	0.86	0.83	0.84	0.82	1.00															
7	Leaf fwt	0.63	0.44	0.66	0.38	0.64	0.53	1.00														
8	Plant height	-0.38	-0.38	-0.33	-0.38	-0.32	-0.41	-0.25	1.00													
9	Time to anthesis	-0.45	-0.60	-0.59	-0.59	-0.59	-0.60	-0.46	0.22	1.00												
10	Grain fill duration	0.25	0.14	0.13	0.13	0.13	0.18	0.26	-0.36	-0.60	1.00											
11	Peduncle %N	0.28	0.59	0.55	0.64	0.60	0.60	0.30	-0.59	-0.37	0.09	1.00										
12	N/peduncle	0.26	0.27	0.35	0.29	0.40	0.33	0.50	0.24	-0.44	0.13	0.21	1.00									
13	Grain %N	0.18	0.33	0.38	0.39	0.41	0.34	0.30	-0.28	-0.31	0.17	0.35	0.12	1.00								
14	N/grain	0.18	0.28	0.38	0.30	0.39	0.26	0.36	0.05	-0.46	0.16	0.21	0.39	0.74	1.00							
15	Grain N/ear	0.22	0.15	0.27	0.10	0.23	0.13	0.34	0.36	-0.29	-0.04	-0.07	0.52	0.13	0.00	1.00						
16	Grain N/plant	0.11	-0.02	-0.02	-0.10	-0.07	-0.06	-0.01	0.10	-0.19	0.19	-0.25	-0.03	-0.26	-0.32	0.27	1.00					
17	Grain weight/plant	-0.07	-0.26	-0.28	-0.33	-0.33	-0.28	-0.23	0.24	0.12	-0.01	-0.38	-0.11	-0.82	-0.61	0.05	0.75	1.00				
18	Grain wt/ear	0.09	-0.07	0.01	-0.14	-0.04	-0.09	0.13	0.48	-0.05	-0.14	-0.28	0.40	-0.41	-0.36	0.84	0.37	0.48	1.00			
19	ear # / plant	-0.15	-0.14	-0.25	-0.13	-0.24	-0.15	-0.33	-0.33	0.19	0.10	-0.02	-0.55	-0.29	-0.14	-0.83	0.26	0.36	-0.62	1.00		
20	Grain#/ear	-0.02	-0.19	-0.17	-0.26	-0.21	-0.18	-0.08	0.34	0.27	-0.25	-0.32	0.09	-0.60	-0.47	0.46	0.25	0.55	0.77	-0.29	1.00	
21	Thousand grain weight	0.12	0.11	0.22	0.10	0.20	0.09	0.27	0.30	-0.41	0.10	-0.01	0.47	0.21	0.07	0.65	0.20	-0.02	0.48	-0.58	-0.18	1.00

Positive: 5% 0.21; 1% 0.27; 0.1% 0.34; 0.01% 0.39

Negative: 5% -0.21; 1% -0.27; 0.1% -0.34; 0.01% -0.39

Colours and intensity of cell shading indicate significant positive (green) and negative (red) correlations from $P < 0.05$ to $P < 0.0001$

QTLs for peduncle N

This study showed that the amount of nitrogen present in the peduncle at the time of grain fill (11, 12) was strongly positively correlated with flag leaf GS activity (1, 2, 3), soluble protein (4, 5), extract colour (6) and leaf fresh weight (7) as shown in the correlation matrix (Table 1) and by the positive co-localisation of QTLs for these traits such as on chromosomes 2BS, 3A, 5A, 5D, 7AS and 7AL (Fig. 1). This study also demonstrated the interaction of plant height (8) and peduncle %N (11) during grain fill. The population varied for plant height, with the largest QTL for height (8), accounting for 30.5% of the phenotypic variance (Table 2), being located at the major dwarfing gene *Rht-B1* on 4BS (Fig. 1). QTLs for peduncle N (12) were also invariably positively co-localised with N/grain (14) or grain N/ear (15), such as on chromosome 1A and 6B (Fig. 1).

