Mol Breeding (2013) 31:587–599
DOI 10.1007/s11032-012-9817-5

Identifying wheat genomic regions for improving grain protein concentration independently of grain yield using multiple inter-related populations

Matthieu Bogard · Vincent Allard · Pierre Martre · Emmanuel Heumez · John W. Snape · Simon Orford · Simon Griffiths · Oorbessy Gaju · John Foulkes · Jacques Le Gouis

Received: 24 July 2012 / Accepted: 17 November 2012 / Published online: 29 November 2012

Abstract Grain yield (GY) and grain protein concentration (GPC) are two major traits contributing to the economic value of the wheat crop. These are, consequently, major targets in wheat breeding programs, but their simultaneous improvement is hampered by the negative correlation between GPC and GY. Identifying the genetic determinants of GPC and GY through quantitative trait loci (QTL) analysis would be one way to identify chromosomal regions, allowing improvement of GPC without reducing GY using marker-assisted selection. Therefore, QTL detection was carried out for GY and GPC using three inter-connected doubled haploid populations grown in a large multi-environment trial network. Chromosomes 2A, 2D, 3B, 7B and 7D showed co-location of QTL for GPC and GY with antagonistic effects, thus contributing to the negative GPC–GY relationship. Nonetheless, genomic regions determining GPC independently of GY across experiments were found on chromosomes 3A and 5D and could help breeders to move the GPC–GY relationship in a desirable direction.

Keywords Grain protein concentration · Grain yield · QTL · MCQTL · *Triticum aestivum* L

Abbreviations

GPC	Grain protein concentration
GY	Grain yield
QTL	Quantitative trait loci

Introduction

Grain yield (GY) and grain protein concentration (GPC) are major traits in wheat as they largely determine wheat productivity and quality (e.g. Shewry 2009; Oury et al. 2010). However, due to the negative genetic relationship

Electronic supplementary material The online version of this article (doi:10.1007/s11032-012-9817-5) contains supplementary material, which is available to authorized users.

M. Bogard (✉) · V. Allard · P. Martre · J. Le Gouis
INRA, UMR 1095 Génétique, Diversité et Ecophysiologie des Céréales, 5 chemin de Beaulieu, 63039 Clermont-Ferrand, France
e-mail: matthieu.bogard@clermont.inra.fr

M. Bogard · V. Allard · P. Martre · J. Le Gouis
UMR 1095 Génétique, Diversité et Ecophysiologie des Céréales, Université Blaise Pascal, 63177 Aubière Cedex, France

E. Heumez
INRA, UMR 1281 Stress Abiotiques et Différenciation des Végétaux Cultivés, Estrées-Mons, 80203 Péronne, France

J. W. Snape · S. Orford · S. Griffiths
Crop Genetics Department, John Innes Centre, Norwich NR4 7UH, UK

O. Gaju · J. Foulkes
Division of Agricultural Sciences, University of Nottingham, Leicestershire LE12 5RD, UK

between GY and GPC, breeding has resulted in increasing GY at the expense of GPC with potential impacts on quality in bread-making wheats (Oury et al. 2003). This negative relationship might be related to the presence of genes having pleiotropic effects on both GY and GPC through a dilution effect (Pepe et al. 1975; Miezan et al. 1977; McNeal and Berg 1978), such as genes affecting the duration of leaf senescence after flowering (Bogard et al. 2011). However, it has been suggested that, depending on the environment, increasing N remobilization efficiency or post-anthesis N uptake would allow increases in GPC without decreasing GY (Slafer et al. 1990, Bogard et al. 2010). This suggests the presence of genes having independent effects on GY and GPC.

Marker-assisted selection (MAS) has been proposed as an efficient tool to complement conventional breeding programs and has already been used to improve wheat quality and resistance to biotic stress in several parts of the world (Gupta et al. 1999; Dubcovsky 2004). In particular, MAS has been successfully used in wheat to increase GPC through the introgression of the *GPC-B1* quantitative trait locus (QTL) for high grain protein content (Chee et al. 2001; Uauy et al. 2006; Sherman et al. 2008). This QTL was initially mapped on the short arm of chromosome 6B in a population of recombinant inbred lines involving a *Triticum turgidum* ssp *dicoccoides* chromosome substitution line. Fine mapping resulted in identifying a NAC transcription factor (*NAM-B1*) that regulates senescence (Uauy et al. 2006). This gene was shown to have pleiotropic effects, and RNAi transgenic lines with reduced expression levels senesced later and exhibited a decrease in GPC, Zn and Fe content.

MAS technology is based upon the identification, through marker–trait association studies (QTL analysis, association genetics), of genetic markers linked to genomic regions determining the trait of interest. Genetic markers usable in MAS should have some desirable features. In particular, such markers should be closely linked to genes of interest to avoid introgression of undesirable characteristics and should be identified on adapted germplasm to be of practical value in breeding of elite material. Moreover, as MAS might be an expensive technology despite the recent deployment of high-throughput molecular marker technology in wheat [e.g. SNP (Oliphant et al. 2002; Akhunov et al. 2009), DArT® (Akbari et al. 2006) platforms], highly polymorphic markers linked to stable QTL (i.e. identified across different experimental conditions) should be preferably selected in order to maximize the benefits of using MAS.

So far, most of the marker–trait association studies carried out by QTL analysis have been based on the use of bi-parental populations, leading to the exploitation of limited genetic diversity to identify loci possibly useful in MAS. The use of association genetics on varietal sets representing the existing genetic variability of the crop (Balfourier et al. 2007; Bordes et al. 2010), connected populations, "multiparent advanced generation intercross" (Varshney and Dubey 2009) and QTL meta-analysis (Goffinet and Gerber 2000) might partially avoid this drawback and help to validate QTL identified using bi-parental populations. Indeed, the use of several populations derived from diverse parental material not only increases the probability that a QTL will be significant in at least one population, but also provides insight into the available genetic variability that might be used in breeding and a better understanding of the genetic architecture of the phenotype (Verhoeven et al. 2005; Holland 2007). The use of inter-related populations is a powerful approach to mapping QTL and this strategy has been applied in several plant and animal species (Rebaï et al. 1997; Jourjon et al. 2000; Blanc et al. 2006; Pierre et al. 2008; Billotte et al. 2010) but not yet in wheat.

Different strategies may be applied to benefit from the use of multi-population material. A popular approach is based on QTL meta-analysis (Goffinet and Gerber 2000) where QTL are detected in each population independently and then combined together. This approach has already been used in different studies on wheat; in particular on earliness (Hanocq et al. 2007; Griffiths et al. 2009) and grain size (Gegas et al. 2010). Another strategy uses specific statistical methods to carry out QTL detection on the combined multiparental data. This can be achieved using the MCQTL software (Jourjon et al. 2005) which enables linear regression in multi-cross designs. This approach has already been successfully used in *Medicago truncatula* (Pierre et al. 2008) and *Elaeis guineensis* (Billotte et al. 2010). Numerous QTL studies have used bi-parental populations in wheat for genetic analysis of GY or GPC separately (Perretant et al. 2000, Prasad et al. 2003, Marza et al. 2006, Kuchel et al. 2007, Wang et al. 2009, Sun et al. 2010), but only a few have addressed the two traits simultaneously (Blanco et al. 2002, 2006, 2012; Charmet et al. 2005;

Laperche et al. 2007; Pushpendra et al. 2007), and none used inter-related populations.

