Journal of Experimental Botany, Vol. 57, No. 11, pp. 2627–2637, 2006
doi:10.1093/jxb/erl026 Advance Access publication 9 July, 2006
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Journal of Experimental Botany

RESEARCH PAPER

Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes

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Received 25 January 2006; Accepted 13 April 2006

Abstract

Previous studies with 95 bread wheat doubled haploid lines (DHLs) from the cross Chinese Spring (CS)×SQ1 trialled over 24 year×treatment×locations identified major yield quantitative trait loci (QTLs) in homoeologous locations on 7AL and 7BL, expressed mainly under stressed and non-stressed conditions, respectively. SQ1 and CS contributed alleles increasing yield on 7AL and 7BL, respectively. The yield component most strongly associated with these QTLs was grains per ear. Additional results which focus on the 7AL yield QTL are presented here. Trials monitoring agronomic, morphological, physiological, and anatomical traits revealed that the 7AL yield QTL was not associated with differences in flowering time or plant height, but with significant differences in biomass at maturity and anthesis, biomass per tiller, and biomass during tillering. In some trials, flag leaf chlorophyll content and leaf width at tillering were also associated with the QTL. Thus, it is likely that the yield gene(s) on 7AL affects plant productivity. Near-isogenic lines (NILs) for the 7AL yield QTL with CS or SQ1 alleles in an SQ1 background showed the SQ1 allele to be associated with >20% higher yield per ear, significantly higher flag leaf chlorophyll content, and wider flag leaves. Epidermal cell width and distance between leaf vascular bundles did not differ significantly between NILs, so the yield-associated gene may influence the number of cell files across the leaf through effects on cell division. Interestingly, comparative mapping with rice identified *AINTEGUMENTA* and G-protein subunit genes affecting lateral cell division at locations homologous to the wheat 7AL yield QTL.

Key words: *AINTEGUMENTA*, comparative genetics, G-protein β subunit, gene function, wheat, yield components, yield QTL.

Introduction

Grain yield of cereals is a particularly complex trait, reflecting the culmination of all the processes of vegetative and reproductive growth and development, and their interactions with the edaphic and aerial environments; yet yield is usually the trait of most importance to plant breeders. Stably expressed genes leading to higher grain yield are important targets of wheat breeding.

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Abbreviations: AFLP, amplified fragment length polymorphism; *ANT*, *AINTEGUMENTA*; BC, backcross; CIM, composite interval mapping; CS, Chinese Spring; DHL, doubled haploid line; DW, dry weight; EST, expressed sequence tag; FW, fresh weight; HI, harvest index; NFA, non-filtered air; NIL, near-isogenic line; *Ppd*, photoperiod sensitivity; QTL, quantitative trait locus; *Rht*, reduced height; SSR, simple sequence repeat (microsatellite); TGW, thousand grain weight; *Vrn*, vernalization sensitivity.

Indeed, apart from disease resistance genes, which clearly have vital roles to play, two such major classes of gene have increased wheat (*Triticum aestivum* L.) yields over the years in a significant and sustained manner. The first class of genes are those regulating flowering time, which allow the phenology of the plant to be adapted optimally to its environment. Examples of such genes are the vernalization sensitivity (*Vrn*) and photoperiod sensitivity (*Ppd*) genes. Another class of genes that significantly influence yield are those regulating plant height, to improve the distribution of dry matter into the grains. Indeed, the 'Green Revolution' which increased wheat production in the 1960s and 1970s was based to a great extent upon the introduction of the gibberellin-insensitive dwarfing genes *Rht1* and *Rht2* into wheat varieties (Smale *et al*., 2002).

Quantitative genetics, revolutionized by the wide range of molecular markers now available, offers the prospect of dissecting and characterizing the genetic complexity of yield itself within any given genetic background. Moreover, the coincidence of quantitative trait loci (QTLs) for yield and those for other traits provides information on traits likely to be responsible for effects on yield.

Increasing grain yield can be achieved by increasing either the total biomass produced by the crop (bigger plants tend to produce greater yield) or the proportion of the total biomass that is invested in grains (greater harvest index). Thus, a gene that increases yield should do so through one of these two fundamental mechanisms. Grain yield also represents the product of grain number and mean weight per grain. Grain number *per se* can be broken down into its components: ear number per plant and grain number per ear, which are determined by the number of spikelets per ear and grains per spikelet. Therefore, by studying how these yield components vary within a particular genetic background, it is possible to gain an insight into the possible function of a gene that influences yield and when, as well as how, it is likely to exert its effect(s). Thus, Slafer (2003) broke down the determination of yield components into different phases within the plant's life cycle, with some overlap between phases. Generally speaking, spikelets per ear are determined before grains per spikelet, both overlapping in time with the determination of ears per plant, and with weight per grain being the final component to be determined.

A compilation of QTLs for yield from a total of 24 (year×treatment×location) trials of a bread wheat mapping population has recently been presented using a population of doubled haploid lines (DHLs) prepared from the cross Chinese Spring (CS)×SQ1 (Quarrie *et al*., 2005). Amongst 17 yield QTL clusters (coincident QTLs from at least five trials) located around the genome, two clusters were particularly striking: those on the long arms of chromosomes 7A and 7B, in apparently homoeologous locations, with increasing alleles contributed by SQ1 on 7AL and CS on 7BL. In the work presented here, the yield components associated with the 7AL yield QTL are examined in detail, and other morphological, anatomical, and physiological traits associated (i.e. showing close genetic linkage) with this yield QTL are identified.

To gain greater precision in quantifying the effects of these QTLs for yield and its associated traits, a set of near-isogenic lines (NILs) was prepared for the 7AL yield QTL. Preliminary data from trials with these NILs confirm the presence of the yield QTL and provide an insight into the primary function of the gene(s) responsible for the effect on yield.

Materials and methods

Genetic stocks

A mapping population of 95 DHLs was obtained from the cross between two hexaploid wheat (*Triticum aestivum* L.) genotypes CS and SQ1, a high abscisic acid-expressing breeding line extracted at F_7 from the spring wheat cross Highbury×TW269/9/3/4, as described in Quarrie *et al.* (2005). Seeds of the mapping population employed in this study, CS and SQ1, are available from the John Innes Centre, Norwich, UK (contact: Professor John Snape, john.snape@bbsrc.ac.uk).