QTLs for grain yield, yield components, and grain N content

Significant QTLs for grain %N (13) were identified on five chromosomes: 2AS, 4AS, 5BS, 5DL and 7A centromere (Fig. 1), accounting for 6–21% of the variance (Table 2). By far the largest QTL for N/grain (14), accounting for 19.6% of the variance, was established on 6BL (Table 2; Fig. 1). A series of QTLs for N/ear (15) was present close to the centromere on the group 6 chromosomes (Fig. 1), which may represent a homoeologous series, and large effect QTLs for grain N/plant (16) were located on 4DL (detected by CIM only) and 6DL, explaining 19.5 and 10.8% of the variance, respectively (Table 2). A large effect QTL was identified for grain wt/plant (17) on chromosome 7BL (Table 2; Fig. 1), explaining 24.4% of the variation and in the same region as those obtained in several field trials (Quarrie et al. 2005). Two other QTLs for grain wt/plant (17) were established in centromeric locations on 7B and 7A with the increasing allele for both coming from SQ1 and were positively co-localised with QTLs for grain wt/ear (18). The largest additive effect QTL for grain wt/ear (18) was identified on 4BS, coincident with the *Rht-B1* dwarfing gene locus, responsible for 28.0% of the variation (Table 2; Fig. 1). Four other grain wt/ear (18) QTLs were present on 6BL, 6DL, 7A and 7B, explaining 20.5, 7.0, 11.5 and 4.4% of the variation, respectively (Fig. 1). All these QTLs had the increasing allele from SQ1 and were positively co-localised with major QTLs for grain number/ear (20) and TGW (21). A major QTL for grain number/ear (20) was established on 6DL and accounted for 13.5% of the variation with the increasing allele coming from SQ1 and positively co-localised with a QTL for grain wt/ear (18) (Fig. 1). The clustering of these yield component QTLs

Table 2 QTLs and their characteristics identified using CIM with 20 background QTLs

Trait number and name	Chrom#	cM	Nearest marker	LR	*R*-squared	Additive
(1) Leaf GS activity/mg protein	**2B**	**25.9**	**wmc243a**	**26.3**	**8.3**	**0.029**
	2D	82.7	m72p78.4a	22.7	7.0	0.025
	3A[a]	**124.6**	**m69p78.3**	**29.3**	**10.3**	**−0.033**
	3A[a]	**127.8**	**wmc388a**	**29.5**	**9.1**	**−0.033**
	5A[a]	**102.2**	**psr2021.1**	**38.5**	**13.2**	**−0.036**
	5A[a]	**112.1**	**vrn-A1**	**39.6**	**13.6**	**−0.036**
	6A	143.1	mtd12h	34.9	12.5	−0.034
	6B	8.5	ak466.1	32.2	10.1	0.034
	6D	142.1	m69p78.10	36.2	25.0	0.051
	7A	**163.2**	**m68p78.6**	**22.8**	**7.2**	**−0.027**
(2) Leaf GS activity/g FW	**2A**	**187.8**	**GS2_463**	**22.4**	**4.3**	**0.178**
	2B	**31.9**	**wmc25b**	**42.8**	**8.7**	**0.245**
	5A	**98.1**	**wmc388b**	**57.2**	**12.3**	**−0.314**
	5A	170.9	gwm6.2	27.8	5.5	0.210
	5B	213.8	m63p78.3	22.3	5.6	−0.202
	5D	**172.4**	**m77p64.8**	**51.1**	**11.1**	**0.300**
	7A	118.9	wmc488b	28.5	5.2	−0.191
	7D	**67.8**	**barc154**	**26.5**	**6.0**	**0.211**
(3) GS activity/leaf	**2B**	**33.6**	**wmc257**	**24.8**	**5.2**	**0.158**
	4A	153.1	gwm165.3	24.2	4.8	0.163
	5A	**103.3**	**psr575.2**	**32.6**	**6.4**	**−0.207**
	5D	172.4	m77p64.8	48.6	11.2	0.245
(4) Soluble protein/g FW	2A	57.3	m85p65.8	31.3	6.2	−0.658
	2B	**32.5**	**wmc25b**	**51.1**	**11.2**	**0.843**
	3D	**18.3**	**dupw173**	**23.5**	**5.7**	**0.575**
	4A	149.9	mwg58	42.2	8.1	0.736
	5A	**96.0**	**psr967.3**	**31.5**	**5.7**	**−0.647**
	5B	**48.0**	**psr326.2**	**28.7**	**6.6**	**−0.670**
	5D	**172.4**	**m77p64.8**	**78.0**	**19.9**	**1.317**
	6B	0.0	psr167	20.3	3.8	0.540
	7D	**67.8**	**barc154**	**23.4**	**5.1**	**0.571**
(5) Soluble protein/leaf	**2A**	**78.6**	**wmc453a**	**44.7**	**8.4**	**−0.766**
	2A	**187.6**	**GS2_463**	**22.5**	**4.0**	**0.490**
	2B[a]	**29.9**	**wmc25b**	**23.4**	**3.7**	**0.429**
	2B[a]	**38.8**	**wmc257**	**24.1**	**5.4**	**0.496**
	5B	61.9	psr725	35.0	6.2	−0.908
	5B	195.6	psr580	23.1	5.5	−0.475
	5D	137.4	cfd57	29.9	4.9	−0.477
	5D	**175.4**	**m77p64.8**	**67.0**	**14.2**	**0.897**
	7A	0.0	m39p78.4	20.1	3.5	−0.406
	7A	118.4	wmc488b	38.4	6.9	−0.967
(6) Leaf extract colour	**1A**	**130.5**	**m69p78.4**	**42.9**	**10.7**	**−0.674**
	2A	**4.0**	**m87p78.3**	**25.5**	**5.2**	**−0.440**
	2B	**33.6**	**wmc257**	**37.6**	**9.3**	**0.829**
	3B	22.0	gwm493	25.5	6.1	0.506
	3B	91.6	psp3144	23.1	4.6	0.554
	4A	0.0	psr392.1	22.9	4.5	−0.421
	4A	149.9	mwg58	25.9	4.9	0.456
	5A	**76.2**	**m71p77.8**	**38.2**	**7.8**	**−0.731**
	5A	**103.3**	**psr575.2**	**20.7**	**3.8**	**−0.448**
	5D	**193.6**	**gwm212**	**28.3**	**5.9**	**0.516**
	6A	68.7	barc3	20.8	9.4	−0.579
	7A	**56.4**	**wmc283.1**	**55.3**	**14.6**	**−0.725**
(7) Leaf fresh weight	1A	30.6	m71p78.5	29.9	5.5	−0.091
	1B	44.8	m43p78.7	23.2	5.1	−0.056
	2A	**66.5**	**m83p65.2**	**52.9**	**12.0**	**−0.121**
	3A	2.1	psr345.2	47.0	8.2	0.075
	3A	**68.8**	**cfa2234**	**33.8**	**5.6**	**−0.062**
	4A	128.0	psr593.2	21.4	4.3	−0.071
	4A	153.1	gwm165.3	33.3	7.2	0.080

