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ORIGINAL PAPER

Chromosomes 3B and 4D are associated with several milling and baking quality traits in a soft white spring wheat (*Triticum aestivum* L.) population

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Abstract Wheat is marketed based on end-use quality characteristics and better knowledge of the underlying genetics of specific quality parameters is essential to enhance the breeding process. A set of 188 recombinant inbred lines from a 'Louise' by 'Penawawa' mapping population was grown in two crop years at two locations in the Pacific Northwest region of the United States and data were collected on 17 end-use quality traits using established quality analysis protocols. Using an established genetic linkage map, composite interval mapping was used to identify QTL associated with 16 of the 17 quality traits. QTL were found on 13 of the 21 wheat chromosomes. A large number of QTL were located on chromosomes 3B and 4D and coincided with traits for milling quality and starch functionality. Chromosome 3B contained 10 QTL, which were localized to a 26.2 cM region. Chromosome 4D contained 7 QTL, all of which were located on an 18.8 cM region of this chromosome. The majority of the alleles for superior end-use quality were associated with the cultivar Louise. The identified QTL detected remained highly significant independent of grain yield and protein quantity. The identification of these QTL for end-use quality gives key insight into the relationship and complexity of end-use quality traits. It also improves our understanding of these relationships, thereby allowing plant breeders to make valuable gains from selection for these important traits.

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Introduction

Wheat (*Triticum aestivum* L.) is a primary food staple worldwide, with consumption averaging 67 kg of wheat per person per year (FAO 2010). Food products, such as bread, ready-to-eat cereals, cake, noodles and pasta, made from processed wheat grain are consumed daily across the globe. Genetic improvements in wheat focus on three main areas: enhancing yield, overcoming biotic and abiotic stresses, and improving end-use quality (Mann et al. 2009). Wheat grains and flour are divided into soft and hard texture classes. Soft wheat is used for cookie and confectionary products, often made with alkaline-leavened batters, whereas hard wheat is used primarily for bread-baking in yeast-leavened dough and for noodle dough. Soft wheat flours have a smaller particle size distribution and the reduced water absorption needed to achieve the batter flow and product texture associated with cookies, crackers and cakes. Hard wheat flours have a larger particle size distribution, increased water absorption, which enhances yeast

growth, and increased gluten protein cross-linking. This protein network holds carbon dioxide and causes bread dough to rise. Other uses of wheat flour (noodles, thickening agents) require specific starch characteristics (see Morris and Rose 1996; Wrigley and Morris 1996 for further discussion of end-use requirements).

End-use quality parameters such as grain soundness, milling, kernel texture, protein and starch functionality and product performance are predicted using several approved tests (AACCI 2008). Grain soundness is assessed using grain volume weight, grain weight and diameter. Milling components are evaluated with experimental mills that measure flour extraction percentage (flour yield) and break flour extraction percentage (break flour yield). Soft wheat kernels have greater break flour yields upon milling than do hard wheat kernels (Posner 2000). Kernel texture (grain hardness) and crushing resistance, as well as kernel weight and size (outer diameter), are measured with the Single Kernel Characterization System (SKCS) 4100 (Perten Instruments, Springfield, IL). Lower SKCS values are equal to greater softness, thus SKCS hardness values are generally negatively correlated with break flour yield. The two measures of kernel texture are not completely correlated because grain structure, as well as particle size, influences break flour, whereas the SKCS values include crushing resistance (Campbell et al. 2007). Flour ash is measured using thermogravimetric ovens. Flour ash, although a positive nutritional component, is composed of minerals and reduces flour functionality in most batters and dough so that low flour ash is specified in export contracts (Liu et al. 2011; Morris et al. 2009). A milling score index, calculated as described below in "Materials and methods", comprises the three main milling components: flour yield, break flour yield and flour ash.

Protein functionality is a critical determinant of wheat end-use quality. Hard-textured wheat flours destined for bread-baking require high gluten strength, or gluten protein cross-linking, whereas low gluten strength enhances texture of confectionary products made from soft textured wheat flours. Gluten strength measured using the flour sodium dodecylsulfate (SDS) sedimentation test is correlated to dough rheology measured using equipment such as the mixograph and alveograph (Carter et al. 1999; Mondal et al. 2009). Total protein content of grain and flour also influence dough rheology and are critical marketing characteristics.

Soft wheat flour has less starch damage after milling than hard wheat (Posner 2000). Batters made from flours with lower starch damage have lower water absorption, resulting in greater cookie spread (Donelson and Gaines 1998). Flour swelling volume (FSV) is a measure of normal starch composition [normal wheat starch contains 20–30% amylose (Zeng et al. 1997; Morita et al. 2002)]. Higher amounts of amylopectin result in waxy and 'partial' waxy starches and higher FSV values, the latter being desirable for some types of Asian noodles. Product tests evaluate total flour functionality. For soft wheat, the sugar snap cookie test is that standard. Cookie diameter and texture are indications of flour texture, water absorption, starch characteristics and protein strength (Finney et al. 1950).

The tests described above, with the exception of SKCS, require large amounts of grain or flour. The solvent retention capacity (SRC) tests were developed by Slade and Levine (1994) to estimate grain quality parameters on small samples. They all are based on a mixture of flour plus one of four different solutions: 5% lactic acid, 50% sucrose, 5% $NaCO_3$ or water, to assess glutenin quality, pentosan (arabinoxylan) content and gliadin characteristics, starch damage, and overall absorption characteristics, respectively. Results are reported as the percentages of the mass of the flour–solvent pellet after mixing with the solvent, divided by the original flour weight. Greater values are associated with stronger gluten, greater starch damage, and greater pentosan (arabinoxylan) content, greater kernel hardness, lower break flour yield, greater water absorption and smaller cookie diameter (Guttieri et al. 2001; Ram et al. 2005). The ratio of the four solvents can predict flour functionality for specific products. For example, soft wheat for crackers would require high lactic acid values and low sodium carbonate values, whereas desirable soft wheat flour for cakes would have low values for all solvents and bread wheat flour would have high values of all solvents (Slade and Levine 1994).

In addition to the tests listed above, specific genes affecting kernel texture, protein and starch quality have been identified. The high and low molecular weight glutenin subunits on the wheat homoeologous group 1 chromosomes control much of the protein functionality of flour (Shewry et al. 1992; D'Ovidio and Masci 2004). A significant portion of the variation for kernel texture between soft and hard wheat is determined by allelic differences at the puroindoline genes *Pina* and *Pinb* located on chromosome 5DS (Morris 2002; Bhave and Morris 2008a, b). The granule bound starch synthase (GBSS) genes (Epstein et al. 2002) control starch composition. Three major GBSS genes have been identified on chromosomes 4A, 7A, and 7D (McLauchlan et al. 2001). Mutations in any one of these genes will result in the non-expression of that GBSS protein, reducing starch amylose content (Zeng et al. 1997). Loss of all three proteins results in 'waxy' endosperm, or virtually no amylose deposited in the starch granules (McLauchlan et al. 2001). All of the above-mentioned genes have specific impacts on end-use quality performance and are major genetic improvement targets for wheat breeding programs around the globe.

Multiple QTL mapping studies have been performed to further understand the genetic architecture underlying end-use quality. Using a hard by soft kernel texture wheat recombinant inbred line (RIL) population, Campbell et al. (1999, 2001) identified QTL for kernel, milling, and baking traits. QTL for kernel traits were located on chromosomes 1A, 2B, 2D, 3B, 7A, and 7B. The *Pinb* gene for kernel texture, located on chromosome 5DS was the major QTL for milling, hydration, and cookie baking traits, whereas the glutenin genes controlled variation for mixograph peak time, curve height, and tolerance (Campbell et al. 2001). Flour protein quantity genes were detected on chromosome 2B. In a similar study, Breseghello et al. (2005), also working with a soft by hard wheat population, detected 15 QTL on 13 linkage groups, which were putatively assigned to chromosomes 1A, 1B, 1A/D, 2A, 2B, 2D, 3A/B, 4B, 5B, and 6B. These traits were associated with milling traits, protein content, and baking assay traits. McCartney et al. (2006) studied 47 quality traits in the RL4452 by 'AC Domain' hard wheat cross and identified 99 QTL over 18 chromosomes for 41 quality traits. Twenty of those QTL mapped near a major plant height QTL (*Rht-D1b*) on chromosome 4D, and 10 QTL mapped near a QTL for time to maturity on chromosome 7D. These QTL were mainly associated with traits for mixograph and farinograph performance, baking performance, and starch functionality.

