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Genetic dissection of grain weight in bread wheat through quantitative trait locus interval and association mapping

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Abstract Genetic dissection of grain weight in bread wheat was undertaken through both genome-wide quantitative trait locus (QTL) interval mapping and association mapping. QTL interval mapping involved preparation of a framework linkage map consisting of 294 loci {194 simple sequence repeats (SSRs), 86 amplified fragment length polymorphisms (AFLPs) and 14 selective amplifications of microsatellite polymorphic loci (SAMPL)} using a bi-parental recombinant inbred line (RIL) mapping population derived from Rye Selection111 × Chinese Spring. Using the genotypic data and phenotypic data on grain weight (GW) of RILs collected over six environments, genome-wide single locus QTL analysis was conducted to identify main effect QTL. This led to identification of as many as ten QTL including four major QTL (three QTL were stable), each contributing >20% phenotypic variation (PV) for GW. The above study was supplemented with association mapping, which allowed identification of 11 new markers in the genomic regions that were not reported earlier to harbour any QTL for GW. It also allowed identification of closely linked markers for six known QTL, and validation of eight QTL reported earlier. The QTL identified through QTL interval mapping and association mapping may prove useful in marker-assisted selection (MAS) for the development of cultivars with high GW in bread wheat.

Keywords Bread wheat · Grain weight · QTL interval mapping · Association mapping

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Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops worldwide, occupying the largest cultivated area, and supplying 40% of the world's food and 25% of calories for the developing world. Although significant progress has been made during the last 50 years in increasing world wheat production, which reached ~650 Mt in 2010, quality traits including grain weight (GW) did not receive the

desired attention of wheat breeders. GW, an important component of grain yield (GY), has a favorable effect on flour yield, so that improvement in GW continues to be one of the major breeding targets in modern tetraploid (*T. turgidum* ssp. *durum*) and hexaploid (*T. aestivum* ssp. *aestivum*) bread wheat breeding programs (Gegas et al. 2010). Therefore, determination of marker–trait associations for GW through quantitative trait locus (QTL) interval and association mapping, and the subsequent use of associated markers for indirect marker-assisted selection (MAS) for GW is certainly desirable for molecular breeding in wheat.

Only limited studies have been conducted on QTL analysis for GW in the past to identify major QTL suitable for MAS. Several studies involved single marker analysis (SMA; Varshney et al. 2000; Dholakia et al. 2003). In another set of studies, QTL interval mapping was conducted using molecular maps, either for individual chromosomes (Shah et al. 1999; Campbell et al. 2003) or for the whole genome (Groos et al. 2003; Quarrie et al. 2005; Marza et al. 2005). Advanced-backcross QTL (AB-QTL) analysis for different yield components including GW (Huang et al. 2003, 2004) and for the identification of QTL for baking quality traits in two BC_2F_3 populations (Kunert et al. 2007) was also undertaken. A bi-parental mapping population developed especially for GW was earlier used in our laboratory for construction of genetic maps for three chromosomes (1A, 1B and 7A). QTL mapping of these chromosomes led to the identification of three QTL contributing 9.06–19.85% of the phenotypic variation for GW (Kumar et al. 2006). In this paper, we report construction of a whole-genome framework linkage map, and the results of QTL interval mapping undertaken using this map. QTL mapping results were compared with the results of whole-genome association mapping, thus confirming some of the QTL that were either detected by both approaches during the present study or those detected by others elsewhere through QTL mapping.

Materials and methods

Mapping population for linkage mapping

A mapping population consisting of 92 recombinant inbred lines (RILs), derived from a cross between Rye Selection111 (RS; high grain weight) and Chinese Spring (CS; low grain weight), was used in the present study. The above RILs and the two parental genotypes were evaluated at three different locations (Meerut, Pantnagar and Ludhiana) representing three major wheat-growing areas of Northern India over 2 years (2000–2001 and 2001–2002), thus constituting six environments. At each location/year the crop was raised in a replicated randomized complete block design (see Kumar et al. 2006).