Table 2 continued

Trait number and name	Chrom#	cM	Nearest marker	LR	*R*-squared	Additive
	5A	**96.0**	**psr967.3**	**49.7**	**9.4**	**−0.080**
	5D	**193.6**	**gwm212**	**59.8**	**13.1**	**0.096**
	7A	**159.0**	**psp3001.1**	**20.1**	**3.1**	**−0.044**
	7D	**74.1**	**mgl59**	**24.4**	**4.4**	**−0.051**
(8) Plant height	3B	38.3	psr567.2	21.0	3.6	−5.88
	4B	**61.7**	**Rht-B1**	**102.8**	**30.5**	**15.19**
	7A	**41.5**	**wmc479**	**20.6**	**5.3**	**6.01**
(9) Time to anthesis	**1A**	**24.7**	**psp3151**	**32.0**	**4.3**	**1.768**
	2D	**35.5**	**cfd53**	**28.7**	**9.3**	**2.966**
	3B	**70.4**	**m60p64.11**	**83.4**	**15.6**	**3.668**
	4B	**110.8**	**wmc47**	**22.5**	**2.8**	**1.385**
	5A	**110.1**	**vrn-A1**	**106.6**	**22.5**	**4.637**
	5D	**172.4**	**m77p64.8**	**47.1**	**7.3**	**−2.169**
	7B	0.0	m59p78.7	20.8	2.7	1.279
	7D	3.2	gwm635a	24.0	3.0	−1.808
(10) Grain fill duration	**1D**	**64.1**	**wmc429**	**21.6**	**9.4**	**1.294**
	4B	**80.0**	**psp3030.2**	**45.1**	**14.2**	**−1.516**
	4D	21.3	gwm165.2	21.1	5.8	0.969
	4D	97.8	psr375.1	20.3	5.7	−0.865
	5D	**165.7**	**cfd3**	**53.9**	**17.1**	**1.587**
(11) Peduncle %N	**1A**	**86.0**	**psr325.1**	**33.7**	**12.0**	**−0.073**
	1D	52.8	cfd65a	25.4	4.4	−0.050
	2A	**8.8**	**m87p78.3**	**24.9**	**4.7**	**−0.044**
	2B	**32.5**	**wmc25b**	**33.7**	**6.9**	**0.054**
	3D	**0.0**	**cfd35**	**37.9**	**7.7**	**0.064**
	4A	**34.8**	**m92p78.8**	**59.4**	**11.6**	**−0.078**
	4B	**61.7**	**Rht-B1**	**73.3**	**16.2**	**−0.094**
	5A	98.1	wmc388b	26.3	4.2	−0.047
	5D	17.1	cfd18	41.5	7.3	0.079
	5D	**193.6**	**gwm212**	**32.9**	**6.1**	**0.067**
(12) Peduncle N	1B	18.0	barc152	65.7	10.0	0.140
	1B	142.8	m92p78.2	24.7	2.8	0.067
	2B	123.3	psr1870	36.2	5.4	−0.087
	3D	**59.5**	**gwm341**	**46.3**	**6.9**	**−0.103**
	5A	**112.1**	**vrn-A1**	**25.4**	**3.1**	**−0.071**
	5B	73.6	gwm499	21.9	2.7	−0.067
	5D	165.7	cfd3	54.4	7.2	0.122
	6B	**75.7**	**m87p78.5a**	**112.4**	**19.9**	**−0.215**
	7A	163.2	m68p78.6	21.9	2.5	−0.062
(13) Grain %N	**2A**	**31.5**	**m92p78.10**	**34.4**	**9.3**	**0.113**
	4A	**163.1**	**psp3028**	**34.8**	**10.4**	**0.089**
	5B	**43.6**	**m51p65.4**	**41.0**	**10.6**	**−0.168**
	5D	136.4	psr806.3	38.2	9.7	−0.094
	5D	**172.4**	**p77p64.8**	**68.6**	**21.3**	**0.145**
	7A	**98.8**	**psp3050**	**24.5**	**5.8**	**0.068**
(14) N/grain	**1A**	**51.8**	**wmc278**	**26.3**	**5.1**	**−0.039**
	1A	**107.4**	**m92p78.4**	**31.3**	**6.7**	**−0.045**
	2A	28.8	m92p78.10	32.4	7.9	0.047
	2A	77.6	wmc453a	29.8	5.9	−0.042
	2B	127.4	barc101a	35.0	7.3	−0.046
	4A	24.7	wmc313	26.4	5.2	0.040
	5B	**30.8**	**wmc149a**	**43.5**	**9.4**	**−0.051**
	5D	**172.4**	**m77p64.8**	**22.6**	**4.8**	**0.055**
	6B	**75.7**	**m87p78.5a**	**77.4**	**19.6**	**−0.074**
	6D	**4.3**	**psr899.2**	**22.6**	**4.8**	**0.036**
	7D	201.1	awm448	29.0	7.2	0.049
(15) Grain N/ear	3B	157.6	wmc326	44.2	8.5	−2.206
	4A	26.0	m68p78.x	22.8	3.8	1.408
	4B	**68.0**	**Rht-B1**	**41.6**	**8.4**	**2.003**

