Identification and Validation of Stable Quantitative Trait Loci for SDS-Sedimentation Volume in Common Wheat (*Triticum aestivum* L.)

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Sodium dodecyl sulfate-sedimentation volume is an important index to evaluate the gluten strength of common wheat and is closely related to baking quality. In this study, a total of 15 quantitative trait locus (QTL) for sodium dodecyl sulfate (SDS)-sedimentation volume (SSV) were identified by using a high-density genetic map including 2,474 single-nucleotide polymorphism (SNP) markers, which was constructed with a doubled haploid (DH) population derived from the cross between Non-gda3753 (ND3753) and Liangxing99 (LX99). Importantly, four environmentally stable QTLs were detected on chromosomes 1A, 2D, and 5D, respectively. Among them, the one with the largest effect was identified on chromosome 1A (designated as *QSsv.cau-1A.1*) explaining up to 39.67% of the phenotypic variance. Subsequently, *QSsv.cau-1A.1* was dissected into two QTLs named as *QSsv.cau-1A.1.1* and *QSsv.cau-1A.1.2* by saturating the genetic linkage map of the chromosome 1A. Interestingly, favorable alleles of these two loci were from different parents. Due to the favorable allele of *QSsv.cau-1A.1.1* was from the high-value parents ND3753 and revealed higher genetic effect, which explained 25.07% of the phenotypic variation, mapping of this locus was conducted by using BC²F² and BC³F² populations. By comparing the CS reference sequence, the physical interval of *QSsv.cau-1A.1.1* was delimited into 14.9 Mb, with 89 putative high-confidence annotated genes. SSVs of different recombinants between *QSsv.cau-1A.1.1* and *QSsv.cau-1A.1* detected from DH and BC³F² populations showed that these two loci had an obvious additive effect, of which the combination of two favorable loci had the high SSV, whereas recombinants with unfavorable loci had the lowest. These results provide further insight into the genetic basis of SSV and *QSsv.cau-1A.1.1* will be an ideal target for positional cloning and wheat breeding programs.

Keywords: SDS-sedimentation volume, major QTL, wheat, quality, mapping
INTRODUCTION

Common wheat is one of the most widely cultivated food crops in the world and one of the important sources of carbohydrates and proteins for human beings (Osinowo, 2011). For a long time, breeders of common wheat (*Triticum aestivum* L.) have mainly committed to improving wheat yield and disease resistance (Curtis and Halford, 2014). However, high-quality wheat has been demanded by consumers and industries, and wheat quality improvement has attracted increasing attention among breeders (Guzman et al., 2016). Gluten strength has a considerable influence on the end-use quality of wheat (Rubenthaler et al., 1990; Addo et al., 1991; Slade and Levine, 1994; Kweon et al., 2011; Liu et al., 2017b), which can be measured by various tests such as sodium dodecyl sulfate (SDS)-sedimentation volume (SSV), extensograph, farinograph, alveograph, and gluten index (Huang et al., 2006; Elangovan et al., 2008; Li et al., 2009; Kerfal et al., 2010; Krfal et al., 2010; Tsilo et al., 2011). The SSV test is well correlated with gluten strength and bread-making quality of wheat (Axford et al., 1979; He et al., 2004; Ozturk et al., 2008), and exhibits advantages such as simplicity, low cost, small sample size requirement, and high efficiency. Therefore, it has been widely used for evaluating the content and quality of gluten protein and for fast screening desired cultivars in wheat breeding programs (Clarke et al., 2000).