The objectives of the present study were to: (1) identify QTL determining GY and GPC using inter-related populations and (2) suggest genomic regions flanked by molecular markers that could be used in molecular-assisted breeding to improve GPC without reducing GY.

Materials and methods

Plant material and field experiments

Plant material used in this study consisted of three doubled-haploid mapping populations from the crosses Toisondor × Quebon (TORQUE), CF9107 × Quebon (CFQUE) and Toisondor × CF9107 (TOR107). The parents have been shown to contrast for several traits related to N uptake, post-anthesis N remobilization and leaf senescence kinetics (Gaju et al. 2011). These three populations were grown in a multi-environment trial network comprising one location in France (Clermont-Ferrand; 45°46′N, 03°09′E) and two in the UK (Sutton Bonington; 52°50′N, 1°14′W, and Norwich; 52°38′N, 1°18′E), over two seasons in Clermont-Ferrand (2007/8, 2008/9; hereafter referred to as 2008 and 2009, respectively) and one season (2009) for the other locations, and two N treatments (LN, low N; HN, high N), resulting in seven location × year × N treatment combinations referred hereafter as different environments (Table 1). The field experiment carried out at Clermont-Ferrand in 2008 under high N input (abbreviated as cf.8.HN) was a first evaluation of the plant material, so the number of genotypes grown for each population was higher than in the other environments: 316 doubled haploid lines (DHL) for the CFQUE population, 230 for TORQUE and 143 for TOR107. In the other environments, 80 DHL were grown for the CFQUE and TORQUE populations while 140 DHL were grown for TOR107. These DHL were chosen at random after eliminating those which were heavily lodged or which showed low seed purity based on the experiment of the previous year. More lines were chosen in the TOR107 population as a related study on the relationship between grain protein concentration, grain yield and leaf senescence during grain filling was carried out using this population (Bogard et al. 2011).

Two N fertiliser treatments were applied at each site in 2008–2009: a high N treatment (HN) intended to replicate local commercial practice, and a low N treatment (LN) with an N supply corresponding to 25 % of that applied in the high N treatment (Table 1). In each location, 50–60 kg N ha^{-1} were applied around the end of tillering (Zadoks' stage GS25; Zadoks et al. 1974) for both N treatments. For the HN treatment, 60–190 kg N ha^{-1} were applied in two equal split applications during stem elongation (between GS31 and GS32), and between flag leaf tip appearance (GS37) and male meiosis (GS39). Each split was applied on the same calendar date for all genotypes as granules of ammonium nitrate (34.5 % N). Mineral soil N at the end of winter and total N fertilizer applied in each experiment are shown in Table 1.

For each population, field experiments were arranged in an incomplete block design with two blocks each representing an N treatment. The blocks were each divided into eight sub-blocks with four sub-blocks allocated to TOR107 and two to each of the TORQUE and CFQUE populations. Lines were randomly arranged within each sub-block which comprised 50 plots with Toisondor, CF9107 and Quebon as controls. For each population, a subset of DHL was duplicated and was represented in two different sub-blocks. Each sub-block contained a subset of 23 DHL among the ones appearing once, and 24 DHL among the ones appearing twice. Plot sizes were 24 rows × 1.65 m at Sutton Bonnington, 6 rows × 1.50 m at Clermont-Ferrand and 6 rows × 1.20 m at Norwich. Across experiments, different seed rates were used sufficient to establish a target of 200 plants m^{-2} after winter. When necessary, fungicides, insecticides and herbicides were applied to achieve optimal management of the crop. Plant growth regulator (chlormequat) was applied at onset of stem elongation in all the locations except in Clermont-Ferrand.

Phenotyping

All plots of the trials were harvested with a combine harvester to measure grain yield (GY, g m^{-2}). Near-infra-red spectrometry (FOSS NIRS 6500, Höganäs, Sweden) was used to assess grain protein concentration (GPC, %) in whole grains. All values were adjusted to 0 % moisture content. Genotype means for GY and GPC across HN and LN treatments, or across all the environments, were calculated for each

Table 1 Characteristics of the environments studied: name of the locations, coordinates (a.s.l, "above sea level"), plot size, harvest year

Location	Coordinates	Plot size (m)	Harvest year	Cumulative rainfall (mm)	Daily air temperature (°C)	Ndays25	Mineral soil N (kg N ha^{-1})	N fertilizer (kg N ha^{-1})	Environment abbreviation
Clermont-Ferrand (F)	45°78′ N	1.5 × 5	2008	183	18	28	115	180	cf.8.HN
	3°08′ E		2009	151	19	28	75	50	cf.9.LN
	401 m a.s.l							200	cf.9.HN
Norwich (UK)	52°63′ N	1.5 × 4	2009	195	15	1	35	50	nw.9.LN
	1°30′ E							200	nw.9.HN
	14 m a.s.l								
Sutton Bonington (UK)	52°82′ N	1.65 × 5	2009	157	16	5	23	60	sb.9.LN
	1°25′ W							240	sb.9.HN
	38 m a.s.l								

Cumulative rainfall, average daily air temperature and number of days with maximum daily air temperature above 25 °C (*Ndays25*) were calculated between 1 June and 1 August of the harvest year, roughly corresponding to the period between anthesis and physiological maturity. Soil N at the end of winter (from 0 to 90 cm depth), total N fertilizer supplied during the experiments and environment abbreviations are given. N fertilizer was applied using split applications (see "Materials and methods")

population, and were analysed as separate environments named, hereafter, as "HN", "LN" or "mean".

Genotyping

One hundred and forty DHL from the TOR107, 91 from the TORQUE and 90 from the CFQUE populations were genotyped. Genomic DNA was extracted from leaves frozen and ground in liquid N using the Qiagen Biosprint plant DNA kit (Qiagen, USA). DNA concentration was then adjusted to 100 ng μL^{-1} using a microlab Star robot (Hamilton, USA). DArT® typing was obtained from the Diversity Arrays Technology Pty Ltd. Company (http://www.triticarte.com.au) by sending 30 µL of DNA solution. Genomic DNA of the parents and DHL were hybridised to the wheat DArT array v2.3 and polymorphisms were detected and scored as described by Akbari et al. (2006).

SSR markers were chosen in order to complete the DArT® typing of the D genome in particular. SSR genotyping was done using a standard protocol described in Bogard et al. (2011). The photoperiod sensitivity gene *Ppd-D1* was typed according to Beales et al. (2007). The vernalization requirement gene *Vrn-D3* was typed using specific primers based on Bonnin et al. (2008). A set of 384 single nucleotide polymorphism (SNP) loci discovered on a panel of 16 hexaploid wheat lines was genotyped on the TOR107 population using the Illumina Golden Gate technology (Akhunov et al. 2009) as previously described (Oliphant et al. 2002). Genotyping reactions were carried out using 150 ng of genomic DNA according to the manufacturer's instructions.