A set of NILs with either CS or SQ1 alleles in the region of a yield QTL on chromosome 7AL was made by backcrossing five times from one of the DHLs (line 72) using SQ1 as the recurrent parent, and selecting at each generation on the basis of the genotype with microsatellite (simple sequence repeat; SSR) marker PSP3094. Only heterozygotes at the 7AL locus were retained for the next generation. After the final backcross (BC), heterozygotes were self-pollinated and the progeny genotyped to retain both CS homozygotes (allele AA) and SQ1 homozygotes (allele BB) in the SQ1 background according to PSP3094. Analysis of the background genome at five other loci indicated a level of heterozygosity amongst 24 NILs of 6.7%. A parallel backcrossing programme with DHL 105 using PSP3094 and CS as the recurrent parent was also carried out to create AA and BB homozygotes at the 7AL yield QTL in the CS background. From these BCs, 17 NILs were generated having either CS or SQ1 alleles at *Xpsp3094.1* in the CS background.

Molecular marker characterization and map construction

The genetic map for CS×SQ1 comprising 567 loci [constituted from restriction fragment length polymorphisms (RFLPs), SSRs, amplified fragment length polymorphisms (AFLPs), and several biochemical and morphological markers], presented in Quarrie *et al*. (2005), was updated with a further 38 informative markers, mainly SSRs, as described in D Habash, S Bernard, J Schondelmaier, J Weyen and S Quarrie (unpublished data). The updated map was constructed using the Kosambi (1944) mapping function as described in Quarrie *et al*. (2005).

Yield data

Details of 24 trials with the 95 DHLs (Norwich, UK in 1994, 1997, and 1998; Zajecar, Serbia in 1999–2000, 2000–2001; Almaty, Kazakstan in 1998, 1999, and 2000; Zaragoza, Spain in 1998–1999 and 1999–2000) are given in Quarrie *et al*. (2005). An additional field trial in Zajecar in 2001–2002 was carried out as in the previous years, except that only rainfed conditions were used, with two replications.

A pot experiment was carried out in 2003 using open-top chambers situated at Newcastle University's Close House Experimental Station (Heddon-on-the Wall, Northumberland, UK). Chambers were ventilated from anthesis onwards with either non-filtered air (NFA) or

NFA plus 75 ppb ozone, 8 h d^{-1}. Four replicate chambers were used for each treatment. Four plants were grown in pots containing 3 dm^3 of John Innes no. 3 potting compost, with one pot of each DHL per chamber. Plants were watered as required and were subject to agrochemical treatments to keep pests and diseases under control in the experiment.

In all trials, yield components [i.e. ear number per plant, grain number per ear, and thousand grain weight (TGW)] were recorded, as well as grain yield per plant.

A preliminary trial of the 7AL yield QTL NILs into both CS and SQ1 was carried out in Zajecar, Serbia in 2005. All the NILs in CS were derived from one heterozygous line at BC5. Ten CS NILs had the SQ1 allele and seven had the CS allele at *Xpsp3094.1*. Twenty-four NILs in SQ1 were tested, representing four sibling heterozygous lines at BC5, of which 16 had the SQ1 allele and eight had the CS allele at *Xpsp3094.1*. All NILs were trialled in a single 1 m row per NIL at 20 cm spacing between rows and ~70 seeds sown per row. Yield and its components were analysed only on the basis of individual ears: 178 and 257 ears from NILs in CS, and 233 and 459 ears from NILs in SQ1 having either the CS or SQ1 allele at *Xpsp3094.1*, respectively. The numbers of fertile and sterile spikelets and the total number of spikelets per ear were also recorded.

Developmental, agronomical, morphological, anatomical, and physiological traits

Flowering time (heading and/or anthesis date) and final height of DHLs were recorded in every trial, except Norwich 1994. Biomass per plant at maturity and harvest index (HI, grain mass per total plant biomass) were recorded on five replicate plants per DHL in Norwich 1997 and 1998 and in Zajecar (biomass per row divided by plant number per row) in 2000, 2001, and 2002.

At flowering time, biomass per plant of three replicate plants per DHL was recorded in Norwich in 1997 and 1998, and in Zaragoza in 2000 and 2001. Biomass per stem (ear-bearing tiller) was recorded in Norwich in 1997 and 1998, and flag leaf chlorophyll content was assessed for low- and high-N treatments in 1997 and 1998 by scanning one representative leaf per DHL, converting the images to a 256-tone grey scale and quantifying the pixel intensity within a standard area of leaf using Adobe Photoshop. Flag leaf chlorophyll content was assessed with a SPAD meter (Minolta, Tokyo, Japan) in Zajecar, in 2001 (irrigated treatment, ~5–10 d after flowering) and 2002 (2 weeks after flowering). Similar measurements were made in 2005 in a second open-top chamber experiment performed at Close House, Newcastle University, UK, with four plants per DHL grown in a pot containing 3 dm^3 of John Innes no. 2 potting compost, with one pot of each DHL per chamber. Chambers were ventilated from approximately mid-stem extension onwards with either NFA or NFA plus 75 ppb ozone, 8 h d^{-1}, and four replicate chambers used per treatment. SPAD meter readings of flag leaf chlorophyll content were taken over 6 weeks starting from around flowering time in two chambers of each ozone treatment. Maximum chlorophyll content was calculated from fitted polynomial curves for each DHL. Flag leaf widths were measured on three plants per DHL in both NFA and ozone fumigation treatments and from five plants per DHL in the Zajecar 2002 trial. Scanned images of flag leaves collected from trials conducted in Norwich in 1997 and 1998 were used to measure leaf width.

During the vegetative phase, plant dry weight and length (shoot base to the tip of the longest leaf) were measured in Norwich in 1997, with five replicate plants per DHL and three replicate plots per nitrogen treatment (Zadoks scale ~25–28; Zadoks *et al*., 1974). The width, fresh weight (FW) and dry weight (DW) of a 10 cm segment of leaf 5 (fifth leaf to emerge) were measured on five plants per DHL in a field trial in Zajecar, 2004–2005, sampled on 24–25 April 2005, around Zadoks growth stage 24. Plants were grown as in previous years and subjected to either rainfed conditions or drought using a rain shelter (described in Dodig *et al*., 2003) erected over droughted plots on 28 March (average Zadoks growth stage 22).

Flag leaf length, width, and chlorophyll content (SPAD meter) were measured 3–4 weeks after anthesis on each of the 24 NILs backcrossed into SQ1 and 17 NILs backcrossed into CS. Measurements were made on 10 plants per NIL, grown in 10 dm^3 pots of John Innes no. 2 potting compost (20 plants per pot) in an unregulated greenhouse at Close House, Newcastle University in 2005. Flag leaf lengths and widths of 10 plants per NIL were also collected during a parallel experiment conducted in the field in Zajecar.

