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ORIGINAL ARTICLE

Identification of milling and baking quality QTL in multiple soft wheat mapping populations

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Abstract
Key message **Two mapping approaches were use to identify and validate milling and baking quality QTL in soft wheat. Two LG were consistently found important for multiple traits and we recommend the use marker-assisted selection on specific markers reported here.**
Abstract Wheat-derived food products require a range of characteristics. Identification and understanding of the genetic components controlling end-use quality of wheat is important for crop improvement. We assessed the underlying genetics controlling specific milling and baking quality parameters of soft wheat including flour yield, softness equivalent, flour protein, sucrose, sodium carbonate, water absorption and lactic acid, solvent retention capacities in a diversity panel and five bi-parental mapping populations. The populations were genotyped with SSR and DArT markers, with markers specific for the 1BL.1RS translocation and sucrose synthase gene. Association analysis and composite interval mapping were performed to identify quantitative trait loci (QTL). High heritability was observed for each of the traits evaluated, trait correlations were consistent over populations, and transgressive segregants were common in all bi-parental populations. A total of

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26 regions were identified as potential QTL in the diversity panel and 74 QTL were identified across all five bi-parental mapping populations. Collinearity of QTL from chromosomes 1B and 2B was observed across mapping populations and was consistent with results from the association analysis in the diversity panel. Multiple regression analysis showed the importance of the two 1B and 2B regions and marker-assisted selection for the favorable alleles at these regions should improve quality.

Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop in the world and has specific quality parameters that are required for particular end-uses. In the broadest terms, major wheat quality traits are defined by gluten strength, water absorption, and milling quality (governed by flour yield and flour particle size). There are two basic classes of wheat, hard and soft. Hard wheat is characterized by strong gluten, extensive starch damage during milling, and high water absorption while soft wheat tends to have weaker gluten and low starch damage and water absorption.

Smith et al. (2011) reviewed some of the biochemistry and genetics of wheat quality. Quality parameters in soft wheat tend to have moderate-to-high heritability (Reif et al. 2011a; Smith et al. 2011; Souza et al. 2012) and are controlled by a few major genes such as the high molecular weight glutenin loci (*Glu*) (Shewry and Halford 2002) and genes with small effects (Reif et al. 2011a; Smith et al. 2011). Several mapping studies have been conducted to locate QTL associated with milling and/or baking quality of wheat (Arbelbide and Bernardo 2006; Breseghello et al. 2005; Breseghello and Sorrells 2006; Campbell et al. 2001; Groos et al. 2003; Huang et al. 2006; Kuchel et al. 2006; Kunert et al. 2007; Ma et al. 2007; Mares and Campbell 2001; McCartney et al. 2006; Nelson et al. 2006; Parker et al. 1999; Prasad et al. 2003; Schmidt et al. 2004). Collectively, these studies identified QTL on 20 of the 21 wheat chromosomes. However, nearly all of these studies were conducted using hard wheat populations. In soft wheat, Breseghello and Sorrells (2006) reported few significant marker associations with milling traits, with the most significant association located in chromosome 5B. Smith et al. (2011) reported large effect QTL for quality on chromosomes 1B and 2B in a Foster by Pioneer 25R26 population. Their QTL effects from chromosome 2B were located in a region that contains the *TaSus2-2B* (*Sus2*) locus which encodes a sucrose synthase enzyme (Jiang et al. 2011) and may be the cause of the reported QTL effects in that population.

Soft wheat quality is commonly assessed by solvent retention capacity (SRC, AACC 56-11; AACC 2000) tests in order to predict commercial baking performance (Souza et al. 2012). The SRC values are the weight of a solvent retained by the flour after centrifugation and draining, and are expressed as a ratio of the original sample weight. The water SRC (WA) is a test of water absorption, the sodium carbonate SRC (SO) is a test for the level of damaged starch, the sucrose SRC (SU) is a test for arabinoxylan and partially hydrated gliadin content, and the lactic acid SRC (LA) is a measure of gluten strength. The SRC tests in combination with tests for flour yield (FY, a percentage of flour extracted during milling) and softness equivalent (SE, flour particle size test that is correlated to break flour yields) have been shown to be highly heritable, repeatable, and of reliable use for making genetic gains in soft wheat quality (Souza et al. 2012; Walker et al. 2008).

Multiple mapping studies are required to identify and validate QTL for complex traits. The genetic architecture of wheat quality traits has been frequently studied through classical linkage mapping in bi-parental populations (Campbell et al. 2001; Elangovan et al. 2008; Li et al. 2013; McCartney et al. 2006; Nelson et al. 2006; Parker et al. 1999; Prasad et al. 2003; Reif et al. 2011b; Schmidt et al. 2004; Sun et al. 2008, 2010). Mapping in these populations provides unequivocal identification of the parental source of a QTL. However, time and resources are required to develop these populations, which often still have extensive linkage disequilibrium (LD) resulting in identification of marker loci distant from the functional loci. In addition, only two alleles are contrasted providing a small frame of inference in a limited genetic background. As an alternative, association analysis can elucidate the genetic basis of complex traits (Yu et al. 2006). It allows the assessment of multiple alleles using existing breeding populations, often with lower LD due to multiple recombination events. Despite, higher probability of type I errors due to population structure (Yu et al. 2006), insufficient marker coverage due to low LD, and the inability to identify the parental source of the desired alleles (Sneller et al. 2009), association analysis takes advantage of populations already developed within a breeding program to identify QTL (Ishikawa et al. 2014).

Very few studies have been done on QTL discovery of quality traits in soft wheat. In this study we integrated two mapping approaches to study the genetic architecture of soft wheat quality. Our objectives were to: (1) screen for potential QTL in a panel of diverse lines using an association mapping approach; (2) further validate and identify new QTL in multiple bi-parental populations, and (3) assess the value of a marker for the *Sus2* locus in mapping QTL for soft wheat quality. For objectives 2 and 3, we report results from three new populations and remapping of chromosome 2B with *Sus2* marker in two populations previously described by Smith et al. (2011). The results from this study will provide breeders with robust tools to facilitate selection of progenies with better quality traits.

Materials and methods

Populations

We integrated the mapping of quality traits in a diversity panel of 187 lines selected as described by Souza et al. (2012) and characterized by Cabrera et al. (2014); and five soft red winter wheat (SRWW) bi-parental populations (Table 1). The results obtained were compared to published results (Smith et al. 2011). Three of the five bi-parental populations have Foster, a good quality cultivar, as one parent and were specifically generated to map quality traits. The parent Pioneer 25R26 has strong gluten. The parents Kanqueen, Hopewell and Pioneer 25R26 present different degrees of quality traits (poor, moderate and good quality, respectively). Foster is known to carry both the 1BL.1RS rye translocation (McKendry et al. 1996) on chromosome 1B and the *Triticum timopheevi* translocation on chromosome 2B (Tsilo et al. 2008). Both translocations segregate in all three Foster-derived populations. Pioneer 25R26 carries the *Glu-B1al* allele, which over expresses the Bx7 peptide (Ragupathy et al. 2008) and increases gluten strength. The F/P and F/H populations were first evaluated by Smith et al. (2011). In this work, both populations were genotyped with *Sus2* marker to perform new QTL analysis on chromosome 2B and assess the importance of *Sus2* marker across all segregating populations. The F/K population was developed to validate the Foster QTL identified in the F/P population (Table 1). The fourth population (9/9 in Table 1) was created by crossing two Purdue university SRWW breeding lines with moderated quality to map QTL for resistance to Stagonospora norodum blotch (SNB) (Uphaus et al. 2007). The fifth population was obtained from the cross of USG 3209 by Jaypee (U/J) and was initially used to map QTL for resistance to powdery mildew and adult plant resistance to leaf rust (Hall et al. 2010). Jaypee is considered to have superior quality to USG 3209 due to its low flour water absorption and high flour yield. USG 3209 carries the 1BL.1RS translocation and both parents have the *T. timopheevi* translocation on chromosome 2B.

Phenotyping

Phenotypic evaluations were conducted by the USDA-Agricultural Research Service (ARS) Soft Wheat Quality Laboratory (SWQL; Wooster, OH). Growing conditions for the association mapping population were presented by Souza et al. (2012). Briefly, all genotypes from this panel where grown at four environments in 2007 and five environments in 2008 (nine environments across Warsaw, VA, Ithaca, NY, West Lafayette, IN and Wooster, OH). Two checks (Roane and Foster) were replicated within each environment.

