RESEARCH ARTICLE

QTL mapping for some grain traits in bread wheat (*Triticum aestivum* L.)

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Abstract Grain traits are important agronomic attributes with the market value as well as milling yield of bread wheat. In the present study, quantitative trait loci (QTL) regulating grain traits in wheat were identified. Data for grain area size (GAS), grain width (GWid), factor form density (FFD), grain length-width ratio (GLWR), thousand grain weight (TGW), grain perimeter length (GPL) and grain length (GL) were recorded on a recombinant inbred line derived from the cross of NW1014 × HUW468 at Meerut and Varanasi locations. A linkage map of 55 simple sequence repeat markers for 8 wheat chromosomes was used for QTL analysis by Composite interval mapping. Eighteen QTLs distributed on 8 chromosomes were identified for seven grain traits. Of these, five QTLs for GLWR were found on chromosomes 1A, 6A, 2B, and 7B, three QTLs for GPL were located on chromosomes 4A, 5A and 7B and three QTLs for GAS were mapped on 5D and 7D. Two QTLs were identified on chromosomes 4A and 5A for GL and two QTLs for GWid were identified on chromosomes 7D and 6A. Similarly, two QTLs for FFD were found on chromosomes 1A and 5D. A solitary QTL for TGW was identified on chromosome 2B. For several traits, QTLs were also co-localized on chromosomes 2B, 4A, 5A, 6A, 5D, 7B and 7D. The QTLs detected in the present study may be validated for specific crosses and then used for marker-assisted selection to improve grain quality in bread wheat.

Keywords Quantitative trait loci · Grain traits · Molecular markers · Linkage map

Introduction

All over the world, bread wheat (*Triticum aestivum* L.) is one of the major staple crop and it provides ∼ 20% calories in human diet (http://faostat.fao.org). A primary objective of all major wheat breeding programs is to enhance grain yield in order to ensure global food security. Grain yield has the following three major components: grain number per spike, spike number per unit area and 1000-grain weight (TGW). TGW is positively correlated with grain size (Wu et al. 2016) and is an important trait due to its phenotypic stability and high heritability (Kuchel et al. 2007). Selection for higher TGW has been carried out during domestication process, and therefore it has been argued that in cereals, grain size is a component of domestication syndrome (Fuller 2007; Brown et al. 2009). Despite polygenic control of TGW, phenotypic selection for this trait is effective (Sidwell et al. 1976). However, phenotypic selection is laborious and time consuming. Therefore, the use of molecular markers for indirect marker-assisted selection (MAS) should be a convenient alternative to phenotypic selection. TGW is mainly

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determined by grain length (GL), grain width (GWid) and grain thickness (GT) (Campbell et al. 1999; Dholakia et al. 2003; Breseghello and Sorrells 2006a, b; Sun et al. 2009). Larger grains generally have favorable effect on TGW, seedling vigor and flour yield characteristics (Chastain et al. 1995). In the past, more than 100 QTLs for TGW, GL and GWid in wheat have been identified, which are distributed on all the 21 chromosomes of wheat (Varshney et al. 2000; Börner et al. 2002; Groos et al. 2003; Huang et al. 2003, 2004, 2006; Narasimhamoorthy et al. 2006; Breseghello and Sorrells 2006a, b, 2007; Kumar et al. 2006; Sun et al. 2009; Ramya et al. 2010; Mir et al. 2012; Williams et al. 2013; Okamoto et al. 2013; Patil et al. 2013; Tyagi et al. 2014; Simmonds et al. 2014; Zhang et al. 2015). However, many of the QTLs for TGW are not detectable across environments, explain only a small proportion of the phenotypic variation for grain weight, and also exhibit high QTL × genotype and QTL × QTL epistatic interactions (Campbell et al. 2003; Gupta et al. 2007; Prashant et al. 2012; Patil et al. 2013; Cabral et al. 2018). Together these factors, make such QTL unsuitable for use in MAS for wheat breeding. Also, QTLs for other important grain traits such as grain length-width ratio (GLWR), grain width (GWid), grain area size (GAS), grain perimeter length (GPL) and factor form density (FFD) have been rarely reported (Giura and Saulescu 1996; Dholakia et al. 2003; Sun et al. 2009; Okamoto et al. 2013; Li et al. 2015). Therefore, the present study was designed to conduct QTL analysis for seven important grain traits (TGW, GL, GLWR, GWid, GAS, GPL and FFD) using a RIL mapping population derived from the cross NW1014 × HUW468, so as to identify important QTL and associated markers for improvement of grain traits using MAS during wheat breeding.

Materials and methods

Plant material

A set of 106 RILs derived from the cross NW1014 × HUW468 was used for QTL analysis of seven grain traits in the present study. The seed for the mapping population was procured from Dr. Arun Joshi and colleagues, who developed the mapping population at the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India.

SSR markers

For construction of genetic maps, a set of 376 SSRs (gwm, wmc, swm, barc and cfd) involving only 8 wheat chromosomes (1A, 2B, 4A, 5A, 5D, 6A, 7B and 7D) was tried during the present study, because only these 8 chromosomes are shown to be associated with QTLs for

grain traits repeatedly (Dholakia et al. 2003; Groos et al. 2003; Huang et al. 2006; Kumar et al. 2006; Wang et al. 2009; Gegas et al. 2010; Ramya et al. 2010; Mir et al. 2012; Tyagi et al. 2014; Zhang et al. 2015; Li et al. 2015). Out of a total of 376 SSR markers, 55 SSR markers that were polymorphic between the parental genotypes of the RIL mapping population were used for preparation of the molecular maps. Out of the 55 polymorphic SSR markers, primer sequences of the 38 SSRs are given in Supplementary Table 1. The aliquots for the remaining 17 polymorphic gwm SSRs were kindly provided by Dr. M.S Röder, Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Correnstrasse-3, Germany and their primer sequences are proprietary and hence are not listed in Supplementary Table 1. The primer aliquots for the remaining SSRs were synthesized by Integrated DNA Technologies, (IDT), USA.