Using clear nail varnish, leaf epidermal imprints were taken of the upper (adaxial) surface of flag leaves from NILs in SQ1, four having the SQ1 allele and four the CS allele at *Xpsp3094.1* (all originating from the same heterozygous BC line, NIL16) growing in the field in Zajecar, 2005. Imprints were transferred with adhesive tape to a sheet of clear plastic (overhead transparency), prior to viewing with a microscope using ×400 magnification. Ten 350 μm×262 μm fields of view were captured for analysis of epidermal cell size. The widths of 7–14 parallel cell files between rows of stomata were measured on each image to calculate mean epidermal cell width.

Each epidermal imprint was also photographed and the images digitized. From these, with appropriate lighting, it was possible to identify the major vascular bundles and inter-vein regions across the leaf as alternating light and dark stripes. The distance between 3–10 vascular bundles in three locations on each imprint was used to calculate the mean distance between vascular bundles. Epidermal imprints were also used to measure maximum flag leaf width.

Statistical analyses

Correlation and linear regression analysis, yield heritabilities on a genotype mean basis, and one-way analysis of variance (ANOVA) were performed using Microsoft Excel 98 (Version 8.0). QTL analysis was carried out using a genetic map containing 449 loci, with duplicated markers and loci of low information content having been excluded. The presence of QTLs was determined with QTLCartographer (Basten *et al*., 1996) version 1.16c (March 2002) for Macintosh using linear regression with LRmapqtl to identify individual markers significantly associated with trait variation. Those QTLs reported here using marker-by-marker linear regression were significant at $P < 0.05$.

Results

The work described here focuses on the chromosome 7AL QTL for yield (designated *Qyld.csdh.7AL*). This region of 7AL and its probable homoeologous location on 7BL (designated *Qyld.csdh.7BL*) both gave QTLs for yield in 11 of 24 yield trials reported by Quarrie *et al*. (2005). The yield QTL on 7AL was also shown by Quarrie *et al*. (2005) to be expressed more frequently under stressed conditions, particularly drought and low nitrogen, based on analysis of yields of DHLs calculated for a site mean yield of only 2 g per plant (equivalent to ~2.5 t ha^{-1}).

The strategy adopted in the present study was to compare the location of the 7AL yield QTL with the location of QTLs for traits that were thought likely to contribute to variation in ultimate grain yield, measured at different stages of development. In this way, it would be possible to assess the likely process involved in the regulation of yield at the 7AL QTL, together with the stage of development at which variation in the process was affected by the QTL.

DHL mapping population

QTL analyses using LRmapqtl identified four broad clusters of yield QTLs on chromosome 7A (Fig. 1). A QTL cluster from five trials was localized on 7AS around loci *Xpsr490.3(Ss1)* to *Xwmc479* (designated *Qyld.csdh.7AS1*). A second broad QTL cluster from five trials was on 7AS, localized around SSR loci *Xwmc283.1* to *Xwmc83* (designated *Qyld.csdh.7AS2*). A cluster of highly significant QTLs from five trials was located at the centromere on 7A, associated with SSR loci *Xpsp3050*, *Xbarc108*, and *Xwmc9* (designated *Qyld.csdh.7AC*). The fourth QTL cluster was associated with SSR loci *Xgwm332*, *Xpsp3001.1*, *Xpsp3094.1*, and *Xwmc273.3*, together with the RFLP locus *Xpsr148(CatO)* within a 4 cM region distal on 7AL (Fig. 1) (*Qyld.csdh.7AL*). In addition to the 11 trials exhibiting yield QTLs on 7AL reported previously, two of

