Main effect and epistatic QTL affecting spike shattering and association with plant height revealed in two spring wheat (Triticum aestivum L.) populations

Firdissa E. Bokore1 · Richard D. Cuthbert1 · Ron E. Knox1 · Heather L. Campbell1 · Brad Meyer1 · Amidou N’Diaye2 · Curtis J. Pozniak2 · Ron DePauw3

Received: 7 May 2021 / Accepted: 18 October 2021 / Published online: 20 March 2022
© Crown 2022, corrected publication 2022

Abstract

Key message A major QTL on chromosome arm 4BS was associated with reduced spike shattering and reduced plant height in coupling phase, and a second major QTL associated with reduced spike shattering was detected on chromosome arm 5AL in the same wheat variety Carberry.

Abstract Spike shattering can cause severe grain yield loss in wheat. Development of cultivars with reduced shattering but having easy mechanical threshability is the target of wheat breeding programs. This study was conducted to determine quantitative trait loci (QTL) associated with shattering resistance, and epistasis among QTL in the populations Carberry/AC Cadillac and Carberry/Thatcher. Response of the populations to spike shattering was evaluated near Swift Current, SK, in four to five environments. Plant height data recorded in different locations and years were used to determine the relationship of the trait with spike shattering. Each population was genotyped and mapped with the wheat 90 K Illumina iSelect SNP array. Main effect QTL were analyzed by MapQTL 6, and epistatic interactions between main effect QTL were determined by QTLNetwork 2.0. Correlations between height and shattering ranged from 0.15 to 0.49. Carberry contributed two major QTL associated with spike shattering on chromosome arms 4BS and 5AL, detected in both populations. Carberry also contributed two minor QTL on 7AS and 7AL. AC Cadillac contributed five minor QTL on 1AL, 2DL, 3AL, 3DL and 7DS. Nine epistatic QTL interactions were identified, out of which the most consistent and synergistic interaction, that reduced the expression of shattering, occurred between 4BS and 5AL QTL. The 4BS QTL was consistently associated with reduced shattering and reduced plant height in the coupling phase. The present findings shed light on the inheritance of shattering resistance and provide genetic markers for manipulating the trait to develop wheat cultivars.

Introduction

Seed shattering of wheat refers to loss of grain from the spike and to the loss of entire spikes from wheat standing in the field or prior to harvesting operations (Chang 1943; Porter 1959). In this study, the trait seed shattering is distinguished from the brittle rachis trait. Seed shattering results from disarticulation above the glume and brittle rachis results from disarticulation below the glume. Cultivated wheats are morphologically very different from ancestral forms. Domestication of wheat was based on mutations that resulted in a non-brittle rachis and kernel hullessness. Wheat genotypes that have a non-brittle rachis retain spikelets attached to the rachis, which means the spikelets do not disarticulate below the glume at maturity. The genes, Non-brittle rachis 1 (btr1) and Nonbrittle rachis 2 (btr2), are linked and located on chromosome...
3AS (Pournheirandish et al. 2018). All cultivated wheat has a non-brittle spike that remains intact after maturity. Domestication of wheat resulted from other mutations that gave rise to hullessness and a non-brittle rachis. The free-threshing trait was attributed to the Q gene located on chromosome 5AL (Sears 1954). Simons et al. (2006) used ectopic expression analysis of transgenic plants to demonstrate pleiotropic effects of the Q gene on traits such as glume shape and tenacity, rachis fragility, spike length, plant height, and spike emergence time which agreed with previously published cytogenetic analysis.

Shattering has long been known to cause yield losses in cereal crops such as oats, barley and wheat (Clarke 1981). Grain losses due to shattering are of economic importance and reported in different studies (Clarke 1981; Clarke and DePauw 1983; Porter 1959). Pincus (1931) conducted experiments in the western part of Serbia on the degree of shelling or shattering in newly developed Russian wheats and found some cultivars that shattered as much as 19.6% compared with 2.0 to 3.0% for other cultivars. Clarke and DePauw (1983) investigated the dynamics of shattering in maturing wheat and reported a shattering loss, expressed as a percentage of yield, that ranged from 3.2 to 17.3% over a three-week period beyond harvest ripeness (14.5% moisture wet weight basis). The cultivar Stoa expressed 2% shattering while Sumai3 exhibited 68% averaged over five environments in North Dakota (Zhang and Mergoum 2007).

Present day wheat cultivars are resistant to shattering compared with their ancestors, but some shattering still occurs due to varietal differences or weather conditions. Varietal morphological attributes including glume tenacity, kernel size, number of kernels per spikelet, awns, spike compactness, and environmental factors such as wind and humidity influence the ability of a cultivar to hold its grain in a recoverable position for a period of several weeks after maturity (Chang 1943; Clarke and DePauw 1983; Harrington and Waywell 1950). Shattering resistance is clearly of benefit during the post-maturity and pre-combine period and would be particularly desirable where direct combining is practiced (Clarke 1981). Mechanical threshability is a desired trait. There is a fine balance between a genotype holding the seeds firmly in the spike prior to harvest while during mechanical harvest the seed separates from the lemma and palea of the spikelet and glumes from the rachis without requiring expressive energy. Failure to separate, results in the retention of spike tissue, which may or may not be enclosing a kernel, that must be removed prior to milling. This spike tissue in a grain sample is classified as dockage by most international grain grading systems and reduces the value of the harvested grain.

Using inbred wheat lines derived from the population Ning7840/Clark and QTL analysis, Marza et al. (2006) identified six loci associated with shattering resistance across chromosomes 4B, 5A, 6A, 6B and 7D. Using the same Ning7840/Clark population, Li et al. (2016) identified an additional QTL on chromosome arm 2DS positioned in marker interval Xwmc25.1 - Xgwm296.2. Zhang and Mergoum (2007) report four QTL across chromosomes 2B, 3B, and 7A (two loci) associated with high kernel shattering in a Sumai3 derived population. Moreover, Jantasuriyarat et al. (2004) report six QTL that affected threshability on chromosome arms 2AS, 2BL, 2DS, 5AL, 6AS and 6DL in recombinant inbred lines of the International Triticeae Mapping Initiative (ITMI) population, W-7984/Opata 85.

Although no report was found on epistatic interactions of shattering resistance QTL in wheat, epistatic interactions for other traits have been reported such as resistance to wheat rust (Singh et al. 2013, 2014) and common bunt (Bokore et al. 2019; Singh et al. 2016). As epistasis describes genetic interactions in terms of how phenotypic effects of an allele depend on another allele in the genome (Chou et al. 2011), the understanding of such epistatic interactions provides additional information on the most desirable allele combinations (Cheverud and Routman 1995; Singh et al. 2013). Knowledge of epistasis is helpful to understand how certain genes may function synergistically, and the contribution of epistasis to additive genetic or breeding value of interacting genes.

Most of the wheat spike shattering response studies (Jantasuriyarat et al. 2004; Marza et al. 2006; Zhang and Mergoum 2007) are based on simple sequence repeat (SSR) markers. Advancement in next-generation sequencing (NGS) technologies has contributed toward high throughput discovery of large numbers of single nucleotide polymorphisms (SNPs) revolutionizing genetic mapping. Also, the shift in DNA marker technologies from fragment-based polymorphism including amplified fragment length polymorphism (AFLP) and SSR markers to sequence-based SNP markers provides a huge opportunity for constructing high-density genetic maps ultimately resulting in an increase in the number of informative markers. Polymorphisms from differences at a single nucleotide (substitution, deletion or insertion) occur frequently and can be associated with phenotypes (Grover and Sharma 2016). Higher incidence of markers allows better coverage of the genome revealing more trait-related loci with greater resolution. Mapping of many traits has benefitted from SNP mapping, as can be the case for shattering response in wheat for the development of breeder friendly markers.

It is essential to understand the genetic basis of shattering resistance to maintain the balance between threshability and shattering traits through marker-assisted selection. Genes affecting shattering resistance and their epistatic interaction in contemporary Canadian spring wheat cultivars such
as Carberry (DePauw et al. 2011) and AC Cadillac (DePauw et al. 1998) have not been characterized. They derive from different genealogical lineages and under very windy conditions after reaching maturity, shattering has occurred (personal observations by DePauw). The objectives of this study were to determine and map genomic regions controlling spike shattering in Canada Western Red Spring wheat cultivars Carberry and AC Cadillac, to investigate epistatic interactions among these loci and to determine the relationship of the shattering trait with plant height.

Materials and methods

Phenotyping

Two doubled haploid (DH) populations Carberry/AC Cadillac (775 lines) and Carberry/Thatcher (297 lines) were evaluated for spike shattering near Swift Current, SK., in a series of nurseries of differing environmental conditions. Carberry is a semi-dwarf doubled haploid, hard red spring wheat cultivar that derives from the cross Alsen and Superb made in 2000 at the Swift Current Research and Development Centre, AAFC, SK, Canada and registered in 2009. AC Cadillac is a hard red spring wheat adapted to the Canadian Prairies with shattering resistance similar to Katepwa. Thatcher was selected from a double cross Marquis/Iumillo// Marquis/Kanred wheat in 1925 and released in 1935 (Hayes et al. 1936). In studies of other traits, segregation for shattering was observed in the two populations.

