Quantitative Trait Loci Mapping for Spike Characteristics in Hexaploid Wheat

Yaopeng Zhou, Benjamin Conway, Daniela Miller, David Marshall, Aaron Cooper, Paul Murphy, Shiaoman Chao, Gina Brown-Guedira, and José Costa*

Abstract
Wheat (Triticum aestivum L.) spike characteristics determine the number of grains produced on each spike and constitute key components of grain yield. Understanding of the genetic basis of spike characteristics in wheat, however, is limited. In this study, genotyping-by-sequencing (GBS) and the iSelect 9K assay were used on a doubled-haploid (DH) soft red winter wheat population that showed a wide range of phenotypic variation for spike traits. A genetic map spanning 2934.1 cM with an average interval length of 3.4 cM was constructed. Quantitative trait loci (QTL) analysis involving additive effects, epistasis (QQ) and QTL × environment (QE), and epistasis × environment (QQE) interactions detected a total of 109 QTL, 13 QE, and 20 QQ interactions in five environments. Spike characteristics were mainly determined by additive effects and were fine-tuned by QQ, QE, and QQE. Major QTL QSl.cz-1A/QFsn.cz-1A explained up to 30.9% of the phenotypic variation for spike length (SL) and fertile spikelet number, QGsp.cz-2B.1 explained up to 15.6% of the phenotypic variation of grain number per spikelet, and QSc.cz-5A.3 explained up to 80.2% of the phenotypic variation for spike compactness. Additionally, QTL for correlated spike characteristics formed QTL clusters on chromosomes 1A, 5A, 2B, 3B, 5B, 1D, and 5D. This study expands the understanding of the genetic basis of spike characteristics in hexaploid wheat. A number of stable QTL detected in this study have potential to be used in marker-assisted selection. Additionally, the genetic map generated in this study could be used to study other traits of economic importance.

Core Ideas
- A high-density wheat genetic map was generated using GBS and the 9K iSelect Array.
- Spike characteristics were mainly determined by additive effects.
- Major QTL of spike characteristics were identified on chromosomes 1A, 2B, and 5A.

Wheat is a major food crop across the globe. Improving its yield potential is crucial to meeting the food demand from an increasing world population. Grain yield of wheat is largely determined by yield components out of which the three most important are spikes per unit area, grains per spike, and grain weight (Dilbirligi et al., 2006; Mengistu et al., 2012). Previous
studies have shown that grain yield variation is mostly associated with increased grain number where grain number, expressed as grains per square meter, is the product of spikes per square meter and grains per spike. There appears, however, to be less opportunity for genetic yield improvement by selecting heavier grains (Fischer, 2011; Frederick and Bauer, 1999). Increases in grains per spike and spikes per square meter have contributed the most to wheat yield improvement in the past decades (Ma et al., 2007). Spike characteristics, including SL, total spikelet number per spike (TSN), fertile spikelet number per spike (FSN), sterile spikelet number per spike (SSN), spike compactness (SC), and grains per spikelet (GSP) determine the number of grains per spike and thus, to a certain extent, determine the yield potential.

Spike characteristics are quantitative traits under QTL control and subject to environmental influence (Cui et al., 2012; Ma et al., 2007). Genetic dissection of spike characteristics could facilitate improving grain yield potential of wheat. Several domestication genes, such as Q, compactum (C), and sphaerococcum (SI) are related to wheat spike morphology and have been identified on chromosomes 5A, 2D, and 3D, respectively (Faris et al., 2003; Faris and Gill, 2002; Johnson et al., 2007a; Rao, 1977). The Q gene confers a free-threshing spike and pleiotropically influences many other domestication related traits including plant height, glume keeledness, rachis toughness, spike type, and spike emergence time (Faris et al., 2003; Simons et al., 2006; Sormacheva et al., 2014). The C gene is located on the long arm of chromosome 2D near the centromere and affects spike compactness, grain size, grain shape, and grain number per spike (Johnston et al., 2007a). The SI gene confers rigid short culms, straight flag leaves, dense spikes, hemispherical glumes, and small spherical grains (Rao, 1977). In addition to these loci, previous studies have identified other genomic regions associated with spike-related traits on all 21 wheat chromosomes (Bornert et al., 2002; Cui et al., 2012; Deng et al., 2011; Kumar et al., 2007; Ma et al., 2007; Marza et al., 2006; Wang et al., 2011). For example, Cui et al. (2012) detected 190 QTL across all wheat chromosomes for seven spike-related traits in two recombinant inbred line populations. 18 of the detected QTL had major effects and were significant across multiple environments. Ma et al. (2007) investigated the additive, dominant and epistatic effects of QTL for SL, FSN, SSN, TSN, and SC in a recombinant inbred line (RIL) population and also from an immortalized F2 population derived from the same parents and found 18 genomic regions on chromosomes 1A, 1B, 2D, 3B, 4A, 5A, 5B, and 7A associated with spike characteristics. Additionally, Kumar et al. (2007) identified QTL for SL on chromosomes 1A, 1B, 1D, 2B, 2D, 4A, 5A, and 5D and QTL for TSN on 2D, 4A, 4D, 5A, and 6A. These results demonstrated that multiple loci with unequal effects can affect spike traits and that epistasis and dominance effects can also influence genetic architecture of spike characteristics. Furthermore, mapping wheat spike characteristics QTL as Mendelian factors in wheat was also reported. Deng et al. (2011) investigated wheat spike traits in a F3 population, derived from the cross between an elite cultivar Laizhou953 and an introgression line 05210 (in Laizhou953 background). This population showed a clear 3:1 segregation ratio for spike number per plant, SL, and grain number per spike. The underlying QTL was mapped to chromosome 4B, spanned 6.2 cM, and explained 30.1 to 67.6% of the phenotypic variation in two environments.

High-density genetic maps are critical for QTL mapping. Driven by the decreasing cost of next-generation sequencing technology, high-throughput genotyping methods are starting to be employed to dissect quantitative traits in wheat with improved map resolution (Li et al., 2013, 2015a,b; Lin et al., 2015; Spindel et al., 2013). For example, using GBS in a 155 RIL population, Lin et al. (2015) delimited a major preharvest sprouting resistance QTL to a 2.9-cM interval on 4AL. Li et al. (2015b) constructed a consensus map using three RIL populations genotyped by GBS and validated 15 published QTL and three well-characterized rust resistance genes (Sr58/Lr46/Yr29, Sr2/Yr30/Lr27, and Sr57/Lr34/Yr18). Additionally, Li et al. (2013) positioned the major Hessian fly resistance H34 in a 4.5-cM region on 6B in a RIL population genotyped with the iSelect 9K SNP Array.