Year and location of the grain used in analysis of the five bi-parental populations is provided in Table 1. The Wooster Ohio (OH) test location was on the campus of the Ohio Agricultural Research Center. A plot consisted of six rows with a total harvested area of 1.5 × 3 m in size. Standard fertilizer applications were used with 150 kg ha^{-1} of 18-46-0 (N-P-K) applied prior to planting, and 65 kg ha^{-1} of nitrogen applied in the spring. The New York test location was near Ithaca on the Cornell University research station. A plot consisted of six rows with a total harvested area of 1.26 × 3 m in size. Standard fertilizer applications were used with 100 kg ha^{-1} of 10-20-20 (N-P-K) applied prior to planting, and 44 kg ha^{-1} of nitrogen applied in the spring. The Indiana test location was near West Lafayette on the Purdue University research station. A plot consisted of seven rows spaced 15 cM approximately 1.5 m in length for an area of about 1 × 1.5 m. Standard fertilizer applications were used with 200 kg ha^{-1} of 18-46-0 (N-P-K) applied prior to planting, and 100 kg ha^{-1} of nitrogen applied in the spring. The Virginia test location was near Warsaw at a Virginia Tech Agricultural Research and Extension Center. In the 2004–2005 tests, plots consisted of single head rows that were 1.2 m in length with 30.5 cM spacing between

Table 1 Summary of the source, composition, phenotyping, genotyping, and mapping of five recombinant inbred lines analyzed in the study

Parentage of population	Foster/Kanqueen	Foster/Hopewell	Foster/Pioneer 25R26	91193D1/92210D5	USG3209/Jaypee
Code	F/K	F/H	F/P	9/9	U/J
Source of population	Cornell University	The Ohio State University	The Ohio State University	Purdue University	Virginia Tech
Number of RILs	175, F8 derived	138, F4 derived	171, F4 derived	254, F8 derived	130, F7 derived
State and year of test environments	NY2005, NY2006, OH2006	OH2007	OH2005, OH2006, OH2007, NY2006	IN2005, IN2007, OH2005, NY2005	VA2005, VA2006, VA2007, NC2007
Number of markers	574 DArT, 30 SSR	9 SSR	162 SSR	127 DArT, 164 SSR	157 DArT, 72 SSR
#Linkage groups and total cM	32; 2618 cM	2; 95 cM	21; 1700 cM	21; 3566 cM	31; 804 cM
References	None	None	Smith et al. (2011)	Uphaus et al. (2007)	Hall et al. (2010)

rows. Fertilizer application was conducted prior to planting: 30-100-80-5, and 45 kg ha^{-1} of N (24-0-0-3) in spring. In the 2005–2006 tests, plots consisted of seven rows spaced 15.24 cM apart with a total harvested area of 1.5 × 2.7 m in size. Standard fertilizer applications were used with 150 kg ha^{-1} of 30-60-60 (N-P-K) applied prior to planting, and 50.4 kg ha^{-1} of nitrogen applied in the spring. The North Carolina location is located near Raleigh on the North Carolina State University research station. A plot consisted of six rows with a total harvested area of 1.5 × 1.2 m in size. Standard fertilizer applications were used with 150 kg ha^{-1} of 18-46-0 (N-P-K) applied prior to planting, and 65 kg ha^{-1} of nitrogen applied in the spring.

One-grain sample per genotype was obtained from each environment. Samples were pooled over replications in IN, NC and VA while single plot samples were provided from NY and OH. FY and SE were measured using a modified Brabender Quadramat Junior mill after tempering to 15 % moisture, as described by Finney and Andrews (1986). In the 2005 harvest year, 20 g samples were milled while 50 g samples were milled in all other environments. SE was measured as the percentage of total flour that is obtained as fine flour (sifting through sequential 40- and 94-mesh screens) in the Quadramat Junior mill and FY was measured as the percent weight of the grain recovered as total flour from the modified Quadramat Junior mill. Both, SE and FY were adjusted to 14 % moisture basis.

Flour protein percentage (FP) and moisture were estimated using a near infrared analyzer (Unity Spectrastar 2200, Columbia MD). Protein values were calibrated using a nitrogen combustion analyzer (Elementar Nitrogen Analyzer, Hanau Germany) as described previously (Guttieri et al. 2008). The SRC tests (Guttieri et al. 2001) were conducted using water (WA), sodium carbonate (SO, 5 % w/w), sucrose (SU, 50 % w/w), and lactic acid (LA, 5 % w/w) by an adaptation of AACC 56-11 using 1-g flour samples as described in Guttieri et al. (2008).

Genotyping and mapping

DNA was isolated from young leaf tissue of each RIL from the F/K, U/J, 9/9 and the diversity panel populations and genotyped with DArT markers at Diversity Array technology Pty. Ltd Yarralumla, ACT, Australia (http://www.triticarte.com.au). Each population was also genotyped with simple sequence repeat (SSR) markers as described by Smith et al. (2011). The F/H and F/P were only genotyped with SSR markers specific to certain chromosomes (1B and 2B on F/H and F/P), since the F/H was specifically developed to validate QTL from all Foster-derived populations. The 1BL.1RS rye translocation was also genotyped in all populations using the *XScm9* marker (Weng et al. 2007). All RILs from the Foster-derived populations and the 9/9 population were also genotyped with the wheat sucrose synthase gene *Sus2* as previously described (Jiang et al. 2011).

The genome-wide association analysis of the diversity panel was conducted using 583 DArT markers fitting a mixed linear model implemented in TASSEL (TASSEL 5.2) (Bradbury et al. 2007).

$$\boldsymbol{Y} = \boldsymbol{X\beta} + \boldsymbol{Zu} + \boldsymbol{\varepsilon}$$

In this model, $\boldsymbol{Y}$ represents the vector of phenotypes for each trait; $\boldsymbol{X}$ and $\boldsymbol{Z}$ are the design matrices relating $\boldsymbol{\beta}$ and $\boldsymbol{u}$ to the observations in $\boldsymbol{Y}$. $\boldsymbol{\beta}$ is a vector of fixed effects, including mean, the tested genetic markers and population structure; $\boldsymbol{u}$ is a vector of random additive genetic effects; $\boldsymbol{\varepsilon}$ is a vector of random residual error. The K matrix (kinship matrix) was used as the random term, while population structure was fitted using two methods: the Q matrix (Pritchard et al. 2000) from the most likely number of subpopulations previously reported for this population (Cabrera et al. 2014) and principal components (PCs). Two significant levels for marker-trait association was considered: marker wise at $P < 0.01$, and experiment wise significant P value at $P < 0.0001$ (corresponding to Bonferroni's correction test).

The method for creating the linkage maps for 9/9 and F/P populations is described in Uphaus et al. (2007) and Smith et al. (2011), respectively. Linkage maps for the U/J, F/K, and F/H populations, along with a new map of chromosome 2B for the F/P and 9/9 populations, were generated using JoinMap (Van Ooijen and Voorips 2001). A LOD score of three or higher was used to generate linkage groups. Information from the wheat SSR consensus map (Somers et al. 2004) and Diversity Array Technology (http://www.diversityarrays.com) was used to assign linkage groups to their respective chromosome. In an effort to join fragmented linkage groups, markers from the same chromosome were rerun separately.

The genetic position and effects of QTL in all populations were determined by composite interval mapping using QTL Cartographer version 2.5 (Wang et al. 2012). Forward and backward regressions were applied with a walk speed of 2 cM. Composite interval mapping was conducted with the standard model number six, a window size of 10 cM and five markers to control for genetic background. We used a LOD score of 3.0 in the F/K, 9/9, and U/J populations to have a common standard across all traits and populations. A LOD score of 2.0 as the significance threshold was applied to the F/P and F/H populations as we mapped only a small part of the genome in each population and thus needed a lower threshold to control type I error.

