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ORIGINAL PAPER

New QTL alleles for quality-related traits in spring wheat revealed by RIL population derived from supernumerary × non-supernumerary spikelet genotypes

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Abstract

Key message **A population developed from an exotic line with supernumerary spikelets was genetically dissected for eight quality traits, discovering new genes/alleles with potential use in wheat breeding programs.**

Abstract Identifying new QTLs and alleles in exotic germplasm is paramount for further improvement of quality traits in wheat. In the present study, an RIL population developed from a cross of an elite wheat line (WCB414) and an exotic genotype with supernumerary spikelets (SS) was used to identify QTLs and new alleles for eight quality traits. Composite interval mapping for 1,000 kernels weight (TKW), kernel volume weight (KVW), grain protein content (GPC), percent of flour extraction (FE) and four mixograph-related traits identified a total of 69 QTLs including 19 stable QTLs. These QTLs were located on 18 different chromosomes (except 4D, 5D, and 6D). Thirteen of these QTLs explained more than 15 % of phenotypic variation (PV) and were considered as major QTLs. In this study, we identified 11 QTLs for TKW (R^2 = 7.2–17.1 %), 10 for KVW (R^2 = 6.7–22.5 %), 11 for GPC (R^2 = 4.7–16.9 %), 6 for FE (R^2 = 4.8–19 %) and 31 for mixograph-related traits (R^2 = 3.2–41.2 %). In this population, several previously identified QTLs for SS, nine spike-related and ten agronomic traits were co-located with the quality QTLs, suggesting pleiotropic effects or close linkage among loci. The traits GPC and mixogram-related traits were positively correlated with SS. Indeed, several loci for quality traits were co-located with QTL for SS. The exotic parent contributed positive alleles that increased PV of the traits at 56 % of loci demonstrating the suitability of germplasm with SS to improve quality traits in wheat.

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Introduction

Common wheat (*Triticum aestivum* L.) was in 2012 the cereal harvested in the largest area (about 216 million ha.) and the cereal with the third highest production worldwide (about 720 million tons), surpassed only by maize and rice. Wheat is also the main food supply crop in the world (66 kg/capita/year) and the most important source of proteins (16.2 g/capital/day) worldwide (FAO-FAOSTAT 2014). These statistics make wheat breeding a paramount activity for food security in the world and for the dynamics of the global markets. Breeding programs of wheat have the objective of supplying varieties for a diverse market and end users including growers, millers, bakers, and consumers. This is a daunting challenge since selection for optimal characteristics for one of these customers could be detrimental for others (Carena 2009). Wheat breeders

select the best genotypes through the assessment of a large number of agronomic and quality traits. Plant height, grain yield, disease resistance, and days to heading are examples of agronomic characters tested; while grain protein content, flour extraction, dough resistance, and baking performance are quality traits considered in the selection of superior cultivars. The assessment of quality traits, with difference of agronomic traits, requires large samples, time, and specialized personnel to conduct complex procedures. Marker-assisted selection (MAS) has been suggested as a shortcut for the development of premium cultivars (Nelson et al. 2006; Raman et al. 2009; Simons et al. 2012). This approach offers the wheat breeders an opportunity to select genotypes bypassing the assessment of quality traits. Therefore, in the last years, a broad number of genetic studies have been conducted to map QTLs/genes associated with quality traits in wheat and identify suitable markers for use in MAS (Huang et al. 2006; McCartney et al. 2006; Nelson et al. 2006; Mann et al. 2009; Raman et al. 2009; Tsilo et al. 2011; Carter et al. 2012; Simons et al. 2012).

Among the quality traits, GPC has received special attention because it is an indicator of the performance of wheat-derived products (Zhao et al. 2010) and a detrimental factor in wheat markets (Regional Quality Report 2011). Several studies have genetically dissected this trait and reported genes associated on all wheat chromosomes (Prasad et al. 2003; Kulwal et al. 2005; Huang et al. 2006; Nelson et al. 2006; Sun et al. 2010; Zhao et al. 2010; Conti et al. 2011; Li et al. 2012a; Maphosa et al. 2013). In several of these studies, molecular markers associated with genes regulating gluten proteins have been reported. Gluten is the coherent mass formed by the binding of glutenin and gliadin (storage proteins) after water is added to flour (Stone and Savin 1999). Glutenins are responsible for dough strength and are conformed by subunits of high molecular weight (HMW) and subunits of low molecular weight (LMW). The major genes controlling HMW subunits (*Glu-1*, *Glu-A1*, *Glu-B1*and *Glu-D1*) are located on the long arms of the homeologous group 1, while the major genes controlling LMW subunits (*Glu-A3*, *Glu-B3*, and *Glu-D3*) are also in the same homeologous group but in the short arm of these chromosomes.

Wheat varieties with good grading standards such as 1,000 kernel weight (TKW) and kernel volume weight (KVW) usually have more flour extraction (FE) with high quality (Gwirtz et al. 2006). These important traits for millers have also been dissected genetically. Loci controlling TKW have been identified on all wheat chromosomes (Huang et al. 2003, 2004; McCartney et al. 2005; Huang et al. 2006; Cuthbert et al. 2008; Wang et al. 2009; Sun et al. 2009; Tsilo et al. 2010; Heidari et al. 2011; Simons et al. 2012; Bennett et al. 2012). For KVW also, QTLs have been reported on all wheat chromosomes except 1A and 6D (McCartney et al. 2005; Narasimhamoorthy et al. 2006; Huang et al. 2006; Sun et al. 2009, 2010; Bennett et al. 2012; Simons et al. 2012; Carter et al. 2012). In wheat, QTLs for FE have been detected on chromosomes 1A, 1B, 2A, 3B, 4A, 4B, 4D, 5A, 5B, 5D, 6A, 6D, and 7A (Campbell et al. 2001; Kuchel et al. 2006; Nelson et al. 2006; Raman et al. 2009; Carter et al. 2012; Simons et al. 2012; Maphosa et al. 2013).

Rheological properties govern the performance of wheat flour dough during mechanical treatment (Alamri et al. 2009a, b). Mixograph, farinograph, and alveograph are used to assess these rheological characteristics giving an insight about baking performance (Gwirtz et al. 2006). Several studies have also reported QTLs for rheological traits derived from mixograph, farinograph, and/or alveograph assessments (Huang et al. 2006; Mann et al. 2009; Tsilo et al. 2011; Li et al. 2012b; Simons et al. 2012; Mergoum et al. 2013; Maphosa et al. 2013). In the case of mixograph, QTLs have been found on all chromosomes except chromosomes 3D and 6B (Huang et al. 2006; Mann et al. 2009; Tsilo et al. 2011; Li et al. 2012b; Simons et al. 2012; Mergoum et al. 2013; Maphosa et al. 2013).

Although the recent information derived from QTL analysis suggests that the QTLs/genes controlling wheat quality are contributed by whole-wheat genome, several studies have shown that the D genome plays a pivotal role in bread wheat quality attributes (Kerber and Tipples 1969; Nelson et al. 2006). However, due to the relatively recent addition of the D genome into hexaploid wheat, the D genome is less diverse than A and B genomes (Akunov et al. 2010) and, therefore, wheat varieties show low polymorphism for D genome markers. This could jeopardize future improvement of wheat quality traits, particularly for those loci located on the D genome. The use of the genetic diversity present in landraces and exotic wheat has been suggested as a mechanism to enrich wheat genetic pool (Raman et al. 2010; Ogbonnaya et al. 2013). In the past, a combination of molecular markers and breeding approaches have allowed the identification and introgression of novel QTLs present in exotic wheat lines and landraces (Nelson et al. 2006; Huang et al. 2003, 2004; Narasimhamoorthy et al. 2006; Naz et al. 2008). Wheat genotypes with supernumerary spikelets (SS) are an unexplored exotic germplasm for quality traits. Usually, wheat bears one spikelet per rachis node; however, in some landraces, it is possible to find genotypes in which a rachis node has more than one spikelet (Martinek and Bednár 1998). Considering the high number of spikelets in which grains could be developed, SS have been suggested as a means to increase grain yield (Pennell and Halloran 1983, 1984; Hucl and Fowler 1992). The quality performance of genotypes with SS is mostly unknown. In the present study, an RIL population derived from the cross of a white

wheat (WW) genotype and an exotic line with SS was used to genetically dissect eight quality traits. White wheat (WW) is a commercial alternative to hard red spring wheat (HRSW) in the northern plains of the USA (Ransom et al. 2006) and is characterized by good-quality performance. The impact of genetic regions controlling SS on quality traits was also studied.

Materials and methods

Plant material

An RIL population derived from a cross between an elite genotype WCB414 and an exotic genotype WCB617 was used in this study. WCB414 is a white wheat (WW) genotype developed by the Hard White and Specialty Wheat breeding program at North Dakota State University (NDSU), Fargo, ND, USA. This line was chosen for its adaptation and good-quality performance and has conventional spike morphology. The genotype WCB617 is an exotic and pre-breeding line with SS phenotype, glume pubescence (Fig. 1), and hard red grain, maintained by the NDSU wheat Germplasm Enhancement project as a source to enrich genetic diversity in wheat breading programs. Single seed descent method was used to advance the RIL population to F_7 generation. Afterward, plants were grown in greenhouse facilities at NDSU to increase seeds and advance the population to F_8 generation. In the growing seasons of 2009, spikes from each plot were collected and grown in New Zealand winter nursery as head rows to ensure the genetic purity of each RIL. Thus, in the years 2009 and 2010, the generations $F_{7,9}$, and $F_{10,11}$ were included in this study. The HRSW cultivars 'Alsen' (PI 615543) (Frohberg et al. 2006), 'Steele-ND' (PI 634981) (Mergoum et al. 2005), 'Glenn' (PI 639273) (Mergoum et al. 2006), 'Faller' (PI 648350) (Mergoum et al. 2008), 'Barlow' (PI 658018) (Mergoum et al. 2011), 'Briggs' (PI 632970) (Devkota et al. 2007), and WW cultivar 'Alpine' (Agripro® wheat variety, USA) were included as checks in this study.

Fig. 1 Supernumeray spiklets in the pre-breeding line WBC617

Field experiment

During the years of 2009 and 2010, the parents, 163 RILs, and seven checks were planted in a 13 × 13 partially balanced square lattice design with two replicates, at two different locations, Prosper (46.96300 N, 97.01980 W, altitude 274 m, Bearden series soils) and Carrington (47.45000 N, 99.12390 W, 484 m of altitude, Heimdal-Emrick series soils) in North Dakota (ND), USA. Planting and environmental conditions were previously described by Echeverry-Solarte et al. (2014). The four environments were designated as: I = Prosper 2009, II = Carrington 2009, III = Prosper 2010, and IV = Carrington 2010.

Phenotypic data collection

The grain samples collected from the field experiments were cleaned by using a clipper grain cleaner before recording phenotypic data. The quality traits analyzed in this study were TKW, KVW, GPC, FE, and mixograph-related traits which include mixograph envelope peak time (MEPT), mixograph MID line peak time (MMLPT), mixograph MID peak integral (MMLPI), and general mixograph pattern (Mx). TKW (g) was calculated from the number of seeds in 10 g sample, counted using a seed counter (Seedburo Equipment Co., Chicago, IL, USA). KVW (kg m^{-3}) was measured according to the American Association of Cereal Chemist International (AACCI) method 55-10.01 (AACCI 2008). GPC (%) at 12 % moisture basis was measured using near-infrared reflectance following the AACCI standard method 39.25.01 (AACC International 2008). FE was determined using 100 or 150 g grain sample tempered to 16.0 % moisture. Brabender Quadrumat Junior Mill was used to mill the grain sample and bran was discarded from the flour. Flour extraction was reported on clean dry wheat basis (Bass 1988). Mixograph measurements were obtained from 35 g of flour in a national manufacturing mixograph (National Manufacturing, TMCO Division, Lincoln NE) following the AACCI method 54-40.02 (AACC International 2008). The Mixmart software was used to collect information on MEPT (min), MMLPT (min), and MMLPI (% torque × min). General mixograph pattern (Mx) was based on a 0–9 scale (0 = weakest; and 9 = strongest). The traits MEPT, MMLPT, MMLPI, and Mx were referred as mixograph-related traits in this study.