In this study, a DH population of 124 lines derived from two soft red winter wheat genotypes that showed a wide range of phenotypic variation for spike characteristics was used for mapping. The objectives of this research were to construct a genetic map with markers aligned to the wheat chromosomes survey sequence to identify QTL responsible for the observed phenotypic variation, gain a better understanding of the genetic architecture of spike characteristics, and develop DNA markers closely linked to the QTL to be used for marker-assisted selection.

Materials and Methods

Plant Materials

A DH population of 124 lines was established from the cross of the soft red winter wheat germplasm line MD01W233-06-1 = PI 658682 (MD233) (Costa et al., 2010) and soft red winter wheat cultivar Southern States 8641 (SS8641) (Johnson et al., 2007b). MD233 is a breeding line released by the Maryland Agricultural Experiment Station in 2009 with enhanced Fusarium head blight resistance. It was produced by crossing the soft red winter wheat cultivar McCormick [VA92-51-39 (IN71761A4-31-5-48//VA71-54-147//McNair 1813)/AL870365 (‘Coker 747’*2/‘Amigo’)] (PI632691) (Griffey et al., 2005) with ‘Choptank’ (‘Coker 9803’/‘Freedom’) (PI 639724) (Costa et al., 2006). MD233 carries the Rht-D1b dwarfing gene, the Ppd-D1b photoperiod sensitive allele, as well as the 1RS:1AL translocation. SS8641 is a photoperiod insensitive cultivar (Ppd-D1a) released by the University of Georgia Experiment Station in 2007, with high yield and multiple-disease resistance
(Johnson et al., 2007b). It is a medium-maturing, white-chaffed, medium-tall line derived from the cross ‘GA 881130/2’GA 881582’. GA 881130 is the source of Hessian fly resistance gene H13 and the rust resistance genes Lr37/Yr17/Sr38 present on the translocation segment between the short arms of T. ventricosum 2NS and the bread wheat chromosome 2AS (Helguera et al., 2003).

Field Experiments and Phenotyping

The DH mapping population and parents were evaluated in field plots with two replications in a randomized complete block design at five location–year environments in Maryland (Clarksville, MD, 2013 [C13]; Clarksville, MD, 2014 [C14]; Queenstown, MD, 2013 [Q13]; Queenstown, MD, 2014 [Q14]) and North Carolina (Kinston, NC, 2014 [K14]). Plots at Maryland locations consisted of seven rows 3.2 m long and 15.2 cm apart planted at 18 seeds per 0.305-m row. Plots at North Carolina had seven rows 19.1 cm apart with 24 seeds per 0.305-m row. Growing season rainfall and temperature data were obtained from respective research farms for Clarksville, MD, and Queenstown, MD, and from the National Oceanic and Atmospheric Administration measurements for Kinston, NC (http://www.noaa.gov) (Supplemental File S1). Soil fertility management followed recommended management practices for each location. All environments were sprayed with the metconazole fungicide (0.11 mL m⁻²) (Caramba, BASF) at anthesis to reduce potential infection by Fusarium graminearum.

Ten plants in the middle rows from each plot were randomly selected for evaluation of spike traits. Traits examined included SL (cm) measured from the base of the rachis to the top of the uppermost spikelet, FSN, and SSN. A spikelet is sterile if none of its florets set grains. Otherwise, it is fertile. Total spikelet number per spike was equal to FSN plus SSN. Spike compactness was derived by dividing TSN by SL, and GSP was derived by dividing grain number per spike by FSN.

Phenotypic Data Analysis

Phenotypic data analysis was performed using SAS version 9.3 (SAS Institute, 2011). The phenotypic value for SL, FSN, SSN, TSN, SC, and GSP for 10 plants from each DH line in each replication was averaged before analyses. Analysis of variance for SL, FSN, SSN, TSN, SC, and GSP was performed separately for each environment and for the five environments combined by the PROC GLM procedure in SAS. The linear model for ANOVA for single environment analysis was $Y_{ij} = \mu + g_i + r_j + E_{ij}$, where $\mu$ is the overall mean, $Y_{ij}$ is the phenotypic value of the $i$th DH line in $j$th replication, $g_i$ is the fixed effect of the $i$th DH line, $r_j$ is the fixed effects of $j$th replication, and $E_{ij}$ is the random effects of error associated with $Y_{ij}$ and for combined analysis $Y_{ijk} = \mu + g_i + r_j + e_k + E_{ijk}$, where $\mu$ is the overall mean, $Y_{ijk}$ is the phenotypic value of the $i$th DH line in $j$th replication of $k$th environment, $g_i$ is the fixed effect of the $i$th DH line, $r_j$ is the fixed effects of $j$th replication of $k$th environment, $e_k$ is the fixed effect of the $k$th environment, and $E_{ijk}$ is the random effect of error associated with $Y_{ijk}$. Pearson’s correlation coefficients were calculated by the PROC CORR procedure in SAS to detect the association among spike traits. Broad-sense heritability ($H^2$), defined as $H^2 = \sigma^2_G / \left[ \sigma^2_G + \frac{\sigma^2_E}{e} + \frac{\sigma^2_r}{r} \right]$, where $\sigma^2_G$ is the variance of genotypic effect, $\sigma^2_E$ is the genotype × environment variance, and $e$ and $r$ are the number of environments and replicates, respectively, for each trait was calculated on a family-mean basis by the PROC MIXED procedure in SAS, as described by Holland et al. (2003).