Along with the DArT markers we also tested the significance of specific translocations and candidate genes. Genotyping score of 1 and 0 was used to represent the presence

and absence for the 1BL.1RS and 1AL.1RS translocations (detected by marker *XScm9*), for the *Hap-H* and *Hap-L* alleles (detected by the KASP marker RSGGL for Sucrose synthase gene *Sus2*), for the dwarf alleles (*Rht1* from *Rht-B1b* and *Rht2* from *Rht-D1b*), for the photoperiod insensitivity alleles (*Ppd-D1a* from Ppd-D1 and *Ppd-B1a* from *Ppd-B1*) and for the gluten strength alleles *GluD1-Dx2*, *GluD1-Dx5*, GluB1-By8, GluB1-By9 and GluB1-B16 from gluten strength genes *GluD1* and *GluB1*. The significant regions were defined as in Cabrera et al. (2014). Briefly, a region was delineated by presence of a significant marker for any trait (P value <0.01) within 5 cM. The end of the region was determined if there was a gap higher than 5 cM with not significant markers.

Other statistics

The GLM procedure implemented in the software Statistical Analysis System (SAS) v9.1 (SAS Institute Inc., Cary, NC, USA) was used to generate mean squares for genotype, environment, and genotype by environment interaction effects. The latter was used as the error term in this study as each environment served as one replication. Least squares means were obtained for each genotype. Flour protein was used as a covariate in the model that generated the least square means of LA. Broad sense heritability and their 95 % confidence interval were calculated based on the method described by Knapp et al. (1985):

$$\widehat{H} = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{\mathrm{GE}}^2/e} = 1 - (\mathrm{MS}_{GxE}/\mathrm{MS}_G),$$

where σ_G^2 is the genetic variance, σ_{GE}^2 is the genetic by environment variance, e is the number of environments, MS_G is the mean square of genotype, and MS_{GxE} is the mean squares of genotype by environment. The confidence intervals were calculated as:

$$(1 - (F - \text{value} \times F_{0.025 \text{ or } 0.975} : \mathrm{df}_n, \mathrm{df}_d))^{-1},$$

where df_n and df_d refer to the degrees of freedom for the numerator and denominator, respectively. Correlations of genotype means were calculated using least square means over environments. Phenotypes of RILs in the F/H population were only obtained for one environment, so heritability could not be calculated.

Multiple linear regression (MLR) was conducted for each trait and population using markers from chromosomes 1B and 2B in order to assess the independence and total variation explained by these two regions. Stepwise regression was used in PROC REG of SAS v9.1 (SAS Institute Inc., Cary, NC, USA) with a 0.25 level for entry and or exit of markers from the model.

Results

Phenotypes

Previously, Souza et al. (2012) reported significant genotype and environmental variation across several quality traits in the diversity panel. High levels of heritability were observed for the traits considered in that study ranging from 0.86 for FP to 0.97 for SO. In the bi-parental populations, high heritability also was observed for all traits analyzed in the F/K, 9/9 and U/J populations. Entry mean heritability ranged from 0.74 to 0.94 among traits and mapping populations (Table 2). The parents of the F/K, 9/9 and U/J populations were significantly different from each other for FY, WA, SO and SU but not for LA. Population means outside the range of the parental lines were observed for FY and LA in the 9/9 population, FP, LA and SE in the U/J population and FP in the F/H population. Transgressive segregants relative to both the high and low phenotype parent were observed for all traits in each population (Table 2). These were abundant (more than 75 % of all RILs) for LA in each population as the parental phenotypes were not greatly different from each other and were close to the mean of the RILs. The high heritability for all quality traits assessed in this work, population mean outside of parental range for FP and SE and transgressive segregants for quality traits is consistent with results of the F/P obtained by Smith et al. (2011).

Several significant trait correlations were present in the F/K, 9/9 and U/J populations. The traits WA, SO and SU were positively correlated to one another ($r > 0.67$ in all three populations) and all were negatively correlated with FY ($r < -0.46$ in all three populations) (Supplementary Table 1). Flour protein and SE were negatively correlated in all three populations ($r < -0.51$). Other correlations were generally low and inconsistent across populations. The correlations among FY, WA, SO and SU and between FP and SE also were observed by Smith et al. (2011) in the F/P population and by Souza et al. (2012) in the diversity panel.

Association analysis in a diversity panel

Results from association analysis using both models ($Q + K$ and PCs + K) were very consistent despite showing small differences in the significant level, therefore we report on the results from $Q + K$ model as it most likely represent better the population structure previously described for this population (Cabrera et al. 2014). In our association analysis of 583 DArTs and 10 gene-based markers, significant associations at family-wise significant level or false discovery rate (FDR) were not detected. As this was likely due to presence of multiple QTL with

Table 2 Summary of parental and RIL means, range of RIL values, percentage of transgressive segregants (Trans. Seg.), and heritability for seven soft wheat quality traits assessed in four RIL mapping populations

	SE	FP	LA	WA	SO	SU	FY
Foster/Kanqueen							
Foster (%)	59.6*	8.3*	92.7	51.4*	64.5*	85.5*	73.7*
Kanqueen (%)	54.4	10.0	90.7	55.2	71.5	92.4	68.7
RIL mean (%)	55.8	9.2	92.1	52.4	66.6	88.6	72.1
RIL range (%)	43.6–66.4	7.6–12.0	68.4–114.6	48.1–59.8	60.1–85.7	80.2–109.3	67.1–75.3
% Trans. Seg.	30, 13	10, 17	46, 41	33, 4	33, 7	28, 17	1, 15
H^2 (CI)	0.92	0.90	0.86	0.90	0.92	0.88	0.94
Foster/Hopewell							
Foster (%)	59.5	9.4	87.9	53.3	63.5	81.0	70.0§
Hopewell (%)	63.0	8.9	104.3	54.8	71.3	88.4	65.2
RIL mean (%)	59.5	10.4	92.8	53.8	66.8	83.4	67.5
RIL range (%)	45.7–66.7	8.31–14.7	67.2–117.8	50.2–59.7	61.1–74. 6	73.9–95. 2	59.8–71.8
% Trans. Seg.	41, 17	1, 62	28, 11	43, 30	12, 6	27, 10	9, 12
91193D1/92201D5							
91193D1 (%)	58.8*	9.7	87.2	51.6*	64.8*	81.2*	71.7*
92201D5 (%)	54.7	9.3	90.5	53.9	69.5	87.7	70.5
RIL mean (%)	56.8	9.5	91.6	52.6	66.5	83.8	70.2
RIL range (%)	44.4–61.5	8.3–11.8	79.5–109.0	48.8–57.1	62.3–73.5	78.5–92.6	67.1–73.2
% Trans. Seg.	13, 20	36, 37	21, 54	19, 15	19, 7	14, 6	36, 37
H^2 (CI)	0.88	0.82	0.77	0.84	0.86	0.74	0.87
USG3209/Jaypee							
USG3209 (%)	50.9	9.14	97.6	51.6*	65.1*	84.4*	71.2*
Jaypee (%)	51.8	8.68	96.3	58.9	78.6	96.8	67.9
RIL mean (%)	50.6	9.44	93.88	54.5	69.4	88.91	69.5
RIL range (%)	37.3–61.2	8.2–12.3	75.1–113.8	48.3–62.0	60.8–82.2	81–101.7	50.8–72.9
% Trans. Seg.	53, 34	8, 62	65, 31	8, 2	15, 2	8, 5	9, 8
H^2 (CI)	0.88	0.79	0.93	0.89	0.92	0.86	0.89

FY flour yield, *WA* water solvent retention capacity, *SO* sodium solvent retention capacity, *SU* sucrose solvent retention capacity, *LA* lactic acid solvent retention capacity, *FP* flour protein, *SE* softness equivalent

* A parental phenotype that is significantly different from the phenotype of its mate based on an LSD (0.05)

§ There was no error term for the Foster/Hopewell population so the difference between parents could not be tested and heritability could not be estimated

minor effects, we considered potential marker-QTL associations at a cut-off of $P < 0.01$ and validate these associations later in the QTL mapping of bi-parental populations. Among the 10 gene-based markers, *XScm9* marker (targeting the 1BL.1RS translocation) was the most significant ($P = 0.7e{-}3$) although with low minor allele frequency (<6 %). Twenty-six regions associated with potential QTL were identified for at least one of the seven traits in the diversity panel (Table 3). Six were associated with at least two traits. Among these regions, three contained a single marker associated with more than one trait (Table 3). The allelic effects of a marker associated with more than one trait were consistent with the observed phenotypic correlation across the three mapping populations (F/K, 9/9 and U/J) (Supplementary Table 1).