Previous QTL studies using bi-parental populations have been conducted within hard wheat populations or within populations derived from cross-hybridizing hard and soft wheat. Two association mapping strategies were used to detect QTL for quality traits in sets of soft wheat germplasm (Breseghello and Sorrels 2006; Reif et al. 2011). Breseghello and Sorrels (2006) detected significant QTL associated with kernel morphology on chromosomes 2D, 5A, and 5B and weak QTL associated with flour yield and break flour yield on 2D and 5B. Reif et al. (2011) detected QTL for kernel weight, protein content, sedimentation volume, and starch concentration on 15 different chromosomes. One bi-parental population using two soft wheat cultivars detected QTL for flour yield, flour protein, softness equivalent, and solvent retention capacity tests, the majority of which were located on chromosome 1B and 2B (Smith et al. 2011).

The genetic control of flour milling characteristics in soft wheat remains unexplored and unidentified in spite of their value to industry. Improvement of milling traits has been slow because most evaluation methods are destructive and require substantial amounts of grain so that milling quality has typically not been assayed until late in the breeding process and the stringent selection placed on end-use quality has restricted overall improvement. However, other than the negative correlation between grain yield and grain protein content (Groos et al. 2003), few studies reporting the genetic correlations among agronomic and quality traits have been reported. Knowledge of these correlations would allow breeders to make gains from both direct and indirect selection for multiple traits (Carter et al. 2010), thereby facilitating the improvement of these complex traits in regionally adapted wheat cultivars.

The objectives of this study were to identify DNA markers associated with QTL for important end-use quality traits in a RIL population created from two soft white spring wheat cultivars, 'Louise' (Kidwell et al. 2006; PI 634865) and 'Penawawa' (PI 495916), which are adapted and widely grown in the Pacific Northwest (PNW) region of the United States and differ for many important end-use quality traits. A second objective was to use the data previously reported for agronomic traits (Carter et al. 2011) to determine genetic correlations among agronomic and quality traits in this population.

Materials and methods

Plant materials

An $F_{5:6}$ derived RIL population of 188 individuals from a cross between Louise and Penawawa was selected for phenotypic and genotypic analyses. Louise, a soft white spring wheat released in 2005, has moderate grain volume weight, low grain protein concentration, and excellent end-use quality characteristics for soft wheat products such as cookies and cakes (Kidwell et al. 2006). Penawawa, a soft white spring wheat released in 1985, has moderate grain volume weight, moderate grain protein concentration, and average end-use quality characteristics. Penawawa also is known to carry a null allele for GBSS on chromosome 4A, which makes it a "partial-waxy" wheat (Zeng et al. 1997). These two parents also contrast for other disease and agronomic traits that are discussed elsewhere (Carter et al. 2009, 2011).

Field experiment

Field trials were conducted in Pullman, WA (latitude 46°41′N, longitude 117°08′W, elevation 776 m) in 2007, and in Moscow, ID (latitude 46°43′N, longitude 116°57′W, elevation 796 m), and again in Pullman, WA, in 2008. Plots were planted using an alpha lattice design with three replicates per location (Mason et al. 2003). Before planting, the field was cultivated and fertilized with nitrogen (formulated as urea) at a rate of 101 kg ha^{-1}. Due to differences in resistance to the foliar fungal disease stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*) to which Louise is resistant and Penawawa is susceptible, plots were sprayed with Tilt (propiconazole; Syngenta, Basel,

Switzerland) at the rate of 0.3 l ha^{-1} at stem extension (Feekes 10; Feekes 1941) to prevent confounding end-use quality results with disease susceptibility. Plots were harvested using a Wintersteiger NurseryMaster combine (Wintersteiger Co., Salt Lake City, UT) and grain was collected individually for each plot.

Data collection

End-use quality analysis was performed by blending 200 g from each replicated field plot for each line at each location (600 g total). Quality analyses were performed individually on a single composite sample per RIL per the locations described above. Data were also averaged over the three locations and analyzed as a 'combined average'. Quality analyses were conducted at the USDA-ARS Western Wheat Quality Laboratory in Pullman, WA. Samples were tempered to 14% moisture content and milled on a Quadrumat system as modified by Jeffers and Rubenthaler (1979). Approved methods of the AACC International (2008) were used for all quality analyses. Grain soundness was evaluated using grain volume weight (Approved Method 55-10). Grain hardness (Approved Method 55-31.01), kernel diameter, and kernel weight were determined using a Single Kernel Characterization System (SKCS) 4100 (Perten Instruments, Springfield, IL). Flour yield (percentage by weight of the total products recovered as straight-grade white flour), break flour yield (percentage by weight of the total products recovered as flour off the break rolls of the mill), and flour ash (Approved Method 08-01) were measured to evaluate milling and particle size components. A calculated trait, milling score (calculated as: $(\{100 - (0.5 \times [16 - \text{temper level}]) + (80 - \text{flour yield}) + (50 \times [\text{flour ash} - 0.30])\} \times 1.274) - 21.602$), also was evaluated. Flour swelling volume (Approved Method 56-21.01), flour sodium dodecylsulfate (SDS) sedimentation volume (Carter et al. 1999), protein content (Approved Method 39-10 adjusted with Dumas combustion method), and flour protein (Approved Method 39-11) were measured to evaluate flour functionality. Solvent retention capacity (SRC) (Approved Method 56-11.02) was conducted on straight-grade flour for lactic acid (SRC lactic), sucrose (SRC sucrose), carbonate (SRC carbonate), and water (SRC water) and data are presented as a percentage (weight of final vs. weight of original). Cookie diameter was evaluated using Approved Method 10-50. Table 1 details the tests performed for samples from each environment.

DNA isolation and marker analysis

The RIL population and the linkage map were fully described in Carter et al. (2009). Briefly, the linkage map comprised 25 linkage groups with markers spaced an average distance of 9.0 cM. Published mapping data were used to place linkage groups on all 21 wheat chromosomes. The genotype data used for linkage map construction included one single nucleotide polymorphism (SNP) and 300 simple sequence repeat (SSR) markers. The marker Ppd-D1 was used to detect allelic differences at the photoperiod insensitivity gene locus, *Ppd-D1* (Hanocq et al. 2004; Beales et al. 2007).

Fresh leaf tissue of three individuals from each $F_{5:6}$ RIL or parent was collected at the five-leaf stage, and used to extract genomic DNA using the method described by Anderson et al. (1992). Sequences of available SSR markers along with their previously determined chromosomal locations were obtained from Graingenes (http://wheat.pw.usda.gov/). SSR marker analysis was conducted using the PCR conditions described by Röder et al. (1998) except that primers were synthesized to include the M13-tail (Oetting et al. 1995). The 10-μL reaction mixture consisted of 50 ng of template DNA, 1.0 μL Mg-free 10× PCR buffer, 0.5 Unit of Taq DNA polymerase, 1.5 mM $MgCl_2$ (Promega, Madison, WI, USA), 200 μM each dCTP, dGTP, dTTP, and dATP (Fermentas, Glen Burnie, MD) and 0.25 μM of each primer pair synthesized by MWG-Biotech (High Point, NC, USA). Appropriate fluorophores for the Global IR^2 analysis system (LiCor Biosciences, Lincoln, NE, USA) were included in the PCR mix. Amplification conditions were an initial 5 min denaturation at 94°C, followed by 41 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 50–65°C (depending on primers), and a 1 min extension at 72°C. The final extension step was 10 min at 72°C. Initial QTL analysis (see below) identified multiple QTL on chromosomes 3B and 4D. As a result, an additional 40 SSR markers located on these chromosomes were screened for polymorphism between the parents and 15 of these were incorporated into the map. To determine the level of polymorphism of associated markers, 24 PNW wheat cultivars were genotyped with the associated markers using the above methods.