Table 2 contiuned

Trait number and name	Chrom#	cM	Nearest marker	LR	*R*-squared	Additive
	5A	**109.3**	**vrn-A1**	**22.5**	**4.2**	**−1.494**
	5D	2.0	m63p78.1b	22.0	3.9	−1.421
	6A	**124.4**	**m62p64.12**	**36.4**	**7.6**	**−2.105**
	6B	**73.9**	**m87p78.5a**	**83.0**	**19.0**	**−3.273**
	6D	**61.5**	**cfd80**	**41.9**	**13.2**	**−2.664**
	7B	**12.7**	**wmc76**	**30.4**	**5.7**	**−1.739**
(16) Grain N/plant	4D	58.8	gwm165.2	25.3	19.5	−0.020
	6D	**124.1**	**m69p78.10**	**24.1**	**10.8**	**−0.014**
(17) Grain weight/plant	2B	0.0	gwm210.1	20.3	5.4	−0.866
	2D	99.6	gwm30.1	26.1	7.6	−1.060
	4D	68.8	psr375.1	40.9	22.4	−1.811
	6D [a]	113.1	m69p78.10	39.6	15.2	−1.372
	6D[a]	132.1	p69p78.10	39.8	18.3	−1.519
	7A	**98.8**	**psp3050**	**31.9**	**9.2**	**−1.220**
	7B	**35.9**	**psr927.1**	**44.1**	**14.2**	**−1.436**
	7B	**120.3**	**m43p78.14**	**36.7**	**24.4**	**1.824**
(18) Grain weight/ear	1A	111.6	psr967.1	23.8	4.7	−0.084
	1D	50.3	cfd65a	44.3	18.3	0.179
	3A	14.8	wmc532	20.0	5.8	−0.086
	4B	**51.7**	**psp3163**	**87.3**	**28.0**	**0.218**
	5B	**186.7**	**m77p64.3**	**25.9**	**5.2**	**−0.099**
	5D	46.7	GS2-related	54.4	15.8	0.182
	6B	**69.1**	**wmc397**	**72.6**	**20.5**	**−0.182**
	6D	**88.0**	**m63p78.8**	**33.8**	**7.0**	**−0.098**
	7A	**103.0**	**wmc422**	**50.2**	**11.5**	**−0.137**
	7B	35.9	psr927.1	20.1	4.4	−0.079
(19) Ear number/plant	1B	55.4	csu109	34.8	6.9	1.005
	1B	107.4	psr967.2	28.1	5.3	−0.696
	2B	19.8	m72p78.8	39.9	10.0	−0.856
	3D	64.7	wmc533	24.7	5.6	0.662
	4B	**82.2**	**gwm513**	**52.8**	**19.8**	**−1.969**
	4B	**136.9**	**m65p64.8**	**52.2**	**11.3**	**0.975**
	5B	**11.9**	**dupw115**	**27.6**	**5.2**	**0.607**
	6B	**69.1**	**wmc397**	**54.2**	**11.6**	**1.008**
(20) Grain number/ear	3B	8.2	m21p76.3	23.5	7.6	−4.691
	6D	**59.5**	**cfd80**	**24.5**	**13.5**	**−2.994**
(21) Thousand grain weight	2B	125.4	barc101a	21.7	4.1	−1.081
	4A	36.8	m92p78.8	39.2	7.8	1.639
	4B	**62.8**	**Rht-B1**	**34.2**	**6.5**	**1.403**
	5A	**44.3**	**barc141**	**26.1**	**4.9**	**−1.172**
	6A	**90.2**	**rsq805.1**	**23.7**	**4.2**	**−1.182**
	6B	**63.9**	**psp3118**	**74.3**	**20.5**	**−3.481**
	6D	**4.0**	**psr899.2**	**33.1**	**6.3**	**1.470**
	7B	145.1	m62p64.9	23.0	4.1	1.140
	7D	116.3	barc26	29.3	5.3	−1.268