The Carberry/AC Cadillac population was planted in 3-m-long single row nurseries in 2012 and 2013 at a site named South Farm and in 1.5-m rows in 2012 and 2013 at a nursery site named Centre Farm and in 2014 at Field 16 North Farm of the Swift Current Research and Development Centre. The Carberry/Thatcher population was evaluated in 1.5-m-long single rows in Centre Farm nurseries near Swift Current in 2016 and 2018, and 3-m-long single rows in 2018 at Field 17 North Farm and in 2019 at South Farm. South Farm has coordinates lat. 50°16’ N., long. 107°44’ W. The South Farm has a Swinton loam soil (Orthic Brown Chernozem) at 825 m above sea level, while the Centre Farm has a slightly higher clay content, and the North Farm Field 16 and 17 is a heavy clay soil at about 750 m asl. All nurseries were irrigated except South Farm, 2012, 2013 and 2019. The lines were inoculated with common bunt [Tilletia laevis Kühn in Rabenh., and T. tritici (Bjerck.) G. Wint. in Rabenh.] races L16 and T19 (Hoffmann and Metzger 1976) in the nurseries (except the 3 m rows in 2018), but the populations expressed a high level of resistance to bunt such that the effect on shattering was minimal. The shattering score was based on the proportion of the spike with loss of kernels x the proportion of the spikes within a plot with symptoms of seed shattering × 100 where 1 was no shattering, 9 was all spikes with a bare rachis and the rest of the numbers fell in between compressed at the lower tail.

Pearson’s correlation analysis was performed on the shattering data using SAS (SAS Institute, Cary, NC) to determine the repeatability of the shattering scores across environments in each of the two mapping populations. Also, correlation analysis was performed for the shattering response with plant height to assess the relationship between these two traits. Broad sense heritability of the spike shattering expressed as the ratio of the genetic variance and the phenotypic variance was determined.

Genotyping, construction of linkage maps and QTL analysis

The DNA of parents and DH lines was extracted from the first leaf of seedlings with the BioSprint 96 DNA Plant Kit (QIAGEN Science, Maryland, USA). The parents and 297 DH lines of the Carberry/Thatcher and 775 lines of the Carberry/AC Cadillac populations were genotyped with the 90 K Infinium iSelect SNP wheat assay (Illumina Inc., San Diego, CA). Linkage maps of the two populations were built using the two-step mapping strategy as previously described (Bokore et al. 2019; Fowler et al. 2016). Main effect QTL associated with shattering resistance were identified by performing QTL analysis with MapQTL.6 ® (Van Ooijen 2009). The permutation test option (1000 permutations) within MapQTL was applied to determine the significance threshold for the logarithm of the odds (LOD). Genome-wide threshold levels were used to declare significant QTL at the 5% level of significance. Automatic co-factor detection based on backward elimination to identify the co-factor markers as well as manual co-factor selection was performed for Multiple QTL Mapping (MQM). The data were square root and logarithm transformed and QTL analyses were repeated.

Epistasis analysis

Additive x additive epistasis between the main effect QTL was performed using QTLNetwork 2.0 (Yang et al. 2008, 2007). The additive epistatic interactions were estimated using the “map epistasis” option of QTLNetwork. QTL with individual or epistatic effects were determined using the “two-dimensional (2D) genome scan” option. The 2D genome scan option enables mapping epistatic QTL with or without single-locus effects. The critical F values were determined with 1000 permutations.
Results

Response of the genotypes to shattering

Thatcher consistently expressed lower shattering, scores ranging from 1.0 to 1.8 with a mean over environments of 1.5, than Carberry, ranging from 1.8 to 2.8 with a mean of 2.3 (Table 1). Thatcher had significantly lower shattering scores than Carberry in three of four environments. Whereas Carberry and AC Cadillac scores were more comparable with equal means over environments of 2.4. Both Carberry’s shattering scores (range: 1.1–4.9) and AC Cadillac’s (range: 1.6–3.5) were wider ranging in the Carberry/AC Cadillac experiments than the Carberry/Thatcher experiments. However, Carberry’s mean score across environments was similar at 2.3 in the Carberry/Thatcher experiments and 2.4 in the Carberry/AC Cadillac experiments. The phenotypic distributions of the lines were typically continuous and skewed to the right with a preponderance of low shattering types (Fig. 1a and b). The populations displayed a wide range of variation in expression from 1 to 7 for Carberry/Thatcher and 1 to 8 for Carberry/AC Cadillac. Transgressive segregation was observed in the populations particularly with the segregation of high shattering lines. The square root and log transformations of the data resulted in similar patterns to the untransformed data are presented.

Variance components and heritability estimates of the shattering trait are presented in Table 1. Estimates of broad sense heritability based on the ratio of the genetic variance to the total variance among the scores of the lines ranged from 0.22 to 0.64 for Carberry/AC Cadillac population while for the Carberry/Thatcher population it ranged from 0.33 to 0.42. Narrow sense heritabilities were somewhat lower than the broad sense heritabilities. The heritability due to the additive epistatic interactions was much lower than both broad sense and narrow sense heritabilities.

Correlation matrix analysis between shattering determinations and plant height for both populations (Table 2) detected highly significant ($P < 0.001$) positive correlations within both plant height and shattering location-year combinations as well as between trait location-year combinations. The correlations among shattering scores between environments were moderate to high, ranging from 0.46 to 0.80 for the Carberry/AC Cadillac population, and from 0.56 to 0.76 for Carberry/Thatcher. Relative to other test environments, the correlations involving Field 16 in 2014 tended to be lower. The correlations between plant height scores were high ranging from 0.83 to 0.89 for the Carberry/AC Cadillac population and were 0.88 for Carberry/Thatcher. Highly significant ($P < 0.001$) positive inter-trait correlations of shattering and plant height were low-to-moderate ranging from 0.15 to 0.41 in Carberry/AC Cadillac and 0.26 to 0.48 in the Carberry/Thatcher population.

Table 1 Mean, range, standard error (SE) and variance components generated by epistasis analysis using QTLNetwork software on mean of spike shattering scores (1–9 scale) in the Carberry/Thatcher and Carberry/AC Cadillac populations evaluated in different environments

| Location-year       | Shattering score (1–9 scale) a | Variance components b |
|---------------------|--------------------------------|-----------------------|
|                     | Range | Mean | SE  | Thatcher | Carberry | V(G)/V(P) | V(E)/V(P) | V(A)/V(P) | V(AA)/V(P) |
| **Carberry/Thatcher** |       |      |     |          |          |           |           |           |            |
| Centre Farm 2016    | 1–5   | 2.2  | 0.06| 1.4      | 2.7      | 0.35      | 0.65      | 0.31      | 0.04       |
| Centre Farm 2018    | 1–7   | 2.3  | 0.08| 1.8      | 1.9      | 0.42      | 0.58      | 0.34      | 0.08       |
| Field 17 2018       | 1–7   | 2.3  | 0.08| 1.8      | 2.8      | 0.39      | 0.61      | 0.36      | 0.03       |
| South Farm 2019     | 1–7   | 1.5  | 0.05| 1.0      | 1.8      | 0.33      | 0.67      | 0.21      | 0.11       |
| **Carberry/AC Cadillac** |       |      |     |          |          |           |           |           |            |
| Centre Farm 2012    | 1–8   | 4.1  | 0.07| 3.5      | 4.9      | 0.59      | 0.41      | 0.58      | 0.01       |
| Centre Farm 2013    | 1–6   | 2.0  | 0.04| 2.3      | 2.1      | 0.39      | 0.61      | 0.36      | 0.03       |
| South Farm 2012     | 1–8   | 3.4  | 0.07| 2.9      | 2.5      | 0.64      | 0.36      | 0.61      | 0.03       |
| South Farm 2013     | 1–8   | 2.3  | 0.05| 1.9      | 1.5      | 0.54      | 0.46      | 0.48      | 0.07       |
| Field 16 2014       | 1–6   | 1.4  | 0.03| 1.6      | 1.1      | 0.22      | 0.78      | 0.20      | 0.02       |

aThe score was based on the proportion of the spike with loss of kernels x the proportion of the spikes within a plot with symptoms of seed shattering x 100 where 1 was no shattering, 9 was all spikes had a bare rachis, and the rest of the numbers fell in between compressed at the lower tail

bV(G), genotype variance; V(A), additive variance; AA, additive x additive variance; V(P), phenotypic variance, V(E), environmental variance V(G)/V(P), broad sense heritability; V(A)/V(P), narrow sense heritability; V(AA)/V(P), additive x additive epistasis heritability; V(E)/V(P), environmental effect
Table 2  Pearson’s correlation between locations and years for shattering and plant height of the Carberry/Thatcher and Carberry/AC Cadillac populations evaluated near Swift Current, SK