Previous work in QTL discovery and validation of quality traits in soft wheat cultivars indicated the relative importance of chromosomes 1B and 2B (Smith et al. 2011); therefore, we focused on these chromosomes. These chromosomes showed 3 and 2 regions, respectively, associated with at least one trait in the diversity panel. On chromosome 1B, the first region carried a single marker two markers: *wPt-7094* and *wPt-6442* (11–12 cM) showing association with SO and LA (respectively). An additional marker in same region: *wPt-2614* (18 cM) showed the highest association with LA and has significant LD with *XScm9*. No LD was observed between *XScm9* and *wPt-7094* and *wPt-6442*. A second region represented by marker *wPt-1684* at 35 cM was also associated with LA but it was also in LD with *XScm9* and *wPt-2614* therefore likely targeting

Table 3 Genomic regions in the diversity panel showing association to one or more traits and the respective markers

Chr	Genomic region (cM)	Traits	Markers
1A	2.54–7.9	FP; SE	*wPt-7541* (0.64, 0.3e−3; −2.5, 0.7e−2)
	24	LA	*wPt-6122* (−5.0, 0.53e−2)
	64	SE SO	*wPt-8347* (−1.7, 0.63e−2; −1.18, 0.5e−2)
1B	9.0–27	SO; (LA)	*wPt-7094* (1.5, 0.41e−2); [*wPt-6442*† (−5.2, 0.61e−2); *wPt-2614*† (−9.9, 0.79e−3)]
	35	LA	*wPt-1684* (−6.5, 0.91e−2)
	87.9–88.3	SO	*wPt-2526* (−2.8,0.1e−2)
2A	18.83	SU	*wPt-4533* (−2.9, 0.50e−2)
	46	FY	*wPt-1657* (0.7, 0.66e−2)
2B	12	SE	*wPt-6223* (1.55, 0.96e−2)
	85–90	FP; FY	*wPt-1068* (0.33, 0.73e−2); *wPt-9736* (−0.94, 0.2e−2)
2D	43.74	SU	*wPt-0330* (−3.9, 0.19e−3)
3A	98.85	SO	*wPt-2910* (−1.4, 0.15e−2)§
3B	68.58	FP	*wPt-1940* (−0.42, 0.26e−2)
	79.29	LA	*wPt-1171* (5.29, 0.55e−2)
	85.87–87.37	SO	*wPt-6785* (−1.7, 0.86e−2)
4A	50	WA	*wPt-7939* (−0.81, 0.68e−2)
	61.11	SE	*wPt-8841* (2.35, 0.21e−2)
	85	LA	*wPt-9196* (−6.47, 0.65e−2)
	105–107	LA; SE; FP	*wPt-9675*† (7.1, 0.55e−2); *wPt-8091*† (1.7, 051e−2); *wPt-4680*† (−1.2, 0.74e−2)
4D	6.24	LA	*wPt-4572* (5.41, 0.58e−2)
6A	0–12	SU	*wPt-8006* (−2.59, 0.53e−2)
	16	FY; SO; SU	*wPt-8266* (0.9, 0.84e−3; −1.5, 0.17e−2; −2.4, 0.6e−2)
	52	LA	*wPt-5310* (5.97, 0.6e−2)
6B	10	FP	*wPt-2964* (0.3, 0.73e−2)
	112–120	WA	*wPt-5176* (0.08, 0.59e−2)
7A	10.75–15.12	FY	*wPt-5153* (−0.14, 0.13e−2)

Genotype effect (AA) for each marker (respect to the foster allele) is presented in parenthesis followed by the *P* value of the association

§ Effect is respect to the minor allele frequency because of missing data in foster

† Markers in LD ($r^2 > 0.2$)

the same QTL. A third region in 1B spanned from 88 to 95 cM and carried a marker (*wPt-2526*) with moderated association with SO. On chromosome 2B, two regions contained 3 MTAs. First region contained a single marker associated to SE (*wPt-6223*), while the second region contained 2 MTAs (*wPt-1068* associated with FP and *wPt-9736* associated to FY). Although *wPt-9736* was located in a large LD block that accounted for nearly 50 % of all 2B markers (most of them in LD with *Sus2*), it did not show LD with *Sus2* (Table 3; Fig. 1a, b).

Genotyping and QTL mapping in bi-parental populations

A total of 280, 230, and 558 markers were assigned to linkage groups from the 9/9, U/J and F/K populations, respectively (Supplementary Table 2). The 9/9 map covered over 3500 cM while the U/J map covered only 804 cM (Table 1). Considerable deviation of allele frequencies from expected values was observed in all populations (Supplementary Table 2). This was pronounced in the U/J population where allele frequencies for 76 % of mapped loci deviated from expected. Of all loci, 47 % favored the Jaypee allele and 29 % the USG 3209 allele (Supplementary Table 2). Percentage of loci with deviant allele frequencies was more moderate in the 9/9 population (50 %) and was lowest (23 %) in the F/K population (Supplementary Table 2). Averaged over all loci, allele frequencies were close to expected in both populations (e.g., close to 0.5 for each parental allele). Segregation distortion was previously reported for 50 % of all markers mapped in the F/P population with 17 % of markers skewed towards the Foster allele and 33 % towards the Pioneer 25R26 allele (Smith et al. 2011). Loci with deviant allele frequencies were frequently clustered in regions across the genome, often in large blocks consisting of more than 20 cM, where allele

frequencies at all loci were skewed towards the same parent (Supplementary Table 3). In the 9/9, U/J. F/K, and F/P populations, 55, 36, 30, and 65 % of the skewed loci were from such blocks.

The new mapping work identified a total of 74 significant QTL across the seven traits and all five bi-parental mapping populations (Table 4). The three populations in which we scanned the whole genome (F/K, 9/9 and U/J) contained 17, 16 and 23 QTL, respectively. Three QTL regions were identified in the F/P population and all the traits associated with chromosome 2B were consistent with a QTL region previously reported by Smith et al. (2011) on chromosome 2B. The proportion of variance explained by the new QTL detected in this study ranged from 0.04 to 0.48: 72, 54 and 40 % of the QTL had r^2 equal to or greater than 0.10, 0.15 and 0.20, respectively (Table 4). Twenty-two of the 25 QTL with r^2 greater or equal to 0.15 identified in the F/K, 9/9 and U/J populations, where we scanned most of the genome, were located on chromosomes 1B and 2B. Previous QTL mapping efforts in the F/P population (Smith et al. 2011) reported that all QTL with r^2 greater or equal to 0.15 were also located on chromosomes 1B and 2B.

Fig. 2 Synteny of linkage groups 1B (**a**) and 2B (**b**) across different ▶ mapped QTL regions are represented in *colored* and *black boxes*. The population codes are Foster/Kanqueen (F/K), Foster/Hopewell (F/P), Foster/Pioneer 25R26 (F/P), 91193/92201 (9/9), and USG 3209/Jaypee (U/J). *Red* and *blue lines* connect markers of the same homologous QTL regions across populations from chromosome 1B. *Yellow box* in 9/9 showed no synteny with other QTL. *Green lines* connect markers in homologous QTL regions in chromosome 2B. *Markers* in *gray* and *dotted lines* connect markers mapped across populations but outside of QTL regions. *Numbers* close to *boxes* identify (in the legend) the traits they control and *number* in *brackets* represent the homologous regions across maps showed on Table 4

The sign of the additive effect of 61 % of all the QTL corresponded to that predicted by the parental phenotypes (e.g., QTL with negative additive effects came from the parent with lower phenotypic value and vice versa). High consistency was observed for QTL from chromosome 2B, where the sign of nearly 92 % of the allele effects corresponded to their parental phenotype and donor of the 2B translocation. However, only 19 % of the 1B QTL associated with the *XScm9* marker (identifying the 1BL.1RS translocation) corresponded to the phenotype of the *XScm9* donor parent.