Field experiment and recording of data (including genotyping)

RILs of the mapping population (NW1014 × HUW468) and two parental genotypes were evaluated in augmented block design experiments during the crop season 2013–2014 at two locations, namely Ch. Charan Singh University, Meerut located at 28.9664°N and 77.8367°E latitude and longitude and BHU, Varanasi located at 25.3176°N and 82.9739°E latitude and longitude. Standard agronomic practices were followed for raising the crop with application of 120 kg N/ha and the irrigation as per the requirements of the crop.

The data of grain traits were collected on 50 grains each of 106 RILs and the parental genotypes using Smart Grain software ver. 1.2 (Tanabata et al. 2012). Smart Grain uses image to determine grain shape. It automatically recognizes all grains within a digital image, detects outlines, and then calculates all the grain size parameters including grain area size (GAS), grain width (GWid), grain length-width ratio (GLWR), grain perimeter length (GPL) and grain length (GL). TGW in grams (g) was determined by weighing 1000 grains of each of the 106 RILs and the two parental genotypes. The data on grain traits were also utilized for the calculation of factor form density (FFD), $\left[FFD = \frac{TGW}{GL \times GWid} \right]$, which describes the grain density differences and the deviation of the shape from a cylindrical form (Giura and Saulescu 1996).

SSRs were used for genotyping of the mapping population and the two parental genotypes. For this purpose, SSRs were amplified using PCR and the amplified products were resolved on PAGE. The PCR reaction mixture

composition and profile of PCR for amplification of SSRs were the same as given by Röder et al. (1998).

Construction of linkage map

As mentioned above, only 8 wheat chromosomes were used for construction of map and QTL analyses. The linkage maps for individual chromosomes were prepared by MAPMAKER/EXP v3.0b (Lander et al. 1987). The 'Try' and 'Compare' commands were used to anchor additional markers onto the maps.

QTL analysis

QTL Cartographer v 2.5 was used for performing composite interval mapping i.e. CIM (Wang et al. 2005). Threshold LOD was calculated experiment wise on the basis of 1000 permutations (Churchill and Doerge 1994; Doerge and Churchill 1996). QTL were detected for individual environments and also jointly for both the environments. QTL detected in both the environments were considered as stable, and the QTL explaining > 20% PV were considered as major QTL.

Statistical analysis

Values of means, standard deviation, correlation coefficients and box plots showing the distribution of phenotypic data for different traits were determined using SPSS version 20.

Results and discussion

To examine the genetic architecture of the grain weight in wheat, a number of studies involving QTL analyses have been conducted in the past. However, only limited studies involving QTL mapping of other grain traits such as grain area size (GAS), grain width (GWid), grain length-width ratio (GLWR), thousand grain weight (TGW), grain perimeter length (GPL), grain length (GL) and factor form density (FFD) have been conducted (Campbell et al.1999; Ramya et al. 2010; Gegas et al. 2010; Prashant et al. 2012; Williams et al. 2013; Okamoto et al. 2013; Williams and Sorrells 2014; Tyagi et al. 2014; Rasheed et al. 2014; Zhang et al. 2015; Wu et al. 2016). In view of this, the present study involving QTL analyses for seven grain traits in wheat was undertaken. The results pertaining to the phenotypic variation in grain traits in parental genotypes and the RIL population, correlations among different traits in the RIL population and QTL analyses are presented and discussed.

Phenotypic variation in grain traits

The parental genotype HUW468 of the mapping population showed consistently higher mean values for all the grain traits in both the environments except for the GLWR, which was higher in the parental genotype NW1014. The data for different traits in the RIL populations showed a wide range and continuous distributions for each of the seven traits (Fig. 1). This suggested that each of the seven traits is controlled by multiple loci. The range of distribution of data in the first or the fourth quartile of the box

Table 1 Descriptive statistics of the parental genotypes and RILs for different grain traits in two environments

Trait	Environment	Parents		RILs			
		NW1014	HUW468	Range	Average	SD	CV%
Grain area size	E1	12.43	14.09	8.30–13.80	10.85	1.22	11.20
(GAS) (mm^2)	E2	11.90	14.20	4.10–13.30	9.93	1.24	12.49
Grain perimeter length	E1	14.97	16.08	12.30–16.30	14.33	0.81	5.62
(GPL) (mm)	E2	14.50	16.50	6.80–15.30	13.70	0.98	7.19
Grain length	E1	6.01	6.19	4.80–6.40	5.67	0.35	6.23
(GL) (mm)	E2	5.70	6.00	2.60–6.00	5.36	0.41	7.57
Grain width	E1	2.71	3.02	2.10–3.00	2.49	0.19	7.47
(GWid) (mm)	E2	2.80	2.90	1.20–2.90	2.37	0.22	9.39
Grain length–width	E1	2.23	2.06	1.90–3.20	2.30	0.18	7.77
Ratio (GLWR)	E2	2.10	2.00	2.00–2.30	2.01	0.04	2.03
Factor form density	E1	2.70	2.95	1.30–4.10	2.44	0.52	21.43
(FFD)	E2	2.50	2.90	1.30–10.40	2.40	1.07	44.43
1000-grain weight	E1	44.00	55.33	16.00–51.00	34.03	5.67	16.67
(TGW) (g)	E2	43.50	54.30	14.30–65.60	29.80	9.49	31.85