| Carberry/AC Cadillac | SH South Farm 2012 | SH South Farm 2013 | SH Centre Farm 2013 | SH Field 16 SC2014 | PHT Centre Farm 2011 | PHT Centre Farm 2012 | PHT Centre Farm 2013 | PHT South Farm 2013 |
|----------------------|-------------------|-------------------|---------------------|-------------------|----------------------|---------------------|---------------------|---------------------|
| SH Centre Farm 2012  | 0.80<sup>a</sup>  | 0.71              | 0.70                | 0.48              | 0.26                 | 0.30                | 0.29                | 0.31                |
| SH South Farm 2012   | –                 | 0.77              | 0.67                | 0.47              | 0.37                 | 0.41                | 0.41                | 0.43                |
| SH South Farm 2013   | –                 | 0.74              | 0.53                | 0.40              | 0.43                 | 0.46                | 0.46                | 0.49                |
| SH Centre Farm 2013  | –                 | –                 | 0.47                | 0.31              | 0.33                 | 0.35                | 0.35                | 0.36                |
| SH Field 16 SC2014   | –                 | –                 | 0.15                | 0.16              | 0.16                 | 0.16                | 0.16                | 0.19                |
| PHT Centre Farm 2011 | –                 | –                 | 0.84                | 0.86              | 0.86                 | 0.83                | –                   | 0.83                |
| PHT Centre Farm 2012 | –                 | –                 | 0.88                | 0.88              | –                   | –                   | –                   | 0.89                |

| Carberry/Thatcher    | SH Centre Farm 2018 | SH Field 17 2018 | SH South Farm 2019 | PHT Centre Farm 2014 | PHT Centre Farm 2015 | PHT Centre Farm 2016 |
|----------------------|---------------------|------------------|---------------------|----------------------|-----------------------|-----------------------|
| SH Centre Farm 2016  | 0.68                | 0.68             | 0.56                | 0.29                 | 0.27                  | 0.28                  |
| SH Centre Farm 2018  | –                   | 0.76             | 0.66                | 0.42                 | 0.40                  | 0.41                  |
| SH Field 17 2018     | –                   | 0.70             | 0.48                | 0.48                 | 0.48                  | 0.47                  |
| SH South Farm 2019   | –                   | –                | 0.30                | 0.29                 | 0.29                  | 0.26                  |
| PHT Centre Farm 2014 | –                   | –                | 0.88                | 0.88                 | –                     | 0.88                  |
| PHT Centre Farm 2015 | –                   | –                | –                   | –                    | –                     | 0.88                  |

<sup>a</sup>SH, shattering response; PHT, plant height

<sup>b</sup>All correlation values are significant at \( P < 0.0001 \)
Table 3 Closest marker to QTL based on peak LOD, marker position (cM) in the genetic map of respective populations, LOD score, phenotypic variations explained (PVE), mean phenotypic value associated with the parent contributing the allele, and additive value for spike shattering resistance QTL and plant height QTL identified in different environments in Carberry/Thatcher and Carberry/AC Cadillac populations. The analysis was performed by the multiple QTL mapping (MQM) option of MapQTL 6

| Environment       | Trait name | QTL       | Peak marker                     | Position, cM | LOD scorea | Phenotypic mean of allele for parent | PVEb, % | Additive effectc |
|-------------------|------------|-----------|---------------------------------|--------------|------------|--------------------------------------|---------|-----------------|
| **Carberry/Thatcher** |            |           |                                 |              |            | Thatcher                            |         |                 |
| Center Farm 2016  | Shattering | Sh.Sparc-4B | Tdurum_con-tig42229_113         | 106.8        | 3.1        | 2.4                                  | 1.9     | 4.6             |
| Center Farm 2018  | Shattering | Sh.Sparc-4B | Tdurum_con-tig42229_113         | 106.8        | 9.9        | 2.9                                  | 1.9     | 14.2            |
| Field 17 2018     | Shattering | Sh.Sparc-4B | Tdurum_con-tig42229_113         | 106.8        | 12.8       | 2.9                                  | 1.7     | 18.0            |
| South Farm 2019   | Shattering | Sh.Sparc-4B | Tdurum_con-tig42229_113         | 106.8        | 5.8        | 1.7                                  | 1.2     | 8.7             |
| Centre Farm 2014  | Plant height| Pht.Sparc-4B | Tdurum_con-tig42229_113         | 106.8        | 55.9       | 101.5                                | 83.4    | 58.0            |
| Centre Farm 2015  | Plant height| Pht.Sparc-4B | Tdurum_con-tig42229_113         | 106.8        | 51.3       | 88.6                                 | 75.2    | 54.9            |
| Centre Farm 2016  | Plant height| Pht.Sparc-4B | Tdurum_con-tig42229_113         | 106.8        | 58.6       | 106.5                                | 89.0    | 59.7            |
| Centre Farm 2016  | Shattering | Sh.Sparc-5A | Kukri_rep_c102608_599           | 189.67       | 18.1       | 2.7                                  | 1.5     | 24.5            |
| Centre Farm 2018  | Shattering | Sh.Sparc-5A | Kukri_rep_c102608_599           | 189.67       | 11.0       | 2.9                                  | 1.8     | 15.7            |
| Field 17 2018     | Shattering | Sh.Sparc-5A | Kukri_rep_c102608_599           | 189.67       | 11.7       | 2.9                                  | 1.7     | 16.6            |
| South Farm 2019   | Shattering | Sh.Sparc-5A | Kukri_rep_c102608_599           | 189.67       | 7.3        | 1.8                                  | 1.1     | 10.6            |
| South Farm 2019   | Shattering | Sh.Sparc-7A.1 | BS00092805_51/Tdurum_con-tig56417_2381 | 125.4       | 3.0        | 1.7                                  | 1.3     | 4.6             |

| **Carberry/AC Cadillac** | AC Cadillac | Carberry |            |         |            | | | |
| Centre Farm 2012 | Shattering | Sh.Sparc-1A | RAC875_c60514_90            | 119.46       | 5.5        | 3.7                                  | 4.5     | 3.2             | −0.4 |
| South Farm 2012  | Shattering | Sh.Sparc-1A | RAC875_c60514_90            | 119.46       | 3.4        | 3.1                                  | 3.7     | 2.0             | −0.3 |
| South Farm 2013  | Shattering | Sh.Sparc-1A | RAC875_c60514_90            | 119.46       | 2.8        | 2.1                                  | 2.5     | 1.6             | −0.2 |
| Centre Farm 2013 | Shattering | Sh.Sparc-1A | RAC875_c60514_90            | 119.46       | 5.1        | 1.9                                  | 2.2     | 3.0             | −0.2 |
| Centre Farm 2012 | Shattering | Sh.Sparc-2D | RAC875_c5016_314            | 0            | 4.0        | 3.8                                  | 4.4     | 2.4             | −0.3 |
| South Farm 2012  | Shattering | Sh.Sparc-2D | RAC875_c5016_314            | 0            | 2.4        | 3.2                                  | 3.6     | 1.4             | −0.2 |
| Field 16 2014    | Shattering | Sh.Sparc-2D | RAC875_c5016_314            | 0            | 4.3        | 1.3                                  | 1.5     | 2.5             | −0.1 |
| Centre Farm 2012 | Shattering | Sh.Sparc-3A | Wsnp_Ku_C44716_51926415    | 101.45       | 3.1        | 3.8                                  | 4.4     | 1.8             | −0.3 |
| Centre Farm 2013 | Shattering | Sh.Sparc-3A | Wsnp_Ku_C44716_51926415    | 101.45       | 3.3        | 1.9                                  | 2.2     | 1.9             | −0.1 |
| Centre Farm 2012 | Shattering | Sh.Sparc-3D | Wsnp_Ex_c1032_1972537       | 11.87        | 4.6        | 3.8                                  | 4.4     | 2.7             | −0.3 |
| Centre Farm 2012 | Shattering | Sh.Sparc-4B | wsnp_BF482960B_ Ta_1_4      | 111.2        | 8.8        | 4.6                                  | 3.6     | 5.1             | 0.5  |
| Carberry/AC Cadillac |  | AC Cadillac | Carberry |
|----------------------|-----------------|-------------|----------|
| South Farm 2012      | Shattering      | Sh.Sparc-4B | wsnp_BF482960B_Ta_1_4 | 111.2 | 17.5 | 4.0 | 2.8 | 9.8 | 0.6 |
| Centre Farm 2011     | Plant height    | Pht.Sparc-4B | wsnp_BF482960B_Ta_1_4 | 111.2 | 146.5 | 110.4 | 93.1 | 58.1 | 8.6 |
| South Farm 2013      | Plant height    | Pht.Sparc-4B | wsnp_BF482960B_Ta_1_4 | 111.2 | 183.2 | 116.5 | 94.9 | 66.3 | 10.8 |
| Centre Farm 2012     | Plant height    | Pht.Sparc-5A | BS00077855_51 | 139.6 | 4.2 | 92.5 | 89.5 | 2.5 | 1.5 |
| Centre Farm 2013     | Plant height    | Pht.Sparc-5A | BS00077855_51 | 139.6 | 6.4 | 93.9 | 90.4 | 3.7 | 1.8 |
| South Farm 2013      | Plant height    | Pht.Sparc-5A | BS00077855_51 | 139.6 | 6.2 | 103.2 | 98.9 | 3.6 | 2.2 |
| Centre Farm 2012     | Shattering      | Sh.Sparc-5A  | wsnp_Ex_c18107_26909127 | 198.63 | 103.7 | 5.4 | 2.6 | 46.0 | 1.4 |
| South Farm 2012      | Shattering      | Sh.Sparc-5A  | wsnp_Ex_c18107_26909127 | 198.63 | 102.6 | 4.6 | 2.0 | 46.2 | 1.3 |
| Centre Farm 2013     | Shattering      | Sh.Sparc-5A  | wsnp_Ex_c18107_26909127 | 198.63 | 38.2 | 2.5 | 1.5 | 20.3 | 0.5 |
| South Farm 2013      | Shattering      | Sh.Sparc-5A  | wsnp_Ex_c18107_26909127 | 198.63 | 45.0 | 3.0 | 1.5 | 23.6 | 0.7 |
| Centre Farm 2011     | Plant height    | Pht.Sparc-5A | BS00077855_51 | 139.6 | 4.2 | 92.5 | 89.5 | 2.5 | 1.5 |
| Centre Farm 2012     | Plant height    | Pht.Sparc-5A | BS00077855_51 | 139.6 | 6.4 | 93.9 | 90.4 | 3.7 | 1.8 |
| Centre Farm 2013     | Plant height    | Pht.Sparc-5A | BS00077855_51 | 139.6 | 6.2 | 103.2 | 98.9 | 3.6 | 2.2 |
| South Farm 2013      | Plant height    | Pht.Sparc-5A | BS00077855_51 | 139.6 | 5.7 | 107.2 | 102.4 | 3.3 | 2.4 |
| Centre Farm 2012     | Shattering      | Sh.Sparc-7A.2 | wsnp_Ex_c19005_27918129 | 145.27 | 3.0 | 4.4 | 3.8 | 1.8 | 0.3 |
| South Farm 2012      | Shattering      | Sh.Sparc-7A.2 | wsnp_Ex_c19005_27918129 | 145.27 | 4.1 | 3.7 | 3.1 | 2.4 | 0.3 |
| Field 16 2014        | Shattering      | Sh.Sparc-7D  | Ku_c17958_576 | 23.78 | 6.5 | 3.7 | 4.6 | 3.7 | −0.4 |
| Centre Farm 2012     | Shattering      | Sh.Sparc-7D  | Ku_c17958_576 | 23.78 | 6.5 | 3.7 | 4.6 | 3.7 | −0.4 |
| South Farm 2012      | Shattering      | Sh.Sparc-7D  | Ku_c17958_576 | 23.78 | 5.8 | 2.0 | 2.6 | 3.4 | −0.3 |
| Centre Farm 2013     | Shattering      | Sh.Sparc-7D  | Ku_c17958_576 | 23.78 | 5.8 | 1.8 | 2.3 | 3.4 | −0.2 |