Statistical analysis

Levene's test for heterogeneity of variance was used to test for differences among experimental variances across environments. Trait distributions also were compared among environments using univariate statistics and normal plots. Analysis of variance was performed with environment and genotype as main effects using Proc GLM in the statistical package SAS V9.1 (SAS Institute, Raleigh, NC). Because samples were composited within each environment over replications, the genotype by environment interaction term was the denominator for *F* tests of

Table 1 List of 17 end-use quality traits measured in the Louise by Penawawa RIL mapping population in up to three Pacific Northwest environments for use in QTL identification

Abbreviations[a]	Trait	Method of measurement	Environments[b]
GVW	Grain volume weight	Approved Method 55-10	P07, P08, M08
KSIZE	Kernel diameter	Approved Method 55-31.01	P07, P08, M08
KWT	Kernel weight	Approved Method 55-31.01	P07, P08, M08
BKYELD	Break flour yield	Percentage by weight of the total products recovered as flour off the break rolls	P07, P08, M08
FYELD	Flour yield	Percentage by weight of the total products recovered as straight-grade white flour	P07, P08, M08
HA	SKCS hardness	Approved Method 55-31.01	P07, P08, M08
FASH	Flour ash	Approved Method 08-01	P07, P08, M08
MSCOR	Milling score	By calculation	P07, P08, M08
FSV	Flour swelling volume	Approved Method 56-21.01	P07, P08, M08
SEV	SDS sedimentation volume	Carter et al. (1999)	P08, M08
PRO	Grain protein content	Approved Method 39-10	P07, P08, M08
FPRO	Flour protein	Approved Method 39-11	P07, P08, M08
LAC	Solvent retention capacity-lactic acid	Approved Method 56-11.02	P08, M08
SUC	Solvent retention capacity-sucrose	Approved Method 56-11.02	P07, P08, M08
CARB	Solvent retention capacity-carbonate	Approved Method 56-11.02	P07, P08, M08
WAT	Solvent retention capacity-water	Approved Method 56-11.02	P07, P08, M08
CDIA	Cookie diameter	Approved Method 10-52	P07, P08, M08

[a] Abbreviations were paired with trait ontologies in the Gramene website

[b] P07 = Pullman, WA 2007; P08 = Pullman, WA 2008; M08 = Moscow, ID 2008

significance for environments and genotypes from this analysis. Heritability estimates and standard errors were calculated as described by Holland et al. (2003). Heritability estimates for flour SDS sedimentation volume and SRC lactic acid were performed using only two locations. Genotypic and phenotypic correlations and their standard errors were estimated using restricted maximum likelihood estimation, calculated as in Holland (2006).

Linkage maps were constructed using Mapmaker V3.0 (Lander et al. 1987). Additional markers were added to the established genetic linkage map using the "try" command and verified using the "ripple" command. The Kosambi map function was applied to calculate genetic distances in centiMorgans (cM) between the ordered markers (Kosambi 1944). Composite interval mapping (Zeng 1993, 1994) was used to identify markers with significant effects on associated end-use quality traits using the software, WinQTLCart V2.5 (Basten et al. 1997). QTL were identified using composite interval mapping, with a window size of 10 cM, probability in and out of 0.1, a walking speed of 2 cM, five control markers, and the forward and backward regression method. LOD value was set for significant QTL based on a permutation test using 1,000 permutations and a significance value of 0.05. QTL were established using the map position of the peak LOD score in the interval between flanking markers. A one-LOD fall-off (from the QTL peak) was used to estimate the confidence interval of the left and right flanking markers (Chaky 2003). A mixed model with protein content as a covariate was used to assess the effect of that trait on the significance of QTL. The model was $Y = X + M + E + G(M) + P$, where Y is the dependent variable, equal to the value of a genotype for a specific trait and environments, X is the overall mean, and the effects of the independent variables were defined as marker (M), environment (E), and genotype within marker allele $G(M)$; with protein content (P) as a covariate. Marker and environment were considered fixed, whereas genotype and protein content were random effects. Markers were included in the model one at a time and selected from the intervals spanned by significant QTL detected as described above.

Results

Significant differences were detected between the parental lines for all traits except flour yield, SKCS hardness, protein content, flour protein content, and SRC water (Table 2). Environment and genotype effects were significant for all traits in the RIL population. All traits approximated a normal distribution except for grain protein content, flour protein, and flour swelling volume, which were bimodal (Supplemental Figure 1). Transgressive segregation was detected for all traits (Supplemental Figure 1); the mean value for the RIL population was close to the parental mean (Table 2).

Heritabilities were highest for milling traits, flour yield and break flour yield. The lowest heritabilities were

calculated for protein, flour protein and SDS sedimentation, which is a measure of protein quality. The SRC traits had moderately high heritabilities indicating that they would be useful measures of quality traits in breeding programs.

Genotypic and phenotypic correlations were calculated for all end-use quality traits (Table 3) as well as among end-use quality and agronomic traits. In Table 3, genetic correlations that were greater than three times their standard error are highlighted. The correlation results largely reflected the quality trait differences between the two parents (Table 2). The limited recombination and rapid fixation of linkage blocks during the self-pollination process to develop the RIL population preserved important linkage blocks. Milling traits (flour yield and break flour yield) were negatively correlated with sedimentation, grain and flour protein concentration. Milling traits also were negatively correlated with all the SRC tests, which were highly positively correlated among each other. Cookie diameter was positively correlated with milling score and negatively correlated with both grain and protein concentration and with sucrose and water SRC. Measures of kernel morphology (grain volume weight, kernel size and kernel weight) also were positively correlated with flour yield and break flour yield. The SRC tests were correlated with the traits that they were reported to predict (i.e. lactic SRC with sedimentation; carbonate SRC with flour ash).

Correlations among agronomic traits and quality traits also reflected the trait combinations of the parents. Louise was taller and had higher grain yields averaged across environments compared to Penawawa. Although the two parents had similar average heading date, Penawawa is photoperiod insensitive which resulted in earlier maturity. Grain yield was negatively correlated with protein and flour protein. Heading date and maturity were positively correlated with SKCS hardness and flour ash and negatively correlated with lactose SRC. Height was positively correlated with several traits: grain volume weight, kernel weight, flour yield and cookie diameter and negatively correlated with flour ash, flour swelling volume, flour protein, as well as lactic acid, sucrose and carbonate SRC.

QTL analysis identified 34 QTL on 13 wheat chromosomes controlling 16 end-use quality traits (Table 4). QTL were not detected for flour protein. Two major QTL clusters were detected on chromosome 3B and chromosome 4D. Ten QTL mapped to chromosome 3B and spanned a 26.2 cM region flanked by markers *Xbarc68–Xbarc164*. Seven QTL mapped to chromosome 4D and spanned an 18.8 cM region flanked by markers *Xgdm129–Xwmc331*.

One QTL for grain volume weight was identified on chromosome 5B (Table 4) with the allele for higher values inherited from Penawawa. No associations were made between kernel weight and grain volume weight in this population. Three QTL were identified for kernel diameter

Table 2 Means, ranges, and heritabilities of 17 end-use quality traits in a Louise by Penawawa recombinant inbred line (RIL) wheat population using data from three Pacific Northwest locations

Traits	Parental lines				RIL population				Heritability (h^2)	Confidence interval (h^2)
	Louise	Penawawa	Mean	SE	Minimum	Maximum	Mean	SE		
Grain volume weight (kg m^{-3})	785.0	759.3	772.2	0.29	707.9	809.5	767.0	0.54	0.64	0.61–0.67
Kernel diameter (mm)	2.6	2.5	2.5	0.01	2.3	2.8	2.6	0.03	0.62	0.58–0.66
Kernel weight (mg)	37.0	30.5	33.8	0.10	29.7	39.1	34.1	0.75	0.69	0.66–0.72
Break flour yield (%)	50.9	48.7	49.8	0.17	42.2	53.7	49.4	0.43	0.91	0.90–0.92
Flour yield (%)	72.3	68.7	70.5	0.61	65.7	73.8	70.4	0.35	0.82	0.80–0.84
SKCS hardness (unitless)	18.9	13.1	16.0	1.30	2.4	36.9	16.7	2.36	0.63	0.60–0.66
Flour ash (%)	0.35	0.45	0.40	0.01	0.33	0.46	0.38	0.02	0.50	0.46–0.54
Milling score (unitless)	91.1	80.0	85.6	0.50	77.1	93.3	86.5	1.01	0.69	0.66–0.72
Flour swelling volume (mL g^{-1})	19.6	25.1	22.3	0.11	18.4	26.2	22.4	0.57	0.82	0.80–0.84
SDS sedimentation volume (mm)	135.2	161.6	148.4	2.43	69.4	178.1	129.5	3.51	0.24	0.17–0.31
Grain protein content (%)	10.5	10.8	10.6	0.18	10.4	12.3	11.1	0.27	0.23	0.18–0.28
Flour protein (%)	8.9	9.2	9.1	0.20	8.3	9.8	9.1	0.24	0.25	0.20–0.30
SRC lactic acid (%)	127.3	136.9	132.1	0.98	109.8	149.0	131.1	1.54	0.74	0.71–0.77
SRC sucrose (%)	94.0	111.3	102.6	1.55	86.1	114.3	100.3	1.86	0.73	0.70–0.76
SRC carbonate (%)	71.9	78.8	75.3	0.82	68.0	83.9	74.9	1.20	0.68	0.65–0.71
SRC water (%)	53.7	54.9	54.3	0.21	50.0	58.3	53.7	0.60	0.66	0.63–0.69
Cookie diameter (cm)	9.45	9.11	9.28	0.02	8.82	9.73	9.33	0.09	0.49	0.45–0.53