Due to small seed sample of WCB617 in Prosper 2009 and Carrington 2009, different procedures were used for estimating KVW and GPC. A miniature test weight device

was used for KVW. The GPC was calculated from flour following the combustion method 46-30.01 of AACCI (AACC International 2008) using a Leco FP 528 (Leco Corporation 3000 Lakeview Avenue, St. Joseph, MI, USA). The information of flour protein content of these two samples was used as equivalent of GPC considering the close correlation between both traits. Data of FE and mixograph-related traits for WCB617 in Prosper 2009 could not be obtained due to the small seed sample.

Electrophoresis for identification of high-molecular-weight and low-molecular-weight glutenin subunits

The high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits in WCB414 and WCB617 were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using the procedure as described in Xu et al. (2010). The protein samples were extracted from WCB414, WCB617, and checks ('Chinese Spring', 'Len', 'Norquay', and 'Thatcher') following the procedure of Singh et al. (1991). Electrophoresis was conducted at 30 mA per gel for approximately 18 h using the SE 600 (GE Healthcare, Piscataway, NJ, USA) vertical apparatus with 12 % separating gels. After electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250. The gel images were captured using the Kodak Logic 200 system and were analyzed using Kodak 1D Image Analysis Software Version 3.6.1 (Eastman Kodak Company, Rochester, NY, USA).

Data analysis

Data for FE and mixograph-related traits from Prosper 2009 were subjected to analyses of variance (ANOVA) for a random complete block design using the MIXED procedure of the Statistical Analysis System (SAS 2004). The data for FE and mixograph-related traits from Carrington 2009, Prosper 2010, and Carrington 2010, as well as data for TKW, TW, and GPC from all the environments were subjected to ANOVA for a lattice design using the MIXED procedure of SAS (SAS 2004). Differences in the statistical analysis methodologies used in this study were due to missing data. ANOVA was estimated for each environment separately. To estimate genotype × environment interaction, combined ANOVA over all locations was performed. The F_{max} test (Tabachnik and Fidell 2001), considering a ratio of less than tenfold, was conducted to verify homogeneity of variance before conducting combined ANOVA analysis. In ANOVA analyses, the RILs, parents, and checks were considered as fixed effects, while environments and blocks were considered as random effects. F tests were considered significant at $P < 0.05$. Significant differences between genotypes were assessed using an F-protected least significant difference (LSD) value at $P < 0.05$. Correlations between two traits were assessed using significant Pearson coefficients at $P < 0.05$. Procedures described by Gomez and Gomez (1984) were followed to test homogeneity among correlation coefficients of different environments at $P < 0.005$. Pooled homogeneous correlation coefficients were considered significant at $P \leq 0.05$. Broad-sense heritability for each trait on plot basis (Holland et al. 2003) was calculated excluding the means of parents and checks. The mixed procedure of SAS (2004) was used to conduct this analysis considering all sources of variation as random component. The outputs of the covariance parameter estimates were used in the equation $h_B^2 = \sigma_G^2 / \left[\sigma_G^2 + \left(\sigma_{GE}^2\right) + \left(\sigma_e^2\right)\right]$, where σ_G^2 is the genotype variance, σ_{GE}^2 is the G × E, e is the number of environments, and r is the number of replicates.

The evaluations of penetrance of supernumerary spikelets (PSS), penetrance of pubescences (PP), penetrance of clavate architecture (PC), and other spike-related and agronomic traits reported in Echeverry-Solarte et al. (2014, 2015) were correlated with the traits investigated in this study and co-located in the genetic map to identify the influence of these traits on quality traits.

QTL analysis

The molecular map developed by Echeverry-Solarte et al. (2014) was used in the present study. This map consisted of 939 DArT markers representing 671 unique loci. All these markers were placed in 38 linkage groups covering 3,114.2 cM with an average distance of 4.6 cM between two markers. The identification of QTLs for each environment and across environments (AE) was conducted through composite interval mapping (CIM) using Cartographer V2.5_011 (Wang et al. 2012) under default procedures and forward regression method. A minimum LOD score 2.5 was used to declare a putative QTL. The ± 2 LOD peak method (Lander and Bostein 1989) was used to determine confidence intervals (CI). The QTLs with overlapping CIs were considered as one QTL. Five hundred permutations were performed to determine critical LOD threshold scores ($\alpha = 0.05$). Putative QTLs with LOD scores above this critical LOD threshold ($\alpha = 0.05$) were declared as definitive QTLs. The putative QTLs detected in only one environment with LOD scores lower than the critical threshold were not included in the final analysis. Putative QTLs detected in at least two data sets (including AE) were reported regardless of the critical LOD threshold to avoid type II error (false negatives). A QTL was considered as consistent if it was detected in at least 50 % of the environments studied (AE was not considered as an environment for this purpose). QTLs explaining at least 15 % of phenotypic variance (PV) were called major QTLs. Linkage groups were drawn using Mapchart 2.2 program (Voorrips 2002).

Results

Phenotypic data

The elite parent (WCB414) had better performance than the exotic parent (WCB617) in all the environments for the traits TKW, KVW, and FE (Supplementary Table 1). However, the exotic parent (WCB617) had higher GPC than the elite parent (WCB414) (Supplementary Table 1). For different mixograph measurements (MX, MEPT, MMLPT, MMLPI), although WCB414 had better performance than WCB617 in most of the environments, these differences were not significant at $P < 0.05$ (Supplementary Table 1). Transgressive segregation in the direction of both parents was observed for KVW and GPC (Fig. 2; Supplementary Table 1) in all the environments. A similar segregation was observed for MX, MEPT, MMLPT, and MMLPI in all environments, except at Prosper 2009. In this case, phenotypic values of the exotic parent were missing. Nevertheless, in this environment, the mixograph-related traits showed transgressive segregation in the direction of the WCB414 parent (Supplementary Table 1). For TKW, transgressive segregation in the directions of both parents was observed in all the environments except in Carrington 2009, where the segregation was in the direction of the elite parent only (Supplementary Table 1). For the trait FE, transgressive segregation in both directions was observed at Carrington 2010 and Prosper 2010, while at Carrington 2009 it was observed in the direction of the exotic parent (Fig. 2; Supplementary Table 1). At Prosper 2009, where the phenotypic values of FE for the exotic parent could not be estimated, no transgressive segregation in the direction of the parent WCB414 was observed (Supplementary Table 1).

For all the traits, error variances among the environments were homogenous (Supplementary Table 2) allowing a combined ANOVA to be performed. The eight quality traits had significant G × E interactions (Table 1). Broad-sense heritability on plot means basis ranged from 0.24 for Mx to 0.84 for FE (Table 1). Several inconsistent correlations (positive and negatives correlations for the same pair of traits in different environments) were

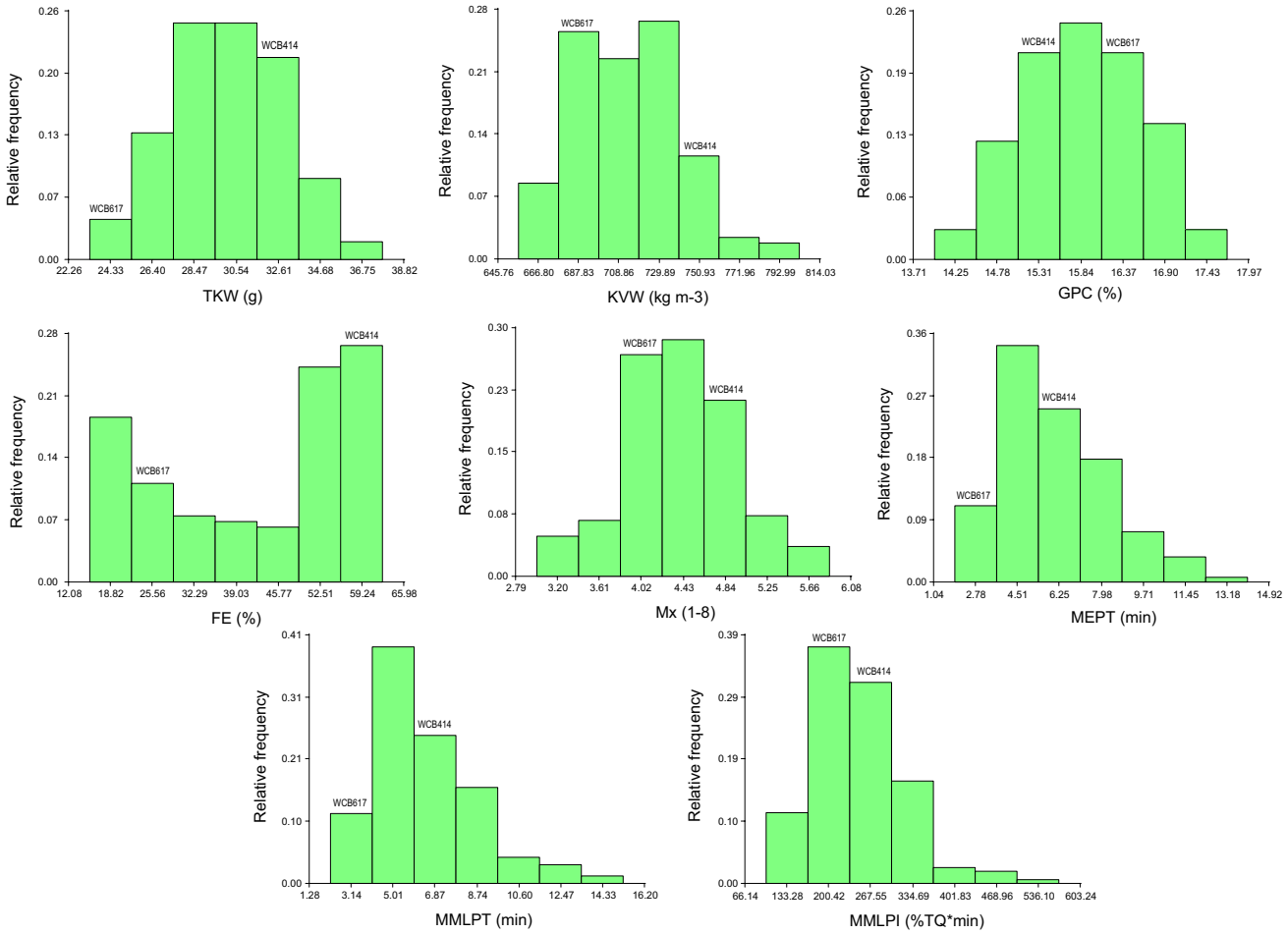

Fig. 2 Frequency distribution of 163 RILs for mean of eight quality traits

Table 1 Mean squares, coefficient of variation, and heritabilities for quality traits of the parents, RILs, and checks evaluated in four environments of North Dakota, USA

Trait	Mean squares		G × E	Error	CV(%)[a]	H[b]
	Genotype	Environment				
TKW	52.8**	3294.73	9.67**	2	4.6	0.5
KVW	6583.2**	593271	1169.6**	130.4	1.6	0.47
FE	1848.8**	4591.3	67.8**	12.4	8.2	0.84
GPC	4.0**	224.9	1.1**	0.2	2.9	0.39
MX	2.3**	25.31	0.7**	0.6	17.1	0.24
MEPT	34.1**	638	6.2**	2.4	26.0	0.48
MMLPT	36.3**	531.2	6.3**	2.6	25.3	0.49
MLPI	38855.0**	354470	47663.1**	1824.3	17.2	0.6