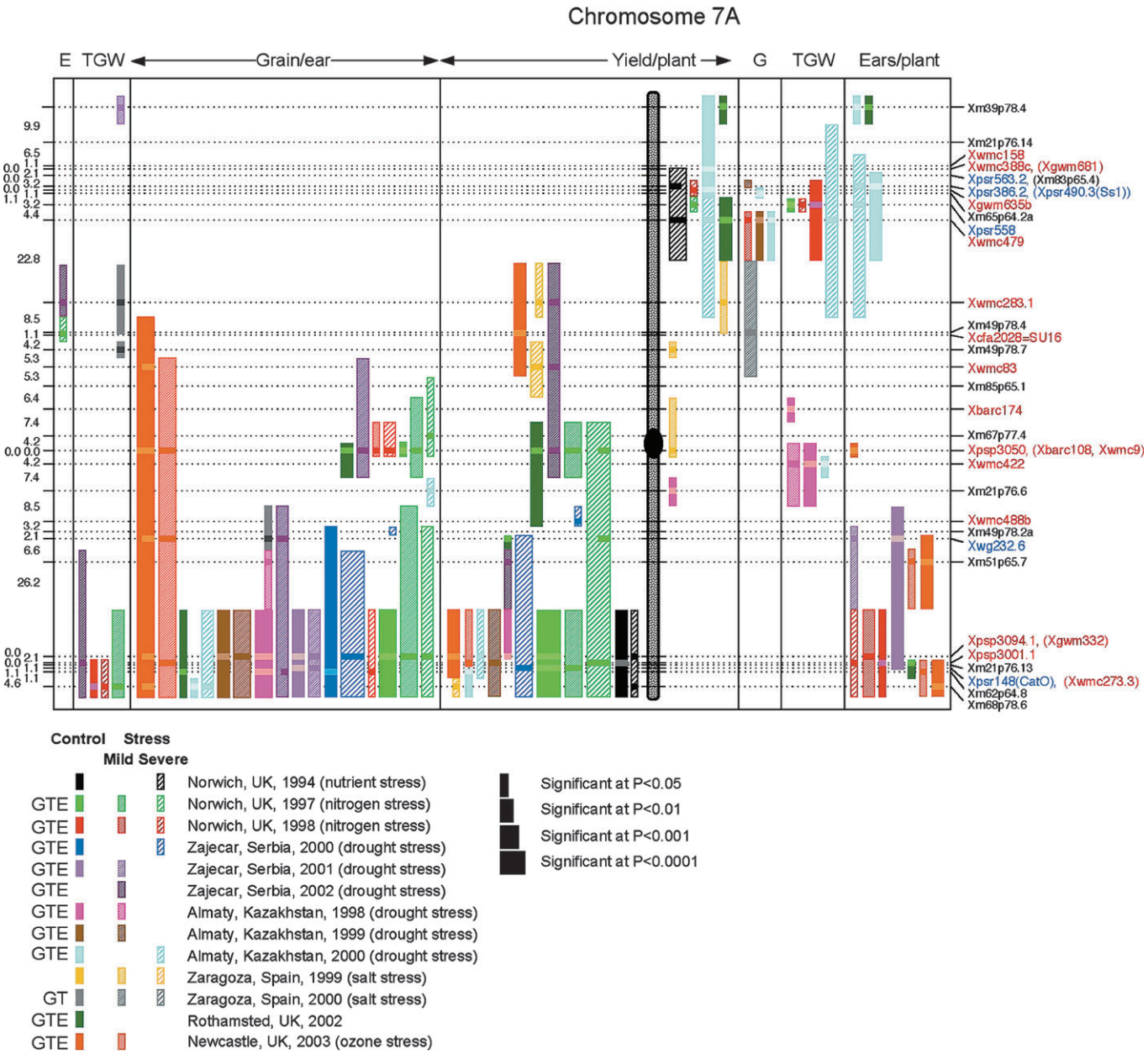

Fig. 1. Location of QTLs for yield and the yield components ears per plant (E), grains per ear (G), and TGW (T) on chromosome 7A from 27 (site×year×treatment) combinations where yield and one or more yield components were measured. Bars to the left of the chromosome indicate that SQ1 contributes the increasing allele. Bars to the right of the chromosome indicate that CS contributes the increasing allele. For each significant (P <0.05) event at one or more markers identified using LRmapqtl, the vertical bars extend either side of the significant markers half-way to the first non-significant adjacent marker. Horizontal bars within QTL bars indicate markers having the most significant association with the QTL (length of horizontal bar proportional to the level of significance). Letters G, T, and E adjacent to trials indicate the yield components measured during that trial. RFLP markers are shown in blue, SSRs are in red, and AFLPs are in black. Distances between adjacent markers are shown in centiMorgans.

the three additional yield trials described here (both ozone treatments in Newcastle in 2003) also resulted in major QTLs for yield in the same region of 7AL. The *Qyld.csdh.7AS1* yield QTL cluster had increasing alleles from CS, while alleles for higher yield in the other three clusters were contributed by SQ1. Heritabilities for yield in the previously reported trials are given in Quarrie *et al.* (2005), and for the 11 trials showing the *Qyld.csdh.7AL* QTL, heritabilities for yield were from 55% to 98%. Heritabilities for yield from the Newcastle 2003 ozone trial were 60.2% (NFA) and 76.3% (NFA+75 ppb ozone).

Yield components (ears per plant, grains per ear, and TGW) were measured in 10 of the 12 year×location experiments (although ears per plant were not recorded in Zaragoza 2000). Analysis of the yield components using LRmapqtl showed a concentration of QTLs coincident with the 7AL yield QTLs (Fig. 1). The component most frequently associated with the yield QTL was grains per ear (16 of the 22 trials in which yield components were measured), with SQ1 contributing the alleles for increased yield and grains per ear. Of the trials in which grains per ear was measured, only those in Zaragoza in 2000 resulted in no co-localization of the QTL for grains per ear with the 7AL yield QTL.

Four of the trials gave QTLs for TGW coincident with the 7AL yield QTL, with the increasing allele coming from SQ1. Six of the trials showed significant effects for ears per plant co-localized with the yield QTL. However, for each of the ears per plant QTLs, the increasing allele came from CS. This indicates that significant increases in grains per ear and/or TGW were occasionally either partially or completely compensated for by significant reductions in ears per plant. Thus, for the Norwich 1998 and Zajecar 2001 irrigated trials, increases in grains per ear and/or TGW were cancelled out by reductions in ears per plant so that no yield per plant QTLs were found.

Because of the well-known association between phenology and yield, and frequent association between dwarfing genes and yield, flowering time and plant height were examined in all trials except Norwich, 1994. Two QTL clusters for flowering time were present on 7A (Fig. 2). One cluster of QTLs from five trials was coincident with the 7AS yield QTL cluster around SSR loci *Xwmc283.1* to *Xcfa2028*, with late flowering alleles coming from CS. The second cluster of flowering time QTLs from three trials was coincident with the 7AL QTL for yield, with late flowering also contributed by CS alleles. However, in only two of these trials (Kazakstan, 2000) were flowering time QTLs also coincident with QTLs for yield in the same trial.

QTLs for plant height were frequently found on 7AS (Fig. 2), associated with RFLP locus *Xpsr558* (13 of 25 trials in which height was measured) and co-localizing with the QTL cluster for yield distal on 7AS. Here, the allele for increasing height came from CS. A second cluster of six height QTLs was located distal on 7AL, within the region containing the yield QTL, with increasing height contributed by SQ1 alleles. Three of these trials showed height QTLs coincident with QTLs for yield, with taller plants resulting in higher yield.

The increasing effect of SQ1 alleles on yield at the 7AL QTL was associated with increases in both harvest index and biomass per plant at maturity (Fig. 2). Of the 11 (year×location×treatment) trials in which HI and biomass per plant at maturity were measured, QTLs for HI were coincident with the yield QTL in five trials, and biomass in four trials. Thus, the increases in yield on 7AL due to SQ1 alleles were associated with both an increase in above-ground biomass per plant and a greater proportional investment of that biomass in grains. The other three QTLs for yield on 7A were associated only with variation in biomass per plant.

At anthesis, QTLs for biomass measured in four (year×location) experiments were frequently co-localized with the yield QTL on 7AL in terms of both biomass per plant and biomass per tiller (measured only in Norwich, 1997 and 1998). The biomass QTLs on 7AL co-localized with QTLs for flag leaf chlorophyll contents for low and high N treatments in Norwich 1997, for the low N treatment in Norwich 1998, the Zajecar irrigated trial of 2001, the rainfed trial of 2002, as well as the ozone trial conducted in 2005. In all cases, greater biomass at anthesis was associated with more flag leaf chlorophyll content. The biomass QTLs on 7AL for the Norwich trials were also associated with QTLs for flag leaf width for three of the four treatments studied (Fig. 2), with the increasing alleles contributed by SQ1 so that wider flag leaves were associated with more biomass. A QTL for flag leaf width was also coincident with the 7AL yield QTL in the Zajecar 2002 trial (Fig. 2). In addition, wider flag leaves were associated with greater biomass at the 7A centromeric yield QTL cluster in the Norwich trials, the Zajecar 2002 trial, and the Newcastle ozone treatment in 2005.

The association between QTLs for yield and biomass on 7AL extended to biomass in vegetative plants (Fig. 2). In the Norwich 1997 experiment, all three N treatments resulted in highly significant QTLs on 7AL for biomass per plant sampled during the phase of rapid tiller production (reflected also in plant length) with QTL peaks co-localizing with the yield QTLs. The QTL effects for biomass per plant at tillering extended to the centromeric region, with secondary QTL peaks coinciding with the centromeric cluster of yield QTLs.