Material for association mapping

A set of 230 elite Indian bread wheat cultivars released during 1910–2006 for commercial cultivation in different agro-climatic regions of the country was procured from the Directorate of Wheat Research (DWR), Karnal and used for association mapping (see Mir et al. 2011 for more details of cultivars used). The material included 35 cultivars developed during the pre-1965 period (the pre-green revolution period), and 195 cultivars developed during the post-1965 period (the post-green revolution period). The data for 1,000-grain weight of the above cultivars were collected under a DUS project by DWR, Karnal (Kundu et al. 2006). The cultivars were evaluated for three consecutive crop seasons (2003–2004, 2004–2005 and 2005–2006) in replicated trials and the means of three environments were utilized for association mapping during the present study.

DNA isolation and SSR/AFLP/SAMPL primers for linkage and association mapping

DNA was isolated from the leaves of 1-month-old plants using a modified CTAB method (Saghai-Maroof et al. 1984). For the construction of the whole-genome framework linkage map, a set of 836 simple sequence repeat (SSR) primer pairs (*Xgwm, Xwmc, Xgdm, Xbarc, Xcfd* and *Xcfa*) spread over all the 21 wheat chromosomes were selected from the genetic maps of wheat published earlier (Roeder et al. 1998; Pestsova et al. 2000; Somers et al. 2004; Song et al. 2005). In addition, amplified fragment length polymorphism (AFLP) and selective amplifications of microsatellite polymorphic loci (SAMPL) markers, which were developed using a set of 15 AFLP primer combinations (two *Eco*RI primers, E35 with seven *Mse*I primers and E36 with eight *Mse*I primers) and 14 SAMPL primer combinations (two SAMPL primers,

S6 and S7 each in combination with seven *Mse*I primers) were also used for detection of polymorphism between the two parents of the mapping population.

For association mapping, a set of 215 SSR markers including 48 markers previously known to be linked/associated with the genomic regions harbouring QTL for GW on chromosomes 1A, 1B, 1D, 2A, 2B, 2D, 3B, 4B, 4D, 7A and 7D were used (see Jaiswal et al. 2011 for more details of these markers). Primer sequences for these SSRs are available on GrainGenes 2.0 (http://wheat.pw.usda.gov/ggpages/SSR/WMC/).

SSR, AFLP and SAMPL analyses

SSR analysis

For the development of the framework linkage map, both labeled and unlabeled SSR primers were used, but for association mapping, only unlabeled SSR primers were used. For labeled SSR primers, details of reaction mixture, PCR amplification and data scoring are available elsewhere (Mohan et al. 2009). In the case of unlabeled SSR primers, amplification products were resolved separately and visualized following silver staining. The composition of PCR reaction mixture and PCR cycling profiles for amplification of SSRs are available elsewhere (Roeder et al. 1998). The amplified products were resolved on 10% polyacrylamide gels on a Mega-Gel High Throughput Vertical Unit model C-DASG-400-5013 (C.B.S. Scientific Company, San Diego, CA, USA) (Wang et al. 2003).

AFLP/SAMPL analysis

Restriction digestion, ligation of adapters, pre-amplification and selective amplification for AFLP/SAMPL analysis were carried out using AFLP system I kit (Life Technologies, USA), following Vos et al. (1995) with a few modifications (for details, see Kumar et al. 2006).

Preparation of whole-genome framework linkage map

After a polymorphism survey of the two parents (RS111 and CS) of the mapping population, the genotyping data on 92 RILs for 158 SSR, 299 AFLP and 120 SAMPL polymorphic markers were used to construct a whole-genome framework linkage map using MAPMAKER/EXP v3.0b (Lander et al. 1987). A total of 194 SSR anchor loci belonging to 158 SSRs were first placed on all 21 chromosomes using previously mapped SSRs (Somers et al. 2004) as anchors within linkage groups, and the best order of other markers within groups was determined using the 'compare' command. Also using the 'try' command, 86 AFLP loci and 14 SAMPL loci were added to the above maps (for more details regarding mapping procedure, see Kumar et al. 2006).

QTL analyses (interval mapping)

Single-locus QTL analysis was carried out by inclusive composite interval mapping (ICIM) using IciMapping v2.0 (Li et al. 2007, 2008). The criteria used by Kumar et al. (2009) were followed for declaring the presence of a putative QTL, and for estimating the relative contribution of an individual QTL (R^2). QTL present in $\geq$50% environments were considered as stable; QTL explaining more than 20% phenotypic variation were considered to be major QTL.