\(^a\)Maximum likelihood LOD score for the QTL
\(^b\)Phenotypic variation explained by the QTL
\(^c\)Positive additive effect indicates an increasing value of the trait from Thatcher and AAC Cadillac; negative additive effect indicates an increasing value of the trait from Carberry
Construction of linkage maps

The summary statics of high-density SNP linkage maps of the Carberry/Thatcher and Carberry/AC Cadillac populations are presented in Supplemental Table 1. For the Carberry/Thatcher population, a total of 8360 polymorphic markers were mapped on 28 linkage groups corresponding to 20 wheat chromosomes, covering 3645.8 cM of the wheat genome with an average density of 0.6 cM per marker. All except chromosome 4D were represented in the map. The minor allele frequency (MAF) ranged from 0.37 to 0.50 with an average of 0.47, whereas the polymorphism information content (PIC) ranged from 0.46 to 0.50 with an average of 0.50. The Carberry/AC Cadillac population map involved a total of 6806 SNP markers mapped on 29 linkage groups, covering 3237.9 cM of the wheat genome and an average density of 0.72 cM per marker. The MAF for the Carberry/AC Cadillac population ranged from 0.41 to 0.50 with an average of 0.48, and the PIC ranging from 0.48 to 0.50 with an average of 0.48.

QTL identified in the Carberry/Thatcher population

A summary of QTL associated peak markers, map position (cM), LOD score, phenotypic variation explained (PVE) and additive effects of shattering response loci identified in Carberry/Thatcher and Carberry/AC Cadillac is presented in Table 3. Figure 2 displays linkage maps of the QTL identified in the population. The analysis by MapQTL detected two consistent shattering resistance QTL, one located on chromosomes 4B (designated as Sh.Sparc-4B) and the other on 5A (Sh.Sparc-5A), along with one sporadic QTL on 7A (Sh.Sparc-7A.1). Carberry was the contributor of the low shattering alleles for the three loci, whereas no QTL was contributed by Thatcher. The 4B and 5A loci appeared in all four test environments, but the 7A locus was marginally significant with a LOD score of 3.0 and identified in one environment only. The QTL interval of Sh.Sparc-5A is quite broad with several markers present, whereas Sh.Sparc-4B is narrow with few associated markers (Fig. 2).

Sh.Sparc-5A was most highly associated with SNP markers Kukri_rep_c102608_599 and wsnp_Ex_c1481_2831499 (Fig. 2) located on chromosome arm 5AL (Wang et al. 2014). This QTL had a LOD score as high as 18.1 (Table 3). Sh.Sparc-4B was associated with IAAV971 and Tdurum_contig42229_113 (Fig. 2 and Table 3) on chromosome arm 4BS (Wang et al. 2014). Sh.Sparc-7A.1 was associated with markers BS00092805_51 and Tdurum_contig56417_2381 located on 7AS (Table 3 and Fig. 2). The phenotypic variation explained by the 4B locus, ranging from 4.6 to 18.0%, was somewhat less than for the 5A ranging from 10.6 to 24.5%. The 4B and 5A loci cumulatively explained 29.2% of the total phenotypic variation at Centre Farm in 2016, 29.9% of the variation at Centre Farm in 2018, 34.6% at Field 17 in 2018 and 19.3% at South Farm in 2019.

QTL identified in the Carberry/AC Cadillac population

A total of eight main effect QTL for shattering resistance were detected on chromosomes 1A (designated Sh.Sparc-1A), 2D (Sh.Sparc-2D), 3A (Sh.Sparc-3A), 3D (Sh.Sparc-3D), 4B (Sh.Sparc-4B), 5A (Sh.Sparc-5A), 7A (Sh.Sparc-7A.2) and 7D (Sh.Sparc-7D) in the Carberry/AC Cadillac population (Table 3). Three of the QTL, on 4B, 5A and 7A, were derived from Carberry, and those QTL on 1A, 2D, 3A, 3D and 7D were contributed by AC Cadillac. The QTL on 4B and 5A were detected in all the five test environments, the QTL on 1A and 7D in four out of five, 2D and 7A in three out of five, 3A in two of five, and 3D in a single environment.

The Carberry/AC Cadillac QTL on 4B and 5A mapped in the same chromosomal region as the Carberry/Thatcher population (Fig. 2) and are designated with the same names. The Sh.Sparc-5A was associated with SNP markers wsnp_Ex_c1880_3545329 and wsnp_Ex_c18107_26909127 located on 5AL (Wang et al. 2014). The QTL, Sh.Sparc-5A, had the highest LOD value (103.7) of any QTL and explained the most phenotypic variation ranging from 13.8 to 46.2% across environments. The Sh.Sparc-4B associated with wsnp_BF482960B_Ta_1_4 and TdurumContig41902_1524 was located on 4BS. The QTL had a maximum LOD value of 38.3 with the phenotypic variation explained ranging from 5.2 to 20.3%. In 2012, the 4B and 5A loci alone explained 51.1% of the phenotypic variation at Centre Farm and 56.0% at South Farm. In 2013 both 4B and 5A explained 30.4% at Centre Farm and 43.9% at South Farm, and in 2014 the two explained 16.6% of the variation at Field 16. The third locus from Carberry, Sh.Sparc-7A.2, explained phenotypic variation ranging from 1.8 to 2.4%. The SNP markers associated
Fig. 2 (continued)
Fig. 2 (continued)
with the 7A locus in Carberry/AC Cadillac, wsnp_Ex_c19005_27918129 and Kukri_rep_c105157_485, mapped to the long arm of the chromosome (Wang et al. 2014) and were located about 448 Mb from Sh.Sparc-7A.1 in the bread wheat reference genome sequence (RefSeq v2.0).

The QTL derived from AC Cadillac tended to explain a smaller proportion of the phenotypic variation than Carberry, with the AC Cadillac variation ranging across environments from 2.4 to 5.5% for Sh.Sparc-1A, 1.4 to 2.5% for Sh.Sparc-2D, 1.5 to 1.9% for Sh.Sparc-3A and explaining 2.7% for Sh.Sparc-3D. Sh.Sparc-7D explained the greatest variation from AC Cadillac ranging from 3.4 to 3.9%. Considering chromosomal locations of AC Cadillac derived QTL, associated markers for the Sh.Sparc-1A were assigned to chromosome arm 1AL (Wang et al. 2014). Similarly markers for Sh.Sparc-2D were assigned to 2DL, Sh.Sparc-3A markers to 3AL, Sh.Sparc-3D markers to 3DL and Sh.Sparc-7D markers to 7DS (Wang et al. 2014).

The 4BS QTL Carberry alleles for reduced spike shattering are in coupling with reduced plant height (designated Pht.Sparc-4B) in both Carberry/Thatcher and Carberry/AC Cadillac populations (Table 3). The 5AL QTL alleles for reduced plant height (Pht.Sparc-5AL) within the Carberry/AC Cadillac population were contributed by Carberry as was the 5AL shattering resistance QTL, but the plant height QTL was not segregating in the Carberry/Thatcher population.