Genetic mapping

The genetic map used in this study was obtained by typing 741 markers comprising 642 DArT, 58 simple sequence repeat (SSR) and 39 SNP markers and specific markers for the *Ppd-D1* and the *Vrn-D3* genes. The map was generated using the Carthagene v1.0 software (de Givry et al. 2005) using the Haldane mapping function. A matrix of genotype scores was created for each population and these were merged using the "merge" command in Carthagene. First, linkage groups (LG) were defined using the "group" command with LOD ≥ 3 and genetic distance ≤ 30 cM. Marker order was then defined by flipping markers and choosing the best order. Although DArT markers are highly clustered (Francki et al. 2009) and markers inside a cluster do not bring any additional information for QTL analysis, all DArT® markers were kept in order to facilitate subsequent comparative studies across populations by meta-analysis. When two LG corresponded to the same chromosome, a number was added at the end of the chromosome name (e.g. 2A1, 2A2).

QTL mapping using the MCQTL software

QTL analysis was carried out using the MCQTL v5.2 software whose architecture and statistical implementation are reported in Jourjon et al. (2005). The iterative QTL mapping procedure implemented in MCQTL (iQTLm) gives an exclusive window of 20 cM around the putative QTL and a forward stepwise method to select genetic cofactors from the whole genome to automatically find a multiple QTL model. A genome-wide significance threshold was obtained for each trait × environment combination using 1,000 permutations. The threshold of inclusion of genetic cofactors was set to 0.95 × genome-wide significance threshold (B. Mangin, personnal communication). The confidence interval for the QTL position corresponded to the genetic map segment included after a LOD drop-off of one unit. The contribution of an individual QTL detected in a given environment to trait phenotypic variance was estimated by the R^2 coefficient. A full model R^2 taking into account all the QTL detected for a given trait × environment combination was obtained to estimate the proportion of total genetic effects of the phenotypic variance. The sum of any QTL allelic effects was null by constraint of the model.

Analysis of QTL colocation

Analysis of QTL co-location over environments was carried out using the MetaQTL software (Veyrieras et al. 2007). This software allows QTL meta-analysis to be performed based on the theory developed by Goffinet and Gerber (2000), who showed that it is robust enough to handle data collected on non-independent experiments (i.e. obtained on a unique mapping population grown in different environments), although the resulting confidence intervals of the meta-QTL might be biased. MetaQTL allows the choice of the best of k models ($k = 1, \ldots, n$) describing the number of QTL clusters on a given LG. This allowed the identification of potentially stable (i.e. identified across different environments) or pleiotropic QTL having an effect on both GPC and GY. MetaQTL analysis was carried out for each LG carrying at least 10 QTL whatever the traits or the environments where these QTL were detected. Graphical representations of the clustering were obtained with the MetaQTL software.

Statistical analysis

Analysis of variance was performed using Rv2.11.1 (R Development Core Team 2005) to test for genetic, environment (nitrogen in particular) and cross effect (genetic background) using the following model:

$$X_{ijkl} = \mu + C_k + \mathrm{SiteYear}_j + N_l + G_i(C_k) + \varepsilon_{ijk}$$

where, X_{ijkl} is the value for a given trait of the ith genotype in the jth site × year for the lth nitrogen treatment for cross k; μ is the general mean, C_k is the effect of cross k, $\mathrm{SiteYear}_j$ is the effect of site × year j; N_l is the effect of nitrogen treatment l, G_i (C_k) is the effect of line i in cross k and ε_{ijk} is the residual variance.

Results

Phenotypic variation

Large phenotypic variation was observed for GY and GPC ranging from 248 to 1,266 g m^{-2} and 5.4–17.3 % across the whole dataset, respectively (Table 2). This range of variation includes genetic, environment and genotype × environment interaction effects and may be attributed to the diversity of climatic conditions, genotypes and N supply represented in the trial network (Table 1). Analysis of variance showed that site × year, nitrogen and genetic background effects were significant for GPC and GY (data not shown). A significant negative GPC–GY relationship was observed in 17 out of the 21 population × environment combinations with large variability for correlation coefficients (r) and slopes (r ranging from −0.64 to +0.11, and slopes ranging from −0.009 to +0.001 % g^{-1} m^2; Table S1).

Genetic map

A set of 741 polymorphic markers were typed across the three mapping populations; 473 polymorphic markers were typed on the TORQUE population, 496 on the TOR107 and 360 on the CFQUE population. Thirty-seven markers were polymorphic in each population, of which seven were DArT® and 30 were SSR, suggesting that three different alleles are present for these markers. Two hundred and forty-two polymorphic markers were common between the TOR107

Table 2 Descriptive statistics of grain yield and grain protein concentration for the three mapping populations (CFQUE, TOR107, TORQUE) used in this study

Environment	Population	Grain yield (g m^{-2})				Grain protein concentration (%)			
		CF	QUE	TOI	Mean (range)	CF	QUE	TOI	Mean (range)
cf.8.HN	CFQUE	868	938	946	842 (604; 1,007)	13.7	13.7	12.6	13.9 (11.9; 17.3)
	TOR107				879 (624; 1,140)				12.4 (10.4; 15.7)
	TORQUE				927 (583; 1,176)				12.7 (9.8; 16.8)
cf.9.HN	CFQUE	620	705	544	641 (384; 748)	10.2	10.3	9.1	10.3 (8.8; 11.9)
	TOR107				631 (444; 765)				10.1 (8.9; 11.6)
	TORQUE				702 (548; 836)				10.5 (8.7; 12)
cf.9.LN	CFQUE	481	568	432	543 (423; 651)	7.6	8.0	7.1	7.8 (6.8; 9)
	TOR107				543 (356; 660)				7.7 (6.2; 9.3)
	TORQUE				590 (451; 663)				7.9 (6.8; 9.4)
nw.9.HN	CFQUE	812	965	800	866 (730; 1,031)	14.2	14.3	12.0	13.9 (11.8; 16.5)
	TOR107				884 (484; 1,154)				12.2 (9.3; 15.2)
	TORQUE				855 (688; 1,016)				12.8 (10.4; 16.2)
nw.9.LN	CFQUE	592	693	630	634 (518; 751)	9.4	9.6	8.1	9 (7; 11.1)
	TOR107				655 (445; 951)				7.9 (5.4; 11.2)
	TORQUE				643 (463; 809)				8.6 (6.9; 11.2)
sb.9.HN	CFQUE	908	970	761	847 (543; 1,266)	13.5	13.0	12.6	12.9 (11.3; 14.8)
	TOR107				858 (275; 1,152)				12.4 (10; 15.2)
	TORQUE				873 (611; 1,129)				12.6 (10.9; 14.5)
sb.9.LN	CFQUE	508	544	598	538 (316; 804)	8.7	9.1	8.6	8.6 (7.5; 10.7)
	TOR107				492 (248; 688)				8.4 (6.9; 9.8)
	TORQUE				594 (308; 894)				8.8 (7.6; 10.1)
LN	CFQUE	527	602	523	573 (472; 674)	8.5	8.9	7.9	8.5 (7.6; 9.8)
	TOR107				564 (451; 708)				8.0 (6.5; 9.4)
	TORQUE				610 (474; 742)				8.4 (7.2; 10.0)
HN	CFQUE	802	895	762	802 (702; 905)	12.9	12.8	11.6	12.7 (11.4; 14.3)
	TOR107				814 (597; 922)				11.7 (10.0; 13.7)
	TORQUE				840 (681; 950)				12.2 (10.4; 14.1)
Mean	CFQUE	684	769	673	704 (607; 784)	11.0	11.2	10.0	10.9 (9.7; 12.2)
	TOR107				707 (557; 827)				10.1 (8.5; 11.6)
	TORQUE				741 (610; 836)				10.6 (9.2; 12.3)