Leaf characteristics were measured in Zajecar, 2005 in vegetative plants, around the time of leaf 5 emergence. A 10 cm segment of leaf 5 was sampled to assess leaf width and weight per unit area. QTLs for segment FW and DW were grouped in two regions of 7A: on the short arm co-localizing with the distal QTL cluster for yield around locus *Xpsr558*, with increasing alleles coming from CS; and highly significantly on 7AL co-localizing

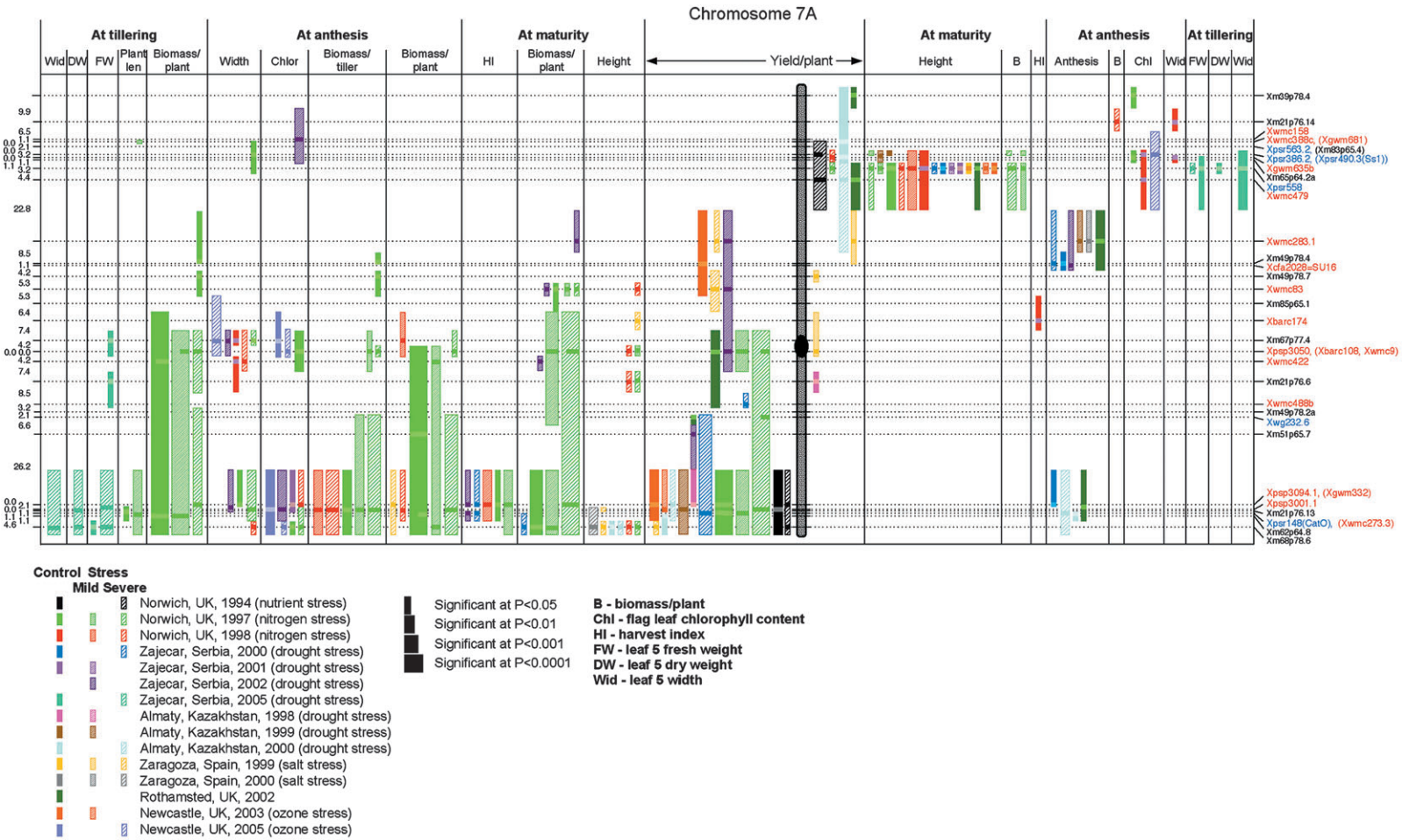

Fig. 2. Location on chromosome 7A of QTLs for yield and other traits measured at tillering, anthesis, or maturity. Other details as in Fig. 1.

with the yield QTL cluster, with increasing alleles coming from SQ1. Leaf 5 segment width also gave highly significant effects in the same regions on 7AS (irrigated treatment) and 7AL (droughted treatment).

NILs for 7AL yield QTL

For clarity, AA and BB refer to alleles at *Xpsp3094.1* corresponding to CS and SQ1, respectively. CS and SQ1 NILs indicate the recurrent parents for the backcrossing programmes used to create the NILs. Significant differences in overall grain yield per ear (P <0.01) and TGW (P <0.001) were found between NILs having AA (178 ears) and BB (257 ears) alleles at *Xpsp3094.1* in the CS background. However, the differences between means were only 8.6% for yield (1.15 and 1.25 g per ear for AA and BB alleles, respectively) and 6.7% for TGW (26.9 and 28.7 g for AA and BB alleles, respectively). Overall differences between AA and BB alleles for these NILs in grain number per ear (1.4%), spikelet number per ear (1.7%), and grains per spikelet (0.5%) were not significant. From the final backcross into CS, of eight lines that were available for screening, only one was shown to be heterozygous (NIL4). Therefore, it is possible that recombination between the marker locus for selection (*Xpsp3094.1*) and the 7AL yield QTL may have taken place in some of these NILs during the backcrossing procedure.

However, yield data for NILs from the backcrossing programme into SQ1 more consistently showed significant differences. After the final backcross, seven of 12 lines were heterozygous at *Xpsp3094.1* and four of those lines (code numbers NIL9, 16, 17, and 18) were retained to identify seeds at the next generation homozygous for either AA (CS) or BB (SQ1) alleles at *Xpsp3094.1*. Thus, for phenotypic analysis, the following NILs into SQ1 were available: NIL9, 1AA+4BB; NIL16, 4AA+7BB; NIL17, 2AA+1BB; and NIL18, 1AA+4BB, giving a total of eight NILs with the AA (CS) allele at *Xpsp3094.1* and 16 with the BB (SQ1) allele at *Xpsp3094.1*. For these NILs into the SQ1 background, phenotypic data were pooled from 233 and 459 ears having AA and BB alleles at *Xpsp3094.1*, respectively.