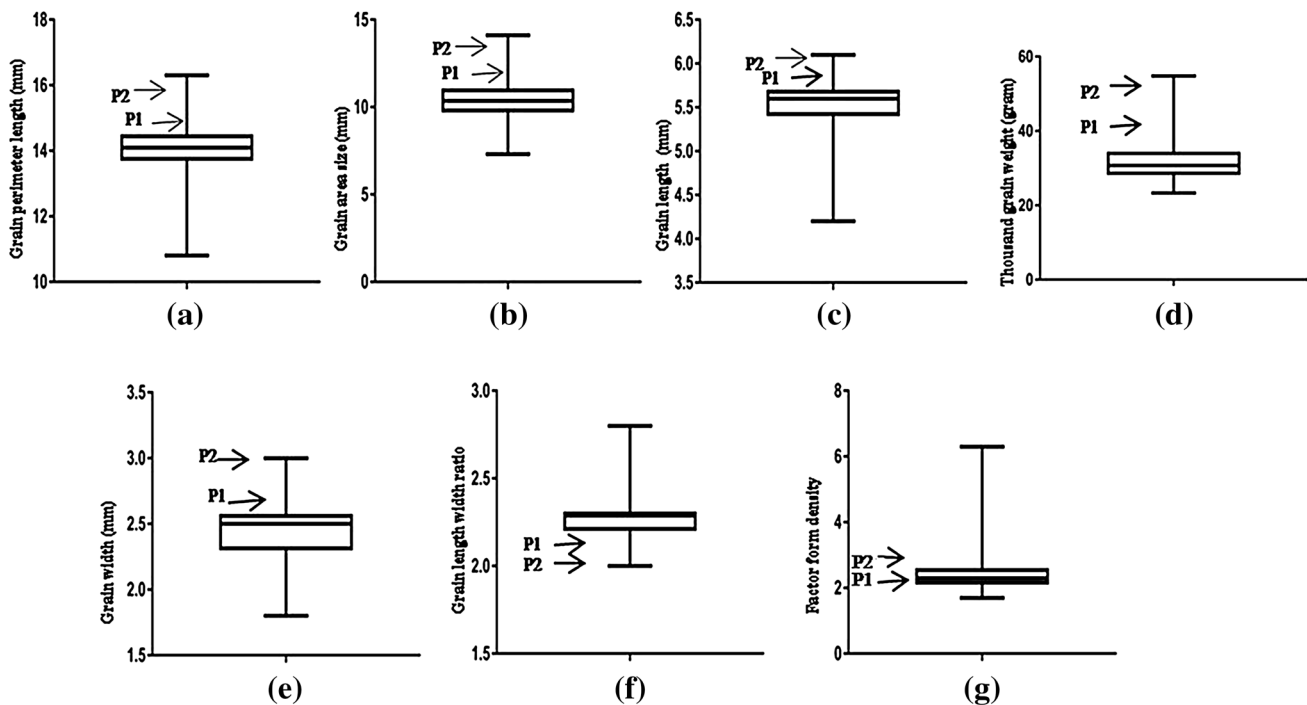*E1* Meerut, *E2* Varanasi, *SD* standard deviation, *CV* coefficient of variance

Fig. 1 Box plots showing the distribution of data of the seven grain traits for 106 RILs derived from the cross NW1014 × HUW468. Arrows labelled as P1 (NW1014) and P2 (HUW468) represent the mean values of the parental genotypes; **a** grain perimeter length; **b** grain area size; **c** grain length; **d** thousand grain weight; **e** grain width; **f** grain length-width ratio; **g** factor form density

plots was much wider for most of the traits, suggesting skewed distribution of the data. Transgressive segregation for most of the traits was observed, where mean values of individual RILs transgressed the mean values of either one or both the parental genotypes (Table 1; Fig. 1). Relatively more variation was witnessed for the following three traits: GAS, FFD and TGW.

Correlations among traits

Details of the correlation coefficient (r) values involving seven grain traits individually at Meerut and Varanasi and also for the pooled data of the two environments are presented in Table 2. Most pairs of traits exhibited significant correlations (negative in some cases) in the individual environments as well as in the pooled data. The correlation of TGW with other traits was inconsistent over the two environments. In the pooled analyses, TGW had positive and significant association with only FFD. Significant positive correlation among GAS, GPL, GL and GWid indicate that these traits together contribute to grain size as reported earlier (Okamoto et al. 2013). However, correlation between these four traits and GLWR were not significant with TGW (except for few weak associations). This suggested that the above five traits and the TGW are each controlled by different sets of loci. This is in agreement with previous reports

indicating that genetic control of these traits is largely independent (Gegas et al. 2010; Kumar et al. 2016).

Significant negative correlation between GLWR and GWid both in the individual environments and in the pooled data indicated that wider grains have reduced GLWR, this type of association may be responsible for grain roundness (Okamoto et al. 2013). Factor form density (FFD) is negatively associated with both GL and GWid, which is in agreement with an earlier report (Dholakia et al. 2003). However, FFD and TGW are positively correlated, which is also in agreement with two earlier reports (Dholakia et al. 2003; Gegas et al. 2010).