TKW 1,000 kernel weight, *KVW* kernel volume weight, *FE* flour extraction, *GPC* grain protein content, *Mx* mixogram score, *MEPT* mixogram envelope peak time, *MMLPT* mixogram MID line peak time, *MLPI* mixogram MID peak integral

** Significance at $P < 0.01$

[a] Coefficient of variation

[b] Broad-sense heritability on plot means basis calculated in the RILs

Table 2 Significant Pearson's correlation coefficients among trait values for the RIL, their parents, and checks grown

	TKW	KVW	GPC	FE	MX	MEPT	MMLPT	MMLPI
TKW	1	0.26**§ 0.67**‡	−0.40**‡ 0.22**†	0.28**	ns	−0.31**‡	−0.31**‡	−0.29**‡
KVW		1	0.37**‡ −0.18* −0.59**	0.41**	0.22**†	0.27**‡ −0.26**†	0.25**‡ −0.25**†	0.24**‡ −0.15*†
GPC			1	ns	0.33**	-0.31**§	−0.30**§	−0.27**†
FE				1	−0.16*†	−0.25**†	−0.26**†	−0.20**†
MX					1	ns	ns	0.26**§
MEPT[a]						1	0.98**‡ 0.99**‡	0.88**‡ 0.92**‡
MMLPT[b]							1	0.93**‡ 0.92**‡

*, ** Significance at $P < 0.05$, 0.01, respectively; *ns* not significant at $P < 0.05$

† *r* from one environment, ‡ *r* pooled from two environments, § *r* pooled from three environments, ¶ *r* pooled from four environments

[a] Alternative pooled correlations were observed between the traits MEPT and MMLPI. The lowest pooled *r* value is presented

[b] Alternative pooled correlations were observed between the traits MMLPT and MMLPI. The lowest pooled r value is presented

observed among several quality traits (Table 2). Flour extraction was positively and significantly associated with TKW and KVW, but negatively associated with mixograph-related traits. Mixograph-related traits, however, showed strong positive correlations among them (Table 2). The presence of spikes with SS (PSS) was positively associated with GPC and mixograph-related traits, but negatively associated with TKW. Meanwhile, spikes with PP trait were positively correlated with GPC, MEPT, MMLPT, and MMLPI, but negatively associated with TKW, KVW, and FE (Supplementary Table 3). The traits PC only showed weakly associations with KVW and GPC (Supplementary Table 3).

High-molecular-weight and low-molecular-weight glutenin subunits in WCB414 and WCB617

SDS-PAGE analysis revealed that WCB414 has the HMW glutenin subunits Ax2*, Bx7+By9, and Dx5+Dy10 coded by alleles *Glu-A1b*, *Glu-B1c*, and *Glu-D1d*, respectively, while WCB617 has subunits Ax1, Bx7+By8, and Dx2+Dy12 coded by *Glu-A1a*, *Glu-B1b*, and *Glu-D1c*, respectively (Fig. 3). For the bread-making quality, the HMW glutenin subunits Bx7+By8 and Dx5+Dy10 are superior over Bx7+By9 and Dx2+Dy12, respectively (Payne et al. 1987). It is expected that certain QTLs for the traits related to bread-making quality are associated with

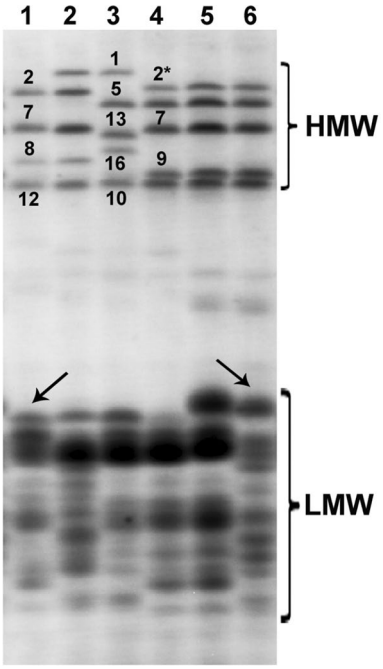

Fig. 3 The high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits of wheat lines on 12 % SDS-PAGE gel. The *lane numbers* at the *top* of the gel image represent the wheat lines and cultivars: *1* 'Chinese Spring', *2* WCB617, *3* 'Norquay', *4* 'Len', *5* WCB414, and *6* 'Thatcher'. All the HMW glutenin subunits in Chinese Spring (Bx7+By8, Dx2+Dy12) and Norquay (Ax1, Bx13+By16, Dx5+Dy10) and three of the HMW glutenin subunits in Len (1Ax2*, Bx7+By9) were labeled using their numerical numbers. In the LMW region, WCB414 (*lane 5*) has a strong band with similar mobility as the band encoded by *Glu-B3h* in Thatcher (*lane 6*, indicated by an *arrow*) and WCB617 has a top band with similar mobility as a band (*lane 1*, indicated by an *arrow*) in Chinese Spring

the loci *Glu-B1* and *Glu-D1* in the population. The LMW glutenin subunits in WCB414 and WCB617 were not clearly identified in this study. But the SDS-PAGE analysis showed that the two lines have different LMW glutenin subunits. WCB414 has a strong band with similar mobility as the band (Fig. 3, lane 6, indicated by an arrow) encoded by *Glu-B3h* present in Thatcher and WCB617 has a top band with similar mobility as a band (Fig. 3, lane 1, indicated by an arrow) in Chinese Spring.

Identification of genomic regions (QTL) associated with eight quality traits

A total of 69 QTLs were detected for eight quality traits investigated in the present study (Table 3). These QTLs were located on 18 different chromosomes (all except chromosomes 4D, 5D, and 6D). A total of nine QTLs were detected on chromosome 6B, seven each on chromosomes 1B and 2D, five each on chromosomes 2B and 3A, four each on chromosomes 1A, 5B, 6A, and 7B, three each on chromosomes 1D, 2A, 3B, 3D, and 4A, two each on chromosomes 4B, and 7D, and one each on chromosomes 5A and 7A. In terms of genome-wide distribution of QTLs, a total of 34, 21, and 15 quality-related QTLs were detected on B, A, and D genome, respectively. Among all the 69 QTLs, a total of 18 QTLs were consistent (identified in at least 50 % of the environments) (Table 3). A total of 13 QTLs explained more than 15 % of PV and were considered as major QTLs, while the remaining 56 QTLs explained less than 15 % of PV and were considered as minor QTLs. The alleles for increased phenotypic values at 31 loci were contributed by WCB414, while exotic parent WCB617 contributed alleles for 39 loci that increased the effect of quality traits.

QTLs for TKW, KVW, and FE

A total of 11 QTLs were associated with TKW, including one consistent QTL (*QTKW.ndsu.4A*) (Table 3; Fig. 4). Only the consistent QTL *QTKW.ndsu.4A* could be considered as major QTL as it explained up to 17.1 % of PV for TKW. The other QTLs explained between 6.2 and 13.9 % of PV for TKW. In individual environments, the number of QTLs identified for TKW ranged from three to five. The PV explained by all the QTLs identified in individual environments ranged from 24 to 47.3 % (Table 3). Both parents contributed the alleles that increased the phenotypic values of TKW. For the consistent QTL *QTKW.ndsu.4A*, elite parent WCB414 contributed the alleles for increased TKW.

For KVW, a total of ten QTLs including three consistent QTLs (*QKVW.ndsu.1A.1*, *QKVW.ndsu.2A.1* and *QKVW.ndsu.6A.1*) were detected (Table 3; Fig. 4). The number of QTLs identified for TKW in individual environments ranged from three to four. The PV explained by all the QTLs in individual environments ranged from 40.8 to 51.2 %. Four QTLs (*QKVW.ndsu.1B.1*, *QKVW.ndsu.2A.1*, *QKVW.ndsu.2A.2*, and *QKVW.ndsu.6A.1*) including two consistent QTLs had major effect (PV >15 %) on KVW, at least in some of the environments. Additive effects indicated that the elite parent WCB414 contributed positive alleles for increasing KVW at most of the loci, including the three consistent QTLs.

A total of six QTLs including four consistent QTLs (*QFE.ndsu.1A*, *QFE.ndsu.1B*, *QFE.ndsu.3D*, and *QFE.ndsu.6A*) were associated with FE in this RIL population (Table 3; Fig. 4). The number of QTLs identified for FE in individual environments ranged from two (Prosper 2009) to six (Prosper 2010) and the PV explained by all the QTLs in individual environments ranged between 15.3 and 51.5 %. The PV explained by individual QTLs ranged from 4.8 to 19.0 %. Only one QTL (*QFE.ndsu.2B*), which was identified in a single environment, explained more than 15 % PV of FE. The elite parent WCB 414 provided the alleles that increased phenotypic values of FE at all loci except *QFE.ndsu.6A*.

Table 3 QTLs identified for eight quality traits in an RIL population derived from the cross WCB414 × WCB617