Grain yield per ear and its components were measured on 20–30 plants of each SQ1 NIL. These data are summarized in Table 1. Heritabilities for grain yield per ear were 86.0% and 73.6% over all NILs having either AA or BB alleles, respectively, at *Xpsp3094.1* on the basis of 26 ears per NIL having the AA and 20 ears per NIL having the BB allele. Over all the NILs, yield and all yield components differed significantly between NILs having AA or BB at *Xpsp3094.1*. For every trait, the BB (SQ1) allele gave higher means, from 3.5% for spikelets per ear to 20.7% for yield per ear. Although the NIL18 lines differed significantly only in spikelets per ear (Table 1), only one line from NIL18 had the AA allele at *Xpsp3094.1*. The increased yield per ear of NILs having the BB allele was due to increases in both grains per ear (12.1%) and TGW (8.2%). Increases in both spikelet production (spikelets per ear) and spikelet fertility (grains per spikelet) contributed to observed increases in grains per ear associated with BB alleles (3.5% and 7.5%, respectively).

Because of the inconsistent differences in yield and its components between AA and BB alleles in CS NILs, flag leaf characteristics are reported only for the SQ1 NILs. Flag leaf dimensions were measured in the trials conducted in both Newcastle, UK and Zajecar, Serbia. In neither location did flag leaf lengths differ between AA and BB SQ1 NILs: 223.8 mm (AA) and 220.1 mm (BB) in Newcastle, and 289.3 mm (AA) and 289.1 mm (BB) in Zajecar. However, in both trials, flag leaves of NILs possessing the BB (SQ1) allele at *Xpsp3094.1* were significantly wider than flag leaves with AA (CS) alleles. In Newcastle, mean leaf widths were 14.9 and 16.4 mm, and in Zajecar 11.4 and 12.7 mm for AA and BB alleles, respectively. Notably, across all 41 CS and SQ1 NILs, leaf widths were significantly correlated between the two sites (r_{39df}=0.67), although leaf lengths showed no correlation between sites. Leaf width of the 24 SQ1 NILs at Zajecar was positively correlated with both grain yield per ear and grain number per ear (Fig. 3): r_{22df}=0.74 and 0.80, respectively, with data for the AA and BB alleles lying in different regions of the fitted lines.

Epidermal imprints collected in Zajecar from the adaxial surfaces of flag leaves of four SQ1 NIL16 lines with AA and four SQ1 NIL16 lines with BB alleles showed no evidence of differences in the mean width of cell files

Table 1. *Yield and yield components of SQ1 NILs having CS (A) or SQ1 (B) alleles at* Xpsp3094.1

Numbers in parentheses indicate the number of families within the NIL. Each family was represented by 20–30 plants. NS, *, **, ***, **** indicate differences between A-allele and B-allele NILs that were non-significant or significant at P <0.05, 0.01, 0.001, and 0.0001, respectively.

Trait	NIL									
	9 A (1)	9 B (4)	16 A (4)	16 B (7)	17 A (2)	17 B (1)	18 A (1)	18 B (4)	All A (8)	All B (16)
Yield per ear (g)	1.28	1.50***	1.15	1.35****	0.94	1.26****	1.08	1.17 (NS)	1.11	1.34****
Grains per ear	35.1	38.6*	32.9	35.6**	29.3	34.3**	32.6	34.8 (NS)	32.2	36.1****
Fertile spikelets per ear	12.9	13.4*	12.8	12.8 (NS)	11.7	12.6*	12.4	13.0 (NS)	12.5	13.0**
Total spikelets per ear	14.6	15.3**	14.6	14.7NS	13.7	14.1 (NS)	14.0	14.8*	14.3	14.8****
Grains per spikelet	2.40	2.52 (NS)	2.25	2.42**	2.14	2.42**	2.34	2.36 (NS)	2.26	2.43****
TGW (g)	36.3	38.7**	34.6	37.7****	31.9	36.7****	32.8	33.2 (NS)	34.0	36.8****

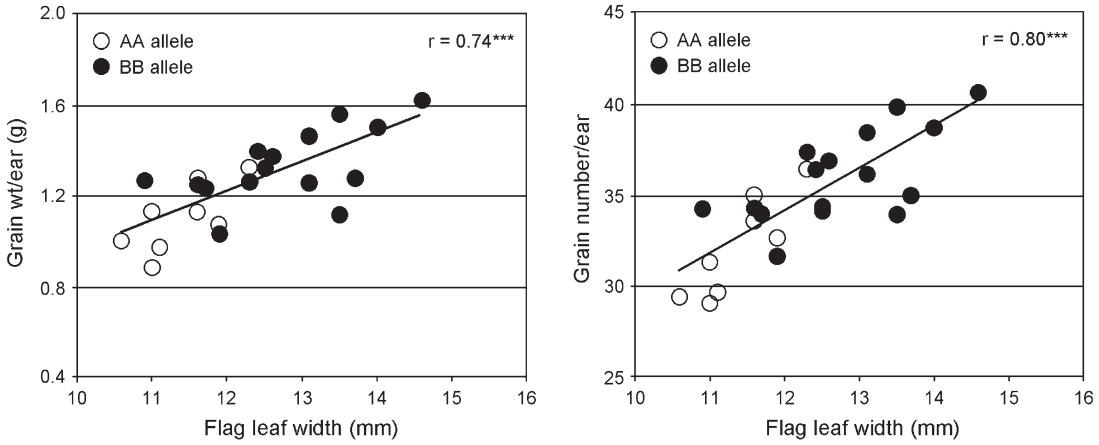

Fig. 3. The relationships between flag leaf width and (left) grain weight per ear and (right) grain number per ear for eight AA and 16 BB NILs in an SQ1 (BB) background. Each point represents the mean of 20–30 measurements of yield and 10 measurements of flag leaf width.

across the leaf between AA and BB alleles, despite allele differences between leaf widths of 7.0%. In total, 1675 and 1480 epidermal cell widths were analysed possessing AA and BB alleles, respectively. Mean cell width for both alleles was 17.63 μm. Interveinal distances averaged over 290 and 262 veins having AA and BB alleles, respectively, were not significantly different: 295 and 302 μm, respectively. Therefore, differences in flag leaf width between SQ1 NILs having AA or BB alleles at *Xpsp3094.1* appeared to be due to differences in cell division in terms of the number of cell files across the leaf and not to differences in lateral cell expansion.

Flag leaf chlorophyll contents, measured with a SPAD meter in Newcastle, also differed significantly between AA and BB alleles in SQ1 NILs, with the BB (SQ1) allele resulting, overall, in 6.5% more chlorophyll (33.9 and 36.1 SPAD units, equivalent to 1.52 and 1.65 μg chlorophyll mg FW^{-1}, respectively).