SSR polymorphism and construction of linkage maps

As mentioned earlier, 376 SSRs were tried for genotyping, but only 120 SSRs were polymorphic between the two parental genotypes. Fifty five SSRs showed a good fit to 1:1 segregation in the RIL mapping population and the remaining 65 SSRs showed huge segregation distortion and thus the genetic maps were prepared using genotyping data of 55 SSRs only. The lengths of genetic maps for individual chromosomes ranged from 154.3 cM (chromosome 4A) to 460.9 cM (chromosome 2B) and number of mapped SSR loci ranged from three (chromosome 4A) to 10 (chromosome 2B).

Table 2 Correlation coefficients among seven grain traits in the RIL population of bread wheat in two different environments as well as in pooled data over the environments

	GAS	GPL	GL	GWid	GLWR	FFD
1. GPL						
E1	0.85**					
E2	0.85**					
AE	0.84**					
2. GL						
E1	0.77**	0.97**				
E2	0.78**	0.97**				
AE	0.76**	0.96**				
3. GWid						
E1	0.92**	0.68**	0.56**			
E2	0.93**	0.76**	0.65**			
AE	0.93**	0.74**	0.61**			
4. GLWR						
E1	− 0.28**	0.20**	0.34**	− 0.51**		
E2	− 0.06	− 0.16	− 0.16	− 0.1		
AE	− 0.40**	0.04	0.21	− 0.56**		
5. FFD						
E1	− 0.55**	− 0.49**	− 0.48**	− 0.52**	0.19*	
E2	− 0.39**	− 0.59**	− 0.58**	− 0.39**	0.15	
AE	− 0.48**	− 0.53**	− 0.52**	− 0.49**	0.12	
6. TGW						
E1	0.04	0.09	0.06	0.01	0.16	0.79**
E2	0.25*	0.12	0.08	0.26**	− 0.02	0.64**
AE	0.09	0.13	0.1	0.09	0.02	0.66**

E1 Meerut, *E2* Varanasi, *AE* pooled data of the two environments, *GAS* grain area size, *GPL* grain perimeter length, *GL* grain length, *GW* grain width, *GLWR* grain length-width ratio, *FFD* factor form density, *TGW* thousand grain weight

*Significant at $P = 0.05$; **significant at $P = 0.01$

The linkage maps for each of the six chromosomes 2B, 4A, 5A, 5D, 6A and 7B carried large gap between the mapped markers making the above maps relatively longer. The large gaps may either represent genomic regions that lack polymorphism among the parental genotypes of the RIL population or these gaps may also be due to exclusion of markers showing segregation distortion. The (unobservable) distorted segregation of these loci causes the observed markers to deviate from the normal Mendelian segregation ratio (1:1) of the RIL population. In all kinds of mapping populations like F_2, DH and RILs, segregation distortion generally occurs. Among these mapping populations, RILs exhibit the maximum probability of distortions due to continued selfing for 5–6 generations (Singh et al. 2007). In addition several studies reported deviation from the expected Mendelian segregation ratios in wheat (Faris et al. 2000; Peng et al. 2000; Kumar et al. 2007), rice (Harushima et al. 1996; Xu 1997; Lyttle 1991), barley (Graner et al. 1991; Kleinhofs et al. 1993; Devaux et al. 1995) and maize (Wendel et al. 1987; Dufour et al. 2001; Lu et al. 2002).

QTLs for grain traits

Eighteen QTLs were detected for the seven grain traits following analyses of data of two individual environments (Meerut and Varanasi) and the data pooled over both the environment (AE) (Table 3; Fig. 2). The LOD score values for the individual QTLs ranged between 1.83 and 10.85, and the 18 QTLs were located on eight chromosomes. Fourteen of these QTLs were detected in Meerut environment and only 4 QTLs were detected in Varanasi environment and none of the QTL was detected in both the environments. This suggested significant QTL × environment interaction, as also reported in some previous studies (Campbell et al. 2003; Kumar et al. 2009).

The number of QTLs mapped on individual chromosomes ranged from two QTLs each on chromosomes 1A, 4A, 5A, 6A, 7B and 5D to three QTLs each on chromosomes 7D and 2B (for details see Table 3; Fig. 3). Individual QTLs explained 7.70–63.66% of PV for the concerned trait. Twelve QTLs with PV exceeding 20% were considered as major QTLs. These included two QTLs

Table 3 QTLs for grain traits detected by composite interval mapping in a RIL population of bread wheat