QTL	Environment	Flanking markers	Position (cM)	CI (cM)	LOD	a^a	R^2(%)
1,000 kernel weight							
QTKW.ndsu.2A	II	wPt2372-wPt2850	70.21	58.5–84.4	4.79	−1.29	13.5
QTKW.ndsu.2B	IV	wPt8760-wPt6158	43.41	32.1–60.8	4.09	1.11	8.8
QTKW.ndsu.2D	II	wPt5014-wPt668017	76.81	66.6–86.8	4.13	0.99	7.8
QTKW.ndsu.3A.1	IV, AE	wPt741078-wPt743858	2.01–2.41	0–12.9	5.03–8.02	−0.91 to −1.56	9.2–13.9
QTKW.ndsu.3A.2	III	wPt6854-tPt-0242	97.51	89.6–115.1	3.83	−1.15	7.9
QTKW.ndsu.3B.1	I	wPt9170-wPt743661	128.81	116.8–147.3	3.36	−0.80	7.8
QTKW.ndsu.3B.2	IV	tPt-6487-wPt2299	44.01	38.3–57.6	4.41	−1.07	7.3
QTKW.ndsu.3B.3	I	wPt9586-wPt741584	4.81	0–17.7	3.56	−0.78	7.2
*QTKW.ndsu.4A**	I, II, III, IV, AE	wPt2247-wPt744614	1.01–22.71	0–38.1	3.58–9.45	1.04–1.66	7.25–17.12
QTKW.ndsu.5A	AE	wPt9094-wPt800131	12.01	0–24.3	4.18	−1.02	12.9
QTKW.ndsu.6B	IV	wPt664250-wPt2297	102.31	96.5–106.4	5.48	1.43	9.2
						RTPVE	24–47.3
Kernel volume weight (KVW)							
*QKVW.ndsu.1A**	I, IV, AE	wPt4065-wPt5167	179.21–180.21	161.0–191.6	4.50–6.04	8.11–9.95	9.5–12.7
QKVW.ndsu.1B	I, AE	wPt0308-wPt8240	22.51	10.1–29.8	5.11–6.62	9.51–10.25	9.3–12.2
*QKVW.ndsu.2A.1**	II, IV, AE	wPt798339-wPt2372	38.01–40.01	30.5–45.7	4.05–8.34	9.71–16.98	10.3–22.5
QKVW.ndsu.2A.2	III	wPt2850-wPt8068	88.71	79.7–106.5	5.81	24.99	17.7
QKVW.ndsu.4B	I	wPt5559-wPt1046	5.51	0.4–6.8	4.19	7.45	7.6
QKVW.ndsu.5B	III	wPt2373-wPt8449	252.81	247.8–260.4	5.52	−21.07	11.9
*QKVW.ndsu.6A.1**	I, II, III, IV, AE	wPt733115-wPt667618	113.61–135.81	98.8–149.3	2.73–8.91	7.39–17.03	7.2–21.0
QKVW.ndsu.6A.2	II	wPt667170-wPt4791	79.71	61.8–85.2	4.39	−9.23	7.8
QKVW.ndsu.6B	III	wPt5408-wPt8412	113.31	102.2–122.1	3.52	14.70	6.7
QKVW.ndsu.7B	II	wPt665428-wPt9299	121.61	96.7–134.3	4.50	−6.76	8.5
						RTPVE	40.8–51.2
Flour extraction (FE)							
*QFE.ndsu.1A**	I, II, III, IV, AE	wPt3698-wPt1906	93.51–94.01	81.1–110.8	2.63–5.60	3.68–5.96	4.9–11
*QFE.ndsu.1B**	III, IV, AE	wPt2389-wPt3451	0.01	0–15.1	2.90–4.48	3.80–5.18	5.0–8.2
QFE.ndsu.2B	III	wPt1813-wPt8404	57.61	42.9–68.8	4.14	7.27	19.0
*QFE.ndsu.3D**	II, III, IV	wPt665093-wPt6169	39.21–42.21	33.1–46.9	2.68–3.32	4.55–4.58	5.8–7.7
QFE.ndsu.4A	III, AE	wPt0817-wPt7926	20.81–30.31	12.5–39.1	2.61–3.36	3.49–4.25	4.8–6.5
*QFE.ndsu.6A**	I, II, III, IV, AE	wPt666773-wPt731592	1.01–2.01	0–12.7	3.16–4.53	−4.36 to −4.92	6.0–9.1
						RTPVE	15.3–51.5
Grain protein content (GPC)							
QGPC.ndsu.1A.1	III, AE	wPt1924-wPt7872	15.41–33.41	12.4–44.4	4.92–8.74	−0.23 to −0.60	8.1–16.5
QGPC.ndsu.1A.2	I	wPt8172-wPt9429	101.91	85.4–113.4	4.11	−0.26	8.2
QGPC.ndsu.1B	II, AE	wPt1684-wPt5899	61.71	51.7–75.7	2.84–3.42	0.17–0.23	4.7–5.3
QGPC.ndsu.2B	IV	wPt744808-wPt4368	30.01	14.4–48.1	3.4	−0.3	10.5
QGPC.ndsu.2D	III	wPt8134-wPt2761	30.41	13.9–38.5	3.69	−0.41	7.8
QGPC.ndsu.3D	III	wPt740945-rPt-1806	0.01	0–9.8	5.51	−0.42	9.9
QGPC.ndsu.4B	II	wPt732423-wPt8892	12.01	3.6–17.8	5.54	0.29	8.8
*QGPC.ndsu.5B**	I, II, IV, AE	wPt1895-wPt6191	111.71–113.31	101.1–127.5	3.63–6.63	−0.19 to 0.33	5.3–13.7
*QGPC.ndsu.6B.1**	I, II, IV, AE	wPt5234-wPt1437	52.51–57.81	41.0–71.5	2.84–9.22	0.27–0.39	5.5–16.9
QGPC.ndsu.6B.2	IV	wPt1241-wPt3605	95.31	88.1–102.2	3.64	0.30	7.7
*QGPC.ndsu.7B**	III, IV, AE	wPt0266-wPt9299	95.51–121.61	90.2–131.7	2.74–6.03	0.25–0.30	4.7–10.4
						RTPVE	38.5–40.3
Mixogram envelope peak time (mixing time) (MEPT)							
QMEPT.ndsu.1B	IV, AE	wPt8320-wPt1560	3.81	0–19.3	2.53–4.55	−0.56 to 0.64	3.2–6.0

Table 3 continued

QTL	Environment	Flanking markers	Position (cM)	CI (cM)	LOD	a^a	R^2(%)
*QMEPT.ndsu.1D**	I, II, III, IV, AE	wPt733835-wPt672077	83.51–85.51	68.8–93.1	7.98–23.61	0.80–1.91	18.7–41.2
QMEPT.ndsu.2B	IV	wPt744808-wPt4368	22.01	11.1–35.6	4.06	0.82	5.6
QMEPT.ndsu.2D.1	II	wPt730568-wPt671914	74.81	64.8–86.7	3.9	−0.53	7.2
QMEPT.ndsu.2D.2	IV	wPt741084-wPt666656	49.51	46.6–55.8	5.63	−1.02	7.9
QMEPT.ndsu.4A	IV	rPt-7285-wPt6728	21.61	16.4–33.6	3.53	−0.76	4.8
*QMEPT.ndsu.5B**	I, IV, AE	wPt1895-wPt6191	110.71–118.31	94.6–135.3	3.01–5.50	0.45–1.12	5.5–10.1
						RTPVE	25.9–64.5
Mixogram MID line peak time (tolerance) (MMLPT)							
QMMLPT.ndsu.1B	IV, AE	wPt8320-wPt1560	3.81	0–20	2.85–4.78	−0.60 to 0.74	4.1–6.5
*QMMLPT.ndsu.1D**	I, II, III, IV, AE	wPt743310-wPt672077	78.61–84.51	67.4–93.3	7.08–22.59	0.79–2.25	15.7–40.3
QMMLPT.ndsu.2D	II	wPt730568-wPt671914	74.81	64.8–86.8	3.99	−0.5895	7.6
QMMLPT.ndsu.3A	AE	tPt-1079-wPt1464	47.31	36.0–59.5	2.56	0.4516	3.5
*QMMLPT.ndsu.5B**	I, III, IV, AE	wPt1895-wPt6191	110.71–127.31	101.1–157.2	2.92–4.07	0.55–1.00	6.0–7.9
QMMLPT.ndsu.6B	IV	wPt0052-tPt-1723	150.41	142.0–164.1	3.31	0.8279	5.6
						RTPVE	23.3–56.2
MMLPI mixogram MID PEAK integral %TQ*MIN							
QMMLPI.ndsu.1B	I, III	wPt8320-wPt1560	3.81	0–17.8	2.88–3.42	−17.51 to 23.13	4.7–6.1
*QMMLPI.ndsu.1D**	I, II, III, IV, AE	wPt743310-wPt672077	79.61–83.51	68.8–94.1	7.01–22.20	28.88–60.49	16.4–37.1
QMMLPI.ndsu.2D.1	II	wPt2675-wPt8134	27.01	18.9–32.2	8.56	−36.65	17.7
QMMLPI.ndsu.2D.2	IV, AE	wPt741084-wPt666656	49.51	38.5–62.7	3.36–3.58	−18.21 to 23.43	4.2–4.8
QMMLPI.ndsu.3D	III	wPt669255-wPt3094	33.71	20.0–42.3	3.39	−26.06	6.3
QMMLPI.ndsu.6B.1	I, AE	wPt745074-wPt6667	72.31	66.7–85.3	3.49–3.74	17.17–18.64	4.7–6.7
QMMLPI.ndsu.6B.2	IV	wPt0052-tPt-1723	148.41	141.3–154.5	7.44	40.31	10.8
QMMLPI.ndsu.7D†	II, AE	wPt744917-wPt664368	0.01	0–10.4	2.76–2.83	−15.49 to 16.21	3.8–4.9
						RTPVE	32.4–52.1
Mixogram (Mx)							
QMx.ndsu.2B2	AE	wPt8404-wPt8398	76.81	63.8–86.4	5.25	−0.19	10.1
QMx.ndsu.3A.1	II	tPt-0242-wPt0286	122.21	108.3–134.1	6.84	−0.38	15.6
QMx.ndsu.3A.2	II	wPt1562-wPt2740	163.41	153.7–165.9	5.56	0.33	11.0
QMx.ndsu.6A	III	wPt666074-wPt733115	109.61	99.0–141.0	3.89	0.23	12.6
QMx.ndsu.6B.1	II	wPt0171-wPt4164	8.51	0–14.6	3.82	−0.25	6.5
*QMx.ndsu.6B.2**	I, II, IV, AE	wPt1756-wPt5234	44.31–45.11	38.5–48.1	5.17–10.48	0.2–0.37	11.3–19.9
QMx.ndsu.7A	IV	wPt3135-rPt-4199	72.21	63.9–85.3	2.82	−0.18	5.8
QMx.ndsu.7B.1	IV	wPt8246-wPt3445	5.71	0–20.3	2.65	−0.18	5.3
QMx.ndsu.7B.2	AE	wPt744769-wPt7108	1.01	0–7.4	3.81	−0.15	6.9
*QMx.ndsu.7D**	I, IV, AE	wPt744917-wPt664368	0.01	0–9.4	3.13–4.27	−0.16 to 0.24	6.6–7.1
						RTPVE	29.0–48.8

RTPVE/env rank of phenotypic variation explained per environment, *I* Prosper 2009, *II* Carrington 2009, *III* Prosper 2010, *IV* Carrington 2010, *V* Prosper 2011, *VI* Carrington 2011 *CI* confidence interval

* Consistent and definitive QTL

† QTL under the empirical threshold

[a] Additive effect

QTLs for GPC

GPC was controlled by 11 different QTLs in this population (Table 3; Fig. 4). These include three consistent QTLs (*QGPC.ndsu.5B*, *QGPC.ndsu.6B.1*, and *QGPC.ndsu.7B*) located on chromosome 5B, 6B, and 7B (Table 3; Fig. 4). The number of QTLs identified in individual environments ranged from three (Prosper 2009) to five (Carrington 2010). The PV explained by individual QTLs ranged from 4.7 to 16.9 % and the PVE explained by all the QTLs in