Mean values for the three parents of the populations are presented (CF9107, *CF*; QUEBON, *QUE*; TOISONDOR, *TOI*). Means and ranges (in brackets) for each population × environment combination are given. Environment abbreviations are given in Table 1

and TORQUE populations (including the *Ppd-D1* gene), 139 were common between TORQUE and CFQUE, and 133 were common between TOR107 and CFQUE (including the *Vrn-D3* gene). The genetic map contained 695 markers (46 markers were discarded as they could not be linked). The total length was 2,510 cM with 34 LG representing the 21 wheat chromosomes and an average length of 74 cM per LG. Of the 695 markers, 269 DArTs were co-segregating in different clusters across the map, leaving 426 informative markers. The average interval between consecutive markers was 6.4 cM after removing clustered DArT markers (i.e. non-segregating markers mapped at the same location that do not bring any additional information for QTL detection). The homoeologous group 4 chromosomes appeared to be under-represented with only 34 markers compared to the 110 markers mapped on average to other

Table 3 Results of QTL detection

Traits	Environment	QTL	Additive effects	R^2g	R^2	1B	1D	2A2	2B2	2D	3A	3B2	3D1	4B2	4D	5A1	5B2	5D1	7A	7B	7D
GY	8	36	(–24.83; 28.81)	(0.13; 0.52)	(0.06; 0.32)	2	3	3	1	7	3	3	–	1	–	–	–	–	1	6	6
GPC	10	53	(–0.32; 0.28)	(0.1; 0.59)	(0.06; 0.25)	–	3	10	–	4	6	6	–	3	–	1	–	6	–	6	8
GY & GPC	–	–	–	–	–	0	0	3	0	4	3	3	0	0	0	0	0	0	0	6	6

QTL analysis was carried out using the MCQTL software on three wheat related mapping populations grown in a large multi-environment trial network for grain yield (*GY*) and grain protein concentration (*GPC*). For each trait, information displayed is the number of environments where QTL were detected (*Environment*), the total number of QTL (*QTL*), the range of variation of the additive effects (*Additive effects*), the range of variation of the global R^2 taking into account all the QTL detected in each environment (R^2g), the range of variation of individual R^2 of each QTL (R^2), and the re-partition of the QTL in the different linkage groups. The last row (*GY & GPC*) indicates for each linkage group the number of environments in which QTL were found both for GY and GPC

homoeologous groups. The D genome was the least represented with only 87 markers compared to the 279 markers on the A genome and the 329 markers on the B genome.

QTL detection for GY and GPC

Eighty-nine QTL were detected in this study. Two to seven QTL were found for GY in each environment, leading to a total of 36 QTL (Table 3). Depending on the environment, one to ten QTL were found for GPC, leading to a total of 53 QTL for this trait (Table 3). The total percentage of variance explained, taking into account all the QTL detected in one environment, ranged from 13 to 52 % for GY and from 10 to 59 % for GPC. The percentage of variance explained by each individual QTL ranged from 6 to 32 % for GY and from 6 to 25 % for GPC. As shown in Table 3, some LG carried only QTL for GY such as 1B (two environments), 2B2 (one environment), and 7A (one environment). Some others carried only GPC QTL such as 5A1 (one environment) and 5D1 (6 environments). The other LG showed QTL for both GY and GPC although some environments showed only GPC QTL or GY QTL (Table 3).

Meta-analysis of QTL co-locations

Meta-QTL analysis was carried out for each LG carrying at least ten QTL whatever the environment where these QTL were detected. This led to the identification of meta-QTL on seven LG.

LG 2A2 carried QTL detected in three environments for GY and in all environments for GPC (Figure S1, Table 4). The most probable meta-analysis model was one unique QTL cluster ("MQTL2A2") with a confidence interval of 0.09 cM (Table 4). Co-location of QTL for GY and GPC were found on this LG for cf.8.HN, mean values across the low N and mean values across all the environments showed co-location of QTL for GY and GPC (Figure S1). The CF9107 and the Quebon alleles had positive effects on GPC (ranging from 0.01 to 0.20 points of GPC) and negative effects on GY (ranging from −0.4 to −19.7 g m^{-2}), while the Toisondor allele had negative effects on GPC (ranging from −0.12 to −0.32 points of GPC) and positive effects on GY (ranging from 8.9 to 15.3 g m^{-2}).

Table 4 QTL clustering on linkage groups 2A2, 2D, 3A, 3B2, 5D1, 7B and 7D

Linkage group	Meta-QTL	Position	CI (cM)	QTL GPC	QTL GY	QTL GPC & GY	Flanking markers
2A2	MQTL2A2	30.91	0.09	10	3	3	*wPt-4197*; *wPt-5245*
2D	MQTL2D_1	67.28	8.8	0	1	0	*FdGogat_2D*; *Ppd-D1*
	MQTL2D_2	78.37	2.92	4	6	4	*Ppd-D1*; *gpw332*
3A	MQTL3A_1	53.9	19.34	0	1	0	*wPt-5125*; *gpw5016*
	MQTL3A_2	84.55	18.58	2	0	0	*gpw5016*; *wPt-6357*
	MQTL3A_3	120.97	10.05	4	0	0	*wPt-6357*; *gwm666*
	MQTL3A_4	163.79	50.37	0	2	0	*K04_3A*; *wPt-7890*
3B2	MQTL3B_1	41.96	6.27	5	2	1	*G16_3D*; *wPt-4364*
	MQTL3B_2	75.95	43.64	1	1	0	*wPt-4364*; *wPt-0773*
5D1	MQTL5D_1	23.5	43.6	1	0	0	*wPt-8030*; *gwm174*
	MQTL5D_2	96.31	1.01	5	0	0	*wPt-0886*; *wPt-1999*
7B	MQTL7B_1	52.39	8.06	5	2	1	*wPt-7934*; *wPt-1826*
	MQTL7B_2	127.9	4.02	0	1	0	*wPt-8921*; *wPt-8040*
	MQTL7B_3	147.7	6.29	1	3	1	*wPt-7720*; *wPt-0408*
7D	MQTL7D	71.87	1.06	8	6	6	*Vrn-D3*; *wPt-3727*

QTL detection was carried out for grain yield (*GY*) and grain protein concentration (*GPC*) on three related wheat populations grown in a large multi-environment trial network using the MCQTL software for each trait × environment combination. QTL were clustered into meta-QTL (MQTL) using the MetaQTL v1.0 software (Veyrieras et al. 2007). For each linkage group, the name of the meta-QTL, its position, the length of its confidence interval (*CI*, $\alpha < 0.05$), the number of environments where QTL were found for GPC, GY and both GY and GPC and markers flanking the confidence interval of the meta-QTL are displayed

LG 2D carried QTL detected in seven environments for GY and in four environments for GPC (Figure S1, Table 4). Meta-analysis identified two QTL clusters, with one being predominant ("MQTL2D_2") as it carried ten out of 11 of the QTL detected on this LG (Figure S1, Table 4). The two clusters flank the *Ppd-D1* gene (Figure S1). Environment cf.8.HN, mean values across the low N treatment, mean values across the high N treatment and mean values across all the environments showed co-location of QTL for GY and GPC in this genomic region (Figure S1). The CF9107 and the Quebon alleles had negative effects on GPC (ranging from −0.02 to −0.27 points of GPC) and positive effects on GY (ranging from 2.4 to 12.5 g m^{-2}) while the Toisondor allele had positive effects on GPC (ranging from 0.09 to 0.24 points of GPC) and negative ones on GY (ranging from −8.6 to −24.8 g m^{-2}).