Some recent studies have found that the quantitative nature of SSV is closely correlated to multiple genes encoding glutenins and gliadins, such as *Glu-1*, *Glu-A3*, *Glu-B3*, and *Gli-B1* (Payne and Lawrence, 1983; Payne et al., 1984; Shewry et al., 2003; Maucher et al., 2009; Reif et al., 2011; Deng et al., 2015; Guo et al., 2020). Glutenins and gliadins are not only the most important storage proteins of wheat but also the main components of gluten protein (Gianibelli et al., 2001; Krfal et al., 2010). Glutenins are related to the extensibility of gluten, while gliadins are associated with the elasticity of gluten (MacRitchie, 1995; Veraverbeke and Delcour, 2002; Van Der Borght et al., 2005; Rasheed et al., 2014). The content and ratio of glutenins and gliadins are the main factors that determine the wheat processing quality (Yang et al., 2014). In addition, puroindolines are a component of wheat grain protein and are closely related to grain hardness. *Puroindolino h* (*Pinb-D1*) gene was found to be related to the variation of SSV in recent studies (Park et al., 2010, 2012; Ahn et al., 2014; Wrschum et al., 2016).

Sodium dodecyl sulfate (SDS)-sedimentation volume (SSV) is a complex quantitative trait affected by both environmental and genetic factors. Quantitative trait locus (QTL) analysis is an effective approach for examining the genetic basis of quantitative traits (Doerge, 2002). Many studies have analyzed QTLs for SSV. To date, QTLs for SSV have been detected on almost all chromosomes, explained 2.2–41.4% of the phenotypic variation (Li et al., 2009; Kerfal et al., 2010; Reif et al., 2011; Deng et al., 2015; Wrschum et al., 2016; Liu et al., 2017a; Mir Drikvand et al., 2018; Goel et al., 2019; Guo et al., 2020; Yang et al., 2020). However, most of the previously identified QTLs were detected only in one or two environments and could not be detected in multiple genetic backgrounds, which were not ideal targets for fine mapping and map-based cloning.

Here, to understand the genetic basis underlying SSV and provide molecular markers linked to QTL for wheat quality breeding, a doubled haploid (DH) population derived from a cross between Non-gda3753 (ND3753) and Lianxing99 (LX99) was employed to detect the QTLs associated with SSV variation. The genetic effect of two major SSV QTLs was validated.

MATERIALS AND METHODS

Plant Materials

The DH population consisting of 123 individuals was developed through *in vitro* anther culture (De Buyser and Henry, 1980) of the F$_1$ hybrids from a cross between ND3753 and LX99. The DH population and two parents were used for genome-wide identification of QTLs related to SSV.

For mapping of the possible QTL, ND3753 that carried the positive allele of the QTL in the confidence interval was crossed with LX99. F$_1$ plants were backcrossed with LX99 for three generations with insertion-deletion (InDel) marker-assisted selection to generate a BC$_3$F$_1$ population containing 418 plants. Subsequently, 126 heterozygotes lines at the QTL-anchored region were self-pollinated to BC$_3$F$_2$ containing 1,081 plants. This population with LX99 background is presented as BC$_3$F$_2$-L in the present paper.

In addition, in order to evaluate the effects of two QTL, LX99 was crossed with recurrent parent ND3753 and 64 BC$_3$F$_1$ heterozygotes lines at the QTL-anchored region were self-pollinated to construct another BC$_3$F$_2$ population containing 387 plants with marker-assisted selection. This population with the background of ND3753 is presented as BC$_3$F$_2$-N.

Field Trials

The DH population and two parents were planted in seven environments during the wheat-growing seasons of 2016, 2017, and 2018 in Beijing (BJ) (40°08′N, 116°10′E), Linfen (LF) (36°04′N, 111°31′E), Xi’an (XA) (34°16′N, 108°55′E), and Changzhou (CZ) (38°18′N, 116°49′E), China. The seven environments, namely 2016BJ, 2017BJ, 2017LF, 2017XA, 2018BJ, 2018LF, and 2018CZ were presented in this study as E1, E2, E3, E4, E5, E6, and E7, respectively. The field trials were conducted following a complete random block design with three biological replicates. However, only one biological duplication was harvested in E7 due to an accident of field management. Then 60 seeds for each of the lines and two parents were planted in two rows of 1.5 long and the row space was 20 cm.