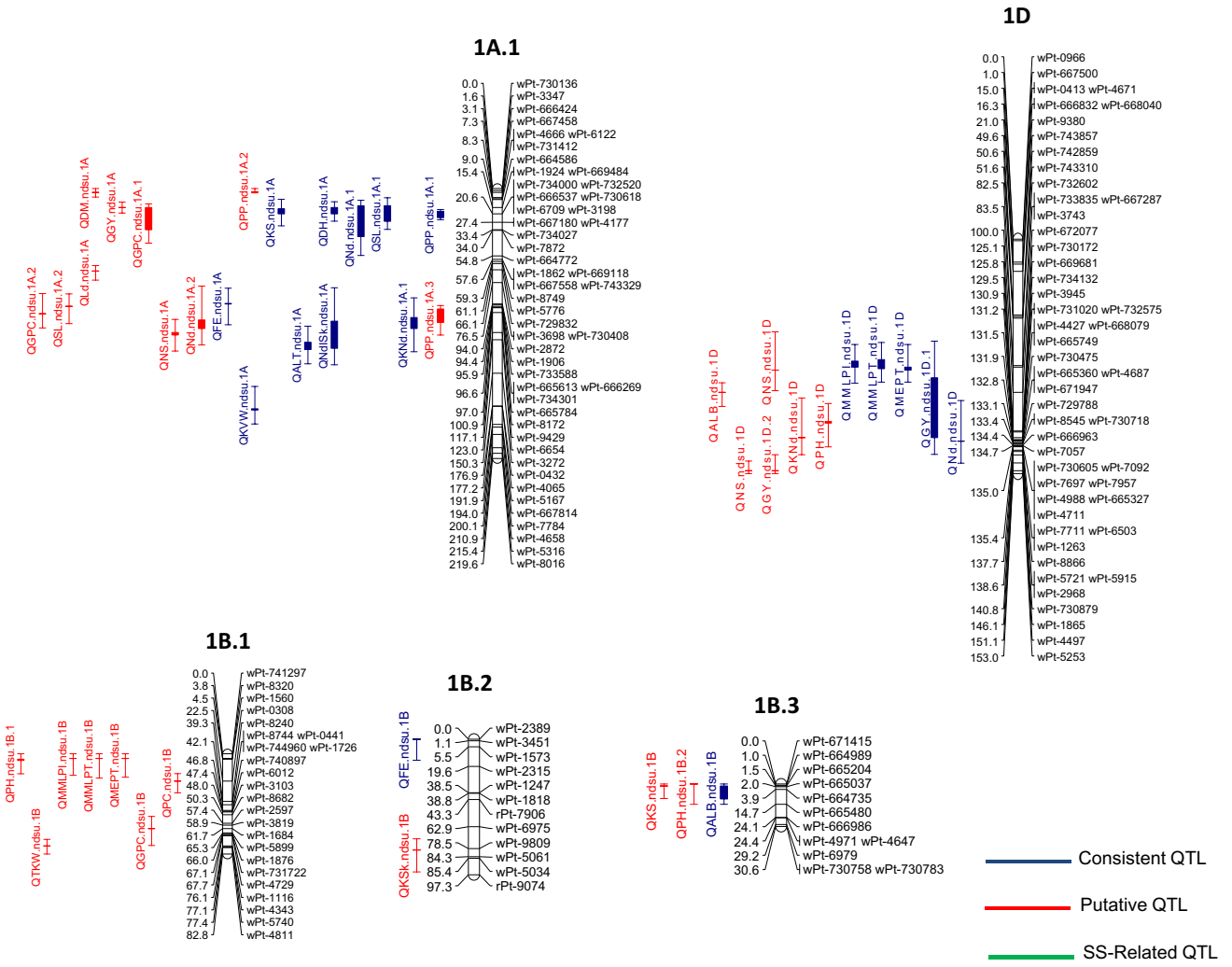

Fig. 4 Genetic map and QTLs for 28 spike-related, agronomic, and quality traits of an RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, evaluated over four to six locations in ND, USA, during 2009, 2010, and 2011. (Abbreviations for each trait: *ALM* awn length at middle of the spike, *AAL* awn averaged length total, *ALB* awn length at the bottom of the spike, *ALT* awn length at the top of spike, *DH* days to heading, *DM* days to maturity, *FE* percentage of flour extraction, *GPC* grain protein content, *GY* grain yield, *KSk* kernels per spikelet, *KNd* kernels per node, *KS* kernel spike, *KVW* kernel volume weight, *LD* lodging susceptibility, *MEPT* mixograph envelope peak time, *MMLPT* mixograph MID line peak time, *MMLPI* mixograph MID peak integral, *Mx* general mixograph pattern, *NdISk* number of nodes with immature spikelets at the spike base, *NNd* number of nodes, *NdD* node density, *NS* number of spikes, *PC* penetrance of clavate architecture, *PH* plant height, *PP* penetrance of pubescences, *SL* spike length, *SS* supernumerary spikelets, *TKW* 1,000 kernels weight)

individual environments ranged from 38.5 to 40.3 %. Two QTLs had major effect and explained up to 16.5 % (*QGPC.ndsu.1A.1*) and 16.9 % (*QGPC.ndsu.6B.1*) of PV for GPC. The other nine QTLs were minor and explained between 4.7 and 13.7 % of PV. The elite parent contributed alleles for increased GPC at five loci (*QGPC.ndsu.1B*, *QGPC.ndsu.4B*, *QGPC.ndsu.6B.1*, *QGPC.ndsu.6B.2*, and *QGPC.ndsu.7B*), while the exotic parent contributed alleles for increased GPC at six loci (*QGPC.ndsu.1A.1*, *QGPC.ndsu.1A.2*, *QGPC.ndsu.2B*, *QGPC.ndsu.2D*, *QGPC.ndsu.3D*, and *QGPC.ndsu.5B*).

QTLs for mixograph-related traits

The genetic dissection of MEPT resulted in the detection of two consistent QTLs (*QMEPT.ndsu.1D*, and *QMEPT.ndsu.5B*) and five putative QTLs (*QMEPT.ndsu.1B*, *QMEPT.ndsu.2B*, *QMEPT.ndsu.2D.1*, *QMEPT.ndsu.2D.2*, and *QMEPT.ndsu.4A*) (Table 3; Fig. 4). In individual environments, the number of identified QTLs ranged from one (Prosper 2010) to six (Carrington 2010). The PV explained by all the QTLs identified in individual environments ranged from 25.9 to 64.5 %. The QTL located

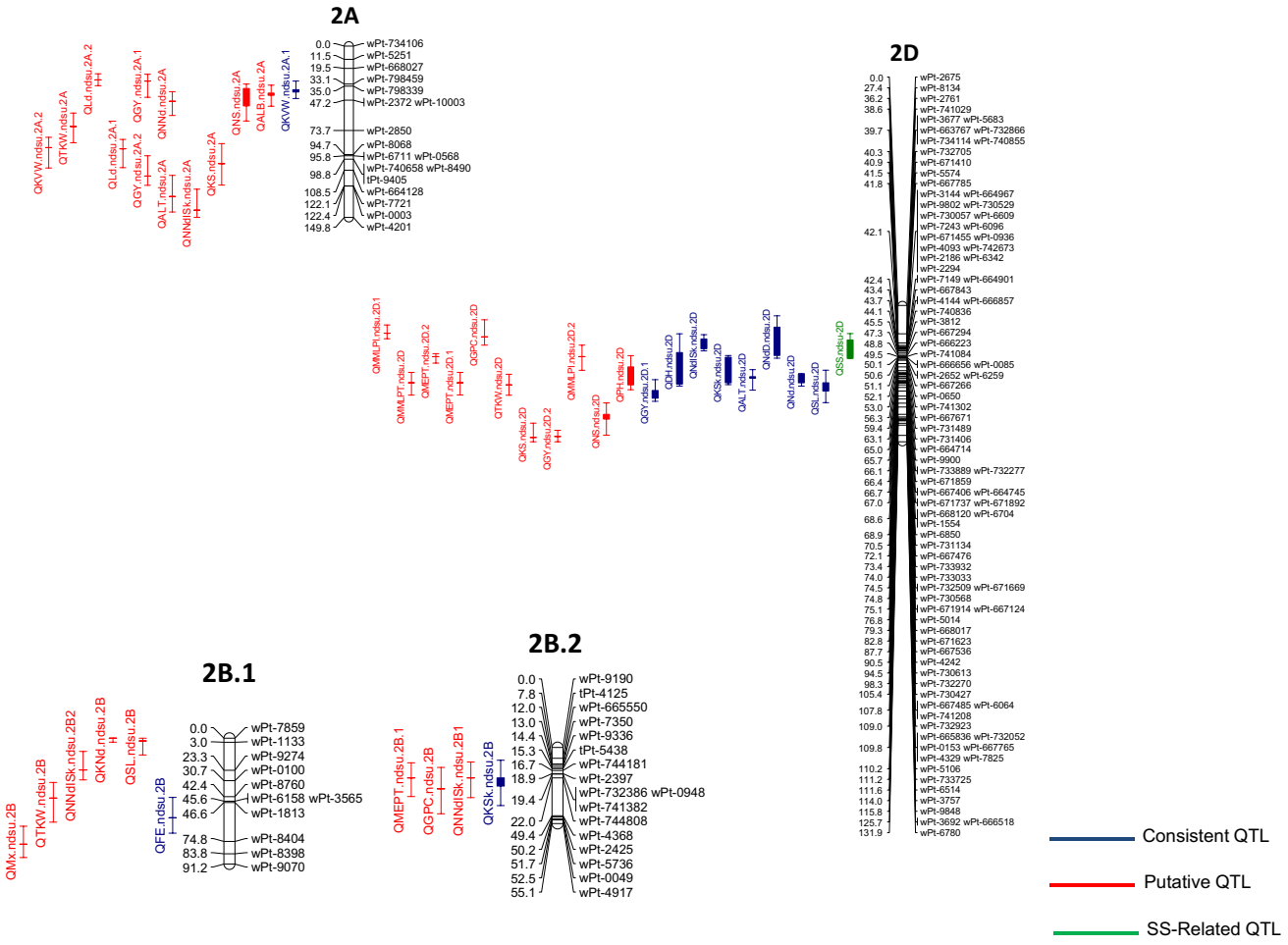

Fig. 4 continued

on 1DL (*QMEPT.ndsu.1D*) is the most important locus in controlling MEPT, as it was detected in all environments and explained up to 41.2 % of PV. The remaining six QTLs were minor and explained between 3.2 and 10.1 % of PV (Table 3). Both parents contributed QTL alleles to increase MEPT; however, for both consistent QTLs (*QMEPT.ndsu.1D*, and *QMEPT.ndsu.5B*), the alleles for increased values of MEPT were contributed by the elite parent (Table 3).

The QTL mapping of MMLPT resulted in the identification of six QTLs including two consistent QTLs (*QMMLPT.ndsu.1D*, and *QMMLPT.ndsu.5B*) (Table 3; Fig. 4). The number of QTLs identified in individual environments for MMLPT ranged from two (Prosper 2009; Carrington 2009; and Prosper 2010) to four (Carrington 2010). The PV explained by all the QTLs identified in individual environments ranged from 23.3 to 56.2 %, while the PV explained by individual QTLs ranged from 3.5 to 40.3 %. Among all these QTLs, *QMMLPT.ndsu.1D* had a major effect on MMLPT as it explained up to 40.3 % of PV and was consistently detected in all the environments. The other five minor QTLs explained between 3.5 and 7.9 % of PV (Table 3). The elite parent WCB414 contributed alleles for increased values of MMLPT at four loci, including both the major (*QMMLPT.ndsu.1D*) and minor consistent QTL (*QMMLPT.ndsu.5B*) (Table 3).

The trait MMLPI was associated with two consistent QTLs (*QMMLPI.ndsu.1B*, and *QMMLPI.ndsu.1D*) and six putative QTLs in this population (Table 3; Fig. 4). The PV explained by individual QTLs ranged from 3.8 to 37.1 % and the PV explained by all the QTLs in individual environments ranged from 32.4 to 52.1 %. A major QTL (*QMMLPI.ndsu.1D*) explaining up to 37.1 % of PV for MLPI, located on 1DL, was consistently detected in all the environments. Another major QTL which explained up to 17.7 % PV for MMLPI was located on 2DS (*QMMLPI.ndsu.2D.1*), but was identified in only one environment. The remaining minor QTLs explained 3.8 to 10.8 % of PV (Table 3). The elite parent WCB414 contributed the alleles for increased values of MMLPI at *QMMLPI.ndsu.1D*,