LG 3A carried QTL detected in six environments for GPC and in three environments for GY (Figure S1, Table 4). Meta-analysis identified four QTL clusters, one cluster including only one QTL for GY, another one including two QTL for GPC, one major cluster including four GPC QTL ("MQTL3A_3") and the last one including two QTL for GY (Figure S1, Table 4). The CF9107 allele had positive effects on GPC (ranging from 0.03 to 0.17 points of GPC) while the effect of the Toisondor allele was negative (ranging from −0.26 to −0.09 points of GPC).

LG 3B2 carried QTL detected in six environments for GPC and in three environments for GY (Figure S1, Table 4). QTL meta-analysis allowed the identification of one major QTL cluster ("MQTL3B_1") including seven QTL for GY and GPC (Figure S1, Table 4). This genomic region showed co-location of QTL with antagonistic effects on GY and GPC in cf.9.LN, using mean values across the LN treatment or across the whole dataset, thus potentially contributing to the GPC–GY negative relationship. The CF9107 and the Quebon alleles had negative effects (ranging from −0.14 to −0.04 points of GPC) and the Toisondor allele had positive effects (ranging from 0.10 to 0.28 points of GPC). Regarding GY, the Quebon allele had positive effects (ranging from 10.1 to 12.1 g m^{-2}), the Toisondor allele negative effects (ranging from −9.7 to −9.2 g m^{-2}) and the CF9107 allele was intermediate (ranging from −2.4 to −0.4 g m^{-2}).

LG 5D1 carried only GPC QTL detected in five environments, predominantly under high N (cf.8.HN, mean, nw.9.HN, sb.9.HN, HN; Figure S1, Table 4). The LOD score ranged from 4 to 7 and the percentage of

variance explained by this QTL from 0.06 to 0.11 correspond to values of the determination coefficient (R^2). In percentage, this is 6 to 11 % depending on the environment (data not shown). Additionally, a QTL analysis carried out only on the Quebon × Toisondor population confirmed the presence of this QTL between markers *Xgpw5174* and *XwPt-1999* (data not shown). Thus, this region potentially affects GPC but not GY under high N. Meta-analysis clearly indicated the presence of a unique QTL cluster ("MQTL5D_2") with a confidence interval of 1 cM (Figure S1, Table 4). The Quebon allele had a positive effect on GPC (ranging from 0.1 to 0.2 points of GPC), the Toisondor allele had a negative effect (ranging from −0.15 to −0.09 points of GPC), and the CF9107 allele was intermediate.

LG 7B carried QTL detected in six environments for GY and GPC (Figure S1, Table 4). Meta-analysis identified two main clusters; a third one contained only one QTL for GY. The first cluster ("MQTL7B_1") contained QTL detected in four environments for GPC only, QTL detected in environment cf.9.HN for GY only, and QTL detected using mean values for the low N treatment for both GPC and GY (Figure S1, Table 4). The Toisondor allele had a positive effect on GPC (ranging from 0.1 to 0.21 points of GPC) but a negative effect on GY (ranging from −13.1 to −2.2 g m^{-2}). The CF9107 and the Quebon alleles had negative effects on GPC (ranging from 0 to −0.11 points of GPC). Regarding GY, the Quebon allele always had positive effects (ranging from 8.9 to 12.5 g m^{-2}) while the CF9107 allele had a positive effect in cf.9.HN (+4.2 g m^{-2}) and a negative effect in the LN environment (−10.2 g m^{-2}).

The second QTL cluster on LG 7B ("MQTL7B_3") contained QTL detected for GY in three environments, and in one environment for GPC (Figure S1, Table 4). Concerning GY, the CF9107 allele had a negative effect (ranging from −8.6 to −18.1 g m^{-2}), the Toisondor allele had a positive effect (ranging from 6 to 9.3 g m^{-2}) and the Quebon allele was intermediate. Regarding the GPC QTL contained in this cluster, the Toisondor allele had a negative effect (−0.21 points of GPC), while the CF9107 and the Quebon alleles had positive effects (0.13 and 0.08 points of GPC, respectively).

LG 7D carried QTL detected in six and eight environments for GY and GPC, respectively (Figure S1, Table 4). Meta-analysis allowed the identification of a unique cluster ("MQTL7D") containing all the QTL detected for this LG. The 1.1 cM confidence interval of this meta-QTL includes the *Vrn-D3* vernalization gene (Figure S1, Table 4). QTL were detected in this region in six environments and the antagonistic effects of all three alleles on GY and GPC were high. This indicates that this region is probably contributing the most to the GPC–GY negative relationship in this study. The Toisondor and the Quebon alleles had a positive effect on GY (ranging from 6.9 to 13.9 g m^{-2}) and a negative effect on GPC (ranging from −0.26 to −0.01 points of GPC), while the CF9107 allele had a negative effect on GY (ranging from −26.2 to −14.8 g m^{-2}) and a positive effect on GPC (ranging from 0.12 to 0.28 points of GPC).

Discussion

QTL analysis for GY and GPC was carried out on three inter-related wheat populations grown in a large multi-environment trial network, using the MCQTL software (Jourjon et al. 2005). Eighty-nine QTL were detected over all trait × environment combinations. QTL meta-analysis allowed the identification of genomic regions having antagonistic effects on GY and GPC (LG 2A2, 2D, 3B2, 7B and 7D), thus contributing to the negative GPC–GY relationship, and genomic regions determining GPC independently of GY (LG 3A, 5D1) that may be useful in MAS for improving GPC without decreasing GY.

Genomic regions contributing to the GPC–GY relationship: candidate genes and ecophysiological properties

Meta-analysis showed co-location of QTL with antagonistic effects on GPC and GY on chromosomes 2A, 2D, 3B, 7B and 7D, although the resolution of this QTL analysis does not allow distinguishing between true pleiotropic effect and tight linkage. QTL clusters on 7D and 2D had the most frequently antagonistic effects on GY and GPC (in six and four environments, respectively). These clusters were close to mapped genes known to be involved in the regulation of flowering time [*Ppd-D1* on 2D (Beales et al. 2007) and *Vrn-D3* on 7D (Yan et al. 2006)]. Regarding chromosome 2A, QTL for grain protein content (Groos et al. 2003; Prasad et al. 2003; Laperche et al. 2007) and dough quality parameters (Kerfal et al. 2010) have already been reported on this chromosome. A chloroplastic glutamine synthetase

gene (*GS2*), an enzyme associated with primary nitrogen assimilation, has been recently mapped on the homoeologous group 2 chromosomes between markers *Xgwm294* and *Xwmc181* (Li et al. 2011). Moreover, these authors reported a significant association with a polymorphism in the gene and different agronomic traits including GPC. According to the consensus map integrating DArT and SSR markers from Crossa et al. (2007), one of the markers (*XwPt-2087*) in the vicinity of this QTL is closely linked to *Xgwm294* and *Xwmc181* (1.3 and 2.7 cM away from these markers, respectively) near to the *GS2* map location.