Trait/QTL	Environment[a]	Flanking markers[b]	Position[c]	LOD	A[d]	R² (%)[e]
1. Grain length						
QGl.ccsu-4A.1	E1	*Xgwm397-Xgwm601 (Xgwm397)*	25.00	2.40	− 0.26	53.00
QGl.ccsu-5A.1	E1	*Xgwm126-Xwms1171 (Xgwm126)*	33.00	3.36	0.26	53.84
2. Grain width						
QGwid.ccsu-7D.1	E1, AE	*Xgwm635-Xgwm37 (Xgwm37)*	372.50, 379.50	2.28, 2.45	− 0.13, 0.10	47.12–33.29
QGwid.ccsu-6A.1	E2	*Xwmc398-Xgwm169 (Xwmc398)*	0.00	2.08	0.06	7.66
3. Grain length width ratio						
QGlwr.ccsu-1A.1	E1	*Xgwm99-Xgwm633 (Xgwm99)*	228.70	2.96	− 0.09	13.87
QGlwr.ccsu-2B.1	E2	*Xgwm972-Xgwm148 (Xgwm972)*	99.20	2.30	− 0.08	17.80
QGlwr.ccsu-2B.2	E1, AE	*Xgwm410-Xgwm429 (Xgwm410)*	110.10, 109.10	2.08, 2.22	− 0.05, 0.04	7.98–7.74
QGlwr.ccsu-6A.1	E2	*Xwmc398-Xgwm169 (Xwmc398)*	18.00	2.45	− 0.12	36.70
QGlwr.ccsu-7B.1	E1	*Xgwm146-Xgwm573 (Xgwm146)*	130.60	3.28	0.44	21.83
4. Grain perimeter length						
QGpl.ccsu-4A.1	E1	*Xgwm397-Xgwm601 (Xgwm397)*	27.00	2.50	− 0.60	52.50
QGpl.ccsu-5A.1	E1	*Xgwm126-Xwms1171 (Xgwm126)*	43.00	2.53	0.61	55.86
QGpl.ccsu-7B.1	E2	*Xgwm146-Xgwm573 (Xgwm146)*	130.60	10.85	3.45	41.62
5. Grain area size						
QGas.ccsu-5D.1	E1	*Xwmc318-Xcfd3 (Xcfd3)*	335.90	2.29	− 0.94	58.15
QGas.ccsu-7D.1	E1	*Xbarc 0092-Xgwm635 (Xgwm635)*	321.60	2.58	− 0.91	52.91
QGas.ccsu-7D.2	E1	*Xgwm635-Xgwm37 (Xgwm635)*	368.50	2.57	− 0.84	63.66
6. Factor form density						
QFfd.ccsu-1A.1	E1	*Xwmc611-Xwmc93 (Xwmc611)*	119.5	1.83	0.33	7.70
QFfd.ccsu-5D.1	E1	*Xwmc318-Xcfd40 (Xcfd40)*	379.9	1.85	0.31	7.90
7. Thousand grain weight						
QTgw.ccsu-2B.1	E1	*Xgwm972-Xgwm148 (Xgwm972)*	84.7	2.98	− 3.14	30.13

[a]*E1* Meerut, *E2* Varanasi, *AE* pooled data of the two environment

[b]Markers in parentheses represent closest marker of the QTL

[c]Position = distance (cM) between the QTL and the first marker of the relevant chromosome

[d]Additive effect of the QTL (a positive value indicates the HUW468 allele having a positive effect on the trait and negative value indicate that the NW1014 allele having a positive effect on the trait), [e] Percentage of phenotypic variation explained (PVE) by the QTL

each for both GL and GLWR, three QTLs each for both GPL and GAS and one QTL for both TGW and GWid (Table 3).

In pooled data as well as in Meerut environment, two QTLs, one each for both GWid and GLWR were detected. Out of these two QTLs, QTL for GWLR (*QGlwr.ccsu-2B.2*) is a minor QTL, since it explained only up to 7.98% PV, but the QTL for GWid (*QGwid.ccsu-7D.1*) located in marker interval *Xgwm635-Xgwm37* is a major QTL (range of PVE = 33.29–47.12%). This particular QTL is the most important among all the QTLs identified in this study. The allele for higher GWid for this QTL was contributed by the parental genotype HUW468.