Many markers were associated with QTL for more than one trait. For such markers the traits and the sign of the

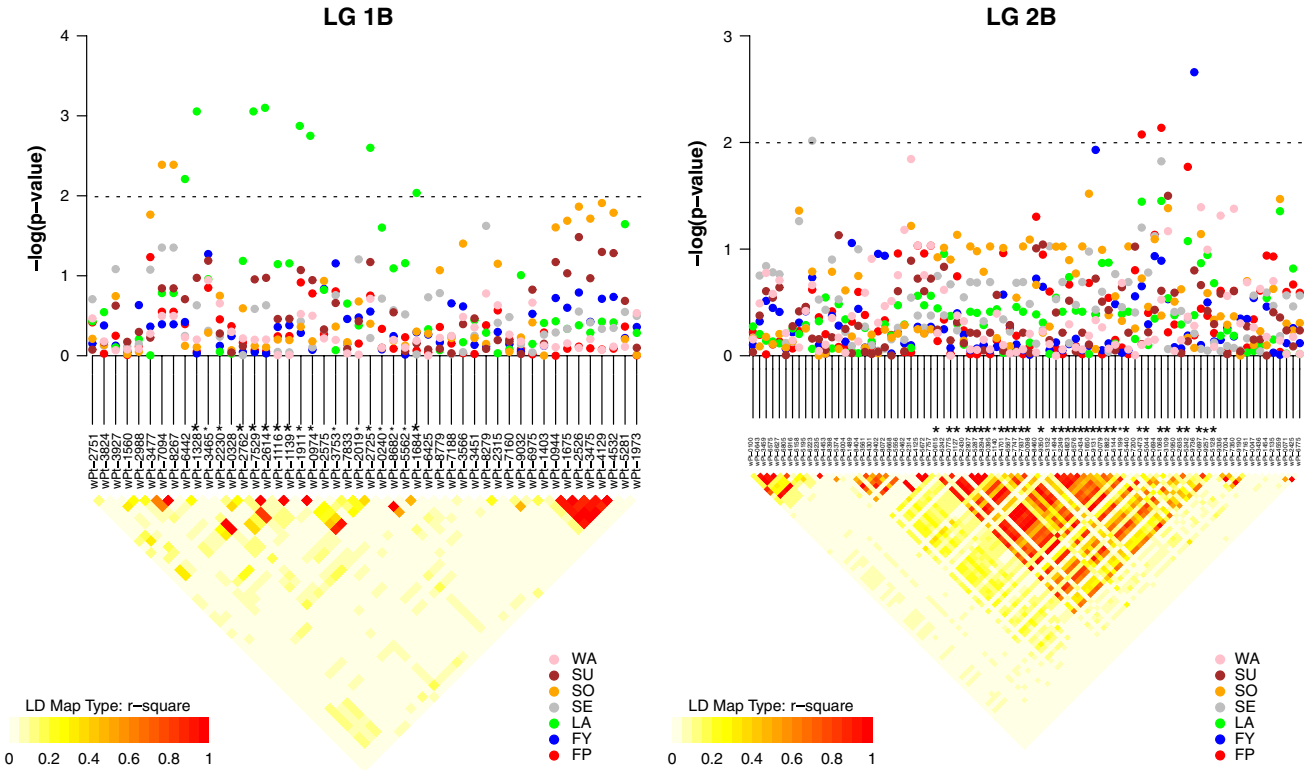
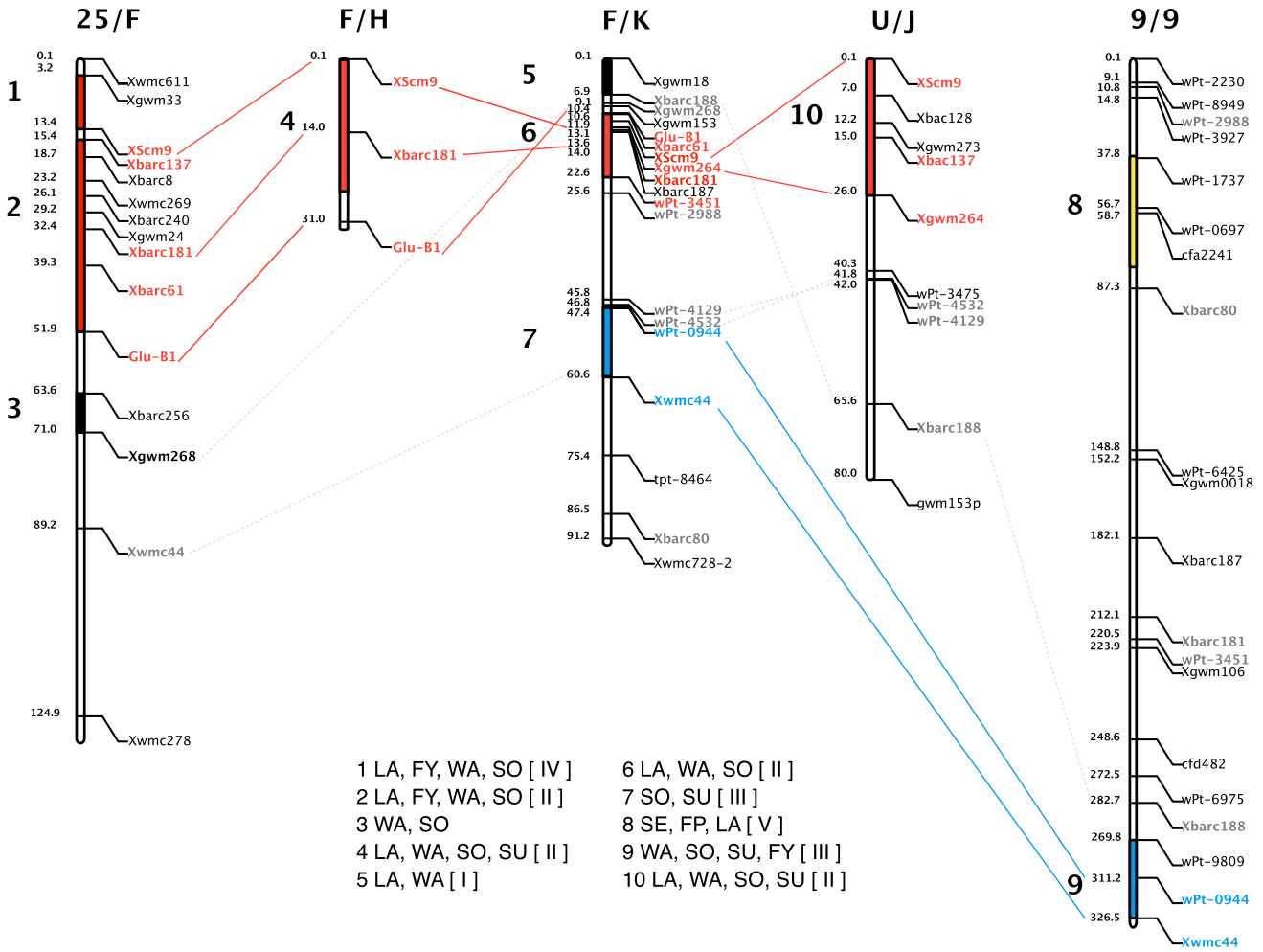


Fig. 1 LD heat map and Manhattan plot for traits showing significant association to DArT markers in Table 3 for linkage group 1B (**a**) and linkage group 2B (**b**). *One line* is drawn at the *Y* axis [−log(*P* value)] for the $P = 0.01$. Level of LD with markers *XScm9* and *Sus2* (in chromosomes 1B and 2B, respectively) is represented by de size of *filled star*. The largest has $r^2 > 0.3$ and the smallest has $0.2 > r^2 > 0.1$


25/F
F/H
F/K
U/J
9/9
1
2
3
4
5
6
7
8
9
10
Xwmc611
Xgwm33
XScm9
Xbarc137
Xbarc8
Xwmc269
Xbarc240
Xgwm24
Xbarc181
Xbarc61
Glu-B1
Xbarc256
Xgwm268
Xwmc44
Xwmc278
XScm9
Xbarc181
Glu-B1
Xgwm18
Xbarc188
Xgwm268
Xgwm153
Glu-B1
Xbarc61
XScm9
Xgwm264
Xbarc181
Xbarc187
wPt-3451
wPt-2988
wPt-4129
wPt-4532
wPt-0944
Xwmc44
tpt-8464
Xbarc80
Xwmc728-2
XScm9
Xbac128
Xgwm273
Xbac137
Xgwm264
wPt-3475
wPt-4532
wPt-4129
Xbarc188
gwm153p
wPt-2230
wPt-8949
wPt-2988
wPt-3927
wPt-1737
wPt-0697
cfa2241
Xbarc80
wPt-6425
Xgwm0018
Xbarc187
Xbarc181
wPt-3451
Xgwm106
cfd482
wPt-6975
Xbarc188
wPt-9809
wPt-0944
Xwmc44
1 LA, FY, WA, SO [IV]
2 LA, FY, WA, SO [II]
3 WA, SO
4 LA, WA, SO, SU [II]
5 LA, WA [I]
6 LA, WA, SO [II]
7 SO, SU [III]
8 SE, FP, LA [V]
9 WA, SO, SU, FY [III]
10 LA, WA, SO, SU [II]