The BC$_3$F$_1$, BC$_3$F$_2$-L, and BC$_3$F$_2$-N populations were all planted in Beijing. The BC$_3$F$_1$ population was planted in the wheat-growing seasons of 2018, while the BC$_3$F$_2$-L and BC$_3$F$_2$-N populations were planted in 2019. All of these backcross populations were sown in rows of 1.5 m long and 30 cm row space with a sowing density of 20 seeds per row. The BC populations were all planted in one trial and designed as a single replicate. During the whole growing season, the local standard field management methodologies were adopted for plant cultivation.
Evaluation of Traits
For DHs, 80 plants were harvested in each line of the seven environments and wheat flour of each line planted in E1 was obtained with a CD1 Quadrumat Junior laboratory mill (Chopin Technology, Paris, France), while the whole wheat flour of each line in the other six environments was produced by an XF-98B experimental mill (Zhenxing Electromechanical Instrument Factory, Cangzhou, China). SSV was determined according to a modified protocol of Axford et al. (1979) and Preston et al. (1982) using 2 g of samples. The specific procedure of the SSV test was similar to that described by Li et al. (2009). In particular, SSVs of each DH line with only one biological duplication harvesting from E7 were measured. SSV of the BC$_2$F$_1$, BC$_3$F$_2$-L, and BC$_3$F$_2$-N populations was measured with whole wheat flour from a single plant.

In addition, 300 g of grains of 30 randomly selected DH lines based on the minimum sample required for the Pearson’s correlation coefficient calculated according to the formula provided by Mangard et al. (2007) and Chen et al. (2011) in E5 were ground into flour with a flour yield of approximately 60% in all samples. Their farinograph parameters (GB/ICC) were recorded by a Farinograph (DongFu Jiuheng, Beijing, China) to evaluate the correlation with SSV (Chicago, IL, United States) (ICC, 1996; Luo et al., 2018).

Genetic Map Construction
Deoxyribonucleic acid (DNA) was extracted from fresh leaves of individual DH lines and two parents using the hexadecyltrimethy ammonium bromide (CTAB) method (Allen et al., 2006). The 15 K Axiom® Wheat Breeder single-nucleotide polymorphism (SNP) Genotyping Array (China Golden Marker Co., Beijing, China) containing 13,947 SNP markers was used to genotype the DH population and parents. SNP markers with a missing data rate > 20% were removed, and the remaining polymorphic markers were used to construct a wheat genetic map based on the inclusive composite interval mapping (ICIM) method using IciMapping v4.1 (Chinese Academy of Agricultural Sciences, China) and MapChart v2.32 (Plant Research International, P.O. Box 16, 6700 AA Wageningen) (Voorrips, 2002). The physical locations of unique SNP markers were obtained from the International Wheat Genome Sequencing Consortium (Appels et al., 2018).

Quantitative Trait Locus Mapping
The average value of SSV in each environment and the BLUP were employed for QTL analysis using inclusive composite interval mapping (ICIM) method in software IciMapping v4.1 (Meng et al., 2015). A QTL with LOD $\geq$ 2.5 was defined as a significant QTL. The confidence intervals (±2 LOD away from the peaks of likelihood ratios) of several QTLs were coincident, which were preliminarily considered as the same QTL. In this study, the QTL that can be detected in three or more environments is defined as an environmentally stable QTL.

Re-sequencing and InDel Markers Development
High-quality genomic DNA of ND3753 and LX99 was extracted to construct paired-end-sequencing libraries. According to the procedures described by Li et al. (2020), the parents were re-sequenced with an average sequencing depth of 6 × and paired-end reads of length 150 bp for two parents using the Illumina HiSeq X Ten platform (Illumina, California, United States), and the re-sequencing data were processed. The InDels were identified using the HaplotypeCaller module of the Genome Analysis Toolkit (GATK). The InDel markers were developed based on the sequence difference between the parents around the target region. Primer3 version 0.4.0 was used to design the sequences of InDel primers.