All QTLs with LR maximum over 20 (LOD = 4.34) are listed. CIM QTLs similar to QTLs identified by linear regression ($P < 0.05$) are shown in bold

[a] Denote the same broad QTL

near the centromeres of both 6B and 6D using CIM, and by linear regression also on 6A suggests a homoeologous set of genes on the group 6 chromosomes controlling aspects of grain yield. Other strong QTLs for grain number/ear (20) were identified by linear regression on 2BL and 5BL, with LR maxima of 18.2 and 12.1, respectively for CIM (Fig. 1), the latter of which coincided negatively with QTLs for TGW (21). By far the largest QTL for TGW (21), accounting for 20.5% of the variance, was identified near the centromere on 6B with the increasing allele from SQ1 (Fig. 1), and this association was also established in several field trials (Fig. 1 ESM).

Competition for assimilate

We studied the competition for assimilate needed to support ear development. Thus, QTLs were identified for ear number/plant (19) with the largest QTL on 4BS accounting for 19.8% of the variation (Table 2; Fig. 1). This QTL had an LR maximum about 20 cM from the dwarfing

gene *Rht-B1*, though with sufficient overlap to consider pleiotropy between the *Rht-B1* gene and the QTL such that taller plants, with the CS allele, had fewer ears/plant but higher grain wt/ear (18) and TGW (21). Another major QTL accounting for 11.6% of the variation for ear number/plant (19) was established proximal on 6BL (Table 2; Fig. 1). This QTL was co-localised negatively with a major QTL for grain wt/ear (18) and with the largest QTL for yield component, TGW (21) accounting for 20.5% of the variation, and for which SQ1 contributed the increasing allele (Fig. 1). In a follow-up study (D. Z. Habash unpublished results), we found that this region of 6B was highly significantly associated with variation in flag leaf area, such that large leaf area was associated with fewer tillers, suggesting competition between the two for available assimilates during organogenesis. Similar QTLs for ears/plant have also been identified in the same position on chromosome 6B from ten other yield trials under field conditions with these CS × SQ1 DHLs (Quarrie et al. 2005, unpublished data; Fig. 1 ESM).