**Epistatic interaction**

Apart from epistatic interactions, QTLNetwork detected the same main effect QTL as MapQTL except for the 7AS QTL that was identified by MapQTL at a single environment in Carberry/Thatcher. The analysis revealed nine significant digenic interactions (Table 4). A1 x A2 epistatic interaction effects with positive additive values contributed to reduced shattering responses while those with negative values contributed to increased shattering responses. Only Sh.Sparc-4B/5A was significant in the Carberry/Thatcher population whereas all nine digenic interactions were significant in Carberry/AC Cadillac. The Centre Farm 2012 environment revealed only one significant epistatic interaction (Sh.Sparc-3A/Sh.Sparc-4B) compared with other environments for which at least two significant interactions were detected. Two of the nine pairs of epistatic interactions contributed to reduced shattering scores (A1 x A2 effect is positive), whereas the remaining seven pairs represented antagonistic interactions (Table 4). The first positive epistatic interaction was between Sh.Sparc-4B and Sh.Sparc-5A observed in both populations across multiple environments. The second positive interaction was between Sh.Sparc-5A and Sh.Sparc-7A.2, which occurred in the single environment Field 16, 2014 in the Carberry/AC Cadillac population. The Field 16 2014 test was characterized by the lowest Carberry/AC Cadillac shattering scores.

Epistatic interactions contributing to increased (A1 x A2 effect is negative) shattering in the Carberry/AC Cadillac population were: Sh.Sparc-1A/Sh.Sparc-4B, Sh.Sparc-2D/Sh.Sparc-5A, Sh.Sparc-3A/Sh.Sparc-4B, Sh.Sparc-3A/Sh.Sparc-5A, Sh.Sparc-4B/Sh.Sparc-7D, Sh.Sparc-5A/Sh.Sparc-7D and Sh.Sparc-7A/Sh.Sparc-7D (Table 4). Among these interactions, Sh.Sparc-5A/Sh.Sparc-7D was the most consistent appearing in four out of five environments. An example graphical depiction of individual effects in an epistatic interaction is presented in Fig. 3a and b between QTL Sh.Sparc-4B and Sh.Sparc-5A for the two populations and selected environments. For Carberry/Thatcher considering the Centre Farm 2018 trial, the shattering score was 3.9 when 4B and 5A resistance alleles were absent compared with 1.6 for the simultaneous presence of these QTL alleles (Fig. 3a). With the simultaneous occurrence of the 4B and 5A resistance alleles, the mean shattering score at the Centre Farm 2012 trial was 2.5 compared to 6.0 in the absence of both these alleles in the Carberry/AC Cadillac population (Fig. 3b). When the effects of individual QTL were compared, the score was 2.8 with the presence of 5A alone, and 4.9 with 4B alone. The presence of the 5A QTL alone resulted in a 2.1 shattering score and 2.0 for the 4B locus alone.

**Discussion**

The continuous distribution of the response to spike shattering in the studied populations suggests polygenic inheritance of the shattering trait. The wide range of heritability values of the trait over environments and populations similarly suggests this trait is under complex genetic control with gene networks influenced by environment. Nevertheless, the broad sense heritability of the resistance to shattering observed in both populations indicated the opportunity to maintain a desirable expression of the trait through selection. The values of the environmental component as the converse to the broad sense heritable component similarly suggested the resistance to shattering is a complex trait. Previous studies indicate traits such as glume tenacity and kernel size coupled with environmental factors of wind and humidity can greatly influence the ability of a cultivar to hold its grain in a recoverable position for the period after maturity until harvest which can be several weeks (Clarke and DePauw 1983; Harrington and Waywell 1950). The moderate to moderately strong positive correlations (P < 0.0001) in the shattering scores observed among the environments is another indication of heritable genetic expression in the studied populations. Edaphic conditions have not been reported to influence seed shattering, although available soil moisture affects
Table 4 Additive x additive (A1 x A2) epistatic interactions detected by QTLNetwork for spike shattering scores (1–9 scale), interacting QTL intervals (QTL1 and QTL2), and levels of significance ($P$ value) for QTL identified in the Carberry/Thatcher and Carberry/AC Cadillac doubled haploid populations evaluated near Swift Current, Canada in different years

| Location          | QTL1 Interval 1 | Position, cM | QTL2 Interval 2 | Position, cM | A1 x A2 effect | $P$ Value |
|-------------------|-----------------|--------------|-----------------|--------------|----------------|-----------|
| Centre Farm 2016  | 4B BS00081631_51-Tdurum_Contig55414_154 | 96.16–120.58 | 5A Wsnp_Ex_C1880_3545329 - Wsnp_Ex_C18107_26909127 | 187.62–189.67 | 0.25 | < 0.001 |
| Centre Farm 2018  | 4B IACX557-Tdurum_Contig64772_417 | 109.91–121.5 | 5A Wsnp_Ex_C18107_26909127 - Wsnp_Ex_C1481_2831499 | 189.67–198.41 | 0.39 | < 0.001 |
| Field 17 2018     | 4B IACX557-Tdurum_Contig64772_417 | 109.91–116.24 | 5A Wsnp_Ex_C16715_25264080 - Wsnp_Ex_C1880_3545329 | 186.59–187.62 | 0.25 | < 0.001 |
| South Farm 2019   | 4B IACX557-Tdurum_Contig64772_417 | 109.91–116.24 | 5A Wsnp_Ex_C18107_26909127 - Wsnp_Ex_C1481_2831499 | 189.67–198.41 | 0.33 | < 0.001 |
| Location-year mean| 4B IACX557-Tdurum_Contig64772_417 | 109.91–116.24 | 5A Wsnp_Ex_C16715_25264080 - Wsnp_Ex_C1880_3545329 | 186.59–187.62 | 0.28 | < 0.001 |
| Centre Farm 2012  | 3A Wsnp_Ku_C44716_51926415-Wsnp_Rfl_Contig2699_2402527 | 101.45–104.65 | 4B Bs00021984_51-Bs00021984_51926415 | 107.87–111.2 | − 0.16 | < 0.001 |
| South Farm 2012   | 4B Ex_C101685_705-Tdurum_Contig41902_1524 | 111.33–121.56 | 5A Wsnp_Ex_C3772_6866645 - Wsnp_Ex_C31672_40435001 | 180.73–190.13 | 0.13 | < 0.005 |
| Centre Farm 2013  | 1A Rac875_C60514_90-Bobwhite_C7337_737 | 119.46–120.12 | 4B Ex_C101685_705-Tdurum_Contig41902_1524 | 111.33–121.56 | − 0.07 | 0.013 |
| South Farm 2013   | 2D Rac875_C10626_2089-Bs00063458_51 | 98–104.81 | 5A Wsnp_Ex_C18107_26909127 - Wsnp_Ex_C1481_2831499 | 198.63–205.24 | 0.17 | < 0.001 |
|                  | Rac875_C10626_2089 - Bs00063458_51 | 22.39–23.07 | 5A Wsnp_Jd_C43389_30288993 - Cap8_Rep_C4852_130 | 206–206.16 | − 0.08 | 0.028 |
seed size and weight. Within a genotype, conditions that produce larger kernels place more stress on glumes (Chang 1943; Porter 1959). Damage by birds was not evident in any of the years. The range in maturity of the genotypes was about seven days. The variation in the correlations observed among shattering scores indicated the primary difference between environments, was likely wind speed after maturity. The average maximum daily wind speed for the period 1–10 September over the seven years varied from 24.2 to 32.4 km hr\(^{-1}\) (https://climate.weather.gc.ca/historical_data/search_historic_data_e.html).

The continuous nature of the phenotypic distributions is consistent with results of the QTL analysis which revealed multiple quantitative loci. This finding agrees with other studies that report multiple genes with quantitative control of spike shattering in wheat (Jantasuriyarat et al. 2004; Marza et al. 2006; Zhang and Mergoum 2007). With both parents of the Carberry/AC Cadillac population contributing positive and negative alleles for shattering, the apparent transgressive segregation of lines in two directions was expected. Transgressive segregation can occur because of the action of loci with complementary additive effects differentially present in parental lines combining in progeny (Rieseberg et al. 1999).

The observation that Thatcher was more resistant to shattering than Carberry, but that this resistance was not reflected in the results of the QTL mapping, with no resistance alleles attributed to Thatcher, is difficult to explain. Based on a shattering test conducted near Saskatoon, SK in 1948, Harrington and Waywell (1950) described Thatcher as a highly resistant wheat with a score of 1% compared with

| Location-year mean | 4B | Excalibur_ C29141_864 - Excalibur_ C17607_542 | 98–104.81 | 5A | Wsnp_Ex_ C18107_26909127 - Wsnp_Ex_ C1481_2831499 | 198.63–205.24 | 0.11 | <0.001 |
|-------------------|----|---------------------------------------------|---------|----|---------------------------------------------|------------|-------|--------|
|                   | 5A | Wsnp_Ex_ C3772_6866645 - Wsnp_Ex_ C31672_40435001 | 180.73–190.13 | 7D | Ku_C17958_576 - Excalibur_ C13094_523 | 23.78–30.35 | 0.08 | <0.001 |
|                   | 7A.2 | Bs00063458_51 - Tplb0036a12_207 | 156.82–157.34 | 7D | Ku_C17958_576 - Excalibur_ C13094_523 | 23.78–30.35 | 0.06 | 0.015 |

Table 4 (continued)
other cultivars such as Marquis at 2%, and Prelude at 23% shattering. Given no QTL for resistance was detected from Thatcher, it is possible several genes are present but lack sufficient expressivity to produce statistically significant effects on the phenotype. The other possibility could be sparse marker placement near genes making them undetected by QTL analysis or some combination of these two scenarios. That genetic differences occur between the two cultivars is supported by the occurrence of transgressive segregation in the progeny. There may also be loci not segregating between Thatcher and Carberry given the level of transgressive segregation appeared to be lower in the Carberry/Thatcher population than the Carberry/AC Cadillac population.