Genomic regions on 2A, 2D and 7D have been shown to affect leaf senescence duration in the TOR107 population (Bogard et al. 2011). Leaf senescence duration during the grain-filling period is a possible candidate trait to explain the GPC–GY negative correlation. In wheat, grain nitrogen mostly comes from N taken up during the vegetative period and remobilised to the grain after anthesis (Sanford and MacKown 1986; Barbottin et al. 2005; Kichey et al. 2007), while C assimilated after flowering is the main source of C for starch synthesis in the grain (Triboi et al. 2006). Therefore, the duration of leaf activity during the post-anthesis period largely affects the capacity for C assimilation but also for N remobilisation efficiency. Later leaf senescence promotes C assimilation and generally is associated with low N remobilisation efficiency, the inverse being generally true for early senescent genotypes (Gregersen et al. 2008). Moreover, QTL for leaf senescence duration after flowering found on chromosomes 2D and 7D were associated with flowering time QTL related to the *Ppd-D1* (Beales et al. 2007) and *Vrn-D3* genes (Yan et al. 2006). The impact of flowering time on leaf senescence duration may be related to modifications of the partitioning of the N uptake between the pre- and post-anthesis period, of the source:sink ratio, or of the perceived environment resulting in reduced post-anthesis C assimilation of late genotypes (Bogard et al. 2011).

Genomic regions determining GPC independently of GY possibly useful in MAS

Two chromosomes showed co-location of QTL for GPC detected in different environments but no QTL for GY, suggesting these regions affect GPC constitutively and independently of GY, and might be useful in MAS to shift the GPC–GY relationship.

The region on chromosome 3A is located at 119 cM, presumably on the long arm (Sourdille et al. 2004), with a confidence interval of 10 cM. The closest genetic marker is *Xgwm666*, 7 cM away from the most probable position of the QTL. This region has already been identified as influencing GPC, dough strength (Groos et al. 2003; Groos et al. 2004; Reif et al. 2010), low molecular weight glutenin, total gliadin content (Charmet et al. 2005), flour protein content (Zhao et al. 2010) and bread-making quality (Groos et al. 2007). The relatively large confidence interval of the QTL limits its usefulness in MAS. Further fine mapping is needed to reduce the uncertainty about the location and develop genetic markers closer to the QTL.

Associations between markers on chromosome 5D and variation for GPC (Reif et al. 2010), dough strength and dough tenacity (Kerfal et al. 2010) have been already reported. The region on chromosome 5D determining GPC independently of GY in the present study is located at 96 cM. The closest SSR marker (*Xgwm639*), 20 cM away from this meta-QTL, was also mapped using Chinese Spring deletion lines by Sourdille et al. (2004), and is located on the long arm of chromosome 5D. This marker was located at the same distance from a QTL for GS activity found on another mapping population under high N (Fontaine et al. 2009). This meta-QTL shows a confidence interval of 1 cM but the use of non-independent data in QTL meta-analysis may have biased the calculation of the meta-QTL confidence interval, so this should be considered with caution. The closest marker to the meta-QTL was DArT *XwPt-1999* mapped at 96.9 cM. This marker might be useful for diagnostics in MAS programs to improve GPC in wheat, if converted to a more suitable marker type. Unfortunately, the sequence of this marker was not available from the Triticarte company (http://www.diversityarrays.com/sequences.html). Alternatively, the closest SSR marker to this QTL (*Xgpw5174*) may be used, though 6 cM away from the most likely position of this QTL.

Finally, some chromosomes containing co-located QTL for GPC and GY had an effect only on one of these two traits in some environments. For instance, the major QTL cluster for GPC found on 2A affected both GY and GPC in three environments, while it solely affected GPC in seven environments. It probably contributes moderately to the negative GPC-GY relationship, and might be useful for improving GPC.

Further environment characterization is needed to determine in which type of environment these regions might be useful for improving GY or GPC independently.

Benefits of using connected populations in QTL detection for GY and GPC in wheat

The main advantage of using inter-related populations in QTL detection is the increased chance of having polymorphic QTL and increasing the number of genotypes per allelic class, thus increasing the statistical power of QTL detection and estimation of allelic effects (Rebaï and Goffinet 1993; Muranty 1996). As a consequence, QTL may be finely mapped, showing a reduced confidence interval compared to single cross studies (Pierre et al. 2008). In comparison with the results of a companion study carried out on the TOR107 population only (Bogard et al. 2011), the number of QTL identified for GPC and GY was increased from 31 in Bogard et al. (2011) to 89 in the present study, presumably due to the increased number of polymorphic loci and possibly to the identification of supplementary small effects loci. Moreover, confidence intervals of the QTL for GY and GPC were reduced. For example, confidence intervals of QTL for GY on 7D belonging to meta-QTL "MQTL7D" ranged from 25 to 62 cM in Bogard et al. (2011) but ranged from 10 to 19 cM in the present study. Regarding QTL detection for such integrative traits as GY or GPC, these properties are of interest as these traits largely depend on multiple loci with small effect that are generally not detected in single cross studies. Moreover, precise estimation of QTL allelic effects and QTL confidence intervals for these traits provides better estimates of their value in breeding and allows precise targeting of the most promising QTL for MAS.

Conclusion

Combined QTL analysis for GY and GPC was carried out on three inter-related wheat mapping populations grown in a large multi-environment trial network using the MCQTL software. Analysis of QTL co-location allowed the identification of genomic regions contributing to the GPC–GY relationship on chromosomes 2A, 2D, 3B, 7B and 7D. The regions on 2D and 7D are close to the *Ppd-D1* and *Vrn-D3* genes involved in the control of flowering time. Based on map comparisons with other studies, the region on 2A appeared close to a glutamine synthetase gene involved in N assimilation. These different regions do not contribute equally to the GPC–GY relationship. In particular, the region on 2A affected both GY and GPC only in a few environments, while in most of the cases this region had a strong impact on GPC only. Meta-analysis also allowed the identification of other genomic regions on chromosomes 3A and 5D determining GPC independently of GY across different environments, possibly useful for improving GPC without reducing GY.

Acknowledgments This work was supported by the "NUE traits" project [IN-BB-06] (http://www.rothamsted.bbsrc.ac.uk/bab/mas-projects/NUE.html); funded by the Institut National de la Recherche Agronomique (INRA) and the UK Biotechnology and Biological Sciences Research Council (BBSRC). Authors gratefully acknowledge the experimental work carried out by Joëlle Messaoud, Séverine Rougeol, Jean-Louis Joseph and Pascal Lemaire (INRA, Clermont-Ferrand), Dominique Brasseur (INRA, Estrées-Mons), Rob Perdue (University of Nottingham, Sutton Bonington), and Lesley Fish (John Innes Centre, Norwich).