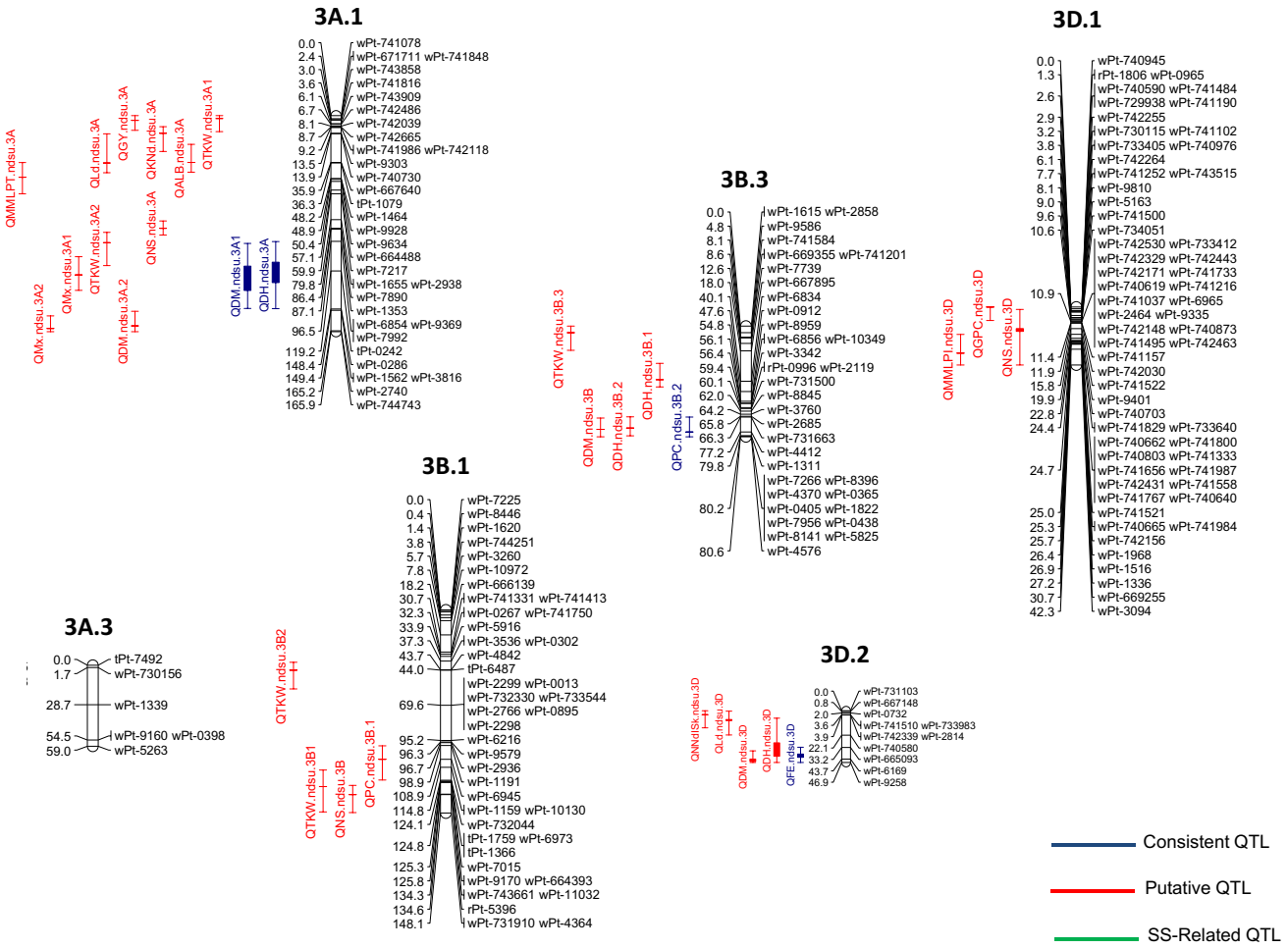

Fig. 4 continued

QMMLPI.ndsu.6B.1, and *QMMLPI.ndsu.6B.2*), while the exotic parent WCB617 provided the alleles that increased the values of this trait at the remaining loci (Table 3).

CIM for Mx resulted in the detection of a total of ten QTLs which included two consistent QTLs (*QMx.ndsu.6B.2* and *QMx.ndsu.7D*) (Fig. 4). The PV explained by individual QTLs for Mx ranged from 5.3 to 19.9 %, while PV explained by all the QTLs in individual environments ranged from 29.0 to 48.8 %. Among all QTLs, the consistent QTL located on 6BS (*QMx.ndsu.6B.2*) explained the highest PV by 19.9 % for Mx. Another major QTL of Mx explaining up to 15.6 % PV was located on 3A (*QMx.ndsu.3A.1*), but could be detected in single environment only. The remaining eight QTLs explained a minor portion of the PV, ranging from 5.3 to 11.0 %. WCB 414 alleles increased the trait values at three loci (*QMx.ndsu.3A.2*, *QMx.ndsu.6A*, and *QMx.ndsu.6B.2*), while WCB 617 alleles contributed to increase in the phenotypic values of Mx at the remaining seven loci (*QMx.ndsu.2B.2*, *QMx.ndsu.3A.1*, *QMx.ndsu.6B.1*, *QMx.ndsu.7A*, *QMx.ndsu.7B.1*, *QMx.ndsu.7B.2*, and *QMx.ndsu.7D*).

Discussion

Phenotypic variations

A better performance of WCB414 for TKW, KVW, FE, and mixograph-related traits was expected, considering that this genotype is an elite line. However, in most of the environments, the RIL population showed transgressive segregation (for all the traits, Fig. 2; Supplementary Table 1), demonstrating the allelic contribution of both parents and the formation of new combinations in recombinant lines. As was reported in previous studies (Huang et al. 2006; Raman et al. 2009; Sun et al. 2009; Wang et al. 2009; Tsilo et al. 2010; Heidari et al. 2011; Tsilo et al. 2011; Simons et al. 2012), in the present study too, the quality traits showed

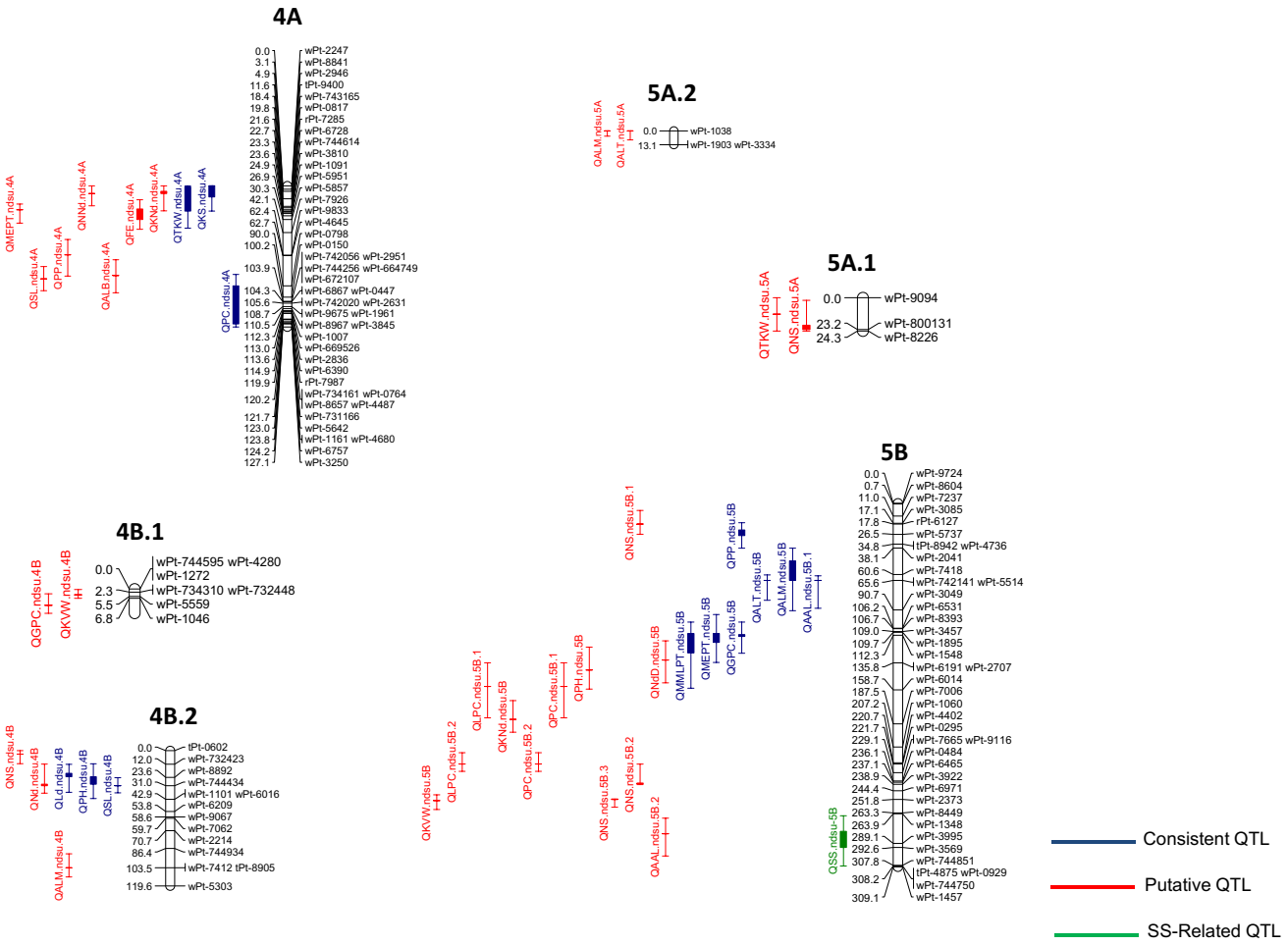

Fig. 4 continued

genotype × environment interaction (Table 1). The influence of the environment on the expression of the quality traits was also confirmed by the inconsistent correlations between some traits. For instance, TKW and GPC were negatively associated in two environments, but positively associated in one environment (Table 2). Likewise, the low values of heritability observed for most of these traits (except FE) indicate a strong influence of the environment on the phenotypic values (Table 1).

The positive significant associations observed between FE and TKW, as well as between FE and KVW (Table 2), are expected considering the impact of grain characteristics on milling characteristics. Similarly, the associations between GPC and mixograph-related traits (Table 2) were expected considering that dough mixing properties are influenced by the quality and quantity of proteins (Finney 1997). The positive and strong associations among several of the mixograph-related traits suggest the presence of common genes controlling these traits.

QTLs for TKW, KVW, and FE

The detection of several QTLs associated with TKW, KVW, and FE is in agreement with the quantitative genetic control and high environmental influence on these traits, as demonstrated in previous studies (Huang et al. 2004, 2006; McCartney et al. 2005; Kuchel et al. 2006; Cuthbert et al. 2008; Sun et al. 2009; Wang et al. 2009; Bennett et al. 2012; Simons et al. 2012; Maphosa et al. 2013). Breeders and millers use both TKW and KVW to determine the potential phenotypic values of FE in their genotypes (Wheat Marketing Center 2008). Indeed, in this study, these three traits were positively correlated (Table 2). Consequently, it is reasonable to expect QTL with pleiotropic effects or close linkage for these traits. CIM indicated that 1B is the only chromosome bearing QTL for TKW (*QTKW.ndsu.1B*), KVW (*QKVW.ndsu.1B*), and FE (*QFE.ndsu.1B*). However, these QTLs were located at different positions and could be considered different loci. Nevertheless, other