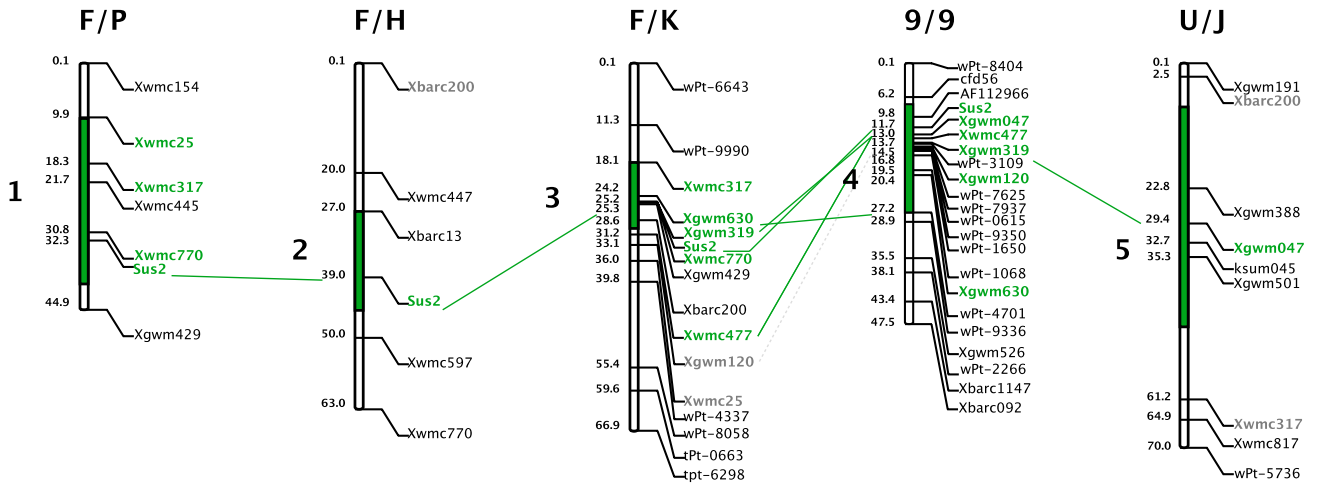

F/P
F/H
F/K
9/9
U/J
1
2
3
4
5
Xwmc154
Xwmc25
Xwmc317
Xwmc445
Xwmc770
Sus2
Xgwm429
Xbarc200
Xwmc447
Xbarc13
Sus2
Xwmc597
Xwmc770
wPt-6643
wPt-9990
Xwmc317
Xgwm630
Xgwm319
Sus2
Xwmc770
Xgwm429
Xbarc200
Xwmc477
Xgwm120
Xwmc25
wPt-4337
wPt-8058
tPt-0663
tpt-6298
wPt-8404
cfd56
AF112966
Sus2
Xgwm047
Xwmc477
Xgwm319
wPt-3109
Xgwm120
wPt-7625
wPt-7937
wPt-0615
wPt-9350
wPt-1650
wPt-1068
Xgwm630
wPt-4701
wPt-9336
Xgwm526
wPt-2266
Xbarc1147
Xbarc092
Xgwm191
Xbarc200
Xgwm388
Xgwm047
ksum045
Xgwm501
Xwmc317
Xwmc817
wPt-5736
1 SE, FP, WA, SO, SU, FY, LA
2 SE, SO, SU
3 SE, FP, WA, SO, SU, FY, LA
4 SE, FP, WA, SO, SU
5 WA, SO, SU, FY

Table 4 Summary of significant (LOD > 3.0) QTL for soft wheat quality traits from each of the bi-parental populations

Trait	Chromosome-region (syntenic region)	Best or flanking markers	cM of LOD peak	LOD	r^2	a‡
F/K population						
LA	1B-1 (I)	*Xgwm18–Xbarc188*	0	3.2	0.4	−2.3
WA	1B-1 (I)	*Xgwm18–Xbarc188*	3	4.1	0.4	0.7
LA	1B-2 (II)	*XScm9*	14	5.7	0.1	−2.4
WA	1B-2 (II)	*XScm9*	14	9.9	0.2	0.8
SO	1B-2 (II)	*XScm9*	14	9	0.17	1.6
SO	1B-3 (III)	*wPt-4532*	50	3.3	0.48	−1
SU	1B-3 (III)	*wPt-4532*	48	4.6	0.43	−1.6
FY	2A	*wPt-5865*	26	3.1	0.05	0.3
SE	2B (VI)	*Sus2*	25	11.1	0.25	−1.7
FP	2B (VI)	*Sus2*	25	4.7	0.12	0.3
WA	2B (VI)	*Sus2*	25	11.5	0.23	−0.9
SO	2B (VI)	*Sus2*	25	21.6	0.4	−2.6
SU	2B (VI)	*Sus2*	25	17.3	0.38	−3.1
FY	2B (VI)	*Sus2*	25	16.7	0.39	1.1
LA	2B (VI)	*Sus2*	25	11.4	0.2	−4.1
HD	3A	*wPt-745076*	83	3.5	0.08	−0.5
FY	3B	*wPt-4048*	53	4.9	0.08	0.4
F/H population (1B and 2B only)						
LA	1B (II)	*XScm9–Xbarc181*	8	8.1	0.36	−6.5
WA	1B (II)	*XScm9–Xbarc181*	11	5.1	0.4	0.8
SO	1B (II)	*XScm9–Xbarc181*	9	3.7	0.31	0.8
SU	1B (II)	*XScm9–Xbarc181*	7	4.8	0.21	1.2
FY	1B (II)	*XScm9–Xbarc181*	9	3	0.04	−0.3
SE	2B (VI)	*Sus2*	36	2.9	0.18	−1.3
WA	2B (VI)	*Sus2*	39	2.2	0.31	−0.5
SO	2B (VI)	*Sus2*	39	4.1	0.37	−1.5
SU	2B (VI)	*Sus2*	38	4.8	0.27	−1.6
F/P population (1B and 2B only, see Smith et al. 2011 for other QTL)						
LA	1B-1 (IV)	*Xwmc611–Xbarc137*	8	8.1	0.15	5.6
WA	1B-1 (IV)	*Xwmc611–Xbarc137*	12	7.9	0.25	−0.7
SO	1B-1 (IV)	*Xwmc611–Xbarc137*	8	8	0.25	−1.3
FY	1B-1 (IV)	*Xwmc611–Xbarc137*	12	8.2	0.23	0.8
LA	1B-2 (II)	*Xbarc8–Glu-B1*	26	19.5	0.3	7.5
WA	1B-2 (II)	*Xbarc8–Xbarc181*	23	8	0.18	−0.6
SO	1B-2 (II)	*Xbarc8–Xbarc181*	23	11	0.21	−1.2
FY	1B-2 (II)	*Xbarc8–Xbarc181*	23	12.1	0.23	0.8
FP	2B (VI)	*Sus2*	32	7.5	0.25	0.3
SE	2B (VI)	*Sus2*	33	4.4	0.15	−1.3
LA	2B (VI)	*Sus2*	32	2.6	0.09	−3.6
WA	2B (VI)	*Sus2*	33	3.8	0.21	−0.4
SO	2B (VI)	*Sus2*	32	8.1	0.27	−0.8
SU	2B (VI)	*Sus2*	33	5.8	0.3	−1.3
FY	2B (VI)	*Sus2*	32	4.9	0.23	0.5
9/9 population						
SE	1B-1 (V)	*wPt-0697*	57	10.2	0.2	−1.4
FP	1B-1 (V)	*wPt-0697*	57	14.1	0.29	0.4
WA	1B-2 (III)	*wPt-0944*	315	5.3	0.15	−0.4