References

Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden M, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A (2006) Diversity arrays technology (DArT®) for high-throughput profiling of the hexaploid wheat genome. Theor Appl Genet 113:1409–1420

Akhunov E, Nicolet C, Dvorak J (2009) Single nucleotide polymorphism genotyping in polyploid wheat with the Illumina GoldenGate assay. Theor Appl Genet 119:507–517

Balfourier F, Roussel V, Strelchenko P, Exbrayat-Vinson F, Sourdille P, Boutet G, Koenig J, Ravel C, Mitrofanova O, Beckert M (2007) A worldwide bread wheat core collection arrayed in a 384-well plate. Theor Appl Genet 114:1265–1275

Barbottin A, Lecomte C, Bouchard C, Jeuffroy M (2005) Nitrogen remobilization during grain filling in wheat: genotypic and environmental effects. Crop Sci 45:1141–1150

Beales J, Turner A, Griffiths S, Snape J, Laurie D (2007) A pseudo-response regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (*Triticum aestivum* L.). Theor Appl Genet 115:721–733

Billotte N, Jourjon MF, Marseillac N, Berger A, Flori A, Asmady H, Adon B, Singh R, Nouy B, Potier F, Cheah SC, Rohde W, Ritter E, Courtois B, Charrier A, Mangin B (2010) QTL detection by multi-parent linkage mapping in oil palm (*Elaeis guineensis* Jacq.). Theor Appl Genet 120:1673–1687

Blanc G, Charcosset A, Mangin B, Gallais A, Moreau L (2006) Connected populations for detecting quantitative trait loci and testing for epistasis: an application in maize. Theor Appl Genet 113:206–224

Blanco A, Pasqualone A, Troccoli A, Di Fonzo N, Simeone R (2002) Detection of grain protein content QTLs across environments in tetraploid wheats. Plant Mol Biol 48: 615–623

Blanco A, Simeone R, Gadaleta A (2006) Detection of QTLs for grain protein content in durum wheat. Theor Appl Genet 112:1195–1204

Blanco A, Mangini G, Giancaspro A, Giove S, Colasuonno P, Simeone R, Signorile A, De Vita P, Mastrangelo AM, Cattivelli L, Gadaleta A (2012) Relationship between grain protein content and grain yield components through quantitative trait locus analyses in a recombinant inbred line population derived from two elite durum wheat cultivars. Mol Breed 30:79–92

Bogard M, Allard V, Brancourt-Hulmel M, Heumez E, Machet J, Jeuffroy M, Gate P, Martre P, Le Gouis J (2010) Deviation from the grain protein concentration–grain yield negative relationship is highly correlated to post-anthesis N uptake in winter wheat. J Exp Bot 61:4303–4312

Bogard M, Jourdan M, Allard V, Martre P, Perretant MR, Ravel C, Heumez E, Orford S, Snape J, Giffiths S, Oorbessy G, Foulkes J, Le Gouis J (2011) Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield and grain protein concentration in a winter wheat population segregating for flowering time QTLs. J Exp Bot 62:3621–3636

Bonnin I, Rousset M, Madur D, Sourdille P, Dupuits C, Brunel D, Goldringer I (2008) FT genome A and D polymorphisms are associated with the variation of earliness components in hexaploid wheat. Theor Appl Genet 116:383–394

Bordes J, Ravel C, Le Gouis J, Lapierre A, Charmet G, Balfourier F (2010) Use of a global wheat core collection for association analysis of flour and dough quality traits. J Cereal Sci 54:137–147

Charmet G, Robert N, Branlard G, Linossier L, Martre P, Triboï E (2005) Genetic analysis of dry matter and nitrogen accumulation and protein composition in wheat kernels. Theor Appl Genet 111:540–550

Chee P, Elias E, Anderson J, Kianian S (2001) Evaluation of a high grain protein QTL from *Triticum turgidum* L. var. dicoccoides in an adapted durum wheat background. Crop Sci 41:295–301

Crossa J, Burgueno J, Dreisigacker S, Vargas M, Herrera-Foessel SA, Lillemo M, Singh RP, Trethowan R, Warburton M, Franco J, Reynolds M, Crouch J, Ortiz R (2007) Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. Genetics 177:1889–1913

De Givry S, Bouchez M, Chabrier P, Milan D, Schiex T (2005) CarhtaGene: multipopulation integrated genetic and radiation hybrid mapping. Bioinformatics 21:1703–1704

Dubcovsky J (2004) Marker-assisted selection in public breeding programs: the wheat experience. Crop Sci 44:1895–1898

Fontaine J, Ravel C, Pageau K, Heumez E, Dubois F, Hirel B, Le Gouis J (2009) A quantitative genetic study for elucidating the contribution of glutamine synthetase, glutamate dehydrogenase and other nitrogen-related physiological traits to the agronomic performance of common wheat. Theor Appl Genet 119:645–662

Francki M, Walker E, Crawford A, Broughton S, Ohm H, Barclay I, Wilson R, McLean R (2009) Comparison of genetic and cytogenetic maps of hexaploid wheat (*Triticum aestivum* L.) using SSR and DArT markers. Mol Genet Genomics 281:181–191

Gaju O, Allard V, Martre P, Snape JW, Heumez E, Le Gouis J, Moreau D, Bogard M, Griffiths S, Orford S, Hubbart S, Foulkes J (2011) Identification of traits to improve the nitrogen use efficiency of wheat genotypes. Field Crops Res 123:139–152

Gegas VC, Nazari A, Griffiths S, Simmonds J, Fish L, Orford S, Sayers L, Doonan JH, Snape JW (2010) A genetic framework for grain size and shape variation in wheat. Plant Cell 22:1046–1056

Goffinet B, Gerber S (2000) Quantitative trait loci: a meta-analysis. Genetics 155:463–473

Gregersen PL, Holm PB, Krupinska K (2008) Leaf senescence and nutrient remobilisation in barley and wheat. Plant Biol 10:37–49

Griffiths S, Simmonds J, Leverington M, Wang Y, Fish L, Sayers L, Alibert L, Orford S, Wingen L, Herry L, Faure S, Laurie D, Bilham L, Snape J (2009) Meta-QTL analysis of the genetic control of ear emergence in elite European winter wheat germplasm. Theor Appl Genet 119:383–395

Groos C, Bervas E, Charmet G (2004) Genetic analysis of grain protein content, grain hardness and dough rheology in a hard×hard bread wheat progeny. J Cereal Sci 40:93–100

Groos C, Robert N, Bervas E, Charmet G (2003) Genetic analysis of grain protein content, grain yield and thousand-kernel weight in bread wheat. Theor Appl Genet 106:1032–1040

Groos C, Bervas E, Chanliaud E, Charmet G (2007) Genetic analysis of bread-making quality scores in bread wheat using a recombinant inbred line population. Theor Appl Genet 115:313–323

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breed 118:369–390

Hanocq E, Laperche A, Jaminon O, Lainé A, Le Gouis J (2007) Most significant genome regions involved in the control of earliness traits in bread wheat, as revealed by QTL meta-analysis. Theor Appl Genet 114:569–584

Holland JB (2007) Genetic architecture of complex traits in plants. Curr Opin Plant Biol 10:156–161

Jourjon M, Durel C, Goffinet B, Laurens F (2000) An example of application for MCQTL software: fine characterisation of a QTL for apple scab resistance. Plant and Animal Genome Conference VIII, San Diego, USA