The shattering response of Carberry was significantly higher than that of Thatcher. However, the shattering resistance of Carberry is in large part due to the consistently and strongly expressed resistance alleles of the Sh.Sparc-4B and Sh.Sparc-5A QTL and minor alleles at Sh.Sparc-7A.1 and Sh.Sparc-7A.2. Apart from the main effects, the desirable epistatic interactions detected between Sh.Sparc-4B and Sh.Sparc-5A across two populations and multiple environments contributed to Carberry’s shattering resistance. The Sh.Sparc-5A interaction with Sh.Sparc-7A.2 in a single environment would have also contributed sporadically to Carberry’s shattering resistance. The year the interaction was discovered was a year Carberry expressed it’s highest level of resistance among the Carberry/AC Cadillac experiments.

The effect of the absence of Carberry resistance alleles is shown in its progeny by the most shattering susceptible lines of the Carberry/Thatcher population rated a relatively high seven out of nine. The QTL identified in the Carberry/Thatcher population were confirmed in the Carberry/AC Cadillac population, with additional QTL contributed by AC Cadillac. The wider distribution of Carberry/AC Cadillac compared to Carberry/Thatcher, with lines scoring as high as eight out of nine is consistent with the greater segregation of QTL in Carberry/AC Cadillac population.
The \textit{Sh.Sparc-4B} chromosomal region not only is important in controlling shattering, but is an important genomic region for other agronomic traits such as yield, plant height, and disease resistance (Dhariwal et al. 2020; Duan et al. 2020; Garcia et al. 2019; Pandey et al. 2015; Toth et al. 2018). In both populations, \textit{Sh.Sparc-4B} was consistently associated with shattering and plant height in coupling phase. Using Ning7840/Clark wheat population, Marza et al (2006) reported two SSR markers, \textit{Barc163} and \textit{Barc20}, which mapped close to \textit{Sh.Sparc-4B} marker, \textit{Sh.Sparc-5A}. The markers associated with the second consistently expressed Carberry locus, \textit{Sh.Sparc-5A}, reside in a similar region as a QTL on chromosome arm 5AL that consistently affected threshability traits in the W-7984/Opata 85 wheat population (Jantasuriyarat et al. 2004). The W-7984/Opata 85 threshability locus is believed to represent the free-threshing wheat gene \textit{Q}. The marker \textit{Xgwm126} for the 5AL QTL reported by Jantasuriyarat et al (2004) was located 10 cM from \textit{Xwmc110} on the high-density SSR consensus map of Somers et al (2004). The marker \textit{Xwmc110} was located only 0.6 cM from the \textit{Sh.Sparc-5A} markers, \textit{Kukri_rep_c102608_599} and \textit{wsnp_Ex_c18107_26909127} in an SSR and SNP integrated map by Wen et al (2017). The physical distance on the bread wheat reference genome sequence (IWGSC RefSeq v2.0) assembly of \textit{Xwmc110} to \textit{Kukri_rep_c102608_599} was 1.2 Mb, and it was 0.73 Mb from \textit{Xwmc110} to \textit{wsnp_Ex_c18107_26909127} (https://urgi.versailles.inrae.fr/blast_iwgsc/blast.php). The close proximity of markers between studies suggest the \textit{Q} gene could be responsible for the \textit{Sh.Sparc-5A} QTL. As all three cultivars are expected to have the \textit{Q} gene, \textit{Sh.Sparc-5A} might represent some subtle base pair difference. The broader interval observed in the \textit{Sh.Sparc-5A} QTL region compared with that of \textit{Sh.Sparc-4B}, and the association of the \textit{Sh.Sparc-5A} with several markers is helpful in the development of diagnostic markers for marker-assisted breeding. Two high confidence genes \textit{TraesCS5A01G463600} and \textit{TraesCS5A01G465200} were located in the \textit{Sh.Sparc-5A} shattering QTL region (IWGSC 2018).

Another study (Marza et al. 2006) reported a shattering resistance QTL on chromosome 5A in the United States soft red winter wheat Clark, but it is different from the Carberry \textit{Sh.Sparc-5A} QTL because the location of markers associated with the two QTL are too far apart. In the consensus map that integrates SNP and SSR markers (Bokore et al. 2020), QTL associated markers \textit{Kukri_rep_c102608_599} for \textit{Sh.Sparc-5A} and \textit{Xbarc180} for the 5A threshability QTL of Clark were 118 cM from each other. The expression of the two QTL also suggests they are different with the 5A QTL in Clark (Marza et al. 2006) being highly inconsistent over environments compared to the consistent expression of \textit{Sh.Sparc-5A}.

The \textit{Sh.Sparc-5A} locus appears to hold a complex of genes controlling multiple traits. For example, the \textit{Sh.Sparc-5A} marker \textit{Kukri_rep_c102608_599} is in the interval of the QTL that increases seed weight and spike length in the Chinese wheat Zhou 8425B (Gao et al. 2015). An allele having a positive effect on the harvest index in another Chinese wheat also lies in this interval (Chen et al. 2019). Other studies in
which the \textit{Xwmc110} marker is involved include Fusarium head blight resistance in the Canadian durum wheat line DT696 (Singh et al. 2008), ear emergence in elite European winter wheat germplasm (Griffiths et al. 2009), and the pasta quality mixogram parameter time-to-peak (Zhang et al. 2008).

The two Carberry QTLs, \textit{Sh.Sparc-7A.1} and \textit{Sh.Sparc-7A.2}, are different as markers associated with each QTL are located in different genomic regions. Furthermore, the QTL behaved differently. \textit{Sh.Sparc-7A.1} associated markers are located on chromosome arm 7AS, whereas the \textit{Sh.Sparc-7A.2} markers are on arm 7AL in the high-density SNP map by Wang et al. (2014). Additionally, a physical distance of 448 Mb observed between markers of \textit{Sh.Sparc-7A.1} and \textit{Sh.Sparc-7A.2} in the bread wheat reference genome sequence (IWGSC RefSeq v2.0) suggests they are distinct loci (https://urgi.versailles.inrae.fr/blast_iwgsc/blast.php). The expression in only one out of four environments and marginally significant LOD score for \textit{Sh.Sparc-7A.1} compared to the relatively stable QTL at \textit{Sh.Sparc-7A.2} that expressed in three out of five environments supports the hypothesis that the two loci represent different genes. In addition to its consistency over environments, the epistasis of \textit{Sh.Sparc-7A.2} with \textit{Sh.Sparc-5A} resulting in reduced shattering compared with either locus alone makes \textit{Sh.Sparc-7A.2} more appealing in breeding than \textit{Sh.Sparc-7A.1}.

Based on the hexaploid wheat consensus map of Bokore et al. (2020) that integrates SSR and SNP markers, markers for \textit{Sh.Sparc-7A.2}, \textit{Kukri_rep_c105157_485} and \textit{wsnp_Ex_c19005_27918129}, were within 0.04–0.29 cM of \textit{Xbarc108} that tagged a shattering resistance QTL in the Clark wheat cultivar (Marza et al. 2006). Additionally, \textit{Xbarc108} is associated with grain protein (\textit{QGpc.usw-A3}) with little effect on grain yield in Strongfield durum wheat (Suprayogi et al. 2009). The shattering resistance region \textit{Sh.Sparc-7A.2} could be combined with grain protein (\textit{QGpc.usw-A3}) using marker assisted selection. Zhang and Mergoum (2007) reported a major kernel shattering resistance QTL near the centromere of chromosome 7AL and a minor locus on the distal end of 7AL both of which were contributed by a hard red spring wheat cultivar Stoa. The map distance from \textit{Sh.Sparc-7A.2} associated marker \textit{wsnp_Ex_c19005_27918129} to \textit{Xwmc633}, a marker associated with the minor 7AL QTL in Stoa was 108.5 cM (Wen et al. 2017), suggesting the region is different from \textit{Sh.Sparc-7A.2}. Overlapping markers were not found to compare if the second Stoa 7A QTL was located in a similar region as either of the Carberry loci.