Simple-Sequence Repeat Genotyping and 9K iSelect Array

Approximately 25 mg of leaf tissue of the parents and 124 DH lines were collected from 2- to 3-wk-old seedlings for genomic DNA extraction, which was performed according to the protocol of Pallotta et al. (2003). For all SSR markers, the polymerase chain reaction (PCR) master mix consisted of 2 μL of 20 ng μL⁻¹ genomic DNA template, 0.40 μL of a 10 μM mixture of forward and reverse primers, 0.18 μL (0.9 U) of Taq polymerase, 1.20 μL of 10× buffer (10 mM Tris-HCl, 50 mM KCl, and 1.5 mM MgCl₂, pH 8.3), 0.96 μL of a 100-μM mixture of deoxyribonucleotide triphosphates (dNTPs), and 7.26 μL of water, bringing the total reaction volume to 12 μL. A touchdown profile was used that consisted of an initial denaturation at 95°C followed by 15 cycles of 95°C for 45 s, 65°C for 45 s decreasing by 1°C each cycle, and 72°C for 60 s, followed by 25 cycles of 50°C annealing temperature. The forward primers were 5’-modified to include one of the following fluorescent dyes: 6-FAM, VIC, NED, or PET. Amplifications were performed using an Eppendorf Mastercycler (Eppendorf AG). Sizing of PCR products was performed by capillary electrophoresis using an ABI3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Analysis of PCR fragments was performed using GeneMarker 1.60 software (SoftGenetics, LLC).

The SNP genotyping was performed on the 9K iSelect SNP genotyping array containing 9000 wheat SNP markers developed by Illumina Inc. This assay was designed under the protocols of the International Wheat SNP Consortium (Cavanagh et al., 2013). In addition, 24 Kompetitive Allele Specific PCR (KASP) markers derived from SSR markers were also used in genotyping.

Genotyping-by-Sequencing

Genotyping-by-sequencing libraries were prepared according to Poland et al. (2012) using the Ptssl-MspI two-enzyme combination for genome complexity reduction. A set of 96 barcodes having the Ptssl overhang was used to barcode samples. The population was sequenced in combination with samples from another project in two lanes on the Illumina HiSeq2500 each having 95 samples and one blank control.

The SNP identification and calling was performed using the TASSEL 4 GBS pipeline (Glaubitz et al., 2014). Reads were aligned using the Burrows–Wheeler aligner...
version 0.6.2 against a pseudo reference genome developed from the *T. aestivum* Chinese Spring chromosome survey sequence (International Wheat Genome Sequencing Consortium, 2014). The pseudo reference consisted of 41 molecules, one for each chromosome arm except for chromosome 3B, which was a single molecule. Each pseudo molecule consisted of concatenated sequences for chromosome specific contigs with a string of 64 Ns inserted between contigs. A text file having the start and end position of each contig within the pseudo molecule was created and used to identify SNP containing contigs and to determine location of SNPs on contigs. The SNPs were named in the following format: ChromArm_contig number_SNPs position within the contig. The SNP in tags aligning to contigs having map positions from the POPSEQ map (International Wheat Genome Sequencing Consortium; http://wheat-urgi.versailles.inra.fr/Seq-Repository/Publication-IWGSC) were used to compare order of the MD233 × SS8641 linkage map with the POPSEQ map.

**Linkage Map Construction**

Markers with >20% missing rate and those that were monomorphic and distorted (differing significantly from the expected 1:1 segregation ratio) were eliminated from the analyses. The remaining polymorphic markers were clustered into linkage groups using the MAP function in software IciMapping version 4.0 with a logarithm of odds (LOD) score of 10 (Li et al., 2008). Linkage groups from the same chromosome were merged referencing the marker chromosomal location on the 9K SNP consensus map (Cavanagh et al., 2013), the SSR consensus map (Somers et al., 2004), and the POPSEQ map. Markers from the same chromosome were reordered and the genetic distance recalculated with RECORD and COUNT algorithm in IciMapping version 4.0. Recombination frequencies were converted to centimorgans (cM) using the Kosambi mapping function.

**Quantitative Trait Loci Detection**

Two methods were used to map QTL for spike characteristics. First, inclusive composite interval mapping (ICIM) was performed separately for each environment. Quantitative trait loci with additive effects were detected by the ICIM-ADD module of IciMapping version 4.0 (Li et al., 2008). The walking speed for all traits under study was 1 cM. Reference LOD values were determined by 1000 permutations (Doerge, 2002). Type I error to determine the LOD from the permutation test was 0.05 and the LOD threshold to declare the presence of a significant QTL was 3.0. Second, mixed-model-based composite interval mapping (MCIM) or a full-QTL model was used to estimate additive, QE, QQ, and QQE interaction effects with QTLNetwork version 2.1 (Wang et al., 1999; Yang et al., 2007). Additive, QE, QQ, and QQE effects were estimated by the Monte Carlo Markov chain method with a scanning speed of 1-cM step with the experiment-wise type I error for putative QTL detection of 0.05. In both methods, the position at which the LOD score curve reached its maximum was used as the estimate of the QTL location. Quantitative trait loci were named following the Catalogue of Gene Symbols for Wheat (McIntosh et al., 2013). Quantitative trait loci with .cz and .czm in their names were detected by ICIM and MCIM, respectively.

**Comparative Mapping**

Flanking markers of QTL detected in multiple environments were used to obtain their associated contig sequences in the wheat survey sequence database (http://wheat-urgi.versailles.inra.fr/). Contig sequences were blasted against genomic sequence of *Oryza sativa* L. subsp. *japonica* Kato and *Brachypodium distachyon* (L.) Beauv. using BLASTn of Ensemble Plants (http://plants.ensembl.org/index.html). An E-value of $1 \times 10^{-10}$ was used to retain the best match. A list of orthologous genes are listed in the Supplemental File S10.

**Results**

**Phenotypic Analysis**

Phenotypic distribution for spike traits is shown in Fig. 1. Mean values of evaluated traits at each environment are shown in Supplemental File S2. SS8641 had longer spikes, also more fertile and total spikelets per spike as well as more grains per spikelet; whereas MD233 had more sterile spikelets per spike. The compactness was similar between the parents. In all environments, the DH population showed significant variation and transgressive segregation was observed with data distributed beyond the parental values (Supplemental File S2). ANOVA results showed that significant differences existed among DH lines and among environments at $p < 0.001$ level for six spike traits (Supplemental File S3). Estimates of heritability (on a family-mean basis) of the traits were high, ranging from 0.88 to 0.95. The TSN had the highest heritability of 0.95, whereas GSP had the lowest (Supplemental File S3). Significant Pearson correlation coefficients were observed among the spike traits in different environments (Table 1). Spike length showed significant positive correlation with fertile and total spikelets numbers and negative correlation with compactness across all five environments. There was a high positive correlation between the number of fertile and total spikelets ($r = 0.87–0.91$). In almost all of the environments, SC was positively correlated with number of total and sterile spikelets. A significant positive correlation between SC and FSN ($r = 0.27$) only occurred in the K14 environment. Grains per spikelet was negatively correlated with SSN and had no significant relationships with either SL or FSN, except in Q13. Significant negative correlations were also observed between GSP and TSN in C13 and C14 as well as GSP and SC in C13, C14, and Q13.