Table 4 continued

Trait	Chromosome-region (syntenic region)	Best or flanking markers	cM of LOD peak	LOD	r^2	a‡
SO	1B-2 (III)	*wPt-0944*	315	10.3	0.25	−0.7
SU	1B-2 (III)	*wPt-0944*	315	4.7	0.13	−0.6
FY	1B-2 (III)	*wPt-0944*	308	3.9	0.12	0.4
LA	1D	*Xgwm642*	72	4.5	0.11	1.9
FP	2A	*cfd50.1*	244	3.4	0.06	−0.1
SE	2A	*wPt-9797*	246	6.2	0.13	1.1
SE	2B (VI)	*Sus2–Xgwm120*	15.3	5.4	0.15	−1.1
FP	2B (VI)	*Sus2–Xgwm120*	12.7	7	0.25	0.3
WA	2B (VI)	*Sus2–Xgwm120*	15.3	5.1	0.14	−0.6
SO	2B (VI)	*Sus2*	10.8	11.6	0.38	−1.4
SU	2B (VI)	*Sus2*	12.7	5.4	0.21	−1.1
SE	5A	*Xgwm205*	39	3.7	0.09	0.8
SE	6A	*Xbarc113*	15	3	0.07	−0.2
SO	6B	*wPt-1241*	0	4	0.08	−0.2
SU	6B	*wPt-1241*	0	7.1	0.18	−0.6
U/J population						
LA	1B (II)	*XScm9–Xgwm273*	10	9.2	0.32	−4.6
WA	1B (II)	*XScm9–Xgwm273*	6	10.1	0.24	1.2
SO	1B (II)	*XScm9–Xgwm273*	6	17	0.33	2.5
SU	1B (II)	*XScm9–Xgwm273*	6	12.3	0.37	2.5
FY	1B (II)	*XScm9–Xgwm273*	7	3.2	0.05	−0.3
FP	2A	*Xbarc1015*	24	4.5	0.11	0.3
WA	2B (VI)	*Xwmc047*	29	3.9	0.07	−0.7
SO	2B (VI)	*Xwmc047*	29	6.1	0.1	−1
SU	2B (VI)	*Xwmc047*	29	7.7	0.19	−1.9
FY	2B (VI)	*Xwmc047*	33	3.1	0.05	0.6
FP	4B	*wPt-2141*	14	3.1	0.08	0.2
FP	4D	*Xbarc048*	6	5.5	0.16	0.3
SE	4D	*Xbarc048–Xgwm149*	20	2.7	0.09	−0.9
FP	5A-1	*wPt1712*	29	3.4	0.1	0.2
SU	5A-2	*Xbarc056*	51	3.7	0.08	1.2
SE	5B-1	*wPt-7006*	12	3.4	0.11	1.3
WA	5B-1	*wPt-7006*	12	3.6	0.07	−0.7
SE	5B-2	*wPt-7400*	22	3.4	0.11	1.3
SE	6A	*wPt-2573*	0	3.6	0.1	1.3
FP	6B	*wPt-9256*	4	3.1	0.08	−0.2
FY	7B-1	*wPt-5228*	9	4.8	0.07	−0.4
WA	7B-1	*wPt-5228*	9	5	0.1	0.8
SE	7B-2	*wPt1066*	22	6.1	0.2	−1.3

Numbers in () correspond to QTL in same syntenic region across maps as in Fig. 2

FY flour yield, *WA* water solvent retention capacity, *SO* sodium carbonate solvent retention capacity, *SU* sucrose solvent retention capacity, *LA* lactic acid solvent retention capacity, *FP* flour protein, *SE* softness equivalent

‡ Additive effect of 91193D1, Foster, and USG 3209 alleles

QTL allele effects were mostly consistent with the phenotypic correlation across populations (Table 4). Across all populations, a total of 19 QTL were identified for at least one of four correlated traits (WA, SO, SU, FY). Two QTL in 1B and one QTL in 2B showed synteny among populations, therefore 12 different regions were observed for the above-mentioned traits. Seven out of these 12 regions contained at least two of the correlated traits and the sign

Table 5 Summary of r^2 values from multiple regression analyses involving markers for QTL regions from chromosome 1B and a single marker for the QTL region on chromosome 2B

	F/K		F/H		F/P		9/9		U/J	
	1B	2B	1B	2B	1B	2B	1B	2B	1B	2B
SE	ns	0.21	0.06 (2)	0.24	0.16 (2)	0.19	0.11 (1)	0.08	0.03 (1)	0.01
FP	0.10 (2)	0.06	ns	ns	0.07 (2)	0.22	0.21 (1)	0.10	0.03 (1)	0.01
LA	0.22 (2)	0.25	0.33 (1)	0.03	0.67 (2)	0.01	0.06 (1)	ns	0.13 (1)	0.01
WA	0.28 (2)	0.15	0.26 (3)	0.03	0.12 (2)	0.10	0.05 (1)	0.14	0.25 (2)	0.08
SO	0.22 (3)	0.41	0.12 (1)	0.40	0.23 (1)	0.16	0.19 (2)	0.21	0.40 (2)	0.12
SU	0.07 (2)	0.36	0.15 (1)	0.19	0.07 (2)	0.17	0.05 (1)	0.17	0.21 (2)	0.09
FY	0.05 (2)	0.31	0.08 (2)	0.13	0.24 (2)	0.08	0.13 (2)	ns	ns	0.06

ns no significant correlation

of the additive effects was as predicted by the correlations. QTL regions with highest additive effect were located in chromosomes 1B and 2B. There was a negative correlation between FP and SE that was consistent across all populations (Supplementary Table 1). Fourteen different regions across the wheat genome contained a QTL for at least one of traits: FP and SE, though only four regions affected both traits. The allelic effects for FP and SE in these four regions had the opposite sign that was consistent with the negative correlation (Table 4).

Multiple regression analysis using the most significant markers from chromosomes 1B and 2B including the *XScm9* for the 1BL.1RS translocation and the marker for *Sus2* from chromosome 2B was used to assess the independence and total variation explained by these two regions. Markers from both 1B and 2B were retained in the model in 30 of 35 trait/population combinations (Table 5). In 19 trait/population combinations, chromosome 1B regions accounted for more variation than 2B while the opposite occurred in 15 instances. Among traits, markers from both QTL regions were retained for WA, SO, and SU in all five populations. Across all traits and populations, the 1B and 2B regions retained in the model combined accounted for an average of 28 % of the phenotypic variation (range from 0 % for FP in F/H to 67 % for LA in F/P). Across populations, the regions retained in the model accounted for over 20 % of the variation for all traits but FP and over 28 % for LA, WA, SO and SU. Markers from chromosome 1B, averaged across populations, accounted for 4.7 times more variation for LA and nearly twice as much variation for WA than the 2B region. However, the 2B region explained more variation for SE and SU. The F/P, F/H, F/K, and U/J populations all segregate for the 1BL.1RS translocation and two QTL regions from the proximal end of chromosome 1B were modeled in F/P and F/K. In 56 % of these trait/population combinations, the model retained markers for the 1BL.1RS and the adjacent second region.

Discussion

Quality parameters determine the final use of soft wheat. Good understanding of the genetic architecture of the several components regulating important traits is fundamental for crop improvement. In this work, moderate-to-high heritability estimates were noted for all traits and populations while transgressive segregation was widely present for most traits in all bi-parental populations.

Trait correlations were consistent across mapping populations. QTL for one trait were frequently coincident with QTL for the correlated traits and the signs of the effects matched those predicted by the correlations. The traits SE and FP were correlated as were WA, SO, SU, and FY. However, the coincidence of QTL effects was less pronounced for SE and FP than for WA, SO, SU and FY, suggesting less genetic dependence between SE and FP than the other traits. Explanation for some of these correlations has been proposed elsewhere (Smith et al. 2011). All these trait correlations were consistent with trait correlations observed in the diversity panel (Souza et al. 2012).

The results obtained from the association analysis were compared to previous QTL studies in chromosome 1B and 2B. In our association analysis, high heritability was observed for all traits (ranging from 0.7 to 0.94); however, the lack of evidence for major effect QTL observed in this study suggest that in our diversity panel genetic control was complex and determined by minor QTL effects. Nevertheless, we cannot rule out the possibility that our panel might have been too small to detect major QTL. In any case, combining consistent results from association analysis and bi-parental populations can provide sufficient evidence for identifying QTL regions and use for marker-assisted selection.