The low shattering QTL identified from AC Cadillac, \textit{Sh.Sparc-7D} is located on chromosome arm 7DS. No QTL has been previously reported on 7DS, but a 7DL linkage group carries a shattering resistance factor that segregated in the Ning7840/Clark wheat population (Marza et al. 2006). The \textit{Sh.Sparc-2D} QTL from AC Cadillac, located on chromosome arm 2DL, appears to be novel, although QTL for shattering resistance were reported on 2DS in the two different wheat populations W-7984/Opata 85 (Jantasuriyarat et al. 2004) and Ning7840/Clark (Li et al. 2016). Our report of the remaining AC Cadillac shattering resistance QTL located on 1AL, 3AL and 3DL appears to be a first. Markers associated with the 1AL and 3AL shattering resistance have been associated with other agronomic traits. For example, the 1AL QTL marker \textit{Kukri_c58155_786} was associated with wheat proteins (Taranto et al. 2020). One of the markers which tagged the 3AL shattering resistance allele, \textit{Wsnp_Ku_C44716_51926415}, is associated with flag leaf traits such as length, width, angle, and area (Wu et al. 2016), highlighting the importance of this region in trait improvement.

Results of the present study indicated that the additive genetic effect is a major component of heritability, although epistatic interactions contributed to a significant portion of the heritable variation which is consistent with other research findings (Ma et al. 2006; Zhou et al. 2017). The consistent detection of epistasis between the two major QTL \textit{Sh.spa-4B} and \textit{Sh.spa-5A} in the present study contrasts with the sporadic occurrences of interactions between major and minor effect QTL. According to Zhou et al. (2017), significant epistasis is possible between QTL that individually have low phenotypic effects, but no epistasis was detected between minor QTL in our study. The epistatic interactions between pairs of Carberry alleles \textit{Sh.spa-4B / Sh.spa-5A} and \textit{Sh.spa-5A / Sh.spa-7A.2} are desired for improving shattering resistance. This reduction in shattering can be illustrated by results of the Centre Farm 2012 trial that involved the Carberry/AC Cadillac population, among other examples. Similar favorable epistatic combinations are likely to be common because breeders select lines with reduced-shattering while retaining threshability. Conversely, the increased level of shattering observed with the remaining digenic interactions that involved the 4B or 5A with the QTL from AC Cadillac suggest caution may be needed when planning crosses to take into account unfavorable combinations of loci.

In summary, the shattering trait showed intermediate heritability with medium to high correlations observed between the scores in different environments. Nine main effect QTL were identified from Carberry and AC Cadillac using MapQTL that demonstrated the complex inheritance of the shattering trait. Despite having low shattering scores compared to Carberry, no QTL were detected from the heritage cultivar Thatcher, likely due to the lack of sufficient expressivity of QTL or sparse marker placement near shattering genes or a combination of these two scenarios. Of the nine QTL we identified, four desirable Carberry alleles were located on chromosome arms 4BS, 5AL, 7AS and 7AL, and
five QTL desirable AC Cadillac alleles were located on 1AL, 2DL, 3AL, 3DL and 7DS. The QTL on 4BS and 5AL with consistent expression across populations and environments are major QTL responsible for the control of seed shattering. The 4B QTL was consistently associated with reduced shattering and reduced plant height in the coupling phase. Based on proximity, there might be some modification to the Q gene that may be responsible for the 5AL QTL, as the three cultivars would have the Q gene. The two remaining Carberry QTL and the other five AC Cadillac loci represent minor QTL having weak and variable expressions across environments. Analysis by QTL Network demonstrated the importance of epistasis with nine significant additive x additive epistatic interactions between main effect loci. The interactions between main effect QTL Sh.Sparc-4B and Sh.Sparc-5A, and between Sh.Sparc-5A and Sh.Sparc-7A.2 are synergistic and thus beneficial in breeding for improved shattering resistance. In contrast, the other seven pairs of interacting QTL Sh.Sparc-1A/4B, Sh.Sparc-2D/5A, Sh.Sparc-3A/4B, Sh.Sparc-3A/5A, Sh.Sparc-4B/7D, Sh.Sparc-5A/7D and Sh.Sparc-7A/7D were detrimental by increasing the expression of shattering. SNP markers closely associated with the QTL will be helpful in characterizing parents and for the identification of detrimental alleles and combinations of alleles across loci for culling early generation breeding lines.

Funding This study was funded by the Canadian Wheat Research Coalition through the Canadian Agricultural Partnership AgriScience Program, and CTAG2 through Genome Canada with the additional support of the Western Grains Research Foundation, Government of Saskatchewan, Saskatchewan Wheat Development Commission, Alberta Wheat Commission, Manitoba Agriculture, DuPont Pioneer, Viterra, Manitoba Wheat and Barley Growers Association, and SeCan. We also acknowledge the financial support of the Western Grains Research Foundation and their funders: Alberta Wheat Commission, Atlantic Grains Council, Ducks Unlimited, Grain Farmers of Ontario, Manitoba Wheat and Barley Growers Association, Producteurs de grains du Québec, Saskatchewan Wheat Development Commission, Saskatchewan Winter Cereals Development Commission, and Winter Cereals Manitoba Inc. Open access funding provided by Agriculture & Agri-Food Canada.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00122-021-03980-2.

Acknowledgements We are grateful for the financial support of the Canadian Wheat Research Coalition through the Canadian Agricultural Partnership AgriScience Program, and CTAG2 through Genome Canada with the additional support of the Western Grains Research Foundation, Government of Saskatchewan, Saskatchewan Wheat Development Commission, Alberta Wheat Commission, Manitoba Agriculture, DuPont Pioneer, Viterra, Manitoba Wheat and Barley Growers Association, and SeCan. We also acknowledge the financial support of the Western Grains Research Foundation and their funders: Alberta Wheat Commission, Atlantic Grains Council, Ducks Unlimited, Grain Farmers of Ontario, Manitoba Wheat and Barley Growers Association, Producteurs de grains du Québec, Saskatchewan Wheat Development Commission, Saskatchewan Winter Cereals Development Commission, and Winter Cereals Manitoba Inc. Open access funding provided by Agriculture & Agri-Food Canada.

References

Bokore FE, Cuthbert RD, Knox RE, Singh A, Campbell HL, Pozniak JC, N’Diaye A, Sharpe AG, Ruan Y (2019) Mapping quantitative trait loci associated with common bunt resistance in a spring wheat (Triticum aestivum L.) cultivar Lillian. Theor Appl Genet 12:3023–3033

Brown PD, Randhawa HS, Mitchell Fetch J, Meiklejohn Fox, Singh AK, Spaner D, Fowler DB, Ruan Y, Berriaies S, Meyer B (2020) Mapping quantitative trait loci associated with leaf rust resistance in five spring wheat populations using single nucleotide polymorphism markers. PLoS ONE 15(4):e0230855

Bokore FE, Knox RE, Cuthbert RD, Pozniak CJ, McCallum BD, N’Diaye A, DePauw RM, Campbell HL, Munro C, Singh A, Hiebert CW, McCartney CA, Sharpe AG, Singh AK, Spaner D, Fowler DB, Ruan Y, Berriaies S, Meyer B (2020) Mapping quantitative trait loci associated with leaf rust resistance in five spring wheat populations using single nucleotide polymorphism markers. PLoS ONE 15(4):e0230855

Bokore FE, Cuthbert RD, Knox RE, Singh A, Campbell HL, Pozniak JC, N’Diaye A, Sharpe AG, Ruan Y (2019) Mapping quantitative trait loci associated with common bunt resistance in a spring wheat (Triticum aestivum L.) cultivar Lillian. Theor Appl Genet

Chang SC (1943) Morphological causes for varietal differences in shattering of wheat. J Am Soc Agron 35:435–441

Chen J, Zhang F, Zhao C, Lv G, Sun C, Pan Y, Guo X, Chen F (2019) Genome-wide association study of six quality traits reveals the association of the TaRPP13L1 gene with flour colour in Chinese bread wheat. Plant Biotechnol J 17(11):2106–2122

Cheverud JM, Routman EJ (1995) Epistasis and its contribution to genetic variance components. Genetics 139(3):1455–1461

Chou HH, Chiu HC, Delaney NF, Segrè D, Marx CJ (2011) Diminishing returns epistasis among beneficial mutations decelerates adaptation. Science 332(6034):1190–1192

Clarke JM (1981) Effect of delayed harvest on shattering losses in oats, barley and wheat. Can J Plant Sci 61:25–28

Clarke JM, DePauw RM (1983) The dynamics of shattering in maturating wheat. Euphytica 32:225–230

DePauw RM, Knox RE, McCaig TN, Clarke FR, Clarke JM (2011) Carberry hard red spring wheat. Can J Plant Sci 91(3):529–534
DePauw RM, Thomas JB, Knox RE, Clarke JM, Fernandez MR, McCAig TN, McLeod JG (1998) AC Cadillac hard red spring wheat. Can J Plant Sci 78(3):459–462

Dharwad R, Henriquez MA, Hiebert C, McCARTney CA, Randhawa HS (2020) Mapping of major fusarium head blight resistance from Canadian wheat. AAC Tenacious J Mol Sci 21(12):1–24

Duean X, Yu H, Ma W, Sun J, Zhao Y, Yang R, Ning T, Li Q, Liu Q, Guo T, Yan M, Tian J, Chen J (2020) A major and stable QTL controlling wheat thousand grain weight: identification, characterization, and CAPS marker development. Mol Breeding 40:68. https://doi.org/10.1007/s10832-020-1471-3

Fowler DB, N’Diaie A, Laudencia-Chingcuanno D, Pozniak CJ (2016) Quantitative trait loci associated with phenological development, low-temperature tolerance, grain quality, and agronomic characters in wheat (Triticum aestivum L.). PLoS ONE 11(3):e0152185. https://doi.org/10.1371/journal.pone.0152185