**Genotyping**

Approximately 207 million reads were obtained for the population from the Illumina HiSeq 2500. The number of reads assigned to each DH line ranged from 998,050 to
2,888,396 with an average of 1,682,947. Six samples of the population parents were included to give a higher depth of coverage and the average number of reads for parents was 5,565,484. For the population and parents, 94% of reads mapped to the pseudo reference genome. The GBS SNP genotypes from the TASSEL 4 pipeline were filtered for markers homozygous and polymorphic between the parents and segregating in the mapping population. A total of 3160 SNPs with <20% missing data that did not significantly deviate from the 1:1 segregation ratio expected for a DH population were retained for linkage mapping. Of the 8,632 functional SNPs from the iSelect Assay, 1,791 were polymorphic between the parents and had <20% missing data. These SNP markers, in conjunction with 24 SSR, four KASP markers, and the morphological red coleoptile trait, were used for construction of the linkage map.

**Linkage Map Construction and Quality Assessment**

Using 4,980 polymorphic markers, a linkage map was constructed assigning a total of 4,973 markers to 21 wheat chromosomes (Table 2). After placing cosegregating markers into bins, the linkage map contained 860 unique loci (Table 2; Fig. 2). The genetic linkage map spanned 2934.1 cm with an average interval length of 3.4 cm, which was the average distance between two adjacent unique loci. The A, B, and D genomes covered total distances of 1023.3, 965.9, and 944.9 cm, respectively. Distribution of mapped markers and unique loci varied across the three genomes. The D genome had the fewest markers and loci, with only 7.6% of mapped markers and 15.6% of unique loci, respectively, compared with 45 and 43.7% of mapped markers and unique loci, respectively, for the A genome and 47.1 and 40.7% for the B genome. Similarly, the A and B genomes had higher map resolution (smaller average interval length) than the D genome. Addition of GBS markers greatly improved resolution of the linkage map. Of the 860 mapped unique loci, 449 (52.2%) were located by GBS markers only and 115 (13.4%) by iSelect markers only (Fig. 3; Supplemental File S4). In particular, the number of unique loci located on D-genome chromosomes increased by 60% with the inclusion of GBS markers.

During map construction pairwise recombination fractions between loci and their associated LOD scores were calculated. Closely linked loci that showed small recombination fractions and large LOD scores were placed in the same linkage group. The assignment of marker to linkage groups was examined by the plot of estimated recombination fractions vs. LOD scores (Supplemental File Fig. 1. Phenotype distribution for spike length (cm), sterile spikelets per spike, fertile spikelets per spike, total spikelets per spike, spike compactness, and grains per spikelet at (A) Clarksville, MD, 2013; (B) Clarksville, MD, 2014; (C) Queenstown, MD 2013; (D) Queenstown, MD 2014; and (E) Kinston, NC, 2014.
Table 1. Pearson correlation coefficients among spike length (SL; cm), fertile spikelet number (FSN), sterile spikelet number (SSN), total spikelet number (TSN), spike compactness (SC), and grains per spikelet (GSP) at Clarksville, MD, 2013 (C13) and 2014 (C14), Queenstown, MD, 2013 (Q13) and 2014 (Q14), and Kinston, NC, 2014 (K14).

<table>
<thead>
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<th>Environments</th>
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<th>FSN</th>
<th>TSN</th>
<th>SC</th>
<th>GSP</th>
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<td>−</td>
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<td>−0.22*</td>
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</tr>
<tr>
<td></td>
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<td>−</td>
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<td>−</td>
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<td>−0.16</td>
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</tbody>
</table>

* Significantly different from zero at the 0.05 probability level.
** Significantly different from zero at the 0.01 probability level.
*** Significantly different from zero at the 0.001 probability level.

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The 1RS.1AL translocated chromosome segregating in this population affected observed recombination in the 1A linkage group. All of the recombination on the 1A linkage group was associated with markers located on the long arm of the chromosome. A total of 213 markers associated with sequences from the short arm and the proximal portion of the long arm were cosegregating in our population, indicating lack of recombination between the 1RS arm from rye (Secale cereale L.) with wheat 1AS. Less than 6% (31) of the 521 polymorphic markers on this chromosome represented unique loci compared with 13 to 26% of markers representing unique loci on other A-genome chromosomes (Table 2).

Quantitative Trait Loci with Additive Effects

Combined QTL analysis using the MCIM method detected a total of 45 regions with significant additive effects on the six spike traits in this study (Table 3). The number of QTL for each trait ranged from five for SC and GSP to 10 for TSN. The QTL analysis performed for each individual environment using the ICIM method detected a total of 109 putative additive QTL for the six spike traits (Supplemental File S9). These QTL were located on 15 chromosomes and mostly formed QTL clusters (Supplemental File S8). The majority of QTL detected in individual environments were located within 20 cM of those identified across environments by MCIM (Table 3). Quantitative trait loci detected in at least four environments and explaining 10% or more of the variation in an environment were considered to have major effects. Major QTL were identified on eight chromosomes and mostly affected multiple spike traits: 1A (SL, FSN, TSN), 5A (SL, SC), 2B (SSN, SC), 3B (SL), 5B (SC), 2D (SSN, TSN), and 5D (TSN, SC). In all cases, the major QTLs identified also had the largest additive effects for the respective traits (Table 3). The additive effects from MCIM represented the average effect of each QTL across environments and were smaller than those observed in some individual environments.