Association mapping

Structure analysis

Model-based cluster analysis was performed to infer genetic structure and to define the number of clusters (gene pools) in the dataset using the software STRUCTURE version 2.2 (Pritchard et al. 2000). The number of presumed sub-populations or groups (K) was set from 2 to 20, and the analysis was repeated three to five times. For each run, burn-in and iterations were set to 100,000 and 200,000 respectively, and a model without admixture and correlated allele frequencies was used. The run with maximum likelihood was used to place individual genotypes into groups (sub-populations). Within a group, genotypes with affiliation probabilities (inferred ancestry) $\geq$80% were assigned to a distinct group, and those with $<$80% were treated as 'admixture'; i.e., these genotypes seem to have a mixed ancestry from parents belonging to different gene pools or geographical origins.

Marker–GW associations

Association between markers and grain weight was worked out by using both general linear model (GLM)

and mixed linear model (MLM) approaches using software TASSEL 2.1. The population structure (Q-matrix) was inferred by STRUCTURE 2.2, and kinship matrix (K-matrix) for MLM was developed from the marker data using TASSEL 2.1. For GLM, genotypic data, phenotypic data and Q-matrix only were used; in the case of MLM, on the other hand, K-matrix was also used in addition to genotypic data, phenotypic data and Q-matrix. Significance of marker–trait associations were described using P value ($P \leq 0.05$ for significant markers).

Results

Linkage mapping

Frequency distribution, analysis of variance (ANOVA) and correlations

The frequency distribution of GW (six environments) of the RILs in all environments and in the data pooled over the environments exhibited normal distribution (Kumar et al. 2006). The results of analysis of variance (ANOVA) of GW showed that the mean squares due to RILs, environments and RIL × environment interactions were significant (Table 1). Correlation coefficients between the ranks of RILs (based on GW) in different environments were also positive and significant (see Kumar et al. 2006).

Framework linkage map

The framework linkage map prepared during the present study contained 294 loci (194 SSR + 86 AFLP + 14 SAMPL loci), which were spread over all the 21 different chromosomes, covering a map length of 5211.8 cM, with an average chromosome length of 248.2 cM (range, 129.3–297.5 cM) (ESM table 1). Out of 294 mapped loci, the number of loci on sub-genome A (110 loci) and B (103 loci) did not differ, but were higher than that on the D sub-genome (81 loci). This represented an average number of 15.7 loci/chromosome in the A sub-genome followed by 14.7 loci/chromosome in the B sub-genome and 11.57 loci/chromosome in the D sub-genome. On individual chromosomes, a maximum of 18 loci each were mapped on chromosomes 2A, 2B and 5B and a minimum of eight loci each were mapped on chromosomes 3D and 6D (ESM table 1). The tentative positions of centromeres on the linkage groups of all the 21 chromosomes were assigned following an earlier genetic map (Somers et al. 2004). The genetic map constructed as above was used for QTL interval mapping.

Table 1 Analysis of variance for GW in RILs of GW mapping population of bread wheat grown in six environments

Sources of variation	Degrees of freedom	Mean square
RILs	91	749.70***
Environments	5	5.60***
Replications (environments)	6	0.88
RILs × environments	455	9.80***
Pooled error	730	0.76

*** Significant at $P \leq 0.001$

QTL analysis

Single-locus QTL analysis allowed identification of as many as ten QTL at LOD score values that were above the threshold values (3.25–20.5). The phenotypic variation explained (PVE%) by an individual QTL varied from 4.37 to 23.27%. These included four major QTL (*QGw.ccsu-1A.3*, *QGw.ccsu-5A.1*, *QGw.ccsu-6A.2*, *QGw.ccsu-6B.1*), one each located on chromosomes 1A, 5A, 6A and 6B. However, only three (*QGw.ccsu-1A.3, QGw.ccsu-5A.1, QGw.ccsu-6A.2*) of these four major QTL (located on 1A, 5A and 6A) were stable (detected in three or more environments; see Table 2). The important genomic regions harboring QTL for GW are presented in Fig. 1.