Co-localized QTLs for grain traits

In earlier studies also, the QTLs that are co-located for grain related traits were reported (Peng et al. 2003; Groos et al. 2003; Marza et al. 2006; Quarrie et al. 2005). In our study, maximum number of QTLs for different traits were co-located and grouped in particular genomic regions in the chromosomes 4A, 5A, 6A, 2B, 7B, 5D and 7D (except chromosome 1A). For instance, out of the two QTLs for GL, one QTL each was co-located with two separate QTLs for GPL. One QTL for GWid and GPL were co-located with separate QTLs for GLWR and similarly a QTL for GWid was co-located with QTL for GAS. A QTL each for TGW and GAS were co-located with a QTL for GLWR and FFD, respectively. These co-located QTLs may
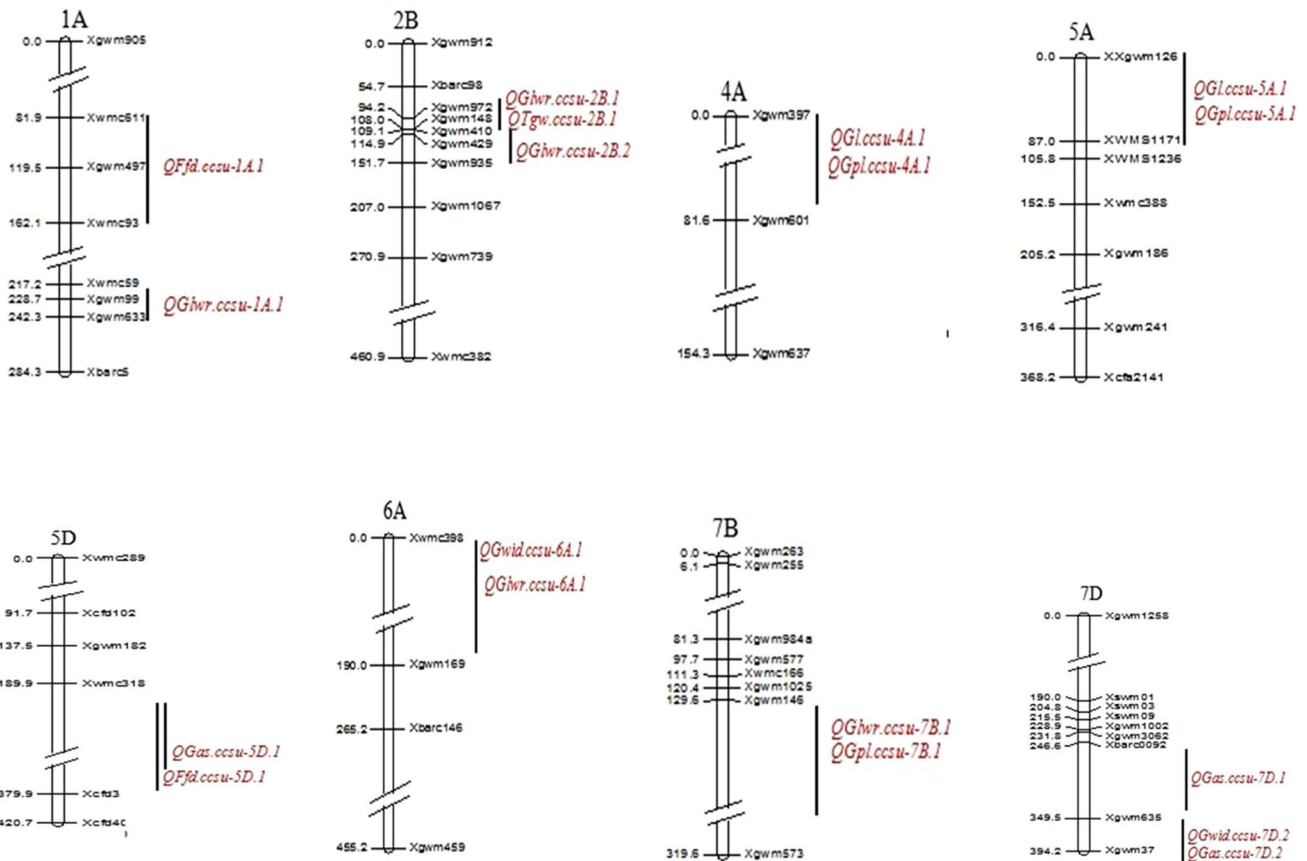
Fig. 2 Important genomic regions harbouring QTLs for grain traits in bread wheat detected using RIL mapping population derived from the cross NW1014 × HUW468. In each case, the genomic region containing the QTL is indicated by black vertical bar followed by the name of the QTL (highlighted with red color). The marker loci are indicated on the right, and the genetic distances (cM) are shown on the left (color figure online)

Fig. 3 A representative QTL Cartographer plot for QTL (*QGPl.ccsu-7B.1*) for grain perimeter length identified on chromosome 7B of bread wheat
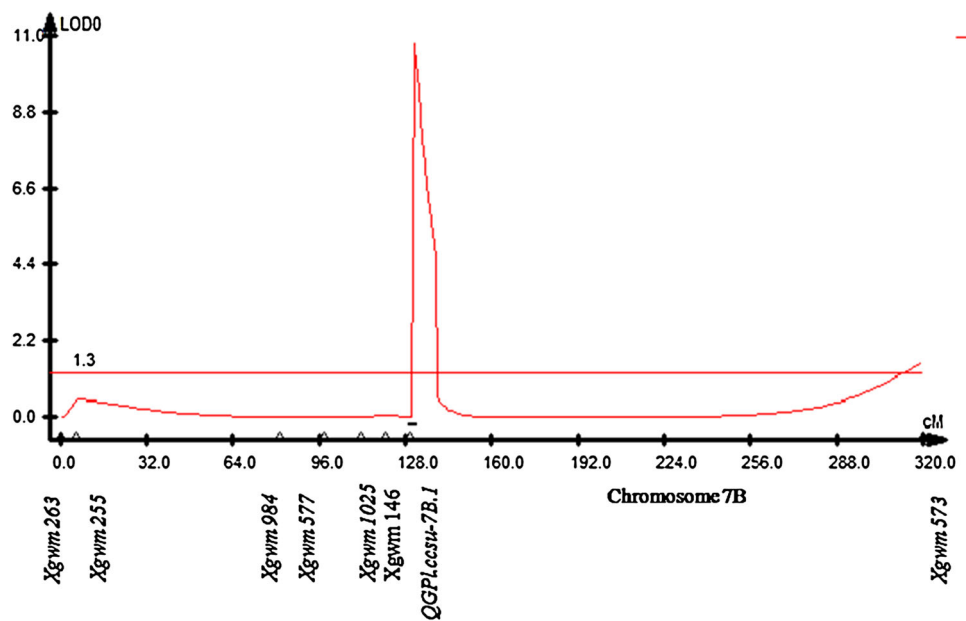
represent pleiotropic QTLs for more than one trait (for details see Table 4).