Quantitative Trait Loci × Environment and Epistatic Interactions: QE, QQ, QQE

In this study, we used a MCIM method to estimate the QE, QQ, and QQE interactions. Thirteen QE interactions involving eight marker intervals were detected for SSN, FSN, and TSN (Table 4). Six intervals were also significant for additive effects. The other two involved nonsignificant QTL (LOD < 3) for additive effects. Results from location–year environments Q14, K14, and Q13 each had six, five, and two QE interactions, respectively. No QE interaction was detected in C13 and C14. The contribution of QE interactions ranged from 0.6 to 2.2%. Twenty pairs of QQ interactions were detected for all six spike traits in the DH population and three QQE interactions were also identified (Table 4; Supplemental File S10). Twelve intervals involved in epistasis were significant for additive effects. The heritability estimates of epistasis...
Table 2. Summary of MD233 × SS8641 linkage map containing iSelect and genotyping-by-sequencing (GBS) single-nucleotide polymorphism, simple-sequence repeat (SSR), Kompetitive Allele Specific polymerase chain reaction (KASP) markers.

<table>
<thead>
<tr>
<th>Chromosome or genome</th>
<th>No. of iSelect markers</th>
<th>No. of GBS markers</th>
<th>No. of SSR or KASP markers</th>
<th>Total markers</th>
<th>No. of unique loci</th>
<th>Genetic length (cM)</th>
<th>Average interval length</th>
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<tr>
<td>1A</td>
<td>286</td>
<td>232</td>
<td>3</td>
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<td>299</td>
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<td>123.9</td>
<td>2.1</td>
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<td>2</td>
<td>333</td>
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<td>3.4</td>
</tr>
<tr>
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<td>272</td>
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<tr>
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<td>2</td>
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<td>58</td>
<td>190</td>
<td>3.3</td>
</tr>
<tr>
<td>6A</td>
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<td>119</td>
<td>0</td>
<td>242</td>
<td>33</td>
<td>95.1</td>
<td>2.9</td>
</tr>
<tr>
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<td>3</td>
<td>365</td>
<td>90</td>
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<td>1.9</td>
</tr>
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<td>2250</td>
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<td>4B</td>
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<td>2</td>
<td>116</td>
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<td>29</td>
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<td>8</td>
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<td>76.8</td>
<td>11.0</td>
</tr>
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<td>1</td>
<td>36</td>
<td>20</td>
<td>179.5</td>
<td>9.0</td>
</tr>
<tr>
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<td>6.6</td>
</tr>
<tr>
<td>7D</td>
<td>16</td>
<td>37</td>
<td>1</td>
<td>55</td>
<td>21</td>
<td>259.8</td>
<td>12.4</td>
</tr>
<tr>
<td>D genome</td>
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<td>7</td>
<td>380</td>
<td>134</td>
<td>944.9</td>
<td>7.1</td>
</tr>
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<td>3155</td>
<td>29</td>
<td>4973</td>
<td>860</td>
<td>2934.1</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Fig. 2. Genetic linkage map of the MD233 × SS8641 cross. Each bar represents one group of cosegregating markers.
ranged from 0.3 to 4.9%, while those for the QQE interactions ranged from 0.9 to 1.4%.

**Discussion**

The genetic architecture of quantitative traits is complex. Quantitative traits are controlled by large-effect QTL and many loci with small effects. The effects of the same QTL allele, however, usually vary in their magnitude and direction with different genetic backgrounds and environments. This is known as genotype \( \times \) genotype (QQ) interactions (epistasis) and genotype \( \times \) environment interactions (Mackay et al., 2009). In this study, we evaluated quantitative traits expressed in a soft red winter wheat DH population in five environments. The QTL analyses were performed to identify QTL with additive genetic effects on six spike traits and to investigate their interaction effects. To date, few QTL mapping studies on wheat spike characteristics have been conducted that integrate additive, QQ, and QE interaction, and QQE interaction effects.

**Quantitative Trait Loci for Spike Characteristics**

Using combined and separate QTL analyses, we detected major QTL for SL, FSN, TSN, and GSP that colocalized to a 5-cM region on chromosome 1A (Table 3; Supplemental File S9). This region contains a common SSR marker, Xbarc28, which was also associated with QTL for spike length (phenotypic variation explained [PVE] = 10.8%) by another study (Marza et al., 2006), implying it is possibly the same underlying QTL. However, in our study, one of the parents (MD233) carries the 1RS:1AL translocation, whereas Marza et al. (2006) used material carrying the 1BL.1RS translocation. The well-known chromosomal segment transferred from rye into cultivated wheat confers superior traits such as improved grain yield and adaptive traits, high grain number per spike, grain weight, as well as better disease and insect resistance (Kim et al., 2004; Qi et al., 2016; Rabinovich, 1998). However, the agronomic benefits of the 1RS segment were not consistent across genetic backgrounds (Villareal et al., 1998). In our population, the translocation contributed to smaller spikes as evidenced by MD233 alleles being associated with reduced length, total number of spikelets, as well as fewer grains per spikelet. Therefore, the genes influencing spike traits found in this region could be, partially if not fully, associated with the 1RS:1AL translocation. Further separation of the underlying QTL for SL, FSN, TSN, and GSP that clustered in this region is hindered by the lack of recombination between the 1RS arm from rye and 1AS of wheat.

Additional major QTL for SL were identified on the long arm of chromosome 5A. The vernalization response gene \( Vrn-A1 \) and the major wheat domestication gene \( Q \) are also located on chromosome arm 5AL (Kato et al., 1998). The wheat \( VRN1 \) genes, together with photoperiod response genes (\( PPD1 \) genes) and earliness per se genes determine flowering time of wheat and hence, in part, confer wide adaptation to diverse growing environments around the world (Snape et al., 2001). The \( Q \) gene is a well-known domestication locus conferring the freethreshing character and is responsible for many other domestication-related traits such as rachis fragility, glume shape and tenacity, spike length, plant height, and spike emergence time (Faris et al., 2003; Simons et al., 2006; Sormacheva et al., 2014). To determine if the SL QTL on chromosome 5AL were in the same regions where \( Vrn-A1 \) and \( Q \) are located, genomic sequences of \( Vrn-A1 \) (Yan et al., 2003) and \( Q \) (Simons et al., 2006) were used to BLAST against the wheat draft genome (version IWGSC 1.0) and Popseq. Two identical sequences that were annotated as \( Traes_5AL_13E2DEC48 \) and \( Traes_5AL_06DC5CDA7 \) were identified for \( Vrn-A1 \) and \( Q \), respectively. However, the contigs on which the annotated genes reside were not assembled into the draft genome and we were not able to locate their physical position on 5AL. BLAST and sequence alignment showed that the flanking markers of QTL identified in this region did not match any annotated genes nor overlap with \( Vrn-A1 \) and \( Q \). Thus, we are not able to determine if one or both of these two genes contributed to SL variation in this study or if a new locus other than \( Vrn-A1 \) and \( Q \) was involved.