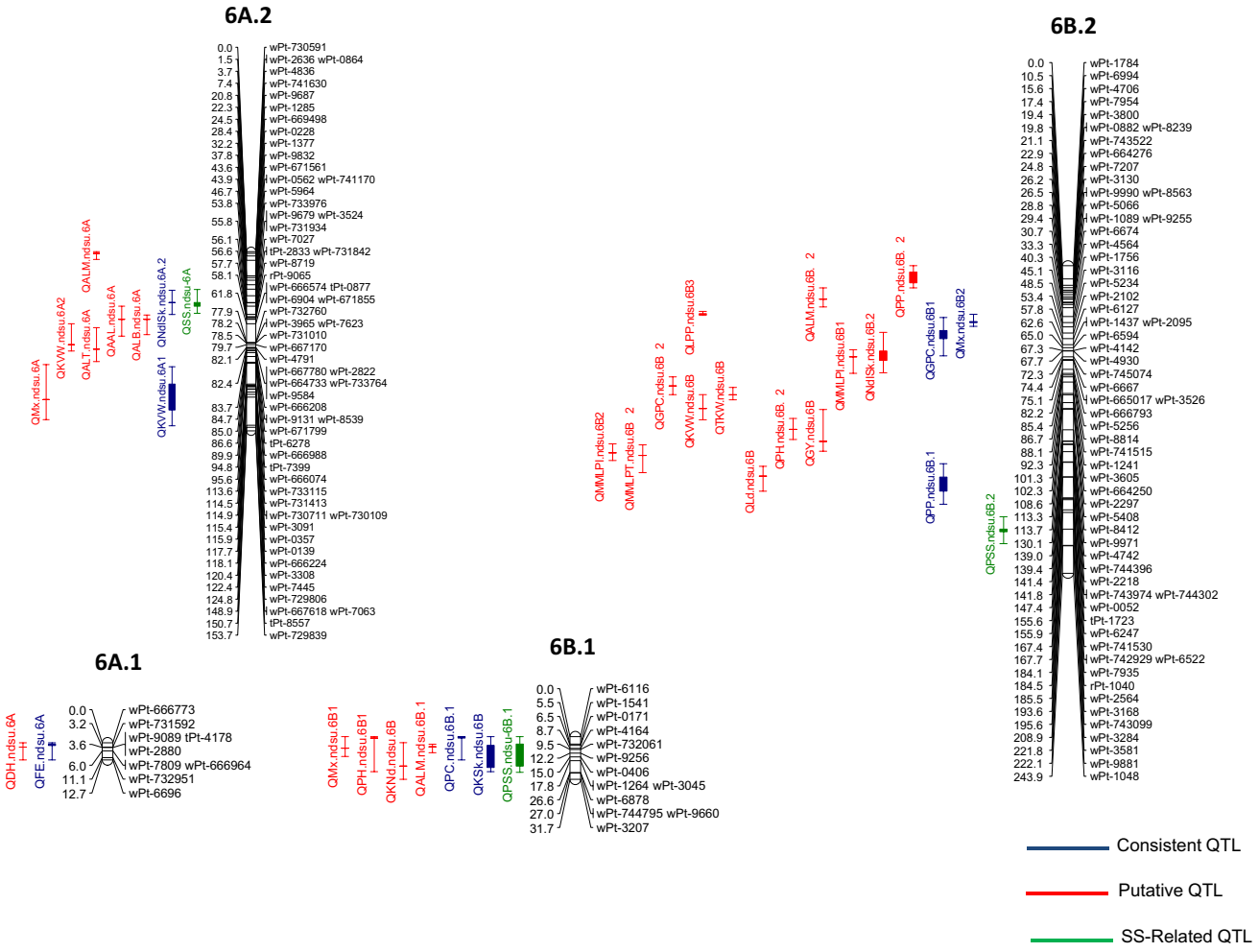

Fig. 4 continued

chromosomes also harbored QTLs for two of these traits as well. For example, chromosomes 2A and 6B had QTLs for TKW (*QTKW.ndsu.2A*, and *QTKW.ndsu.6B*) and KVW (*QKVW.ndsu.2A.1*, *QKVW.ndsu.2A.2* and *QKVW.ndsu.6B*); 1A and 6A had QTLs for KVW (*QKVW.ndsu.1A*, *QKVW.ndsu.6A.1*, and *QKVW.ndsu.6A.2*) and FE (*QFE.ndsu.1A*, *QFE.ndsu.6A*); and 2B had QTLs for TKW (*QTKW.ndsu.2B*) and FE (*QFE.ndsu.2B*). However, in all of these cases, the QTLs for different traits were located at different positions suggesting the absence of pleiotropic effects. An exception to this trend was observed on chromosome 4A, where the positions of *QTKW.ndsu.4A* and *QFE.ndsu.4A* overlapped suggesting a pleiotropic effect of this genetic region on TKW and FE (Table 3; Fig. 4).

QTKW.ndsu.4A was a major QTL and could be an excellent target for the selection of TKW through molecular markers, considering the fact that this QTL was consistently detected in all the environments (Table 3). However, the value of this QTL should be validated on elite wheat germplasm. Previous studies have also reported the presence of a QTL for TKW on 4AL (Araki et al. 1999; McCartney et al. 2005). In addition to the co-localization with *QFE.ndsu.4A, QTKW.ndsu.4A* was also co-located with putative QTL associated with kernels per node (KNd) (*QKNd.ndsu.4A*), number of nodes per spike (Nd) (*QNNd.ndsu.4A*), and kernels per spike (KS) (*QKS.ndsu.4A*) identified earlier in the same population (Echeverry-Solarte et al. 2015) (Fig. 4). This cluster of QTLs suggests a pleiotropic effect of this genetic region on spike and kernel development with impact on grain-quality traits. Kernel volume weight (also called test weight) is a grade-determining factor of wheat quality defined as the weight of grain per unit of volume (Schuler et al. 1994; Wheat Marketing Center 2008). A major QTL, *QKVW.ndsu.6A.1* for KVW, detected consistently in all the environments, was located on the distal region of 6AL. After further validations on wheat populations, the QTL allele from WCB414 for increased KVW, could be considered for MAS in wheat breeding

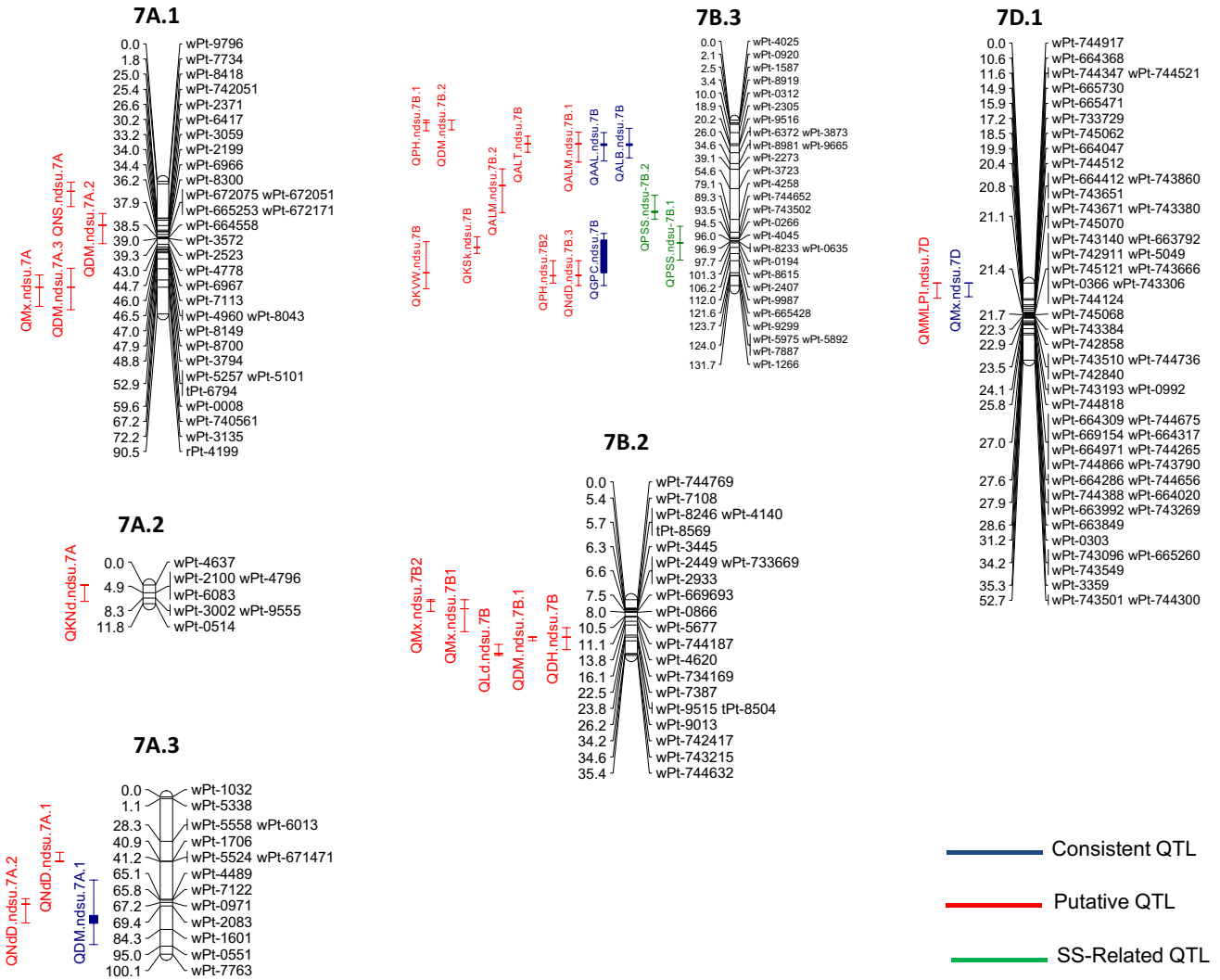

Fig. 4 continued

programs aimed at increasing KVW. In this population, *QKVW.ndsu.6A.1* was also co-located with a minor and putative QTL for Mx detected in Carrington 2010. Indeed, a positive correlation was also observed between KVW and Mx (Table 2) in this environment. A previous study also detected a QTL associated with KVW on the distal region of 6AL in Chinese winter wheat varieties (Sun et al. 2009). Another QTL associated with KVW under a water-limited environment was also detected on 6AL, but in a different position (Bennett et al. 2012). The distal region of 6AL is also known to harbor QTL for grain weight per ear and leaf erectness (Börner et al. 2002).

Another major and consistent QTL for KVW was located on 2A (*QKVW.ndsu.2A.1*), which explained up to 22.5 % of PV for this trait. In this population, this genomic region has also been found to be associated with putative QTL for awn length at the bottom of the spike (ALB), number of spikes m^{-2} (NS), number of nodes (Nd), grain yield (GY), and lodging (Ld) (Echeverry-Solarte et al. 2015) (Fig. 2). Another novel and consistent QTL associated with KVW was located on the distal region of chromosome 1AL (*QKVW.ndsu.1A*). Although this region was associated with only KVW in this population, the distal region of 1AL has been shown to harbor QTL associated with Fusarium head blight (FHB) and deoxynivalenol (DON) toxin produced by FHB (Semagn et al. 2007).

The trait FE has the highest proportion of consistent QTLs among all the eight quality traits studied in this population, which makes them suitable for MAS, once they had been validated in other spring wheat populations. All the chromosomes except 2B, where QTL for FE were identified in this study, have been reported to carry QTL for this trait (Nelson et al. 2006; Kuchel et al. 2006; Simons et al. 2012). The novel QTL located on 2BS (*QFE.*

ndsu.2B), although had major effect, was detected in one environment only and needs further validation before it could be used in MAS. Another important QTL was *QFE.ndsu.1A* and detected in all the four environments studied as well as across environments (Table 3). A previous study (Kuchel et al. 2006) has also reported a QTL on 1A associated with FE in a double haploid population derived from two commercial wheat varieties. *QFE.ndsu.1A* was located on the long arm of chromosome 1A and its confidence interval (CI) overlapped with a QTL-rich region associated with ALT (*QALT.ndsu.1A*), NS (*QNS.ndsu.1A*), KNd (*QKNd.ndsu.1A*), NNdISk (*QNNdISk.ndsu.1A*), Nd (*QNd.ndsu.1A*), and PP (*QPP.ndsu.1A.3*) (Echeverry-Solarte et al. 2015; Fig. 4).

Another consistent QTL for FE was *QFE.ndsu.1B* which was located on 1B. A previous study also reported a QTL for FE in a population derived from the cross of a soft and a hard red spring wheat (Simons et al. 2012). The chromosome 3D also harbors a consistent QTL (*QFE.ndsu.3D*) for FE. Another recent study also reported a QTL for FE on the distal region of 3DL under water-limited conditions (Maphosa et al. 2013). QTLs for other traits like days to heading (*QDH.ndsu.3D*) and days to maturity (*QDM.ndsu.3D*) have also been identified in this population in the same region where *QFE.ndsu.3D* was located (Echeverry-Solarte et al. 2015; Fig. 4). The QTL alleles for higher FE and early heading and maturity at those loci were contributed by WCB414 suggesting that the selection of QTL alleles for higher FE could also result in early heading and maturity. This was also supported by negative correlation observed between FE and DH, as well as between FE and DM (data not shown).