SE standard error term for each measured trait; *ND* no data, values could not be calculated due to missing environments

Table 3 Genotypic correlations (*r*) among 17 end-use quality traits for data collected from recombinant inbred lines from a Louise by Penawawa soft white spring wheat population averaged over three Pacific Northwest locations

	GVW	KSIZE	KWT	BKYELD	FYELD	HA	FASH	MSCOR	FSV	SEV	PRO	FPRO	LAC	SUC	CARB	WAT	CDIA
KSIZE	0.01																
KWT	0.21	**0.78**															
BKYELD	0.09	**0.44**	−0.14														
FYELD	**0.41**	−0.22	0.14	**0.83**													
HA	0.22	−0.02	−0.11	**−0.52**	−0.19												
FASH	−0.36[a]	−0.28	−0.56	−0.11	−0.29[a]	0.12[a]											
MSCOR	**0.47**	−0.03	**0.35**	**0.62**	**0.85**	−0.19	−0.81[a]										
FSV	0.10	−0.14	−0.14	−0.08	−0.10	0.02	0.21[a]	−0.16									
SEV	−0.02	0.21	0.10	**−0.43**	**−0.36**	−0.13	0.06[a]	−0.33	−0.26								
PRO	−0.17	**0.34**	0.04	**−0.56**	**−0.52**	0.12	−0.14	**−0.32**	−0.19	0.33							
FPRO	−0.13	**0.36**	0.03	**−0.44**	**−0.49**	−0.04	−0.23	−0.28	−0.03	0.37	**0.93**						
LAC	−0.04	0.10	−0.03	**−0.37**	**−0.46**	−0.07	−0.25	−0.27	−0.10	**0.68**	**0.53**	**0.56**					
SUC	−0.23	0.18	−0.15	**−0.67**	**−0.85**	0.15	0.30	**−0.74**	0.25	0.33	**0.38**	**0.40**	**0.53**				
CARB	−0.20	0.06	−0.18	**−0.63**	**−0.83**	0.18	**0.37**	**−0.78**	0.17	0.12	0.24	0.19	**0.49**	**0.92**			
WAT	0.01	0.20	0.03	**−0.84**	**−0.68**	**0.67**	0.26	**−0.63**	0.25	0.18	0.26	0.16	**0.32**	**0.71**	**0.76**		
CDIA	0.01	−0.25	0.06	**0.83**	**0.76**	**−0.58**	−0.31	**0.67**	**−0.35**	−0.35[a]	**−0.43**	**−0.34**	−0.43[a]	**−0.78**	−0.80[a]	**−0.93**	
HD	−0.07	−0.13	−0.06	−0.05	0.04	**0.40**	0.32[a]	−0.10	0.03	0.13	0.09	−0.11	**−0.28**	−0.09	0.01	0.12	−0.05
HT	**0.23**	0.17	**0.48**	0.05	**0.35**	**0.27**	**−0.32**	**0.42**	**−0.21**	−0.11	−0.19	**−0.32**	**−0.23**	**−0.36**	**−0.30**	−0.04	**0.23**
MAT	0.05	−0.09	−0.00	0.02	0.16	**0.41**	**0.30**	−0.00	−0.00	0.02	0.02	−0.19	**−0.36**	−0.16	−0.08	0.08	0.03
YLD	0.22	−0.14	0.20	0.13	0.23	0.02	−0.10	0.23	−0.15	0.05	**−0.25**	**−0.45**	−0.08	−0.24	−0.16	0.02	0.26
	GVW	KSIZE	KWT	BKYELD	FYELD	HA	FASH	MSCOR	FSV	SEV	PRO	FPRO	LAC	SUC	CARB	WAT	CDIA

See Table 1 for description of abbreviations. Bold values indicate correlation is three times greater than its standard error

HD heading date, *HT* height, *MAT* maturity, *YLD* yield

[a] Indicates that the REML analysis did not converge

Table 4 Significant quantitative trait loci (QTL) identified through composite interval mapping in a Louise by Penawawa soft wheat spring recombinant inbred line mapping population for end-use quality traits averaged over three Pacific Northwest locations

Chromosome	QTL name	Confidence interval (cM)[a]	Peak position (cM)	Marker nearest peak LOD	Trait	Location[b]	R^2	LOD	Additive effect[c]	Parental allele contributing to higher trait value	Coincident agronomic QTL[d]
1A	*QMscor.wak-1A*	24.0–34.4	28.3	*Xgwm136*	Milling score	M8	0.05	3.3	−0.68	Penawawa	
						Ave	0.06	3.7	−0.72		
	QSuc.wak-1A	45.2–60.7	52.0	*Xwmc278*	SRC sucrose	P7	0.10	4.0	1.83	Louise	
						M8	0.13	4.1	2.18		
						Ave	0.11	4.8	1.93		
2A	*QKsize.wak-2A*	0.0–27.3	22.7	*Xgwm630*	Kernel diameter	P8	0.10	3.3	0.03	Louise	
						M8	0.13	3.0	0.03		
						Ave	0.13	3.4	0.03		
2B	*QHa.wak-2B*	71.5–85.6	78.0	*Xwmc474*	SKCS hardness	P7	0.16	3.4	−2.02	Penawawa	Stripe rust resistance
						P8	0.09	5.8	−2.02		
						M8	0.10	4.0	−2.64		
						Ave	0.13	5.2	−2.21		
	QKsize.wak-2B	116.6–124.6	122.8	*Xwmc441*	Kernel diameter	M8	0.08	4.4	−0.03	Penawawa	
						Ave	0.07	3.2	−0.03		
	QKwt.wak-2B	121.3–132.7	128.2	*Xbarc101*	Kernel weight	P8	0.16	3.0	−0.46	Penawawa	
						M8	0.10	4.7	−0.79		
						Ave	0.11	5.1	−0.71		
2D	*QHa.wak-2B*	50.3–60.8	56.1	*Ppd-D1*	SKCS hardness	P8	0.21	10.2	3.73	Louise	Heading/maturity date
						M8	0.20	8.5	3.13		
						Ave	0.16	7.8	2.42		
3B	*QCdia.wak-3B*	86.8–95.3	90.9	*Xwmc777*	Cookie diameter	P7	0.10	4.9	0.08	Louise	Grain yield/plant height
						P8	0.17	8.3	0.09		
						Ave	0.13	7.7	0.07		
	QMscor.wak-3B	89.8–92.4	91.0	*Xwmc777*	Milling score	P7	0.16	5.4	1.21	Louise	Grain yield/plant height
						P8	0.23	13.4	1.68		
						M8	0.23	13.4	1.41		
						Ave	0.21	13.8	1.36		
	QFyeld.wak-3B	89.8–92.9	91.7	*Xgwm72*	Flour yield	P7	0.19	9.1	0.72	Louise	Grain yield/plant height
						P8	0.30	17.3	1.04		
						M8	0.24	14.3	0.89		
						Ave	0.24	14.9	0.85		

Table 4 continued

Chromosome	QTL name	Confidence interval (cM)[a]	Peak position (cM)	Marker nearest peak LOD	Trait	Location[b]	R^2	LOD	Additive effect[c]	Parental allele contributing to higher trait value	Coincident agronomic QTL[d]
	QBkyeld.wak-3B	94.1–100.3	96.6	*Xwmc751*	Break flour yield	P7	0.17	8.8	0.84	Louise	Grain yield/plant height
						P8	0.16	8.8	0.79		
						M8	0.09	4.9	0.64		
						Ave	0.12	7.0	0.70		
	QSuc.wak-3B	96.0–100.7	97.7	*Xwmc751*	SRC sucrose	P7	0.18	10.0	−2.45	Penawawa	Grain yield/plant height
						P8	0.15	8.0	−2.84		
						M8	0.19	9.4	−2.61		
						Ave	0.24	12.7	−2.78		
	QWat.wak-3B	94.2–102.9	97.9	*Xwmc751*	SRC water	P8	0.16	7.4	−0.78	Penawawa	Grain yield/plant height
						Ave	0.09	4.6	−0.47		
	QLac.wak-3B	95.8–108.4	98.6	*Xwmc751*	SRC lactic acid	P8	0.14	6.9	−3.35	Penawawa	Grain yield/plant height
						Ave	0.15	7.3	−3.43		
	QCarb.wak-3B	95.5–101.7	99.7	*Xwmc751*	SRC carbonate	P7	0.13	6.1	−1.40	Penawawa	Grain yield/plant height
						P8	0.17	8.5	−1.62		
						M8	0.11	5.6	−1.13		
						Ave	0.13	6.1	−1.23		
	QPro.wak-3B	96.6–104.9	100.7	*Xwmc751*	Grain protein content	P7	0.07	3.0	−0.14	Penawawa	Grain yield/plant height
						M8	0.07	3.0	−0.14		
						Ave	0.14	6.8	−0.14		
	QSev.wak-3B	96.4–113.0	106.4	*Xwmc527*	SDS sedimentation volume	P8	0.10	4.8	−8.07	Penawawa	Grain yield/plant height
						M8	0.11	4.5	−12.2		
						Ave	0.10	4.4	−7.28		
4A	*QFsv.wak-4A*	16.9–24.7	19.2	*Xbarc223*	Flour swelling volume	P7	0.73	45.0	−1.98	Penawawa	
						P8	0.79	58.2	−2.12		
						M8	0.81	65.4	−2.02		
						Ave	0.78	67.6	−1.94		
	QCdia.wak-4A	16.9–24.7	20.0	*Xbarc223*	Cookie diameter	P8	0.07	4.5	0.06	Louise	
						M8	0.08	3.0	0.06		
						Ave	0.09	3.0	0.05		
4B	*QHa.wak-4B*	0.0–6.1	0.8	*Xbarc114*	SKCS hardness	P8	0.15	6.7	2.66	Louise	
						M8	0.14	6.9	3.07		
						Ave	0.14	6.4	2.22		