Association mapping

Population structure in Indian wheats

Model-based cluster analysis placed 230 Indian bread wheat cultivars into 13 genetically distinct sub-populations [$K = 13$, having maximum natural log probability (−17,888.6), which is proportional to the posterior probability]. It may be noted that 17 out of the 35 pre-green revolution cultivars were grouped predominantly into two sub-populations (populations

Table 2 QTL analysis for GW of bread wheat by single-locus analysis using QTL IciMapping

Trait/QTL	Environment[a]	Flanking markers[b]	Position	A	LOD	R^2 (%)
QGw.ccsu-1A.1	IV, V	Xgwm136-**Xwmc336**	11–13	2.1	6.90–9.40	4.37–7.05
QGw.ccsu-1A.2	I	Xwmc333-**Xwmc24**	81	2.19	3.95	7.60
QGw.ccsu-1A.3	IV, V, VI	**Xwmc59-**Xgwm99	256	−2.56	5.4–17.8	5.42–23.27
QGw.ccsu-1B.1	I, II, VI, AE	Xwmc419-**E35M4714**	84–85	3.42	3.32–4.64	14.79–19.20
QGw.ccsu-2B.1	IV, V	E35M4710-**E35M479**	78–79	2.57	6.70–18.60	5.00–13.30
QGw.ccsu-5A.1	I, II, III, IV, V, VI	E36M6221-**E36M6211**	281–284	3.21	4.95–20.50	7.65–22.26
QGw.ccsu-6A.2	I, III, IV, V, VI	**Xbarc3-**Xbarc146b	268	−3.92	9.90–18.00	5.77–22.70
QGw.ccsu-6B.1	V	Xbarc24-**Xgwm219**	223	3.66	3.25	21.60
QGw.ccsu-7A.1	I, II, III, IV, VI, AE	E36M6125-**E36M6126**	133–136	−2.92	13.5–13.7	13.10–17.33
QGw.ccsu-7D.1	V	Xgwm295-**Xgwm437**	103	−2.77	20.14	12.41

[a] Environment I = Meerut 2000–01; II = Pantnagar 2000–01; III = Ludhiana 2000–01; IV = Meerut 2001–02; V = Pantnagar 2001–02, VI = Ludhiana 2001–02; AE = pooled data across environments; [b] markers in *bold* represent closest marker of the QTL; Position = distance (cM) between the QTL and the first marker of the relevant chromosome; A = additive effect of the QTL; R^2 (%) = percentage of phenotypic variation explained by the QTL