QFE.ndsu.6A was the only QTL associated with FE, for which the alleles from the branched parent WCB617 were responsible for increased trait values. Considering the high stability in detection of *QFE.ndsu.6A* (Table 3), alleles of the exotic parent at this QTL are excellent candidates to be introduced into the wheat breeding programs for further improvement of FE. *QFE.ndsu.6A* was located on the long arm of chromosome 6A, where QTL for DH (*QDH.ndsu.6A*) was also reported earlier in the same population (Echeverry-Solarte et al. 2015; Fig. 4). QTL for FE has also been reported on 6A in a previous study using a population derived from two commercial wheat varieties (Kuchel et al. 2006).

QTLs for GPC and mixograph-related traits

GPC affects mixing properties of wheat (Finney 1997); therefore, it is important for breeding purpose to recognize genetic regions controlling GPC and dough strength. For this purpose, this study detected a consistent QTL on 5B with pleiotropic effect on GPC (*QGPC.ndsu.5B*), MEPT (*QMEPT.ndsu.5B*), and MMLPT (*QMMLPT.ndsu.5B*) (Table 3; Fig. 4). For this QTL, alleles from the exotic parent increased GPC, but decreased the mixograph-related values. These results are in agreement with the negative correlations observed between GPC and mixograph-related traits in the present study (Table 2) as well as in some previous studies (Huang et al. 2006; Simons et al. 2012). Previous studies for GPC in wheat have reported a QTL associated to this trait on 5BL (Kulwal et al. 2005; Conti et al. 2011; Bordes et al. 2011, 2013). However, similar to our finding, a recent study also reported a common QTL for GPC and MMLPT on 5B (Simons et al. 2012). Another closely linked QTL for GPC and mixograph-related traits also was observed on 6BS. The QTL for GPC (*QGPC.ndsu.6B.1*) and Mx (*QMx.ndsu.6B.2*) had consistent and major effect, while another QTL found in this region for MMLPI (*QMLPI.ndsu.6B.1*) had minor effect. At these loci on 6BS, the elite parent WCB414 contributed alleles that increased phenotypic values of these traits. Probably, the highly consistent QTL, *QGPC.ndsu.6B.1*, is associated with gliadin genes located on 6BS (Payne et al. 1987).

In the past, several mixograph-related traits like mixograph midline peak value, mixograph line peak integral, mixograph weakening slope, and mixograph total energy have been studied. However, there is no consensus on the merits of each of these mixograph-related traits in the selection of the best dough properties (Ingelin 1997). In this study, to the best of our knowledge, this is the first time that QTL for MEPT have been reported. However, QTLs for MMLPT and MMLPI have been identified in few earlier studies (Campbell et al. 2001; Huang et al. 2006; Tsilo et al. 2011; Li et al. 2012b; Simons et al. 2012; Mergoum et al. 2013). QTLs controlling MEPT (*QMEPT.ndsu.1B*), MMLPT (*QMMLPT.ndsu.1B*), and MMLPI (*QMMLPI.ndsu.1B*) were detected on chromosome arm 1BS, which coincides with the location of *Glu-B3* for LMW glutenin subunits (McCartney et al. 2006; Mann et al. 2009; Simons et al. 2012; Maphosa et al. 2013). The SDS-PAGE analysis confirmed that WCB414 and WCB617 have different LMW glutenin subunits. Therefore, the QTLs controlling MEPT are most likely associated with the *Glu-B3* locus in the population. The positive alleles responsible for increasing MEPT, MMLPT, and MMLPI for loci located on 1BS were contributed by the exotic parent, suggesting the possibility of using WCB617 in improving wheat quality traits. This locus on 1BS controlling mixograph-related traits also overlaps with a cluster of other QTLs associated with GPC (*QGPC.ndsu.1B*), and KVW (*QKVW.ndsu.1B*) (Fig. 4), as well as with penetrance of clavate architecture (PC) (*QPC.ndsu.1B*) identified in the same RIL population previously (Echeverry-Solarte et al. 2015). The chromosome 1B is well documented to play an important role in wheat quality as it harbors several genes for gluten strength and other

quality traits (Campbell et al. 2001; Huang et al. 2006; Mann et al. 2009; McCartney et al. 2006; Tsilo et al. 2010; Kumar et al. 2013; Maphosa et al. 2013). Comparison of map position, however, suggests that the QTL identified in the present study on 1BS should be different from *Glu-B1* on 1BL. However, further investigations are needed to completely rule out any such possibilities.

The most consistent genomic region associated with mixograph-related traits was identified on chromosome 1D, where major QTLs for MEPT (*QMEPT.ndsu.1D*), MMLPT (*QMMLPT.ndsu.1D*), and MMLPI (*QMMLPI.ndsu.1D*) were identified. The high PV explained by these QTLs (15.4–41.2 %) and their stability across environments confirm previous studies which also identified major QTLs/QTL clusters associated with mixograph properties as well as with other quality traits in this region (Campbell et al. 2001; Huang et al. 2006; Nelson et al. 2006; Mann et al. 2009; Tsilo et al. 2010; Simons et al. 2012). The position of these QTLs coincides with the position of *Glu-D1* locus (Mann et al. 2009) that encodes for HMW glutenin subunits. The SDS-PAGE analysis from this study revealed that WCB414 and WCB617 have the HMW glutenin subunits Dx5+Dy10 and Dx2+Dy12, respectively, suggesting that these QTLs are associated with the *Glu-D1* locus in the population.

As there were high correlations between MEPT, MMLPT, and MMLPI, it was expected to identify common loci controlling these traits. However, some of the QTLs specific to only one mixograph-related traits were also identified. For example, two QTLs located on 2BL (*QMEPT.ndsu.2B*) and 4AL (*QMEPT.ndsu.4A*) were specific to MEPT; one QTL located on chromosome 3A (*QMMLPT.ndsu.3A*) was specific to MMLPT; and three QTLs located on 3DS (*QMMLPI.ndsu.3D*), 6BS (*QMMLPI.ndsu.6B.1*), and 7DS (*QMMLPI.ndsu.7D*) were specific to MMLPI. Most of these QTLs, although reported for the first time, have minor effects and were unstable across environments. This suggests a need for further study of these QTLs before any recommendations could be made for their use in improving wheat quality.

Interestingly, the trait Mx shared only one common QTL with MMLPI, which was located on chromosome 7DS at 0.01 cM (Table 3; Fig. 4). The lack of additional common QTLs among Mx and mixograph-related traits could be due to difference in the methodologies conducted to assess each trait. Mx is assessed through a visual scale; while the information on MEPT, MMLPT, and MMLPI is collected from the Mixmart software (National Manufacturing, TMCO Division, Lincoln NE). Although Mx is broadly used by the breeders to select germplasm with optimal dough strength, this trait has not been subjected to detailed genetic dissection in the past. In one study, the presence of four QTLs located on chromosomes 1B, 1D, 3B, and 6D controlling Mx was reported (Tsilo et al. 2011). However, all those QTLs differ from the QTLs described in our study, which were located on 2BS, 3A, 6AL, 6BS, 6BL, 7AS, 7BL, and 7DS. Two consistent QTLs associated with Mx were identified; one on chromosome 6B (*QMx.ndsu.6B.2*) and the other on 7D (*QMx.ndsu.7D*). Once again, the exotic parent WCB617 played an important role in providing alleles that increased the values of Mx at seven QTLs, including the consistent QTL located on 7D (Table 3). This shows the potential use of WCB617 as a source for improving some important wheat quality traits.

Association of QTLs for GPC, TKW and KVW

TKW and KVW may also be considered as yield components. In the past, several studies have shown a negative relationship between GPC and yield components in wheat (Groos et al. 2003; Blanco et al. 2012). The few studies which used molecular markers to understand the relationship between loci controlling GPC and yield or yield components have shown that although there are some common genomic regions associated with both GPC and yield (or yield traits), these studies found several other genomic regions which do not show a negative association between these two traits (Groos et al. 2003; Tsilo et al. 2010; Blanco et al. 2012). For example, in hexaploid wheat, Tsilo et al. (2010) found two (out of 3) stable QTLs for GPC located on 5A and 6D which were independent of grain yield, grain weight, and grain size, while Blanco et al. (2012) identified four QTLs for high GPC (located on 1AL, 2AS, 2BL and 4AL) which had no effect on grain yield (Blanco et al. 2012).

In the present study, out of the total 11 QTLs associated with GPC, two (*QGPC.ndsu.2B, QGPC.ndsu.6B.2*) were located in the same region where QTLs for TKW (*QTKW.ndsu.2B*, *QTKW.ndsu.6B*) were identified and one QTL for GPC (*QGPC.ndsu.4B*) was identified in a region where a QTL for KVW ((*QKVW.ndsu.4B*) was identified. The remaining eight QTLs for GPC were independent of TKW and/or KVW. Only one of the common QTLs (*QGPC.ndsu.2B* and *QTKW.ndsu.2B*) have different parental alleles contributing to increased trait values, meaning that increase in GPC at this locus was associated with decrease in TKW. These results suggest that the negative correlation observed between GPC and TKW or KVW for some environments is not because of co-localized or pleiotropic QTL with opposite effects. However, a significant outcome in the present study was that all of the consistent QTLs for GPC, TKW, and KVW were independent of each other. Such QTLs should be incorporated in breeding programs through MAS to improve GPC without decreasing TKW and KVW or vice versa.

Influence of SS loci on quality traits

The exotic parent (WCB617) used in the present study has SS phenotype. To understand any role of loci controlling SS on quality traits, genomic locations of QTLs identified for SS (Echeverry-Solarte et al. 2014) were compared with the locations of QTL for quality traits identified in this study. This study identified QTLs for TKW (*QTKW.ndsu.2D*), GPC (*QGPC.ndsu.2D*), MEPT (*QMEPT.ndsu.2D.1*, *QMEPT.ndsu.2D.2*), MMLPT (*QMMLPT.ndsu.2D*), and MMLPI (*QMMLPI.ndsu.2D.1*, *QMMLPI.ndsu.2D.2*) on chromosome 2D. These QTLs have overlapping CI with a major QTL for the SS phenotype and some other spike-related and agronomic traits mapped in the same region and/or nearby on 2D (Echeverry-Solarte et al. 2014, 2015) (Fig. 4). This 2D region has been identified as a rich gene region previously (Erayman et al. 2004). Other genomic regions where minor QTLs for quality (KVW, Mx and GPC) were co-located/linked with QTL for SS, were on chromosome 6B and 7B. Interestingly, the co-localization of QTLs for SS and quality-related traits was also supported by significant correlations observed between SS and quality-related traits (TKW, GPC, mixograph-related traits) (Supplementary Table 3). Except for TKW QTL on the 2D genomic region, the positive QTL alleles for SS and quality traits were contributed by the exotic parent WCB617. In agreement with our findings, another study also identified a cluster of QTLs for mixograph and baking traits on 2D and reported that the favorable QTL alleles were derived from wild wheat specie at those loci (Li et al. 2012). This clearly suggests the influence and potential usefulness of exotic germplasm for quality trait improvement in wheat.

Conclusion

The use of an RIL population generated from an exotic germplasm with SS and a white elite wheat line in this study resulted in the identification of a large number of QTLs for eight important wheat quality traits. A total of 69 QTLs were detected for these traits, in which the exotic parent provided alleles with increasing effect at 51 % of these QTLs. These results suggest that germplasm with SS is a valuable resource to improve quality traits in wheat. Therefore, after previous validations on other elite breeding populations, identifying molecular markers associated with consistent and/or major QTLs detected in this study could be of great interest for wheat breeding programs.

Author contribution statement MES and MM, with the help of SK, ELD, EE, PEM, MSA and SK, designed the research and MES and MM executed the research; SK provided the F_2 population from which MM developed the RILs; EEM, MSA, BS, and SSX contributed to laboratory and data collection; MES and AK analyzed the data; MES and AK drafted the manuscript with comments from all authors. All authors read and approved the final manuscript.

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

Ethical standards The authors declare that the experiments complied with the current laws of the USA.

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