Deoxyribonucleic acid (DNA) amplification was programmed for an initial 5 min at 94°C, then followed by 35 cycles of 30 s at 94°C, 30 s at 56°C, and 30 s at 72°C, and finally 5 min at 72°C. A 10 µL PCR reaction system was used, containing 5 µL of 2 × Taq PCR StarMix (GenStar, Beijing, China) (for PAGE), 1.5 µL of DNA template (about 50–100 ng), 1.5 µL of each InDel primer, and double-distilled H$_2$O. The PCR products were analyzed on 8% non-denaturing polyacrylamide gels with silver staining.

Validation and Mapping and Annotation of Putative Genes
Insertion-deletion (InDel) markers tightly linked to QSsv.cau-1A.1.1 and QSsv.cau-1A.1.2 were used to genotype DH, BC$_2$F$_1$, BC$_3$F$_2$-L, and BC$_3$F$_2$-N populations. The putatively annotated high confidence (HC) genes located between the flanking markers of QSsv.cau-1A.1.1 were acquired based on the reference genome of T. aestivum cv. Chinese Spring$^3$.

Statistical Analysis
To conduct phenotypic statistical analysis and calculate correlation coefficients between SSV and farinograph parameters, IBM SPSS Statistics 21.0 (SPSS, Chicago, United States) was used. R software v3.6.2$^4$ was used to perform the Shapiro-Wilk test across seven environments and the best linear unbiased prediction (BLUP), as well as to estimate the broad-sense heritability ($h^2B$) following the formula:

$$h^2_B = \frac{\sigma^2_h}{(\sigma^2_S + \sigma^2_G + n + \sigma^2_{ge})/nr},$$

as described by Liu et al. (2014).

RESULTS
Phenotypic Analysis
Descriptive statistics for SSV of two parents and the DH population in the seven environments are shown in Table 1. SSV of ND3753 was significantly higher than that of LX99 in all environments. SSV showed bi-directional transgressive

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1 http://www.isbreeding.net/software/?type=detail&id%20=18

2 http://bioinfo.ut.ee/primer3-0.4.0/

3 https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Annotations/v1.0/

4 https://www.r-project.org/

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**Table 1:** SSV of ND3753 was significantly higher than that of LX99 in all environments. SSV showed bi-directional transgressive...
TABLE 1 | Descriptive statistics of two parents and DH population for SDS-sedimentation volume (SSV) under seven environments.

| Trait  | Environment | ND3753 | LX99 | Range | Mean | SD | h2 B<sup>c</sup> |
|--------|-------------|--------|------|-------|------|----|----------------|
| SSV (mL) | E1          | 23.8 ± 0.9 | 20.0 ± 1.6 | 15.6–26.3 | 21.6 | 2.5 | 0.86 |
|        | E2          | 23.4 ± 2.6  | 19.4 ± 0.5  | 15.3–25.8 | 21.2 | 2.3 | 0.91 |
|        | E3          | 20.6 ± 0.8  | 15.8 ± 0.4  | 13.2–24.7 | 18.2 | 2.1 | 0.80 |
|        | E4          | 23.1 ± 1.4  | 16.4 ± 1.9  | 15.0–26.1 | 19.8 | 2.2 | 0.88 |
|        | E5          | 20.9 ± 3.3  | 17.2 ± 1.1  | 13.6–24.5 | 18.5 | 2.3 | 0.86 |
|        | E6          | 21.0 ± 0.4  | 14.2 ± 0.8  | 12.0–25.3 | 17.5 | 2.5 | 0.86 |
|        | E7          | 22.4 ± 0.4  | 17.0 ± 0.4  | 13.7–24.4 | 19.3 | 2.4 | – |

<sup>a</sup>E1, 2016–2017 (Beijing); E2, 2017–2018 (Beijing); E3, 2017–2018 (Linfen); E4, 2017–2018 (Xi’an); E5, 2018–2019 (Beijing); E6, 2018–2019 (Linfen); E7, 2018–2019 (Cangzhou).