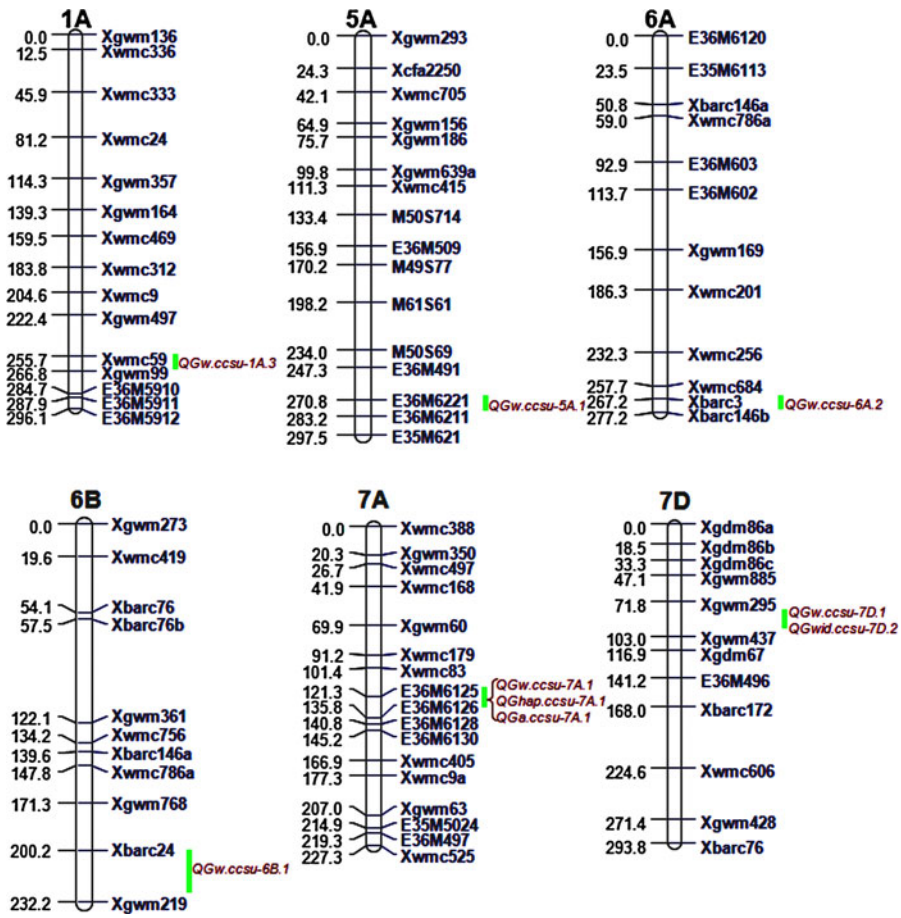

Fig. 1 Important genomic regions harbouring QTL for grain weight. The genomic regions are indicated by a *vertical bar* followed by the name of the QTL/QTL cluster for GW or GW and related traits including grain width (Gwid), grain area (Ga) and grain horizontal axes proportion (Ghap). The marker loci are indicated on the *right*, and the genetic distances (cM) are shown on the *left*

1 and 12), while the remaining 18 cultivars were grouped with post-green revolution cultivars in four of the 11 sub-populations. Of the total number of 230 cultivars, only 13 (5.6%) showed admixtures (membership probability <0.8) and were distributed in five sub-populations (ESM Fig. 1).

Marker–GW associations

During the present study, both whole-genome association and targeted regional association mapping were conducted. The main focus was on some important genomic regions, which were identified through QTL mapping during the present study (see Table 2) and through other earlier studies conducted on GW. Association mapping for GW led to the identification of 25 significant QTL ($P < 0.05$) on 12 chromosomes (Table 3). Fourteen of these QTL (including seven QTL identified during the present study) for GW were also reported through QTL mapping (Table 3). The remaining 11 QTL, to the best of our knowledge, were reported for the first time. In summary, the study allowed validation of eight known markers linked with QTL for GW, identification of six new markers (with relatively tighter linkage) in the genomic regions/marker intervals previously reported to harbour QTL for GW, and 11 markers in genomic regions that were not known to carry any QTL for GW (for details see Table 3).

Discussion

In bread wheat, GW is an important trait contributing to grain quality and yield and has, therefore, drawn major attention from the wheat breeding community all over the world. Genetic dissection of grain traits through QTL interval mapping and association mapping, followed by the use of associated molecular markers for MAS is an actively pursued area of research in wheat genetics and breeding (Kumar et al. 2006; Breseghello and Sorrells 2006; Sun et al. 2009). Several M-QTL have already been identified in earlier studies, either through bi-parental linkage mapping (see Reif et al. 2010) or through association mapping using a worldwide panel of 96 accessions in one study (Neumann et al. 2011) and a collection of 207 European soft winter wheat (*Triticum aestivum* L.) lines in another (Reif et al. 2010). The present study in wheat is the first of its kind where both linkage and association mapping for GW was conducted. This is the first time that wheat germplasm comprising Indian cultivars has been used for association mapping, although similar limited efforts using US, Chinese and European germplasm were made earlier (Breseghello and Sorrells 2006; Yao et al. 2009; Reif et al. 2010; Neumann et al. 2011).