Table 4 continued

Chromosome	QTL name	Confidence interval (cM)[a]	Peak position (cM)	Marker nearest peak LOD	Trait	Location[b]	R^2	LOD	Additive effect[c]	Parental allele contributing to higher trait value	Coincident agronomic QTL[d]
4D	*QFyeld.wak-4D*	0.0–2.9	0.1	*Xgdm129*	Flour yield	M8	0.05	3.6	0.49	Louise	
						Ave	0.07	3.6	0.38		
	QSuc.wak-4D	0–2.7	0.9	*Xgdm129*	SRC sucrose	P7	0.06	3.0	−1.46	Penawawa	
						M8	0.08	3.6	−1.75		
						Ave	0.07	3.3	−1.47		
	QBkfeld.wak-4D	0–8.5	3.0	*Xwmc52*	Break flour yield	P8	0.05	3.1	0.45	Louise	
						M8	0.07	3.4	0.55		
						Ave	0.06	3.6	0.48		
	QCdia.wak-4D	1.0–12.0	7.0	*Xbarc288*	Cookie diameter	P8	0.05	3.0	0.05	Louise	
						Ave	0.07	4.1	0.05		
	QCarb.wak-4D	0–18.8	12.9	*Xbarc288*	SRC carbonate	P7	0.10	3.5	−1.24	Penawawa	
						P8	0.16	5.9	−1.56		
						M8	0.11	6.4	−1.12		
						Ave	0.17	6.8	−1.38		
	QWat.wak-4D	0–18.8	12.9	*Xbarc288*	SRC water	P7	0.08	3.9	−0.46	Penawawa	
						M8	0.08	3.8	−0.50		
						Ave	0.10	4.3	−0.50		
	QMscor.wak-4D	0–18.8	12.9	*Xbarc288*	Milling score	P8	0.08	4.8	1.02	Louise	
						M8	0.06	4.0	0.70		
						Ave	0.06	4.2	0.78		
5B	*QTwt.wak-5B*	29.6–38.7	33.9	*Xgwm133*	Grain volume weight	P7	0.10	3.6	−0.50	Penawawa	
						P8	0.10	4.4	−0.51		
						M8	0.07	4.4	−0.42		
						Ave	0.11	5.2	−0.46		
6A	*QFash.wak-6A*	25.9–37.3	31.8	*Xwmc256*	Flour ash	M8	0.09	3.0	−0.01	Penawawa	
						Ave	0.09	3.7	−0.01		
	QHa.wak-6A	37.4–43.4	38.8	*Xwmc684*	SKCS hardness	P8	0.07	3.2	1.75	Louise	
						M8	0.08	3.2	2.38		
						Ave	0.07	3.7	1.61		
6B	*QBkyeld.wak-6B*	6.8–28.4	18.4	*Xgwm508*	Break flour yield	P7	0.10	3.6	0.63	Louise	
						P8	0.11	4.1	0.66		
						M8	0.10	4.2	0.66		
						Ave	0.12	4.6	0.68		

Table 4 continued

Chromosome	QTL name	Confidence interval (cM)[a]	Peak position (cM)	Marker nearest peak LOD	Trait	Location[b]	R^2	LOD	Additive effect[c]	Parental allele contributing to higher trait value	Coincident agronomic QTL[d]
6D	*QFyeld.wak-6D*	97.2–109.4	103.6	*Xcfd80*	Flour yield	Ave	0.06	4.3	0.63	Louise	
	QBkyeld.wak-6D	97.2–109.4	103.6	*Xcfd80*	Break flour yield	P7	0.12	4.9	0.71	Louise	
						P8	0.10	3.0	0.63		
						M8	0.12	4.1	0.71		
						Ave	0.10	4.3	0.63		
7D	*QKsize.wak-7D*	68.9–79.0	74.0	*Xgwm130*	Kernel diameter	Ave	0.10	3.7	0.03	Louise	Heading/maturity date

[a] Confidence interval based on a one LOD drop-off from the peak LOD position

[b] Locations: P7 = Pullman, 2007; P8 = Pullman, 2008; M8 = Moscow, 2008; Ave = averaged trait value from all three locations

[c] Additive effect of the Louise allele

[d] Based on results from Carter et al. (2009, 2011)

and one QTL for kernel weight. The QTL for kernel weight coincided with the QTL for kernel diameter on chromosome 2B (Table 4; Fig. 1) with higher values inherited from Penawawa. Conversely, the Louise allele at the QTL on chromosomes 2A and 7D contributed to larger kernel diameter. On average, Louise had a larger and heavier kernel compared to Penawawa (Table 2).

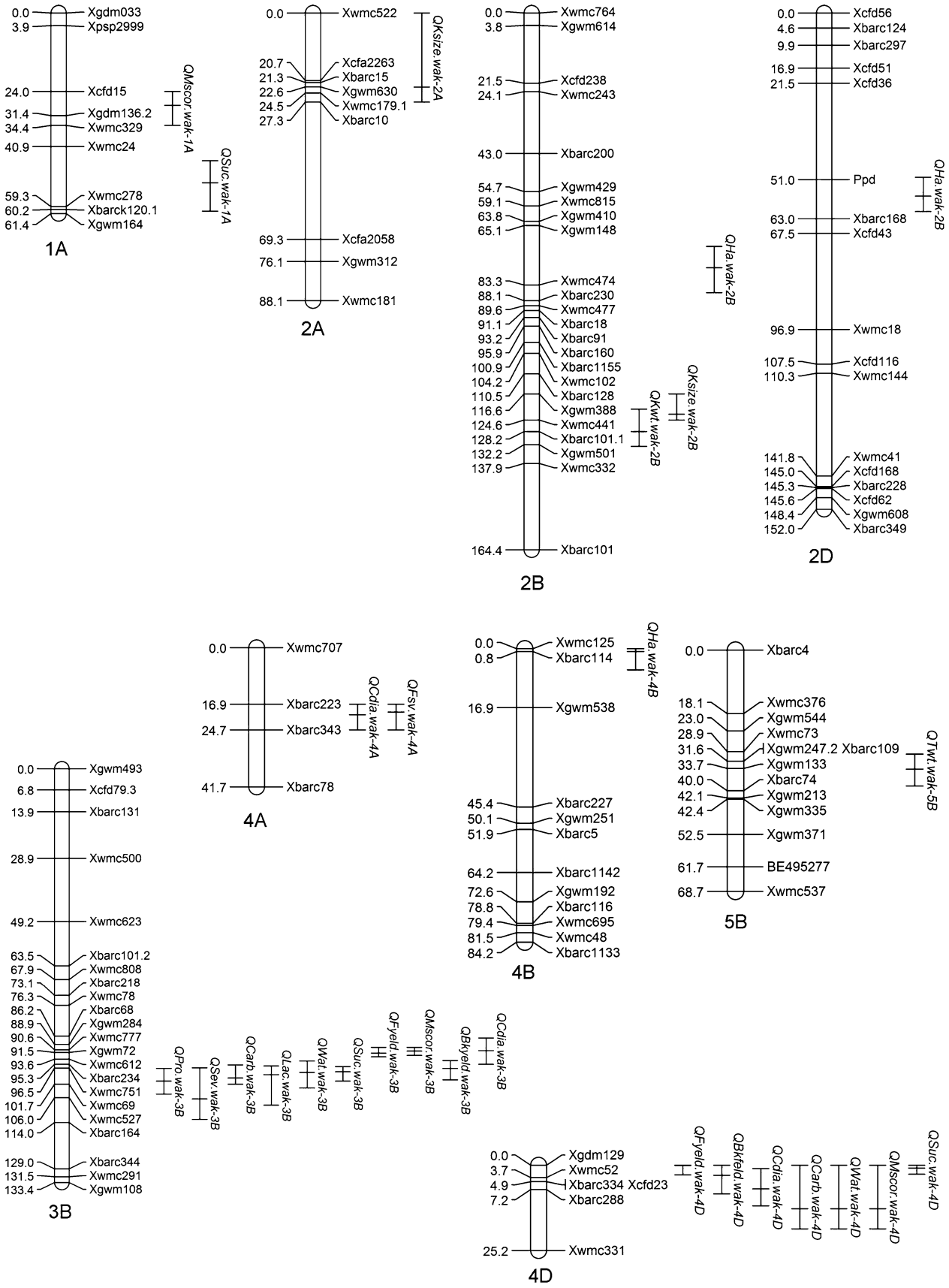
Four QTL were identified for break flour yield and three QTL were identified for flour yield in this population. QTL for both traits were identified on chromosome 3B, 4D, and 6D (Table 4). The Louise allele at all of these QTL contributed to the higher flour and break flour yields. The most significant increase in break flour and flour yield occurred at the 3B locus, where the Louise allele contributed to 2% greater yields (increased from 48 to 50%), whereas the Louise allele at the 4D and 6D loci contributed to increases of 1% (percentages are expressed in yield vs. total products).