Additionally, a consistent QTL for SL (\( QSl.czm-3B \)) was identified on chromosome 3B in C13, C14, Q13, and K14 environments. Quantitative trait loci for FSN, TSN, and GSP were also identified in the same region in some environments (Supplemental File S9). Li et al. (2007) detected QTL for grain yield and grain number per...
Fig. 4. Marker order relationship between genetic map from the MD233 × SS8641 cross and the 9K iSelect consensus map for all 21 chromosomes of hexaploid wheat. Unique loci are represented by spots.

Fig. 5. Marker order relationship between genetic map from the MD233 × SS8641 cross and the POPSEQ map for all 21 chromosomes of hexaploid wheat. Unique loci are represented by spots.
Table 3. Quantitative trait loci (QTL) detected for spike length (SL; cm), sterile spikelet number per spike (SSN), fertile spikelet number (FSN), total spikelet number per spike (TSN), spikelet compactness (SC), and grain number per spikelet (GSP) in the MD233 × SS8641 population using a mixed-model-based composite interval mapping method.

<table>
<thead>
<tr>
<th>QTL name</th>
<th>Peak position</th>
<th>Left marker</th>
<th>Right marker</th>
<th>Additive effect</th>
<th>P-value</th>
<th>Environments†</th>
<th>LOD‡ range</th>
<th>PVE§ range</th>
</tr>
</thead>
<tbody>
<tr>
<td>QSc.czm-3A</td>
<td>2.8</td>
<td>3AS_3398062_644</td>
<td>IAMW677</td>
<td>0.1</td>
<td>&lt;0.001</td>
<td>C13, K14</td>
<td>3.7–5.0</td>
<td>6.5–7.3</td>
</tr>
<tr>
<td>QSc.czm-5A</td>
<td>78.9</td>
<td>SDL_4584184_14443</td>
<td>SAL_460944_596</td>
<td>0.19</td>
<td>&lt;0.001</td>
<td>ALL</td>
<td>4.0–8.2</td>
<td>6.7–13.1</td>
</tr>
<tr>
<td>QSc.czm-6A</td>
<td>88.2</td>
<td>6AS_5833998_2845</td>
<td>6AL_5828239_2771</td>
<td>0.08</td>
<td>&lt;0.001</td>
<td>C13, Q14</td>
<td>3.7–5.0</td>
<td>6.5–7.3</td>
</tr>
<tr>
<td>QSc.czm-2B</td>
<td>45.3</td>
<td>3B_1070647_9184</td>
<td>3B_10416442_2670</td>
<td>0.12</td>
<td>&lt;0.001</td>
<td>C13, C14, Q13, K14</td>
<td>3.0–6.6</td>
<td>6.0–12.1</td>
</tr>
<tr>
<td>QSc.czm-20.1</td>
<td>28.5</td>
<td>2DS_536510_5409</td>
<td>2DS_555730_1417</td>
<td>−0.1</td>
<td>&lt;0.001</td>
<td>C13, K14</td>
<td>2.7–9.4</td>
<td>3.7–5.5</td>
</tr>
<tr>
<td>QSc.czm-20.2</td>
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<td>2DL_9813904_943</td>
<td>IAMW3571</td>
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<td>0.003</td>
<td>C14</td>
<td>3</td>
<td>5.6</td>
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<tr>
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<td>Xgdm136</td>
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<td>&lt;0.001</td>
<td>C14</td>
<td>6.1</td>
<td>13.5</td>
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<td>Q14, K14</td>
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<td>5.8–12.0</td>
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<tr>
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<td>7</td>
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<td>C13, Q14</td>
<td>3.9–8.1</td>
<td>9.8–13.8</td>
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<td>K14</td>
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<td>8.4</td>
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<td>&lt;0.001</td>
<td>C13, Q13, K14</td>
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<td>7.5–30</td>
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<td></td>
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<td>&lt;0.001</td>
<td>C13, Q14</td>
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<td>Xgwm319</td>
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<tr>
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<td>72.5</td>
<td>3B_1076925_4136</td>
<td>3B_107604_6083</td>
<td>0.03</td>
<td>&lt;0.001</td>
<td>C14</td>
<td>4.3</td>
<td>7.7</td>
</tr>
<tr>
<td>QFsp.czm-5B</td>
<td>60.1</td>
<td>5AL_101554_8072</td>
<td>—</td>
<td>−0.07</td>
<td>&lt;0.001</td>
<td>ALL</td>
<td>3.4–7.7</td>
<td>7.9–14.5</td>
</tr>
<tr>
<td>QFsp.czm-20</td>
<td>118.5</td>
<td>2DL_9813904_943</td>
<td>IAMW3571</td>
<td>0.05</td>
<td>&lt;0.001</td>
<td>C13</td>
<td>7.5</td>
<td>15.5</td>
</tr>
<tr>
<td>QFsp.czm-3D</td>
<td>16.6</td>
<td>3DS_257014_1698</td>
<td>3DS_200133_61</td>
<td>−0.04</td>
<td>&lt;0.001</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

† Environments having a QTL peak within 20 cM according to inclusive composite interval mapping results.
‡ LOD, logarithm of odds.
§ PVE, phenotypic variation explained.
¶ Major QTL noted in bold were significant in at least four environments and explained at least 10% of the variation in one environment.
spike on 3B in two environments using a population of recombinant inbred lines derived from two winter wheat cultivars. In addition, Wang et al. (2009) also found this region to be significant for grain filling rate and yield-related traits over multiple environments.