Gao F, Wen W, Liu J, Rasheed A, Yin G, Xiao X, Wu X, He Z (2015) Genome-wide linkage mapping of QTL for yield components, plant height and yield-related physiological traits in the Chinese wheat cross Zhou 8425B/Chinese spring. Front Plant Sci 6:1099. https://doi.org/10.3389/fpls.2015.01099 (DEC)

Garcia M, Eckermann P, Haefele S, Satija S, Szajner B, Timmins A, Baumann B, Wolters P, Mather DE, Fleury D (2019) Genome-wide association mapping of grain yield in a diverse collection of spring wheat (Triticum aestivum L.) evaluated in southern Australia. PLoS ONE 14(2):e0211730

Griffiths S, Simmonds J, Leverington M, Wang Y, Fish L, Sayers L, Allibert L, Orford S, Wingen L, Henry L, Faure S, Laurie D, Bilham L, Snape J (2009) Meta-QTL analysis of the genetic control of ear emergence in elite European winter wheat germplasm. Theor Appl Genet 119(3):383–395

Grover A, Sharma PC (2016) Development and use of molecular markers: past and present. Crit Rev Biotechnol 36(2):290–302. https://doi.org/10.3109/07388551.2014.959891

Harrington JB, Waywell CG (1950) Testing resistance to shattering and lodging in cereals. Sci Agric 30:51–60

Hayes, HK, Ausemus, ER, Stakman, EC, Bailey, CH, Wilson, HK, Bamberg, RH, Markley, MC, Crim, RF, Levine, MN (1936) Lodging in cereals. Sci Agric 30:51–60

Jantasuriyarat C, Vales MI, Watson CJW, Riera-Lizarazu O (2004) Identification and mapping of genetic loci affecting the free-threshing habit and spike compactness in wheat (Triticum aestivum L.). Theor Appl Genet 109(6):1105–1114

Li C, Bai G, Carver BF, Chao S, Wang Z (2016) Mapping quantitative trait loci for plant adaptation and morphology traits in wheat using single nucleotide polymorphisms. Euphytica 208(2):299–312

Ma HX, Bai GH, Zhang X, Lu WZ (2006) Main effects, epistasis, and environmental interactions of quantitative trait loci for fusarium head blight resistance in a recombinant inbred population. Physiologia 96(5):534–541

Marza F, Bai GH, Carver BF, Zhou WC (2006) Quantitative trait loci for yield and related traits in the wheat population Ning7840 x Clark. Theor Appl Genet 112(4):688–698

Pandey M, Singh AK, DePauw RM, Bokore FE, Ellouze W, Knox RE, Cuthbert RD (2015) Coleoptile length, gibberellin sensitivity, and plant height variation of durum wheat in Canada. Can J Plant Sci 95:1259–1264

Pincus JW (1931) New Russian wheats which will not shell or shatter easily. J Amer Soc Agron 23:328–328

Porter KB (1959) The inheritance of shattering in wheat. Agron J 51:173–177

Pourkheirandish M, Dai F, Sakuma S, Kanamori H, Distelfeld A, Wilcox G, Kawahara T, Matsumoto T, Kiliyan B, Komatsuda T (2018) On the origin of the non-brittle rachis trait of domesticated einkorn wheat. Front Plant Sci 8:2031

Riaz A, Kock Appelgren P, Hehir JG, Kang J, Meade F, Cockram J, Melbourne D, Spink J, Mullins E, Byrne S (2020) Genetic analysis using a multi-parent wheat population identifies novel sources of septoria tritici blotch resistance. Genes 11:887. https://doi.org/10.3390/genes11080887(8)

Rieseberg LH, Archer MA, Wayne RK (1999) Transgressive segregation, adaptation, and speciation. Heredity 83(4):363–372

Sears ER (1954) The aneuploids of common wheat. MO Agr Exp Sta Res Bull 572:1–59

Simons KJ, Fellers JP, Trick HN, Zhang Z, Tai SY, Gill BS, Faris JD (2006) Molecular characterization of the major wheat domestication gene Q. Genetics 172(1):547–555

Singh A, Knox R, Clarke F, Clarke J, Somers D, Fedak G, Singh A, DePauw R (2008) Fusarium head blight QTL mapping in durum wheat and Triticum carthlicum sources of resistance. Eds: Rudi Appels, et al. In: Proceedings of the of the 11th international wheat genetics symposium 24–29 August 2008, Brisbane, QLD, Australia, pp. 845–847

Singh A, Knox RE, DePauw RM, Singh AK, Cuthbert RD, Campbell HL, Shorter S, Bhavani S (2014) Stripe rust and leaf rust resistance QTL mapping, epistatic interactions, and co-localization with stem rust resistance loci in spring wheat evaluated over three continents. Theor Appl Genet 127(1):2465–2477

Singh A, Knox RE, DePauw RM, Singh AK, Cuthbert RD, Campbell HL, Singh D, Bhavani S, Fetch T, Clarke F (2013) Identification and mapping in spring wheat of genetic factors controlling stem rust resistance and the study of their epistatic interactions across multiple environments. Theor Appl Genet 126(8):1951–1964

Singh A, Knox RE, DePauw RM, Singh AK, Cuthbert RD, Kumar S, Campbell HL (2016) Genetic mapping of common bunst resistance and plant height QTL in wheat. Theor Appl Genet 129(2):243–256

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (Triticum aestivum L.). Theor Appl Genet 109(6):1105–1114

Suprayogi Y, Pozniak CJ, Clarke FR, Clarke JM, Knox RE, Singh AK (2009) Identification and validation of quantitative trait loci for grain protein concentration in adapted Canadian durum wheat populations. Theor Appl Genet 119(3):437–448

Taranto F, D’Agostino N, Rodriguez M, Pavan S, Minervini AP, Pecchioni N, Papa R, De Vita P (2020) Whole genome scan reveals molecular signatures of divergence and selection related to important traits in durum wheat germplasm. Front Genet 11:217. https://doi.org/10.3389/fgene.2020.00217

Toth T, Pandurangan S, Burt A, Fetch JM, Kumar S (2018) Marker-assisted breeding of hexaploid spring wheat in the Canadian prairies. Can J Plant Sci 99(2):111–127

Van Ooijen JW (2009) MapQTL® 6: software for the mapping of quantitative trait loci in experimental populations of diploid species. Kyazma BV, Wageningen, The Netherlands

Wang S, Song D, Forrest K, Allen A, Chao S, Huang BE, Maccarelli M, Salvi S, Milner SG, Cattivelli L, Mastrangelo AM, Whan A, Stephen S, Barker G, Wieseke R, Plikske J, Lilemo M, Mather D, Appels R, Dolfers R, Brown-Guedira G, Korol A, Akhunov EA, Kyazma BV, Wageningen, The Netherlands

Yang R, Cheng C, Wang Y, Li H, Wang Y, Ausemus E, IWGSC (2014) Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. Plant Biotechnol J 12(6):787–796

Wen W, He Z, Gao F, Liu J, Jin H, Zhai S, Qu Y, Xia X (2017) A high-density consensus map of common wheat integrating four...
mapping populations scanned by the 90k SNP array. Front Plant Sci 8:1389. https://doi.org/10.3389/fpls.2017.01389

Wu Q, Chen Y, Fu L, Zhou S, Chen J, Zhao X, Zhang D, Ouyang S, Wang Z, Li D, Wang G, Zhang D, Yuan C, Wang L, You M, Han J, Liu Z (2016) QTL mapping of flag leaf traits in common wheat using an integrated high-density SSR and SNP genetic linkage map. Euphytica 208(2):337–351

Xu D, Wen W, Fu L, Li F, Li J, Xie L, Xia X, Ni Z, He H, Cao S (2019) Genetic dissection of a major QTL for kernel weight spanning the Rht-B1 locus in bread wheat. Theor Appl Genet 132:3191–3200

Yang J, Hu C, Hu H, Yu R, Xia Z, Ye X, Zhu J (2008) QTLNetwork: mapping and visualizing genetic architecture of complex traits in experimental populations. Bioinformatics 24:721–723

Yang J, Hu C, Ye X, Zhu J (2007) QTLNetwork-2.0 user manual: software for mapping qtl with epistatic and qe interaction effects. Copyright 2005–2006 ©. Zhejiang University, China. http://ibi.zju.edu.cn/software/qltnetwork/QTLNetworkUserManual.pdf.

Zhang G, Mergoum M (2007) Molecular mapping of kernel shattering and its association with Fusarium head blight resistance in a Sumai3 derived population. Theor Appl Genet 115(6):757–766

Zhang W, Chao S, Manthey F, Chicaiza O, Brevis JC, Echenique V, Dubcovsky J (2008) QTL analysis of pasta quality using a composite microsatellite and SNP map of durum wheat. Theor Appl Genet 117(8):1361–1377

Zhou Y, Conway B, Miller D, Marshall D, Cooper A, Murphy P, Chao S, Brown-Guedira G, Costa J (2017) Quantitative trait loci mapping for spike characteristics in hexaploid wheat. Plant Genome. https://doi.org/10.3835/plantgenome2016.10.0101

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.