Linkage mapping

GW in wheat is a quantitative trait, controlled by a number of QTL. In earlier studies, although QTL for GW were reported, none of the earlier studies (except those conducted earlier in our own laboratory; see Kumar et al. 2006) involved the use of a mapping population that was specially developed for mapping QTL for GW. Based on this RIL mapping population, a framework linkage map was developed by us for the first time for GW and used for QTL interval mapping. The results of single-locus QTL analysis during the present study are in agreement with the results of earlier studies, suggesting the presence of a few major and many minor QTL for GW in bread wheat (see Kumar et al. 2006). Of the ten QTL identified during the present study, three QTL (*QGw.ccsu-1A.2*, *QGw.ccsu-2B.1*, *QGw.ccsu-7A.1*) were also identified earlier in our laboratory using linkage maps of only three individual chromosomes (1A, 2B, 7A) using the same mapping population (Kumar et al. 2006). Another QTL (*QGw.ccsu-7D.1*) seems to represent a QTL (*QTgw.ipk-7D*) that was earlier identified by Huang et al. (2003, 2004) and later fine-mapped by Roeder et al. (2008), since one of the flanking markers (*Xgwm295*) is common in these studies. The major and stable QTL identified on chromosome 6A (*QGw.ccsu-6A.2*), defined by marker interval (*Xbarc3-Xbarc146b*), has been mapped in the same genomic region where a wheat ortholog (*TaGW2*) of the rice grain development gene (*GW2*) was mapped earlier. *GW2* is responsible for rice grain development (increasing grain width and weight) and its ortholog (*TaGW2*) was recently mapped on wheat chromosome 6A in the marker interval *Xcfd80.2-Xbarc146.1* (Su et al. 2010). The presence of one common marker (*Xbarc146*) with the QTL identified by us during the present study and ortholog of *GW2* clearly suggested that the QTL mapped during the present study may represent *TaGW2*. Three major and stable QTL (*QGw.ccsu-1A.3*, *QGw.ccsu-5A.1* and *QGw.ccsu-6A.2*) identified during the present study may prove useful for MAS for improvement of GW in bread wheat.

From the above, we conclude that the polygenic control of GW in bread wheat, involving only a few major and many minor QTL, limits the chances of success for improvement of GW in bread wheat through classical methods of plant breeding. Hence, the accelerated development of wheat cultivars with

Table 3 Details of markers found to be associated with GW through association mapping and their comparison with present and earlier QTL mapping studies

S. no.	Marker	Chromosome	MLM	GLM		Validation from present/earlier QTL mapping studies
			p-Marker	p-Marker	r^2-Marker	
1	*Xwmc 336*	1A	–	0.017	4.14	The same marker is one of the flanking markers for the QTL (*QGw.ccsu-1A.1*) for GW identified during the present study
2	*Xwmc24*	1A	–	0.0391	4.7	The same marker is one of the flanking markers for the QTL (*QGw.ccsu-1A.2*) for GW identified during the present study
3	*Xgwm99*	1A	0.0334	0.0079	6.81	The same marker is one of the flanking markers for the QTL (*QGw.ccsu-1A.3*) for GW identified during the present study
4	*Xgwm135*	1A	0.0051	0.0121	8.01	New QTL identified for the first time
5	*Xwmc269*	1B	–	0.009	4.67	The marker is mapped in the same genomic region where a QTL (*Gw.ccsu-1B.1*) for GW was identified during the present study
6	*Xgwm413*	1B	–	0.0076	8.31	New QTL identified for the first time
7	*Xgwm425*	2A	0.0439	0.0032	3.65	New QTL identified for the first time
8	*Xbarc164*	3B	0.0023	3.78E–05	9.48	The same marker was also found associated with one of the QTL for TKW by Huang et al. (2006)
9	*Xgwm107*	4B	4.84E–05	3.00E–05	10.24	The same marker was also found associated with one of the QTL for TKW by Huang et al. (2004)
10	*Xwmc48*	4D	0.0058	0.0018	8.15	The same marker is one of the flanking markers for the QTL (*QGwt.crc-4D*) for GW identified by McCartney et al. (2005)
11	*Xwmc516*	4A	0.018	7.83E–04	6.58	The marker is mapped in the same genomic region where a QTL for GW was identified by Quarrie et al. (2005)
12	*Xwmc89*	4D	–	0.0099	2.78	The marker is mapped within the QTL interval of the QTL (*QGwt.crc-4D*) for GW reported by McCartney et al. (2005)
13	*Xwmc 52*	4D	–	0.0098	5.95	The same marker is one of the flanking markers for the QTL (*QTgw.crc-4D*) for GW identified by Huang et al. (2006)
14	*Xwmc399*	4D	0.0362	0.0012	5.66	New QTL identified for the first time
15	*Xgdm109*	5A	–	0.0012	4.27	New QTL identified for the first time
16	*Xgwm415*	5A	0.0086	5.36E–04	8.34	New QTL identified for the first time
17	*Xbarc54*	6D	–	0.0043	6.48	The same marker is one of the flanking markers of the QTL (*QTgw.nfcri-6D*) for GW identified by Wang et al. (2009)
18	*Xwmc593*	7A	0.01	0.0021	5.9	The marker is present within the marker interval of the QTL *(QGw.ccsu-7A.1)* for GW identified by us during present study and QTL (*QTgw.ipk-7A)* for GW identified by Huang et al. (2003)
19	*Xgwm297*	7B	0.0087	5.77E–05	10.49	New QTL identified for the first time
20	*Xwmc335*	7B	6.98E–04	1.22E–05	13.31	New QTL identified for the first time
21	*Xwmc364*	7B	0.0023	6.60E–06	10.65	New QTL identified for the first time
22	*Xwmc396*	7B	0.0362	0.0028	9.05	New QTL identified for the first time
23	*Xwmc475*	7B	0.0063	9.88E–05	9.27	New QTL identified for the first time
24	*Xgwm111*	7D	0.042	–	–	The marker is present within the interval of QTL (*QGw.ccsu-7D*) for GW identified during present study and seems to be the same QTL for GW identified by Huang et al. (2003) and fine mapped by Roeder et al. (2008).
25	*Xgwm44*	7D	0.0044	2.45E–04	8.54	The marker is present within the interval of QTL (*QGw.ccsu-7D*) for GW identified during present study and seems to be the same QTL for GW identified by Huang et al. (2003) and fine mapped by Roeder et al. (2008).