Discussion

Impact of the rate of development, leaf N metabolism and leaf size

This is the first report of the detection of QTLs relating to components of flag leaf N metabolism during grain fill in hexaploid wheat. One striking feature of our results, on the basis of both genotypic and phenotypic correlations, was the consistent negative association between flag leaf biochemical traits (1–6) and anthesis date (9). This is partly due to the major influence of development on the profile of metabolism in leaves since the two vernalisation genes *Vrn-A1* (5AL) and *Vrn-D1* (5DL) had an influence on phenology in this population with additive effects of nearly 5d and 2d, respectively (Table 2). It has been documented that major changes occur to soluble protein in wheat leaves during the growth phases after anthesis (Feller and Fischer 1994; D. Z. Habash unpublished). Furthermore, it has recently been established in wheat, that the activity of GS isozymes changes during leaf development, especially post-anthesis, with the chloroplastic isozyme (GS2) showing a gradual decline and the cytosolic isozyme (GS1) showing a corresponding increase in both amount and extractable activity (Habash et al. 2001). Therefore, the coincidences we established for flag leaf N assimilation at loci affecting anthesis time could relate to the complex interaction between metabolism and the rate of development. Variation in other traits associated with the *Vrn* genes may also be due either to a secondary effect of the *Vrn* genes, independent of the effect on flowering date per se, or to one or more closely linked genes at the *Vrn* loci. Such an example of genes acting independently of the *Vrn* genes, located on group 5 long arms closely-linked to the *Vrn-A1* and *Vrn-D1* genes but not resolvable from them in this mapping population, are the major frost tolerance genes *Fr-A1* and *Fr-D1* (S. A. Quarrie unpublished data; Galiba et al. 1995; Snape et al. 2001). Interestingly, one of the seven genes identified by Yan et al. (2003) adjacent to the *Vrn* gene on $5A^m$ of *T. monococcum*, separated by only 0.2 cM, was phytochrome C, known to be involved in the plant's light response and leaf extension in *Arabidopsis* (Franklin et al. 2003). Figure 1 shows that we have identified a QTL for leaf size (7) on 5A at *Vrn-A1*. Therefore, although many QTLs for GS activity and other flag leaf traits coincided with those for flowering time on 5AL and 5DL, these effects may not all be pleiotropic consequences of the flowering time vernalisation genes.

Our results showed very strong positive correlations between flag leaf metabolic-related traits (1–6) which demonstrates the expected tight co-ordination that exists in the underlying processes regulating light absorption (trait 6 for leaf chlorophyll), carbon assimilation (traits 4 and 5 for soluble protein) and nitrogen metabolism (traits 1–3 for GS activity). Leaf soluble protein is a good measure of the organic N in the leaf representing mainly Rubisco content and our results show a positive correlation between leaf soluble protein and GS activity/leaf. This is not surprising because of the dependency of ammonia assimilation on ATP and carbon skeletons ultimately derived from photosynthesis. However, our results also showed strong positive phenotypic and genotypic correlations between flag leaf weight (7), soluble protein content/fwt (4) and higher GS activity/protein (1–3) (Fig. 1; Table 1) implying shared control over the size of the leaf and its metabolic capacity during grain filling in wheat. Studies on several thousand species, in their natural ecosystems, have established strong positive correlations between leaf size (leaf area/mass or specific leaf area), total leaf N and photosynthetic ability (rate and capacity) (Wright et al. 2004). These studies argue for convergent evolution governing the size of the leaf, its N content and photosynthetic capacity. It is interesting that we obtained qualitatively similar correlations for leaf fwt and assimilatory capacity (soluble protein and GS activity) using a single species with alleles segregating for many traits. Our results demonstrate that the capacity to assimilate carbon and nitrogen in leaves during grain dough stage in this mapping population may be determined by the size of the major assimilatory organ, the leaf.