As stated above, the two QTLs, one each for GL (*QGl.ccsu-4A.1*) and GPL (*QGPl.ccsu-4A.1*) were co-

Table 4 Co-localized QTLs for different grain traits in wheat

Chromosome	Traits for co-localized QTL	Marker interval	Parental allele
2B	GLWR and TGW	*Xgwm972-Xgwm148*	−/−
4A	GL and GPL	*Xgwm397-Xgwm601*	−/−
5A	GL and GPL	*Xgwm126-Xwms1171*	+/+
6A	Gwid and GLWR	*Xwmc398-Xgwm169*	+/−
5D	GAS and FFD	*Xwmc318-Xcfd3–Xcfd40*	−/+
7B	GLWR and GPL	*Xgwm146-Xgwm573*	+/+
7D	Gwid and GAS	*Xgwm635-Xgwm37*	−/−

GLWR grain length-width ratio, *TGW* thousand grain weight, *GL* grain length, *GPL* grain perimeter length, *GWid* grain width, *GAS* grain area size, *FFD* factor form density, + sign = HUW468 allele contributed for increased trait value, − sign = NW1014 allele contributed for increased trait value

localized on chromosome 4A. The closest marker *Xgwm397* associated with these co-localized QTLs was also earlier reported to be the closest marker to the QTL for GL and VP (vertical perimeter) (Williams et al. 2013; Breseghello and Sorrells 2007). Each of these two co-localized QTLs were detected only in the Meerut environment, although each QTL explained > 50% of the PV for the traits. The positive alleles for the above two QTLs were contributed by the parental genotype NW1014 and therefore QTL alleles from the parental genotypes NW1014 have the potential for exploitation in MAS for improvement of GL and GPL traits in wheat. Although none of these two traits are significantly correlated with TGW. This suggested that these traits were controlled by independent loci which is in agreement with an earlier report by Gegas et al. (2010).

Similarly, two other QTLs, one each for GL (*QGl.ccsu-5A.1*) and GPL (*QGPl.ccsu-5A.1*) located on chromosome 5A were also co-located and the marker closest to these QTLs is *Xgwm126*. These two QTLs were also detected at Meerut environment and each QTL also explained > 50% PV for the respective traits. A comparison of the genetic map of chromosome 5A prepared during the present study with that reported by Ganal and Röder (2007) showed that the above two QTLs and the QTL for GL (*QGl.ccsu-5A.1*) earlier identified by Tyagi et al. (2014) and Ramya et al. (Ramya et al. 2010) were present in the adjacent regions, suggesting that the genomic regions harbouring these QTLs possess genes that control GL and GPL in wheat. Although, the marker interval containing the above two co-located QTLs is more than 80 cM. However, this large interval between the two flanking markers may be due to absence of polymorphic markers among the markers tried during the present study or the failure to map polymorphic markers in this region due to segregation distortion. Hence, there is need to screen more markers in future studies so as to find more polymorphic markers for this region in order to map additional markers to more precisely map the QTLs in this region. Similar to above co-localized QTLs for GL and

GPL on chromosome 4A, the co-localized QTLs for these two traits on chromosome 5A are also important for use in MAS for improving grain traits in wheat. However, the positive alleles for these two QTLs were contributed by the parental genotype HUW468.

A QTL each for GWid (*QGwid.ccsu-7D.1*) and GAS (*QGAs.ccsu-7D.2*) were mapped in the marker interval *Xgwm635-Xgwm37*. Marker *Xcfd69* associated with QTLs for GWid and TGW reported by Williams et al. (2013) was located at 4 cM from the marker *Xgwm37* in the consensus map of Somers et al. (2004). Further, a gene for grain size in wheat (*TaGS-D1*), which is an ortholog of rice gene *OsGS3* affecting grain length and weight in wheat is located adjacent to the co-localized QTL discussed above in the present study (Zhang et al. 2014). Thus, the genomic region encompassed by the markers *Xgwm635* and *Xcfd69* seems to be important for controlling grain traits. Further, in the consensus map of Somers et al. (2004), the marker *Xgwm 37* associated with the above co-located QTL for GWid (*QGwid.ccsu-7D.1*) and GAS (*QGAs.ccsu-7D.2*) was placed adjacent to the marker *Xwmc273* at a distance of 2 cM. The marker *Xwmc273* was reported to be associated with the grain filling rate (GFR) in wheat under heat stress using bulked segregant analysis (Barakat et al. 2012). The physiological mechanisms underlying GFR have been widely explored (Egli et al. 1989), and the GFR plays an important role in determining grain size and consequently the grain yield. Therefore, the genomic region harbouring the markers *Xgwm635*, *Xgwm37*, *Xwmc273* and *Xcfd69* could be exploited for improvement of grain traits during wheat breeding.

Two QTLs, one each for GLWR (*QGlwr.ccsu-7B.1*) and GPL (*QGpl.ccsu-7B.1*) were mapped on chromosome 7B in the marker interval *Xgwm146-Xgwm573* during the present study. Two QTLs (*QPv.sdau-7B* and *QPet.sdau-7B*) for starch traits were also reported on chromosome 7B in an earlier study by Sun et al. (2008). On the consensus map reported by Somers et al. (2004), the markers *Xgwm644* and *Xgwm 577* associated with the QTLs

QPv.sdau-7B and *QPet.sdau-7B* for starch traits are at a distance of 6 cM and 13 cM from the markers *Xgwm573* and *Xgwm 146,* respectively, flanking the above QTLs for GLWR and GPL reported during the present study. Hence the genomic region on the chromosome 7B harbouring the above QTLs is important as it controls GLWR, GPL and also starch traits, which contributes to grain processing and end-use quality (Hurkman et al. 2003; Yamamori and Quynh 2000; Tetlow et al. 2004; Yamamori 2009).