GLM general linear model, *MLM* mixed linear model, *TKW* thousand kernel weight, *GW* grain weight, r^2 phenotypic variation explained by the marker (%)

high GW may be realized through the use of molecular markers linked with high GW in marker-assisted recurrent selection (MARS) or following genome-wide selection (GWS) (Charmet et al. 1999, 2001, Bernardo and Yu 2007).

Association mapping

In the present study, we report the results of whole-genome association mapping, which also includes targeted association mapping of those genomic regions which are known to carry important QTL for GW either through earlier QTL studies or through the present study involving interval mapping. Targeted association mapping for different traits in wheat has also been conducted earlier (see Breseghello and Sorrells 2006; Yao et al. 2009). Selected markers linked with QTL in earlier studies were found to improve the power of association mapping studies to identify more closely linked markers and for fine mapping of important QTL (Breseghello and Sorrells 2006). In this manner, exploitation of complementary strengths and weaknesses of both QTL analysis and association mapping will allow development of efficient markers for molecular breeding programs. The structure analysis during the present study showed genetic divergence/differentiation in the Indian bread wheat cultivars released before and after the green revolution, probably as a result of the selection pressure exercised during the breeding process. The presence of population structure may also indicate an intensive use of elite lines as parents of several crosses. The information gained through population structure analysis was used while conducting association mapping.

The identification of marker–GW associations through association mapping largely confirmed the