Marker *wPt-6442* (on the short arm of chromosome 1B) showed association with LA while not in LD with *XScm9* (marker for the 1BL.1RS translocation) that was consistently associated to LA in the four bi-parental populations, suggesting this marker is either targeting an independent

QTL or is flanking the end of the *XScm9* QTL region. The high LD observed between other significant markers in the same region (including *wPt-2614*) and *XScm9* support the presence of a QTL for LA associated to the 1B translocation (Smith et al. 2011) in the diversity panel. Another important region was located on the distal portion of chromosome 1B. In this region, a highly defined LD block represented by *wPt-2526* was associated to SO. This block contained marker *wPt-0944*, which was also linked to SO and SU in the F/K and 9/9 populations. Other studies have reported QTL in this distal region of 1B for a number of dough mixing-related traits (Arbelbide and Bernardo 2006; Breseghello et al. 2005; Huang et al. 2006; Kuchel et al. 2006; McCartney et al. 2006; Schmidt et al. 2004). All together, these results support the importance of the 1BL.1RS translocation for quality traits and a QTL region on the long arm of 1B.

In our diversity panel, 39 lines (including Foster) contain the favorable *Sus2* allele, called *Hap-H*, for high 1000 kernel weight (Jiang et al. 2011) and the linked 2B translocation. The SSR marker, *Xwmc477* is used as a diagnostic marker to identify the 2B translocation region (containing the resistance genes *Sr36* for stem rust and *Pm6* for powdery mildew) that was introduced from *Triticum timopheevi* (Sturbaum et al. 2012). In the diversity panel, *Xwmc477* is in complete LD with *Sus2*. Lack of significance for these markers on any trait suggested that *Sus2* and the translocation did not affect SRWW quality in this panel. However, we cannot discard the possibility that as a result from sampling, the genetic background of the 39 lines with the desired *Hap-H* allele negate the effect on quality of this allele. Among all traits and markers from chromosome 2B, FY showed the most significant association with marker *wPt-9736*. However, different from *wPt-1068*, this association seems to be independent from *Sus2* as no association between both markers was observed in the diversity panel.

Across all five bi-parental populations, all large effect QTL ($r^2 > 0.2$) were consistently located on either chromosome 1B or 2B and associated with important translocations on each of the two chromosomes. Common markers among populations facilitated alignment of these QTL regions and homologous regions were detected. Collectively, the QTL regions from 1B and 2B account for a large portion of phenotypic variance for most traits in most populations. Averaged over all bi-parental populations, these regions accounted for 14 % (FP) to 48 % (SO) of the phenotypic variance for a single trait. Averaged over traits within bi-parental populations, these chromosomes accounted for 17 % (in the 9/9 population that did not segregate for 1BL.1RS) to 38 % (F/K) of the phenotypic variation within a population. These two chromosomes accounted for an average of 34 % of the phenotypic variation in the three Foster-derived populations (F/P, F/H, F/K) that segregated for 1BL.1RS and *Sus2*. Thus marker-assisted selection for favorable alleles from these chromosomes could be effective at improving soft wheat quality.

On chromosome 1B, the 1BL.1RS translocation (through marker *Xscm9*) was clearly associated with some of the aligned QTL effects across the four populations where it segregates. Chromosomal translocation from the short arm of rye chromosome 1R to the 1B chromosome from wheat have been used extensively as a source of resistance for major wheat diseases and adaptation; however, these traits were also accompanied by detrimental effects for some quality traits (Dhaliwal et al. 1987). Among all traits evaluated in this study, undesirable high SO and SU levels but low LA and FY values were consistently associated with 1BL.1RS. In the F/K population, a QTL region (conserved across F/H, F/P and U/J as well) was identified for LA and SO.

The significant negative effect of this translocation on milling and end use quality was previously reported (Zhao et al. 2012). Particularly, negative effect of 1BL.1RS on LA was reported in Canadian spring wheat breeding programs (Fenn et al. 1994). Direct association of the *XScm9* marker with traits in the diversity panel was not reliable due to its extremely low minor allele frequency. However, LD mapping showed a strong association of *XScm9* with *wPt-2614* but not with *wPt-6442* supporting the importance of this region for LA in the proximal region of 1B as shown in F/P and F/K maps. An additional consistent QTL associated to SO and SU was identified on the distal portion of the long arm of chromosome 1B of two populations (F/K and 9/9). Evidence for an additional QTL on 1B (other than the 1BL.1RS region) as observed by Smith et al. (2011) was provided by the results from the multiple regression analyses from this study where markers for two distinct QTL regions were sometimes included and by association of an LD block (carrying wPt-0944) with SO in the diversity panel. In addition a new, non-homologous region in the short arm of chromosome 1B contained the largest effect on FP and SE in the 9/9 population, which did not have the 1BL.1RS translocation. Clearly the region containing the 1BL.1RS rye translocation had an effect on many quality traits. We were unable though to determine whether there is more than one QTL in the proximal region of chromosome 1B in the bi-parental populations as alignment of QTL and mapping precision were hindered by the presence of the rye translocation that limited recombination and skewed segregation of markers.

A major QTL was observed on chromosome 2B in all five bi-parental populations. In all Foster and 9/9 derived populations, a QTL peaked at (or very near) the *Sus2* locus, confirming homology of the QTL across populations. A region in the same arm of chromosome 2B has been previously reported important for traits that relate to FP and SU

(McCartney et al. 2006). The *Sus2* locus codes for sucrose synthase 2, an enzyme that catalyzes the initial step to produce the precursor for conversion of sucrose to starch in the endosperm (Jiang et al. 2011). Since starch comprises 65–85 % of the final dry weight of the grain, it is not surprising that the sucrose synthase gene might affect milling quality in these populations. Our results implicated allelic variation at the *Sus2* as the cause of the 2B QTL effects but it did not prove cause and effect.

Evidence for a second QTL close to *Sus2* was observed in the U/J population. In this population, *Sus2* did not segregate as both parents carried the same allele. The marker *Xgwm047* was found significantly linked to several traits (WA, SO, SU and FY) and USG3209 was considered to carry the favorable allele. The association from the diversity panel (*wPt-9736* with FY) further supports the hypothesis that there are QTL other than *Sus2* in this region of 2B in the in U/J population. Interestingly, USG 3209 has been classified as very poor milling and baking quality cultivar despite carrying the desirable allele in this region of 2B (Souza et al. 2008). It is possible unfavorable genetic background reduces the quality potential in this genotype.

As expected from the presence of transgressive segregants, some parents with desirable phenotypes carried alleles with undesirable QTL effects and vice versa. This was particularly true for QTL residing at the proximal end of chromosome 1B where the sign of the effect of only 19 % of the QTL was consistent with the parental phenotype (compared to 92 % for the QTL in 2B). Foster, used as the parent in three out of the five populations from this study has good milling and baking quality; however, it also carries the 1BL.1RS translocation. Significant reduction in softness equivalent and baking quality has been previously associated to this translocation (McKendry et al. 1996, 2001). However, genetic background rather than 1BL.1RS translocation alone has been suggested as major determinant of quality traits (Johnson et al. 1999). In this study, we observed evidence for a second QTL in the distal portion of chromosome 1B associated to SO and SU in both bi-parental and diversity panel populations. The effects of these QTL were consistent with the parental phenotypes. Thus, for chromosome 1B additional genetic factors probably affect milling and baking quality more than the 1BL.1RS translocation alone (Johnson et al. 1999).

In this study, we identified new regions of the wheat genome with significant effects on soft wheat quality. QTL mapping in five bi-parental and one diversity panel population was conducted. Some regions of chromosomes 1B and 2B displayed consistent effect on the same traits over populations. The QTL regions from chromosome 1B and 2B are important and marker-assisted selection for favorable alleles at these loci should improve selection for good quality. Breeders should select against the 1BL.1RS translocation and for favorable alleles at other regions of 1B and 2B. Despite the lack of direct association observed between any trait and *Sus2* in the diversity panel, the Sus2 marker and that region associated on 2B was still important across populations. Our results strongly implicate allelic variation at the *Sus2* locus as a cause of the QTL effects mapped to chromosome 2B. We recommend selecting for the Hap-H allele at the *Sus2* locus.

Author contribution statement AC analyzed the data and wrote the manuscript. MG and NS collected samples and extracted the DNA. ES provided overall advice and support of this project. AS collected samples, evaluate quality parameters and review the manuscript. DH evaluated quality parameters. CG, MB, PM, HO, JU, MS, EH, GB-G and DVS critically evaluated the analysis of the data and review the manuscript. CS supervised the study and wrote the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The Experiments conducted during this study comply with the current laws of the United States of America.

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