Contribution of leaf ammonia assimilation

Glutamine synthetase function can be described in terms of a matrix of plant cell type, tissue, organ, development, metabolic, nutritional and environmental specificity (Miflin and Habash 2002). During active remobilisation of N, ammonia in leaves is generated via various metabolic processes such as assimilation (ammonia from photorespiration and nitrate reduction), reassimilation from transamination reactions and catabolic senescence driven processes. Most of this ammonia is reassimilated into glutamine via GS and to enable this central and complex role in plant cells, GS exists in several isozymes in the chloroplast and the cytosol. Studies have shown that GS2 is still active in flag leaves during the whole period of grain filling but that the activity and amount of protein gradually declines whilst those of GS1 isozymes increase (D. Z. Habash unpublished; Habash et al. 2001). It was at this stage of leaf metabolism that the activity of total leaf GS, including all the isozymes, was measured in this population. Our studies have identified GS activity (1–3) QTLs at the *GS2* locus on chromosome 2A with the increasing allele coming from CS. This is an interesting result and indicates that there are genetic differences between the parents at these loci for GS2 activity, which contribute to the total GS activity measured. The QTLs established on 2A co-localise with soluble protein content/leaf (4, 5) but with no other plant developmental or yield traits; therefore, one can conclude that even though there were genetic differences at this locus these did not have a major impact on growth or yield under the conditions used for this study. Another noticeable QTL for total GS activity (2,3) in this mapping population was established at the *GSr* (*Gln1-2* in rice, *Gln1* in maize) locus on chromosome 4AS with a small effect but nevertheless co-localised positively with a grain %N (13) QTL contributing 10.4% of the variance for that trait (Fig. 1; Table 2). Genetic studies by Galais and Hirel 2004 established co-localisations of QTLs for leaf total GS activity and N remobilisation at the orthologous maize *Gln1* locus for *GSr* on chromosome 1. Furthermore, work in our laboratories has shown that the expression of *GSr* in wheat increases at later stages of leaf development and this is supported by data from western blots (D. Z. Habash unpublished data). Taken together, these results in wheat suggest the likely involvement of this particular GS isozyme (*GSr)* in N remobilisation from leaves and defines this as an important genomic region for future work. An intriguing result from our study is the lack of GS activity QTLs at the wheat *GS1* (*Gln1–1* in rice, *Gln4/3* in maize) locus on chromosome 6BL and the identification of two large effect QTLs (CIM only) for GS/protein (1) 20–30 cM from the predicted locations of *GS1* (*Gln1–1* in rice, *Gln4/3* in maize) genes on 6A and 6D. Furthermore, large-effect QTLs for grain N (14, 15) and TGW (21) were associated, in our study, with the *GS1* locus on 6B (Figs. 1, 1ESM). Obara et al. (2004) have recently established an orthologous rice locus influencing panicle weight associated with GS1 content close to, but not co-localising with, the *Gln1–1* gene (*GS1* in wheat) and have argued for non-GS elements controlling the amount of GS protein present. Their findings are supported by our data in wheat which shows a region orthologous to *Gln1–1* on rice chromosome 2, namely wheat 6B in our study, controlling grain weight close to the *GS1* mapped gene. Overall, our findings, that most GS activity QTLs were obtained in regions not associated with the mapped GS genes, lends strong support to the conclusion of Moorhead et al. (1999) and Finnemann and Schjoerring (2000) that GS is regulated post-transcriptionally and this remains a crucial area for study in wheat.

Role for peduncle N

A major finding from these studies in wheat has been the very strong positive genetic correlations between the amount of nitrogen present in the peduncle at the time of grain fill and all flag leaf traits (1–7) on chromosomes 2BS, 3A, 5A, 5D, 7AS and 7AL (Fig. 1). This is an expected result as the peduncle N content is mainly comprised of glutamine, asparagine and glutamate at this stage of development and these amino acids are the favoured form of transport from the site of assimilation/remobilisation in the leaf to the sink or grain in wheat (Simpson and Dalling 1981). Therefore the processes involved in their production which are ammonia assimilation via GS (traits 1, 2, 3) and the mobilisation of amino acids from the reserve of organic nitrogen locked up in Rubisco (leaf soluble protein, traits 4, 5) should be positively associated with the amount of nitrogen present in the stem at that particular stage of development. This study also demonstrated the interaction of plant height and peduncle %N (11) during grain fill. This was not surprising since this population varied for plant height and the largest QTL for height was located at the major dwarfing gene *Rht-B1* on 4BS (Fig. 1). It is well established that these *Rht* dwarfing alleles cause a reduced response to the gibberellic acid class of plant hormones which amongst many of their influences target stem elongation (Hedden 2003). Thus, the taller the plants, the less N was present per unit stem weight as seen from the QTLs for peduncle %N and height being on opposite sides of chromosome

4BS where the *Rht* gene resides. QTLs for peduncle N content were also positively co-localised with N/grain (14) or grain N/ear (15) such as on chromosome 6B (Fig. 1), demonstrating the tight relationship between the amount of N in the stem during grain fill and the amount of N in the grain at maturity. This is also consistent with stem function as a storage organ for plant organic N. This study has demonstrated, for the first time in wheat, that peduncle nitrogen content reflects the status between source (leaves) and sink (grain). Thus, it should be a good indicator of the capacity to assimilate nitrogen in the leaf as well as a measure of grain N at harvest.

Relevance of grain-fill duration

Our results showed that a long period of grain filling per se does not necessarily ensure good N and grain yield, at least in this particular cross and environmental conditions. However, major QTLs for grain fill duration (10) were established on 4B (near *Rht-B1*) and 5D (near *Vrn-D1*) (Fig. 1) that co-localised negatively with plant height and days to anthesis, respectively, implying that the period of grain fill is related to the flowering time and size of the plant. A genetic study designed to map QTLs for N remobilisation in barley (Mickelson et al. 2003) concluded that fast N remobilisation from flag leaves is advantageous for grain yield and total grain protein, but not for grain % protein. Our data showed a trend in the opposite direction; those DHLs with yellower leaves, low protein and GS activity or advanced senescence at dough stage had a disadvantage in terms of grain yield and nitrogen.