The QTL for SKCS kernel hardness were detected on different chromosomes than those for break flour yield (Table 4). Higher values for SKCS (harder) were attributed to the Louise allele on chromosomes 2D, 4B, and 6A but not at the QTL on chromosome 2B. In this experiment, Louise was 5 SKCS units harder than Penawawa, although significant differences did not exist for SKCS hardness, or for flour yield, between the two parents (Table 2). Long-term data indicates that the SKCS hardness of these two cultivars is quite similar (data not shown). Although SKCS kernel hardness was negatively correlated to break flour yield and cookie diameter, no QTL for SKCS kernel hardness coincided with QTL for the other two traits. The QTL for break flour yield and flour yield indicate that there are other factors controlling differences in break flour yield and cookie diameter besides kernel hardness as defined by resistance to crushing. Milling involves both a shearing and crushing action, and includes endosperm–bran separation, and flour sifting characteristics.

The QTL for flour ash content was identified on chromosome 6A and corresponded to the same chromosomal region as a QTL for SK hardness (Fig. 1). The Louise allele contributed to lower flour ash content (0.37%) compared to the Penawawa allele (0.39%).

Three milling score QTL were identified in this population and corresponded to the same QTL region identified for the flour yield QTL on chromosomes 3B and 4D (Table 4). The Louise allele at these QTL contributed to the higher milling score for these RIL (on average three points higher). An additional weaker QTL was identified on chromosome 1A with the higher allele inherited from Penawawa.

The QTL for flour swelling volume, indicative of normal vs. reduced amylose starch, was located on chromosome 4A near the *Wx-B1* locus, based on previous mapping


0.0 Xgdm033
3.9 Xpsp2999
24.0 Xcfd15
31.4 Xgdm136.2
34.4 Xwmc329
40.9 Xwmc24
59.3 Xwmc278
60.2 Xbarck120.1
61.4 Xgwm164
QMscor.wak-1A
QSuc.wak-1A
1A
0.0 Xwmc522
20.7 Xcfa2263
21.3 Xbarc15
22.6 Xgwm630
24.5 Xwmc179.1
27.3 Xbarc10
69.3 Xcfa2058
76.1 Xgwm312
88.1 Xwmc181
QKsize.wak-2A
2A
0.0 Xwmc764
3.8 Xgwm614
21.5 Xcfd238
24.1 Xwmc243
43.0 Xbarc200
54.7 Xgwm429
59.1 Xwmc815
63.8 Xgwm410
65.1 Xgwm148
83.3 Xwmc474
88.1 Xbarc230
89.6 Xwmc477
91.1 Xbarc18
93.2 Xbarc91
95.9 Xbarc160
100.9 Xbarc1155
104.2 Xwmc102
110.5 Xbarc128
116.6 Xgwm388
124.6 Xwmc441
128.2 Xbarc101.1
132.2 Xgwm501
137.9 Xwmc332
164.4 Xbarc101
QKwt.wak-2B
QKsize.wak-2B
2B
0.0 Xcfd56
4.6 Xbarc124
9.9 Xbarc297
16.9 Xcfd51
21.5 Xcfd36
51.0 Ppd
63.0 Xbarc168
67.5 Xcfd43
96.9 Xwmc18
107.5 Xcfd116
110.3 Xwmc144
141.8 Xwmc41
145.0 Xcfd168
145.3 Xbarc228
145.6 Xcfd62
148.4 Xgwm608
152.0 Xbarc349
QHa.wak-2B
QHa.wak-2B
2D
0.0 Xwmc707
16.9 Xbarc223
24.7 Xbarc343
41.7 Xbarc78
QCdia.wak-4A
QFsv.wak-4A
4A
0.0 Xwmc125
0.8 Xbarc114
16.9 Xgwm538
45.4 Xbarc227
50.1 Xgwm251
51.9 Xbarc5
64.2 Xbarc1142
72.6 Xgwm192
78.8 Xbarc116
79.4 Xwmc695
81.5 Xwmc48
84.2 Xbarc1133
QHa.wak-4B
4B
0.0 Xbarc4
18.1 Xwmc376
23.0 Xgwm544
28.9 Xwmc73
31.6 Xgwm247.2 Xbarc109
33.7 Xgwm133
40.0 Xbarc74
42.1 Xgwm213
42.4 Xgwm335
52.5 Xgwm371
61.7 BE495277
68.7 Xwmc537
QTwt.wak-5B
5B
0.0 Xgwm493
6.8 Xcfd79.3
13.9 Xbarc131
28.9 Xwmc500
49.2 Xwmc623
63.5 Xbarc101.2
67.9 Xwmc808
73.1 Xbarc218
76.3 Xwmc78
86.2 Xbarc68
88.9 Xgwm284
90.6 Xwmc777
91.5 Xgwm72
93.6 Xwmc612
95.3 Xbarc234
96.5 Xwmc751
101.7 Xwmc69
106.0 Xwmc527
114.0 Xbarc164
129.0 Xbarc344
131.5 Xwmc291
133.4 Xgwm108
QPro.wak-3B
QSev.wak-3B
QCarb.wak-3B
QLac.wak-3B
QWat.wak-3B
QSuc.wak-3B
QFyield.wak-3B
QMscor.wak-3B
QBkyield.wak-3B
QCdia.wak-3B
3B
0.0 Xgdm129
3.7 Xwmc52
4.9 Xbarc334 Xcfd23
7.2 Xbarc288
25.2 Xwmc331
QFyield.wak-4D
QBkfield.wak-4D
QCdia.wak-4D
QCarb.wak-4D
QWat.wak-4D
QMscor.wak-4D
QSuc.wak-4D
4D

◀ **Fig. 1** Partial genetic linkage map from the Louise by Penawawa recombinant inbred line populations depicting the chromosomes where quantitative trait loci (QTL) were identified for end-use quality traits when averaged across three Pacific Northwest locations. *Lines* indicate peak position and confidence interval of the identified QTL

results (Miura and Sugwara 1996). The Penawawa allele at this locus increased flour swelling volume by 4 mL g^{-1}.

SDS sedimentation values ranged from 69.4 to 178.1 mm in the RIL population providing insight into gluten strength (lower values are indicative of weaker gluten). One QTL for flour SDS sedimentation volume was localized to the cluster of end-use quality QTL on chromosome 3B (Fig. 1). The Louise allele at this QTL was associated with a lower SDS sedimentation volume (Louise = 135.2 mm), indicating that it has weaker gluten content than does Penawawa (Penawawa = 161.6 mm). Both Louise and Penawawa are null for *Glu-A1*, 7 + 9 for *Glu-B1*, and 5 + 10 for *Glu-D1*.

Across the three locations, one QTL was identified for grain protein content (Table 4) and the Louise allele at the chromosome 3B locus contributed to a lower protein content of 0.2% (11.0 compared to 11.2%).