Discussion

Genetic variation in grain yield has to be due to either variation in biomass production or HI, or a combination of the two. Our results showed that the yield QTL distal on 7AL (*Qyld.csdh.7AL*) was associated with QTLs for both above-ground biomass and HI, and this would be expected from coincidence with QTLs predominantly for the yield component grain number per ear. A higher biomass at anthesis would lead to more florets successfully fertilized per ear, creating a larger sink demand for assimilates, thereby also increasing HI.

Coincidences of QTLs on 7AL in the mapping population and results for the NILs in SQ1 showed that greater biomass at anthesis was associated with both wider flag leaves and more flag leaf chlorophyll content per unit leaf area. Evidence was also found for variation in leaf width and plant biomass in vegetative plants associated with the 7AL yield QTL, suggesting that the gene(s) regulating yield at the 7AL QTL was active early on in plant development, at least in those trials where seedling traits were measured.

The highly significant positive correlations for the SQ1 NILs between flag leaf width and both grain yield per ear and grain number per ear (Fig. 3) support the hypothesis that the two traits are causally related, although effects of separate genes on 7AL cannot be excluded. The differences in width of flag leaves associated with the yield QTL were not due to variation in epidermal cell width or variation in distances between leaf veins measured in several of the SQ1 NILs. Thus it seems likely that variation in flag leaf width was due to variation in numbers of cell files across the leaf, i.e. variation in cell division during leaf ontogeny. Although flag leaf thickness was not measured in these trials, it is possible that differences in chlorophyll content associated with the 7AL QTL could also be due to variation in mesophyll cell numbers across the leaf, i.e. variation in cell division. In contrast, on no occasion was any QTL for leaf length coincident with the 7AL yield QTL. Therefore, if this series of physiological associations does indeed lead to variation in grain yield on 7AL, then the function of the gene affecting yield appears to be to modify leaf cell division only in the lateral dimension.

Many genes have been identified that influence leaf development and final organ size through modification of cell division, such as p34cdc2 kinase of maize (Granier *et al*., 2000), *RADICLELESS1* (Scarpella *et al*., 2003) that determines vascular patterning of rice, G proteins implicated in a wide range of plant processes including developmental control (Lease *et al.*, 2001; Perfus-Barbeoch *et al.*, 2004), and cyclin-dependent kinases (Schuppler *et al*., 1998) and their inhibitors, such as *ICK1* of *Arabidopsis* (Wang *et al*., 2000). These genes generally affect organ size in all dimensions, although the *Arabidopsis* G-protein β subunit was implicated particularly in the determination of leaf width (Lease *et al.*, 2001). Two

other *Arabidopsis* genes affecting only leaf width are *AINTEGUMENTA* (*ANT*) and *ARGOS*. The *ANT* gene, encoding a transcription factor of the AP2-domain family, was shown by Mizukami and Fischer (2000) to affect cell division during leaf ontogeny mainly in the lateral plane by maintaining the meristematic competence of cells. The *ARGOS* gene also affects organ size in the lateral plane (Hu *et al*., 2003). This gene is highly auxin responsive and was suggested to act upstream of *ANT* in mediating auxin-triggered developmental signals to maintain *ANT* expression during leaf organogenesis.

According to Sourdille *et al*. (2004), the locus *Xgwm332* is located in wheat chromosome 7AL deletion bin 0.86–0.90. As scores for GWM332 and PSP3094.1 are identical in the CS×SQ1 mapping population, it is likely that the yield QTL is also in deletion bin 0.86–0.90 of 7AL. Wheat expressed sequence tags (ESTs) identified in this deletion bin and in homoeologous locations on 7BL and 7DL are associated mainly with rice chromosome 6 distally on the long arm (Francki *et al*., 2004; Hossain *et al*., 2004; www.tigr.org/tigr-scripts/osa1_web/gbrowse/rice). The location on rice chromosome 6 of these wheat EST homologues (www.tigr.org/tigr-scripts/osa1_web/gbrowse/rice) and comparative mapping with RFLP markers on genetic maps of chromosome 6 show that the wheat 7AL yield QTL has a probable homologue in rice. Li *et al*. (2001) identified a QTL for yield per plant between the RFLP markers RZ682 and C236, and other authors have shown coincidence with QTLs also for spikelets per panicle (He *et al*., 2001) and TGW (Ishimaru, 2003), as well as flag leaf width (Mei *et al*., 2003). The same mapping populations were used by Li *et al*. (2001) and Mei *et al*. (2003), and allele effects for yield and flag leaf width QTLs came from the same parent. The relationship between these rice QTLs and the wheat yield QTL on 7AL is shown in Fig. 4. Using NILs for the TGW QTL on chromosome 6 (*Qtgw6*, open bar in Fig. 4), Ishimaru (2003) concluded that a 10% higher TGW, and 15% higher grain yield in NILtgw6 was due to greater storage of carbohydrates in leaf sheaths before anthesis, although net photosynthetic rates and flag leaf areas did not differ.

These QTLs for yield and related traits in wheat and rice coincide with a rice putative Ap2-domain gene (Os06g44750, TC257102, www.tigr.org/tigr-scripts/osa1_web/gbrowse/rice) located between bp 27 024 454 and 27 028 231 (TIGR Rice Genome Annotation—Release 4, January 2006) (arrowed in Fig. 4). BlastP analysis showed 100% similarity with an aintegumenta-like protein of rice. Thus, from the mode of action of ANT in *Arabidopsis*, a wheat homologue of the rice AP2-domain gene appears to be an ideal candidate for the 7AL yield QTL. A second candidate gene for the 7AL yield QTL is the wheat EST BF471156 (shown in bold in Fig. 4), which has homology with a G-protein β subunit (http://wheat.pw.usda.gov/cgi-bin/westsql). However, this candidate is less likely as the *Arabidopsis* G-protein β subunit was shown by Lease *et al.* (2001) to have opposite effects on leaf length and leaf width. Our 7AL NILs showed no difference in