Three QTL on 2D (Supplemental File S9) were of special interest because these were the only three loci where MD233 alleles were associated with a longer spike. In a QTL mapping study for spike-related traits, Ma et al. (2007) detected two QTL on chromosome 2D flanked by marker Xgwm261 for SL and SC in the cross of winter genotypes Nanda 2419 and Wangshuibai, where the QTL linked to Xgwm261 explained 8.8 to 23.2% of the phenotypic variation. In our study, Xgwm261 was 2.3 and 13.3 cM away from QSl.cz-2D.1/QFsn.cz-2D.1 and QSl.cz-2D.2, respectively. In addition, Xgwm261 was reported to flank colocalized QTL and a QTL cluster for yield-related traits including plant height, harvest index, days to maturity, 1000-grain weight, and grain weight per spike (Mason et al., 2013). Taken together, these results suggest that the region near the marker Xgwm261 on chromosome 2D may harbor genes affecting grain yield. Another QTL, QSl.cz-2D.3, mapped to the long arm of chromosome 2D and shared the same interval with major QTL QFsn.cz-2D.3 and QTsn.cz-2D.4 in agreement with the QTL for FSN and TSN report by Ma et al. (2007). The same position and genetic effects suggested the possibility of similar underlying QTL.

A few studies have documented QTL and genes for SSN, FSN, and TSN (Cui et al., 2012; Ma et al., 2007). Some previously reported QTL were confirmed in the present study. A minor QTL, QSSn.cz-6D, was consistent with the QTL detected by Cui et al. (2012) who also located a cluster of QTL for spike characteristics on chromosome 2B corresponding with the major QTL clusters identified in the present study. Quantitative trait loci in this cluster were repeatedly detected in almost all environments evaluated. At these loci, SS8641 contributed positive additive effects for SSN, TSN, and SC, whereas MD233 was associated with positive GSP, suggesting that the SS8641 allele of this cluster may lower spikelet fertility and increase TSN and SC by increasing the number of sterile spikelets. In addition, a QTL cluster for FSN and TSN was identified on chromosome 5D flanked by 5DL_74079_2181-Xgdm136 and was located in the same region as previously reported QTL detected by Li et al. (2007) and Cui et al. (2012). Cuthbert et al. (2008) reported a QTL cluster for grain numbers per spike, grain yield, 1000-grain weight, grain filling time, and days to heading on chromosome 2D, which may correspond to the region of major QTL QSSn.cz-2D.3 identified in this study (Supplemental File S9).

At the distal end of chromosome 2D, we detected three additional QTL, QFsn.cz-2D.4, QTsn.cz-2D.5, and QGsp.cz-2D, which were closely linked. These QTL were not located in the region containing the compactum (C) locus, a spike-compacting gene on the long arm of chromosome 2D (Johnson et al., 2007a). The SS8641 alleles in this region decreased FSN and TSN but increased SSN, whereas MD233 was associated with positive GSP, suggesting that the SS8641 allele of this cluster may lower spikelet fertility and increase TSN and SC by increasing the number of sterile spikelets. In addition, QTsn.cz-2D.4 shared the QTL cluster identified in the present study. These results suggest that the region near the marker Xgwm261 on chromosome 2D may harbor genes affecting grain yield.

### Table 4. Quantitative trait loci \times environment interactions influencing spike length (LS; cm), fertile spikelet number (FSN), sterile spikelet number (SSN), total spikelet number (TSN), spike compactness (SC), and grains per spikelet (GSP) at Clarksville, MD, 2013 (C13) and 2014 (C14); Queenstown, MD, 2013 (Q13) and 2014 (Q14); and Kinston, NC, 2014 (K14).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Chromosome</th>
<th>Right marker</th>
<th>Left marker</th>
<th>Position</th>
<th>Q13†</th>
<th>Q14†</th>
<th>K14†</th>
<th>(H^2(\text{ae}))†</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSN</td>
<td>5A</td>
<td>5AL_2758417_1165</td>
<td>5AL_2755044_1686</td>
<td>92.5</td>
<td>-0.05*</td>
<td>0.05*</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>SSN</td>
<td>2B</td>
<td>2BL_0018724_14746</td>
<td>2BL_799505_9892</td>
<td>69</td>
<td>-0.05*</td>
<td>0.05*</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>SSN</td>
<td>2D</td>
<td>XPpd-D1</td>
<td>2DS_5382880_5243</td>
<td>67.7</td>
<td>-0.12***</td>
<td>0.12***</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>FSN</td>
<td>1A</td>
<td>1AL_3962319_3787</td>
<td>Xbarc28</td>
<td>0.9</td>
<td>-0.22***</td>
<td>0.14**</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>FSN</td>
<td>2A</td>
<td>2AS_5260255_4791</td>
<td>2AS_5307952_3491</td>
<td>44.8</td>
<td>0.10*</td>
<td>0.05*</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>FSN</td>
<td>1D</td>
<td>1DS_1890379_6849</td>
<td>1DL_2227188_3400</td>
<td>52.6</td>
<td>-0.10*</td>
<td>0.11*</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>TSN</td>
<td>1A</td>
<td>1AL_3962319_3787</td>
<td>Xbarc28</td>
<td>0.9</td>
<td>-0.15***</td>
<td>0.13*</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>TSN</td>
<td>2D</td>
<td>2DS_535730_1417</td>
<td>2DS_5375380_1169</td>
<td>34.4</td>
<td>-0.12*</td>
<td>0.19**</td>
<td>-0.16**</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* Significant at the 0.05 probability level
** Significant at the 0.01 probability level
*** Significant at the 0.001 probability level
† The additive \times environment interaction effect at each environment.
‡ The additive \times environment interaction effect across environments.
§ Interval with significant additive effect.
and that it has a major inhibitory effect on paired spikelet formation by regulating the expression of the FLOWER-ING LOCUS T (FT) (Boden et al., 2015).