Jourjon M, Jasson S, Marcel J, Ngom B, Mangin B (2005) MCQTL: multi-allelic QTL mapping in multi-cross design. Bioinformatics 21:128–130

Kerfal S, Giraldo P, Rodriguez-Quijano M, Vazquez JF, Adams K, Lukow OM, Roder MS, Somers DJ, Carrillo JM (2010) Mapping quantitative trait loci (QTLs) associated with dough quality in a soft × hard bread wheat progeny. J Cereal Sci 52:46–52

Kichey T, Hirel B, Heumez E, Dubois F, Le Gouis J (2007) In winter wheat (*Triticum aestivum* L.), post-anthesis nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological markers. Field Crops Res 102:22–32

Kuchel H, Williams KJ, Langridge P, Eagles HA, Jefferies SP (2007) Genetic dissection of grain yield in bread wheat. I. QTL analysis. Theor Appl Genet 115:1029–1041

Laperche A, Brancourt-Hulmel M, Heumez E, Gardet O, Hanocq E, Devienne-Barret F, Le Gouis J (2007) Using genotype × nitrogen interaction variables to evaluate the QTL involved in wheat tolerance to nitrogen constraints. Theor Appl Genet 115:399–415

Li X, Zhao X, He X, Zhao G, Li B, Liu D, Zhang A, Zhang X, Tong Y, Li Z (2011) Haplotype analysis of the genes encoding glutamine synthetase plastic isoforms and their association with nitrogen-use- and yield-related traits in bread wheat. New Phytol 189:449–458

Marza F, Bai GH, Carver BF, Zhou WC (2006) Quantitative trait loci for yield and related traits in the wheat population Ning7840 × Clark. Theor Appl Genet 112:688–698

McNeal F, Berg C (1978) Recurrent selection for grain protein content in spring wheat. Crop Sci 18:779–782

Miezan K, Heyne EG, Finney KF (1977) Genetic and environmental effects on the grain protein content in wheat. Crop Sci 17:591–593

Muranty H (1996) Power of tests for quantitative trait loci detection using full-sib families in different schemes. Heredity 76:156–165

Oliphant A, Barker DL, Stuelpnagel JR, Chee MS (2002) BeadArray technology: enabling an accurate, cost-effective approach to high-throughput genotyping. BioTechniques Suppl 56–58:60–61

Oury F, Bérard P, Brancourt-Hulmel M, Depatureaux C, Doussineaux G, Galic N, Giraud A, Heumez E, Lecomte C, Pluchard P, Rousset M, Trottet M (2003) Yield and grain protein concentration in bread wheat: a review and a study of multi-annual data from a French breeding program. J Genet Breed 57:59–68

Oury F, Chiron H, Faye A, Gardet O, Giraud A, Heumez E, Rolland B, Rousset M, Trottet M, Charmet G, Branlard G (2010) The prediction of bread wheat quality: joint use of the phenotypic information brought by technological tests and the genetic information brought by HMW and LMW glutenin subunits. Euphytica 171:87–109

Pepe J, Heiner JF, Robert E (1975) Plant height, protein percentage, and yield relationships in spring wheat. Crop Sci 15:793–797

Perretant M, Cadalen T, Charmet G, Sourdille P, Nicolas P, Boeuf C, Tixier M, Branlard G, Bernard S (2000) QTL analysis of bread-making quality in wheat using a doubled haploid population. Theor Appl Genet 100:1167–1175

Pierre J, Huguet T, Barre P, Huyghe C, Julier B (2008) Detection of QTLs for flowering date in three mapping populations of the model legume species *Medicago truncatula*. Theor Appl Genet 117:609–620

Prasad M, Kumar N, Kulwal P, Röder M, Balyan H, Dhaliwal H, Gupta P (2003) QTL analysis for grain protein content using SSR markers and validation studies using NILs in bread wheat. Theor Appl Genet 106:659–667

Pushpendra KG, Harindra SB, Pawan LK, Neeraj K, Ajay K, Reyazul RM, Amita M, Jitendra K (2007) QTL analysis for some quantitative traits in bread wheat. J Zhejiang Univ Sci B 8:807–814

Rebaï A, Goffinet B (1993) Power of tests for QTL detection using replicated progenies derived from a diallel cross. Theor Appl Genet 86:1014–1022

Rebaï A, Blanchard P, Perret D, Vincourt P (1997) Mapping quantitative trait loci controlling silking date in a diallel cross among four lines of maize. Theor Appl Genet 95:451–459

Reif JC, Gowda M, Maurer HP, Longin CFH, Korzun V, Ebmeyer E, Bothe R, Pietsch C, Wurschum T (2010) Association mapping for quality traits in soft winter wheat. Theor Appl Genet 122:961–970

Sanford DA, MacKown CT (1986) Variation in nitrogen use efficiency among soft red winter wheat genotypes. Theor Appl Genet 72:158–163

Sherman JD, Lanning SP, Clark D, Talbert LE (2008) Registration of near-isogenic hard-textured wheat lines differing for presence of a high grain protein gene. J Plant Regist 2:162–164

Shewry PR (2009) Wheat. J Exp Bot 60:1537–1553

Slafer GA, Andrade FH, Feingold SE (1990) Genetic improvement of bread wheat (*Triticum aestivum* L.) in Argentina: relationships between nitrogen and dry matter. Euphytica 50:63–71

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsattelite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genomics 4:12–25

Sun X, Marza F, Ma H, Carver BF, Bai G (2010) Mapping quantitative trait loci for quality factors in an inter-class cross of US and Chinese wheat. Theor Appl Genet 120:1041–1051

Triboi E, Martre P, Girousse C, Ravel C, Triboi-Blondel A (2006) Unravelling environmental and genetic relationships between grain yield and nitrogen concentration for wheat. Eur J Agron 25:108–118

Uauy C, Brevis JC, Dubcovsky J (2006) The high grain protein content gene Gpc-B1 accelerates senescence and has pleiotropic effects on protein content in wheat. J Exp Bot 57:2785–2794

Varshney RK, Dubey A (2009) Novel genomic tools and modern genetic and breeding approaches for crop improvement. J Plant Biochem Biotechnol 18:127–138

Verhoeven KJF, Jannink J, McIntyre LM (2005) Using mating designs to uncover QTL and the genetic architecture of complex traits. Heredity 96:139–149

Veyrieras JB, Goffinet B, Charcosset A (2007) MetaQTL: a package of new computational methods for the meta-analysis of QTL mapping experiments. BMC Bioinfo 8:49

Wang RX, Hai L, Zhang XY, You GX, Yan CS, Xiao SH (2009) QTL mapping for grain filling rate and yield-related traits in RILs of the Chinese winter wheat population Heshangmai × Yu8679. Theor Appl Genet 118:313–325

Yan L, Fu D, Li C, Blechl A, Tranquilli G, Bonafede M, Sanchez A, Valarik M, Yasuda S, Dubcovsky J (2006) The wheat and barley vernalization gene VRN3 is an orthologue of FT. Proc Natl Acad Sci USA 51:19581–19586

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

Zhao L, Zhang KP, Liu B, Deng Z, Qu HL, Tian JC (2010) A comparison of grain protein content QTLs and flour protein content QTLs across environments in cultivated wheat. Euphytica 174:325–335