The closest marker (*Xcfd3*) to the QTL for GAS (*QGAs.ccsu-5D.1*) reported during the present study and marker *Xcfd81* closest to the QTL for GAS reported by Okamoto et al. (2013) on chromosome 5D are located at an interval of 29 cM in the consensus map of Somers et al. (2004). Therefore, the QTL for GAS identified in the current study is different than the QTL for GAS identified by Okamoto et al. (2013). However, the marker *Xcfd3* that is closest to the QTL *QGAs.ccsu-5D.1* for GAS is also reported to be associated with a QTL, *QGfd.nfcri-5D* for grain filling duration (GFD) (Wang et al. 2009). Since the grain filling is a crucial and dynamic process, its duration and rate determine the grain related traits such as grain size, grain weight and consequently the GFD indirectly determines grain yield in wheat (Gebeyehou et al. 1982; Li and Pan 2005). Hence, the genomic region harbouring the above QTLs on chromosome 5D seems to be important in controlling grain traits as well as physiological processes such as GFD and are thus valuable for use in MAS.

Two QTLs are located on chromosome 6A, one each for GWid (*QGwid.ccsu-6A.1*) and GLWR (*QGLwr.ccsu-6A.1*) and their closest marker is *Xwmc398*. In an earlier study by Su et al. (2011), a wheat ortholog (*TaGW2*) of rice gene *GW2* was mapped on chromosome 6A and the closest marker to this gene is *cfd 80.2*. This marker is co-located with the marker *Xwmc398* that is closest to the above QTLs for GWid and GLWR and thus, it is possible that the above two QTLs may represent the *TaGW2* for grain weight in wheat. The QTL for GLWR also explained ∼ 37% PV for the trait making this an important candidate for use in MAS.

Relative to the QTLs for above discussed traits, one QTL each for FFD were mapped on chromosomes 1A and 5D, both these QTLs were minor as they explained 7.70 and 7.90% PV. One of these QTLs, namely *QFfd.ccsu-5D.1* was flanked closely by the marker *Xcfd40*. This particular marker was also closest to the QTL (*QKl.ncl-5D.1*) for GL reported by Ramya et al. (2010). The marker *Xwmc93* flanking the QTL (*QFfd.ccsu-1A.1*) detected during present study was co-located with *Xwmc312* in the consensus map of Somers et al. (2004). The marker *Xwmc312* was reported to be linked with QTL for TGW, GWid, GL and GLW (Li et al. 2015). Therefore, the genomic regions close to these QTLs are important in

controlling several grain traits in wheat. Similarly, *Xgwm 99*, the closest marker to the QTL for GLWR (*QGlwr.ccsu-1A.1*) reported in this study was earlier shown to be associated with a QTL for TGW by Elangovan et al. (2011) and Mir et al. (2012). Hence the genomic region on the chromosome 1A is important as it controls different grain traits.

In a region adjacent to QTL for GLWR (*QGlwr.ccsu-2B.2*), a QTL for TGW (*QTkw.ncl-2B.1*) was reported by Ramya et al. (2010). A QTL for TGW (*QTgw.ccsu-2B.1*) was also found on chromosome 2B in a genomic region flanked by markers *Xgwm 972* and *Xgwm148*. This particular QTL explained 30.13% PV and hence is a major QTL controlling TGW. This QTL and the QTL for TGW (*QKw.ncl-2B.2*) reported by Ramya et al. (2010) were present adjacent to each other in the consensus map of Somers et al. (2004). The desirable allele for higher TGW for this QTL was contributed by the parental genotype NW1014, which may be exploited in MAS for improvement of grain traits.

Two QTL for GLWR (*QGlwr.ccsu-2B.1* and *QGlwr.ccsu-2B.2)* and a QTL for TGW (*QTgw.ccsu-2B.1*) were co-located in the genomic region flanked by markers *Xgwm972* and *Xgwm 148* in the present study. A QTL (*QFLL-2B*) for flag leaf length (FLL) earlier reported on chromosome 2B by Liu et al. (2018) was flanked by markers *Xbarc 318* and *Xwmc 344*. Markers *Xgwm972* and *gwm*148 flanking the above three QTL for grain traits and the marker *Xwmc344* associated to the QTL for FLL are located in adjacent region in the consensus maps of Somers et al. (2004) and Ganal and Röder (2007). Hence, the above genomic region of 2B covering QTLs for GLWR, TGW and FLL is an important region as the flag leaf in wheat play an important role in photosynthesis and contributes 45–58% of photosynthates during the grain-filling stage (Khaliq et al. 2008). FLL is also strongly positively correlated with spike length, kernel number, and weight per spike, indicating that larger flag leaves contribute to the yield-related traits (Liu et al. 2018).

Two QTLs, one each for GLWR (*QGlwr.ccsu-7B.1*) and GPL (*QGpl.ccsu-7B.1*) were novel and reported in the present study for the first time.

Conclusions

QTL analysis for grain traits provides a basis for improvement of grain quality in wheat using marker-assisted selection. Present study provides information on genomic regions regulating grain size in wheat. Out of a number of QTLs for different traits reported during the present study, a QTL for GWid (*QGwid.ccsu-7D.1*) was stable and explained 33.29–47.12% PV. This QTL was co-located with a QTL for GAS (*QGas.ccsu-7D.2*), which

explained 63.66% PV for grain area size. Therefore, markers *Xgwm635* and *Xgwm37* flanking these QTLs could be used for MAS for improving the grain quality in bread wheat.

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