Analysis of the SRC data revealed QTL clustering to the chromosome 3B and 4D regions in this population (Table 4). QTL were found on 4D for all SRC tests except SRC lactic acid, which only mapped to chromosome 3B. The Louise allele at the QTL on 3B lowered SRC values by three to seven percentage points. These QTL overlapped the same general region on chromosome 3B, although slight variation was detected (Fig. 1). On chromosome 4D, the Louise allele for all of these QTL contributed to lower SRC values by 2–3%. One additional QTL for SRC sucrose was identified on chromosome 1A (Fig. 1). The Louise allele for the QTL on 1A contributed to higher SRC sucrose score. Averaged over all locations, Louise had lower SRC values than Penawawa (Table 2).

Three QTL for cookie diameter were identified on chromosomes 3B, 4A, and 4D (Table 4). The QTL on chromosomes 3B and 4D correspond to the other QTL clusters that have been associated with these chromosomes for SRC tests, break flour yield, flour yield and milling score. The QTL on 4A is at the *Wx-B1* locus (Miura and Sugwara 1996). The Louise allele at the three QTL increased the cookie diameter of the RIL by 0.14, 0.15, and 0.10 cm, respectively. The cookie diameter of RIL with the

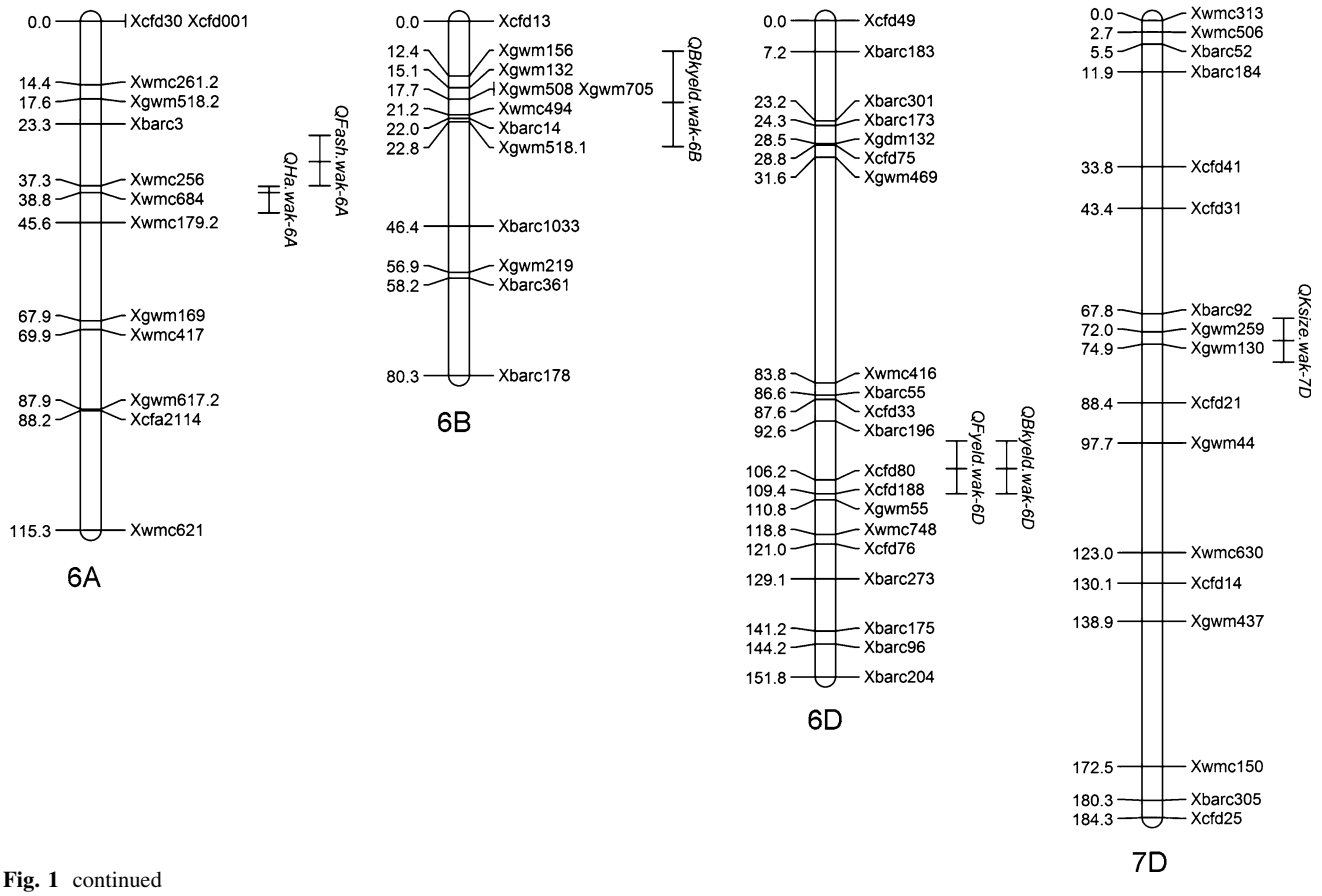


Fig. 1 continued

Louise allele at the 3B, 4A, and 4D loci have an increased cookie diameter of 0.21 cm as compared to RIL with the Penawawa allele at all three loci.

Because we detected the well-known negative correlation between grain yield and protein content, as well as QTL for both traits on chromosome 3B, we used mixed model analyses including either protein content or grain yield as a covariate to determine if grain yield or protein content was underlying our ability to detect significant QTL for the other traits in that cluster. When grain yield was analyzed as a covariate, it was found to be significant for all traits, but the marker effect was still highly significant ($P < 0.0001$) for all end-use quality traits analyzed. The protein covariate was significant for all traits analyzed but the marker effect was still highly significant ($P < 0.0001$) for the SRC tests, milling traits, cookie diameter and for grain yield. Only the QTL for flour SDS sedimentation volume became non-significant with protein in the model, indicating that this QTL was probably due to protein content as much as protein quality at this locus. Although protein concentration has a demonstrated effect on grain quality, the QTL detected in this study are independent of protein quantity.

In addition to Louise and Penawawa, 22 elite soft white wheat (both spring and winter) lines were assayed for seven of the SSR markers on chromosomes 3B and 4D which were associated with end-use quality traits (Table 5). End-use quality scores were compiled from the USDA Western Wheat Quality Laboratory (http://www.wsu.edu/~wwql). Wakanz and Wawawai are the parental lines of Louise and have the same allele profile at all loci for these markers. Diva and WA8089 both have Louise as one of the parents and exhibit excellent end-use quality. Diva has the same allele profile as Louise on chromosome 3B with the exception of *Xwmc751*, but does not share the same alleles on chromosome 4D. WA8089 has the same allele profile as Louise with the exception of marker *Xgdm129* on chromosome 4D. WA8124, which has excellent end-quality, is a spring wheat line which does not have Louise in the pedigree, yet shares six of the seven loci in common. The winter wheat lines, ARS-Amber, which has excellent end-use quality, shares five of the seven loci in common, whereas Brundage 96, a quality standard, shares four of the seven loci in common.

Discussion

In the Louise by Penawawa RIL population, RIL with higher grain volume weights also had higher kernel weight and kernel diameter. Two QTL for kernel diameter identified on chromosome 2A and 7D were associated with the Louise alleles, which contributed to larger kernels. However, the kernel diameter and kernel weight QTL on 2B was associated with the alleles from Penawawa, as was the grain volume weight QTL on 5B. Interestingly, these higher trait values are associated with the alleles from Penawawa, the cultivar with the lower mean values for these traits. Breseghello et al. (2005) found similar QTL with effects opposite to the phenotypes. For example, the hard wheat parent in the population contributed alleles for softness, larger cookie diameter, flour yield, and sucrose retention capacity. These differences are to be expected, especially when parental lines are characterized based on observable phenotypes rather than genetic potential.

Break flour and total flour yield are two traits of economic importance to flour millers. An increase in total flour yield results in a higher profit margin on the total grain milled. When RIL were selected containing the Louise allele at the three QTL on chromosomes 3B, 4D and 6D, there was a 3.5% increase in total flour yield (68.5% with the Penawawa alleles compared to 72.0% with the Louise alleles). There was a 4% increase in break flour yield when all alleles from Louise were present (47.2% with Penawawa alleles compared to 51.2% with Louise alleles). These values were similar to the mean values for each of the parental lines (Table 2). Thus, the full potential for break flour and flour yield from Louise can be captured at these three loci and these QTL appear to be additive in nature.