Grain yield, N content and its components

Of the N-related yield traits presented here (Fig. 1), only grain protein content (grain %N, trait 13) appears to have been previously the subject of genetic analysis in either hexaploid or tetraploid wheats. Amongst 15 QTL locations identified here for grain %N (13), 8 are likely to be coincident with QTLs for grain %N (grain protein content) identified previously. Thus, those on 1AL, 5B and 7A, correspond to those identified by Groos et al. (2003); those on 2AS and 2DL correspond to those identified by Prasad et al. (1999, 2003) and that on 5DL at the *Vrn-D1* locus has been identified previously using single chromosome substitution lines (Law et al. 1978). The 7A centromeric QTL for grain %N also coincided with grain protein content QTLs identified by Turner et al. (2004) and Charmet et al. (2005), and its likely homoeologue centromeric on 7B (identified only by linear regression, Fig. 1) was coincident with a grain protein content QTL identified by Blanco et al. (2002). QTLs for grain N traits were identified at the Glu-A1 and Glu-D1 loci which are consistent with the findings of Charmet et al. (2005) showing an association between the amount of N in the grain and the composition of grain proteins.

The structure of grain yield in these DHLs had a major influence on grain %N (13) and N/grain (14), with those traits showing significant positive associations with grain N/ear (15) and TGW (21) (Table 1). In contrast, grain %N (13) and N/grain (14) were negatively associated with grain wt/plant (17), ear number/plant (19) and grain number/ear (20) on the basis of both phenotypic mean data and QTL co-localisations (Table 1; Fig. 1). These results confirm the generally-accepted view that the fewer the ears and grains/ear, the more N goes into each grain, and the bigger the grain grows, the more N it contains. Grain %N (13) and N/grain (14) are both major determinants of grain quality and our study has also showed that genetic elements controlling flag leaf N metabolism and stem N storage have some association with these traits.

Assimilate partitioning and yield

As ear number/plant is usually determined earlier in the season than both grain number/ear and TGW (Slafer 2003), tillering capacity affects assimilate partitioning and is a major determinant of both grain number/ear and TGW. Therefore, a major method of modifying the amount of N in the grain is by altering ear number/plant and we have identified a major QTL for ears per plant (19) on chromosome 6B (Fig. 1). This QTL was negatively co-localised with the largest QTL for TGW (21), accounting for 20.5% of the variation, and for which SQ1 contributed the increasing allele. Similar QTLs for ears/plant have also been identified in the same position on chromosome 6B from ten other yield trials under field conditions using the same mapping population (Fig. 1ESM). These findings for 6B demonstrate one of the major ways in which plant breeding has increased yield by reducing excessive tiller production and reallocating assimilates to fewer ears carrying a larger number of heavier grain with more N. Results of an in silico search for mapped genes for tillering in cereals included *tin* (1AS), *rcn* (group 2S, group 7S and 7L), *cul2* mutation (group 6 centromeric), and *moc1* (group 7L) (Babb and Muehlbauer 2003; Li et al. 2003; Spielmeyer and Richards 2004; Gramene database). In particular, a wheat homologue of the *cul2* uniculm mutant of barley is a strong candidate for the large QTL for ears/plant near the centromere on 6B. According to the location of *cul2* on the barley genetic map, 8.8 cM from abg458 (Babb

and Muehlbauer 2003), the consensus barley genetic maps of Langridge et al. (1995) and Qi et al. (1996) imply that the wheat homologue of *cul2* would be located on the long arm of group 6, very close to the centromere (Fig. 1ESM). This would correspond to the most proximal of three QTL maxima for ear number/plant (19) on 6B using linear regression (Figs. 1, 1ESM) and 3–4 cM from the QTL maximum with CIM. The wild type CUL2 gene product is known to play a major role in regulating tiller development through promoting the growth of axillary meristems (Babb and Muehlbauer 2003). The strength and consistency of these QTLs for yield and their components near 6B centromere across several environments, together with the identification of a candidate mutation in the form of the barley homologue *cul2*, make this QTL worth targeting to isolate the functional gene in the future.

Conclusion

This study has demonstrated the influence of the dwarfing and vernalisation alleles on wheat N use. New genetic associations amongst traits were also uncovered such as the strong co-localisations between leaf size and flag leaf N metabolism. The contribution of flag leaf total GS activity to grain and stem N was demonstrated. We have also shown that peduncle N is a good marker both for leaf N metabolism and for the status of grain N. A major genomic region on chromosome 6B, established in our study and supported by data from field trials, should receive further work since it harbours genes influencing assimilate partitioning. This study has identified genomic regions regulating aspects of N metabolism and grain N assimilation that should help plant breeders in the search for allelic variation within traits that improve N use in wheat.

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