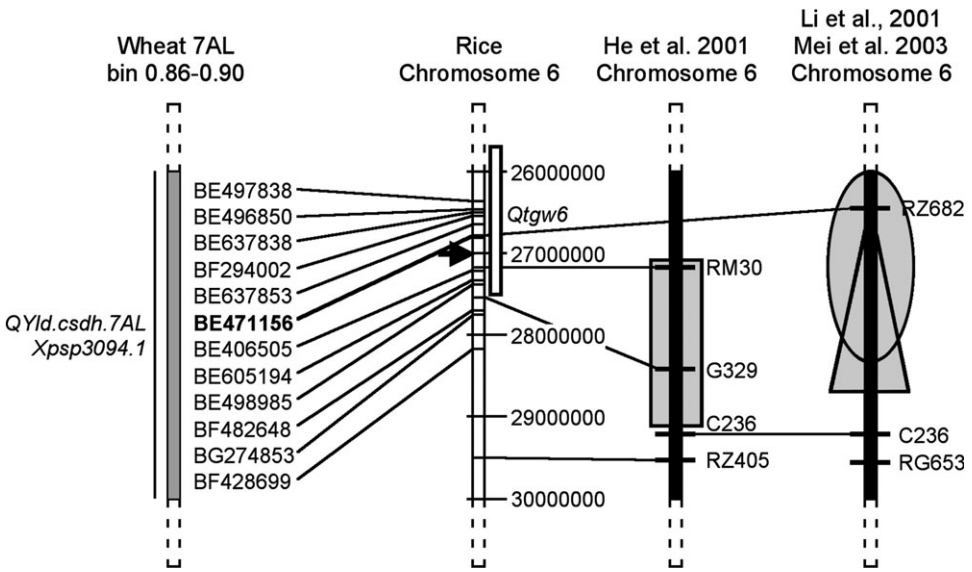

Fig. 4. The relationship between part of wheat chromosome 7AL deletion bin 0.86–0.90 ESTs, and parts of the rice chromosome 6L physical map and two genetic maps of chromosome 6L showing the base pair locations in rice chromosome 6 of wheat ESTs (taken from TIGR Rice Genome Annotation Version 3 and Release 4) as well as selected rice RFLP and SSR markers. The locations of the wheat yield QTL, designated *Qyld.csdh.7AL*, and the selectable marker locus *Xpsp3094.1* are shown with a vertical line. The location of the rice TGW QTL *Qtgw6* of Ishimaru (2003) on rice 6 is shown as an open bar; the QTL for spikelet number on rice 6 identified by He *et al*. (2001) is shown as a shaded rectangle; the QTL for flag leaf width identified by Mei *et al*. (2003) is shown as a shaded triangle; and the QTL for yield identified by Li *et al*. (2001) is shown as a shaded oval. An arrow shows the location on rice 6 of the AP2-domain gene having similarity with *ANT*. BE471156 (bold) is the EST for the G-protein β subunit gene.

leaf length between those with AA and BB alleles. Fine mapping and, ultimately, transformation would be needed to test these candidate genes unequivocally.

Not only do our results with the 7AL NILs confirm the presence of the yield QTL identified using LRmapqtl closely linked with SSR locus *Xpsp3094.1* on 7AL, but they also validate the simple approach to QTL analysis of linear regression marker by marker. The same levels of significance for yield QTLs were also obtained using single-factor ANOVA and allele scores for PSP3094.1 (data not presented). The consistency of identifying QTLs for yield distal on 7AL using linear regression (about half of all trials) amply justified the attempt to create NILs for the QTL.

It is, of course, possible that these coincidences on 7AL of QTLs for physiological, morphological, and agronomic traits are determined independently by closely linked genes, and the TIGR Rice Genome Annotation—Release 4 shows ~200 expressed sequences between BE497838 and BF428699 (Fig. 4). Nevertheless our results, based on physiological measurements, are consistent with the hypothesis that the primary function of the gene responsible for the chromosome 7AL yield QTL is to regulate lateral cell division within the leaves; the high yield allele causing increases in the number of either cell files across the leaf width, leading to wider leaves, or mesophyll cells across the leaf, leading to thicker leaves. Thicker leaves would have a higher density of chloroplasts per unit area, and therefore chlorophyll content per unit leaf area. Wider or thicker leaves would lead to better light interception and photosynthetic productivity, resulting in increased growth rate. Increased growth rates result in greater biomass production and, consequently, by increasing grain number per ear, greater grain yield per plant. The greater biomass might also be associated with greater HI via effects on grain numbers and hence sink demand.

Although the 7AL yield QTL has been found in about half of all trials so far, there was no clear association of the expression of the QTL with any particular environmental treatment (Figs 1, 2), such as water or nutrient availability, although Quarrie *et al*. (2005) showed the QTL to be preferentially expressed under stressed conditions. Nevertheless, the association between flag leaf width and yield at *Qyld.csdh.7AL* suggests that any environmental factors affecting the width of the flag leaf may also influence yield at this locus, and many stresses such as water and nutrient availability can influence flag leaf development, provided they occur at the critical cell division phase of leaf ontogeny. We are currently testing the NILs with stress factors imposed at various stages of flag leaf development to determine whether a particular stress and/or its timing is critical for the expression of the 7AL yield QTL.

Alleles of a homoeologue of this 7AL yield QTL may already be in regular use by breeders at CIMMYT in the form of lines carrying a 7DL–7AgL translocation of the nearly complete long arm of chromosome 7Ag from *Agropyron elongatum* (Knott, 1971; Friebe *et al*., 1996). These translocation lines are characterized by higher yield, more grains per ear, and increased biomass (Reynolds *et al*., 2001, 2005). The increased grain number per ear stimulated higher net photosynthetic rates per unit leaf area during grain filling, although no effects on leaf area duration, chlorophyll distribution, or leaf architecture were found (Reynolds *et al*., 2005). Specific effects of the 7DL–7AgL translocation on flag leaf width do not appear to have been reported.

The highly significant associations found in our 7AL NILs between flag leaf width and both grain yield and grain number, together with the identification of candidate genes for yield, provide immediate opportunities for breeders to exploit the yield QTL on 7AL, and its homoeologue on 7BL, as well as the 7DL–7AgL translocations, to improve the yield of wheat. The approach demonstrated here, using a detailed physiological approach to aid understanding of the control of yield at a particular genetic locus coupled with functional genomics information on the wheat, rice, and *Arabidopsis* genomes to identify candidate genes for yield, is an example of the advances that can be delivered employing the strategy to bridge the gap between QTLs and genes advocated by Tuberosa *et al.* (2002).

Acknowledgements

We are grateful to the Ministry of Science and Technology, Serbia for financial support to DD, the EU for supporting AK [via a combination of Marie Curie Training Site Funding (HPMT-CT-2001-00219) and the Polish Academy's 'Crop Stress' Programme (QLAM-2001-00424)], and to the Ministry of Agriculture and Forestry, Italy (project AMIFRUGAM) for support to CC. We are also thankful to IG Fernandez [Marie Curie PhD student affiliated to the same EU Training Site (Newcastle University)] for processing chlorophyll data resulting from the 2005 Newcastle trial. We acknowledge the helpful comments made on the manuscript by R Tuberosa.

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