The QTL cluster on chromosome 5A for SC included the locus Xgwm304 that is neither close to the Q gene nor the Vrn-A1 gene. However, it has also been related to grain yield and 1000-grain weight by Cuthbert et al. (2008) and SL and SC by Ma et al. (2007). In those two studies, this region was identified as harboring major QTL because of high PVE values similar to our results. Thus, it is possible that this region may contribute to grain yield by increasing spikelet numbers and grain weight. Furthermore, a major QTL (QSc.cz-5A.3) residing in a 0.9-cM region on 5AL stood out since it explained 80.2% of the phenotypic variation for SC in K14. It is flanked by Xgwm304 and IWA4736. IWA4736 is a SNP within the protein coding region of the annotated gene Traes_5AL_4210A8A6E. This gene codes for asparagine synthetase, which plays a crucial role in the synthesis and transport of asparagine in plants (Gaufichon et al., 2010). In addition to its role in N assimilation, asparagine synthetase has also been shown to be required for the defense responses to microbial pathogens in tomato (Solanum lycopersicum L.) (Olea et al., 2004) and pepper (Capsicum annuum L.) (Hwang et al., 2011). Another SNP, IWA3445, was mapped to the same position as IWA4736 but did not hit any annotated gene. Thus, we postulate that Traes_5AL_4210A8A6E is a possible candidate gene for the QSc.cz-5A.3 QTL.

Sourdille et al. (2003) used a DH population derived from the cross Courtot × Chinese Spring to study wheat development traits and detected one QTL on the long arm of chromosome 5D for SC. This QTL explained 13.6% of the phenotypic variation and was similar to the genomic region IWA4274-5DL_1208228–231, where two major QTL for SL and SC were identified in the present study. Another QTL cluster comprising four major QTL for GSP on chromosome 5B (Supplemental File S9) coincided with the interval of the SL QTL QSl.cz-5B.2 identified by Kumar et al. (2007).

Chromosome 3A of wheat is known to contain QTL for grain yield and other important agronomic traits. Using a RIL population derived from the winter wheat cultivar Cheyenne and its single chromosome substitution line Cheyenne (W13A) where chromosome 3A of Cheyenne was substituted for Wichita chromosome 3A, Mengistu et al. (2012) as well as Dilbirligi et al. (2006) detected QTL for grain yield, plant height, spikes per square meter, and grain number per spike and found that most of the detected QTL on 3A were colocalized in two regions. We detected five QTL on chromosome 3A for SC and SSN among which QSSn.cz-3A.1 explained 13.8% of the phenotypic variation while the rest were minor QTL. Based on the mapping positions of SSR markers used in the current and previous studies (Somers et al., 2004), these five QTL and the QTL previously identified by Dilbirligi et al. (2006) and Mengistu et al. (2012) are located in this region.

**Genetic Complexity of Spike Characteristics**

Most important agronomic traits are quantitative in nature controlled by polygenes and influenced by the environment. Understanding the genetic and environmental factors causing the phenotypic variation of quantitative traits is essential for the genetic improvement of crops via knowledge-based breeding (Mackay, 2001; Würschum, 2012). In the present study, the effects of major, minor, and epistatic QTL as well as their interactions with the environment and their relative contributions to spike characteristics were estimated (Fig. 6). Most QTL had additive effects and had the largest genetic contribution to phenotypic variation. This agreed with previous QTL studies involving QQ, QE, and QQE interactions (Kuchel et al., 2007; Wu et al., 2012; Xing et al., 2002; Zhang et al., 2014). Furthermore, QTL for spike characteristics were not evenly distributed within and across chromosomes and tended to cluster (Supplemental File S8). We identified QTL clusters on chromosome 1A, 5A, 2B, 3B, 5B, 1D, 2D, and 5D where QTL for multiple spike characteristics were colocalized or closely linked within a 10-cM region. In most cases, each cluster contained at least one major QTL. The clustering of QTL also partially explained the correlation among spike characteristics. In this study, SL was highly correlated with FSN across environments (Table 1). This could be caused by the colocalization of QSl.cz-3A.1 and QFs.n.cz-3A.1 plus the effects of closely linked QTL QSl.cz-3A.1, QSl.cz-3A.2, and QFs.n.cz-3A.1. Despite the slight difference in interpretation, characterizing the interaction at two or more loci or epistasis is as important in quantitative genetics as in classical genetics. We found that interactions (QE, QQ, and QQE) served as modifiers for spike characteristics determination in this DH population. For example, the interval 5AL_2775235-593–5AL_460494_596 on chromosome 5A was not detected with significant additive effects but contributed to SL through its interactions with 2DL_9813904-943-IWA3571, Xwmc496–1AL_3962319–3787, and 5DS_2782527-6993-Xgdm156, which were associated with significant additive effects for FSN, GSP, and SL, respectively. Significant epistasis was also detected between nonsignificant intervals, such as IBL_3802551_64689280–1BL_3886068_66500558 and 2BL_8084228–12492–2BL_8092402_18755, which increased FSN and accounted for 4.9% of the phenotypic variation. Similar results were reported by Ma et al. (2007), where the interaction of two nonsignificant loci on chromosome 3D decreased TSN and FSN. These results confirmed that loci without main effects may contribute to trait determination through epistasis (Li et al., 2001). Additionally, we found that the SS8641 allele at the interval 1AL_3962319–3787-Xbarc28 increased FSN and TSN in K14 and these effects were enhanced by 21.5% through the QE interaction. Although the effects and contribution from QE, QQ, and QQE interactions were relatively small compared with additive main effects, they were important terms for fine-tuning the expression
locate 190 QTL for spike characteristics in a hexaploid winter wheat DH population. Consistent QTL, such as QGsp.cz-2B.1 that explained 15.6% of the phenotypic variation for GSP, QSl.cz-1A/QFsn.cz-1A that explained up to 30.9% of the phenotypic variation for SL and FSN, as well as QSc.cz-5A.3 that explained 80.2% of the phenotypic variation for SC, have potential to be used in marker-assisted selection. The genetic determinant of spike characteristics was mainly from additive QTL, but was also fine-tuned by QQ and genetic × environment interactions. For example, we found that the contribution of QFsn.cz-1A to FSN was enhanced by 19.9% via QQ interaction with the interval 5DS_2782527_6993-Xgdm136. Additionally, QTL clusters on chromosomes 1A, 5A, 2B, 3B, 5B, 1D, and 5D with synergistic or antagonistic genetic effects partially explained the phenotypic correlation between spike characteristics. These results provide valuable information for manipulating spike morphology for breeding purposes.

Supplemental Information Available

Supplemental information is available with the online version of this manuscript.

Acknowledgments

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