The QTL for grain hardness on chromosome 2B has been reported in other research. Smith et al. (2008), using association analysis of 192 soft winter wheat cultivars, identified grain hardness QTL on chromosome 2B in the same region that we identified. Smith et al. (2011) found a QTL for softness equivalent in a soft white RIL population also located on chromosome 2B. Sourdille et al. (1996) reported QTL affecting grain hardness on 2A, 2D, 5B, and 5D. Giroux and Morris (1997, 1998) showed that grain hardness in wheat was associated with mutations in either of the closely linked *Pina-D1* or *Pinb-D1* loci; however, these genes were not segregating in this population. Both Louise and Penawawa contain the *Pina-D1a/Pinb-D1a* haplotype, which results in soft kernels. Thus, the identified QTL are for grain texture traits that are distinct from these known mutations and potentially of more use to wheat breeders since they rarely make crosses outside of texture classes. Chang et al. (2006) found that several loci for storage protein content were associated with kernel hardness on chromosome 2B. Chang et al. (2006) concluded that other factors such as pentosans, polar lipids, and protein in the endosperm may also affect kernel texture. The QTL identified in this study and others reflecting grain hardness apart from the *Ha* locus on 5DS needs further investigation to determine which of the above-mentioned factors may be influencing kernel texture in this population.

The AACC International sugar snap cookie is the widely used standard for soft wheat baking quality in North

Table 5 Polymorphism in 24 selected soft white wheat germplasm for the molecular markers associated with superior end-use quality traits on chromosome 3B and 4D from the soft white spring wheat cultivar Louise

Germplasm	Growth habit	3B				4D		
		Xwmc777	*Xgwm72*	*Xwmc751*	*Xwmc69*	*Xgdm129*	*Xwmc52*	*Xbarc288*
Louise (PI 634865)[a]	Spring	153[b]	160	140	253	141	235	261
Penawawa (PI 495916)	Spring	134	132	138	251	139	237	257
Wakanz (PI 506352)	Spring	153	160	140	253	141	235	261
Wawawai (PI 574598)	Spring	153	160	140	253	141	235	261
Diva (PI 660663)	Spring	153	160	138	253	139	238	260
Alturas (PI 620631)	Spring	134	132	138	251	139	243	261
Alpowa (PI 566596)	Spring	134	132	136	251	139	237	258
ID377S (PI 591045)	Spring	134	132	138	253	139	235	265
Whit (PI 653841)	Spring	153	160	132	251/253	139	235	265
Babe (PI 656791)	Spring	153	132	136	251	139	236	257
Nick (Westbred)	Spring	154	160	140	249	141	242	260
Eden (PI 630983)	Spring Club	154	160	138	253	139	235	260
JD (PI 656790)	Spring Club	153	160	138	253	139	235	261
WA8089 (WSU)	Spring	153	160	140	253	141	235	260
WA8124 (WSU)	Spring	154	132	140	253	141	235	260
Brundage 96 (PI 631486)	Winter	153	132	132	253	139	235	261
Bruneau (U of I)	Winter	152	132	136	253	139	235	260
Xerpha (PI 645605)	Winter	153	160	135	250	141	234	No data
Eltan (PI 536994)	Winter	153	160	140	251	139	237	261
Madsen (PI 511673)	Winter	153	132	136	253	139	235	261
Legion (Syngenta Cereals)	Winter	152	132	136	251	139	235	No data
Bitterroot (U of I)	Winter	152	132	136	253	139	235	261
WB528 (WestBred)	Winter	152	132	138	253	139	235	261
ARS-Amber (USDA-ARS)	Winter	153	160	140	251	139	235	261
Polymorphism (%)		45.8	50	66.7	37.5	70.8	33.3	52.4

WSU Washington State University, *U of I* University of Idaho

[a] Each germplasm is indicated either with the PI number or the university/company who released the variety

[b] Reported fragment is in base pair length and includes a 19-bp M13 tail

American wheat breeding programs (Morris and Rose 1996). In the present study, the QTL for cookie diameter and flour swelling volume identified on chromosome 4A maps near the *Wx-B1* locus, encoding the GBSS gene (Miura and Sugwara 1996). The *Wx-B1* allele has a dramatic effect on starch properties as evidenced by the bimodal distribution of RIL phenotypes (Supplemental Figure 1) and the high LOD score (45.0–67.6) for the 4A QTL. The clustering of cookie diameter and FSV on 4A may indicate that the partial waxy trait is somewhat detrimental to cookie baking quality. Certainly, starch damage, pentosans and other flour constituents that affect water relations are known to affect cookie diameter by affecting the spread in the oven.

The clustering of QTL may be due to linked genes, or to pleiotropic effects of a common gene. A reasonable model to describe the multiple effects of the QTL on chromosome 3B is that endosperm proteins increase the strength of the endosperm, thereby making it 'harder' (Chang et al. 2006). Harder endosperm would have lower milling performance, reduced break flour and flour yields and reduced milling score (as evidenced by the Penawawa allele contributing negatively to these traits). Harder endosperm also contributes to greater starch damage (although not measured here), which increases solvent retention in SRC tests and reduces cookie diameter. The Penawawa allele contributed to higher SRC values and lower cookie diameters. Higher protein levels would increase SDS sedimentation volume and SRC lactic acid, both direct measures of protein content and strength. Thus, the Louise allele at these loci would contribute to desirable end-use quality traits.

The clustering of QTL on chromosome 3B also may be a pleiotropic effect of the grain yield (Carter et al. 2011) and grain protein concentration QTL found in the similar region. We detected the well-known negative correlation between grain yield and protein content, although it was

moderate in our study. For soft wheat products, lower protein content is generally preferred, and, as was also noted in our study, protein content has been shown to have a negative correlation with cookie diameter (Gaines 2004). For soft wheat products, the effect of protein is due to water holding capacity, rather than formation of gluten networks. Protein quantity is generally not correlated with flour milling traits in soft wheat (Moiraghi et al. 2011). Protein quantity and grain yield are negatively correlated in wheat but neither of those traits influenced our ability to detect QTL for milling, SRC, or end-use quality traits at the 3B locus so we believe that there is a direct effect on grain quality at that locus, apart from the effect on grain yield and protein content.

The clustering of QTL on 4D have a logical connection between a fundamental physical–chemical difference in endosperm composition, and the manifestations observed as break flour and flour yield, milling score, cookie diameter, and SRC water, carbonate and sucrose. The Louise alleles at all these loci contribute to lower SRC values and higher break flour and flour yield, milling score, and cookie diameter. Consequently, the present results suggest that two regions of chromosomes 3B and 4D confer multiple benefits to soft wheat quality in this population. By using a subset of markers dispersed throughout these two regions, lines could be selected to carry favorable alleles for end-use quality traits in wheat.

Although the percentage polymorphism of these markers when tested across spring and winter wheat germplasm is moderate, they will still be useful in marker-assisted selection programs since many of the lines with good end-use quality carry the desired alleles whereas lines with marginal quality do not. Many of the spring wheat lines tested in this panel either have Louise in their pedigree or are newer lines selected to have excellent end-use quality and thus have fixed many of these alleles in newer populations. Since they were found to carry similar alleles at each locus, the overall percentage of polymorphism was lowered. When older lines such as Alturas, Alpowa, and ID377S are examined, the level of polymorphism between these lines and Louise is nearly 100%. The winter wheat lines are less diagnostic, with excellent quality lines like Brundage 96, Bitterroot, and ARS-Amber sharing only 60–72% of loci with similar alleles. In the winter wheat line Xerpha, which has acceptable end-use quality, only 43% of the loci have similar alleles. In the spring and winter wheat germplasm evaluated, the presence of these alleles is associated with desirable end-use quality parameters; however, these QTL still need to be validated in multiple backgrounds to confirm these results. The identified markers may be useful for introgressing genes associated with good quality into lines with desirable attributes that do not have acceptable end-use quality.

The genetic correlations currently existing in the population are favorable for soft wheat breeding where the targets are high milling score combined with low SRC water, carbonate and sucrose values, and greater cookie diameter. The association of kernel size with milling traits has been reported previously (Breseghello and Sorrels 2006), as have inter-correlations among SRC solvents. The negative correlation of yield with protein is desirable in soft wheat; premiums for low protein soft wheat have been available in some years. Although this population resulted from limited recombination, this research identified linkage blocks with trait combinations inherited from parents that should be maintained. Most correlations among traits evaluated here were not significant, however, indicating that simultaneous improvement of agronomic and quality traits can be achieved in soft wheat breeding.

Conclusion

Knowledge of the genetic architecture of end-use quality traits in wheat is essential to making gains from selection during the breeding process. The majority of the positive alleles for end-use quality were associated with the parent Louise on chromosomes 3B and 4D. Utilizing Louise as a parent in wheat cross-hybridization allows breeders to select the desired DNA regions associated with improved end-use quality, thereby allowing for the improvement of end-use quality through marker-assisted selection. These results improve our understanding of the relationships among multiple quality traits and especially milling quality, and identified major QTL for multiple traits, thereby allowing plant breeders to make valuable gains from selection early in the soft wheat breeding process.

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