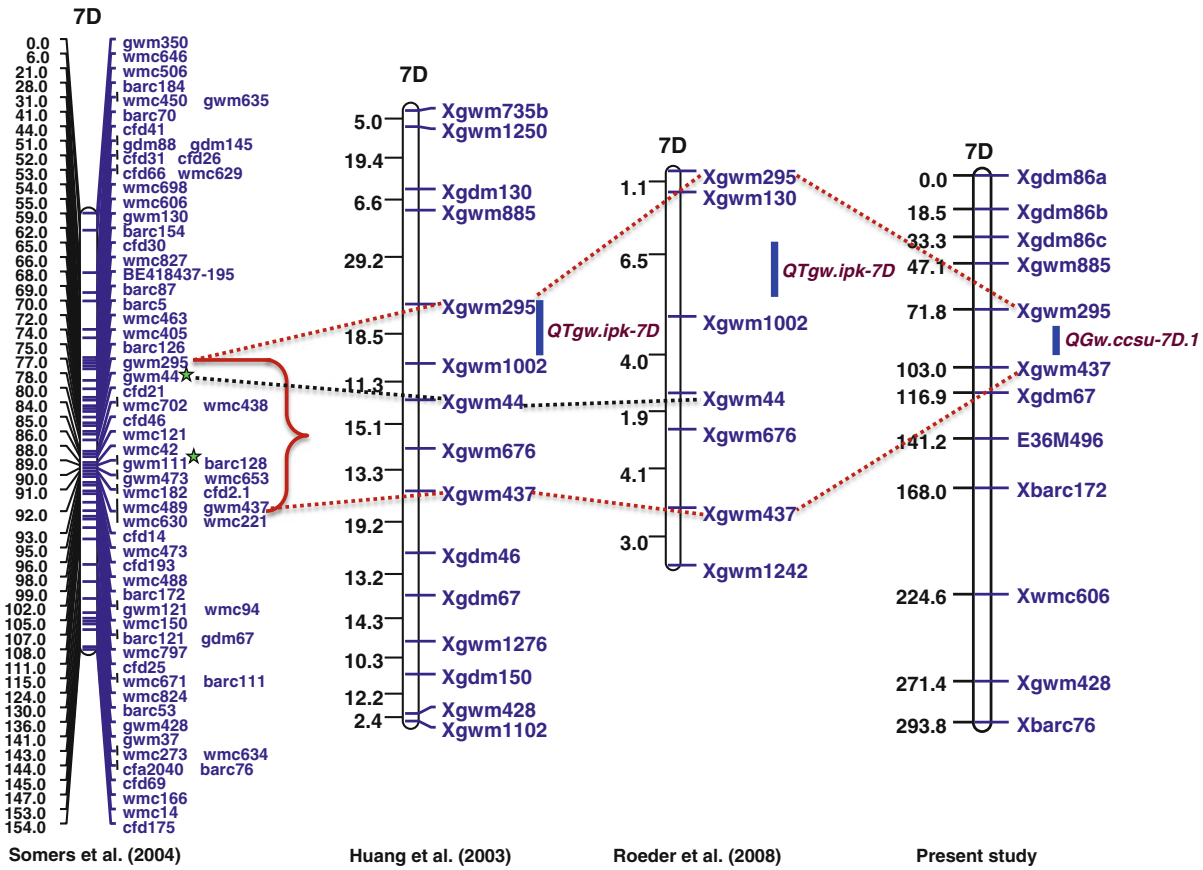

Fig. 2 Comparative map positions of a specific genomic region (indicated by *vertical line*) on chromosome 7D harbouring QTL for grain weight. Using markers from the corresponding genomic region from the map of Somers et al. (2004) in association mapping, two markers (*Xgwm111* and *Xgwm44*) indicated with *stars* showed significant association with GW

results of QTL analysis carried out in our own laboratory and elsewhere, thus validating these earlier results (see Table 3 for details). Of the four associated SSR markers (*Xwmc336*, *Xwmc24*, *Xgwm99* and *Xgwm135*) identified through association mapping on chromosome 1A, *Xgwm99* is a flanking marker of the major and stable QTL (*QGw.ccsu-1A.3*) identified through QTL analysis (Table 2). The marker identified on chromosome 4D (*Xwmc89*) lies within the genomic region that is defined by marker interval *Xwmc617-Xwmc48*, which also contains one QTL each for six important traits (grain weight, test weight, grain yield, plant height, days to maturity and lodging) identified earlier through QTL analysis (McCartney et al. 2005; ESM Fig. 2). Similarly, closely linked markers were also identified for some other QTL including one important QTL on chromosome 7D identified by us (see Tables 2, 3 and Fig. 2). Therefore, these markers not only validated the previously mapped QTL for GW but also provided a more closely linked marker, which may be used fruitfully in MAS. The results of association mapping during the present study confirmed that association mapping has a higher power of resolution than bi-parental linkage mapping. The closely linked markers identified through association mapping may prove efficient for the improvement of GW through MAS. The identification of new QTL for the first time through association mapping may also prove useful for molecular breeding for GW.

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