<sup>b</sup>SD, standard deviation.

<sup>c</sup>h<sub>2</sub>B, Broad sense heritability based on a family mean basis was estimated under individual environments.

FIGURE 1 | Histograms of the ND3753/LX99 DH population for SDS-sedimentation volume (SSV) under BLUP data. The Y-axis represents the density (the ratio of frequency to group distance) of each trait and the X-axis represents the phenotypic data.

segregation, suggesting that both parents have increasing alleles for SSV. The broad-sense heritability values in all environments were greater than 0.8, indicating that SSV was mainly controlled by genetic factors. The result of the Shapiro-Wilk test displayed that SSV exhibited normal distribution under six environments and BLUP value, indicating SSV was determined by many genes (Figure 1). Pearson’s correlation coefficient analysis was carried out between farinograph parameters and SSVs of 30 DH lines planted in E5 which indicated a significant positive correlation between the two (Table 2).

Linkage Map Construction
A total of 2,523 SNP markers showed polymorphisms between the two parents. Finally, 2,474 SNP markers participated in the map construction and were mapped to 21 linkage groups, covering the 21 chromosomes of common wheat (Supplementary Table 1). The total length of the map was 7,349.01 cm, and the average interval distance between two adjacent markers was 7.24 cm (Supplementary Table 1). The A genome contained the most SNP markers (963), followed by the B genome (902), while the D genome had the least (609) (Supplementary Table 1). The total length of chromosome 7D...
was the largest (619.02 cm), while that of chromosome 4B was the smallest (180.86 cm) (Supplementary Table 1). Chromosome 5A harbored the most SNP markers (236), while chromosome 6D contained the least (42) (Supplementary Table 1).

Quantitative Trait Locus Analysis
A total of 15 QTLs were detected on 11 chromosomes (1A, 1B, 1D, 2A, 2D, 4B, 4D, 5A, 5D, 6B, and 6D) in the seven environments (Table 3 and Supplementary Table 2). Four environmentally stable QTLS (QSsv-cau-1A.1, QSsv-cau-1A.2, QSsv-cau-2D and QSsv-cau-5D.1) were identified on chromosomes 1A, 1A, 2D, and 5D, respectively (Table 3). The favorable allele of QSsv-cau-2D came from LX99, while the superior alleles of the other three QTLs were contributed from ND3753. The major QTL QSsv-cau-1A.1 was repeatedly detected in five environments and the BLUP data, explaining 39.67% of the phenotypic variation in the BLUP analysis (Table 3). QSsv-cau-2D and QSsv-cau-5D.1 contributed 3.17 and 4.82% of the phenotypic variation in the BLUP analysis, respectively. QSsv-cau-1A.2 explained 8.17–18.62% of the phenotypic variation. The remaining 11 were putative QTLs (Supplementary Table 2).

Verification of QSsv-cau-1A.1
Considering its stability and the genetic effect, QSsv-cau-1A.1 was chosen to saturate the positioning interval. Then 15 InDel markers near this region were developed according to the resequencing results of two parents (Table 3 and Supplementary Table 3) and a new genetic linkage map of 1A long arm was constructed. QTL remapping detected an additional QTL located next to the original interval. These two QTLs were named QSsv-cau-1A.1.1 and QSsv-cau-1A.1.2 (Figure 2).

QSsv-cau-1A.1.1 was detected in six environments and BLUP, explaining 17.21–26.47% of the phenotypic variation, and the favorable allele was from ND3753. The confidence interval was between the markers 1A248 and 1A332 corresponding CS physical position of 386,222,224–463,923,653 bp (Appels et al., 2018; Figure 2 and Supplementary Table 5). QSsv-cau-1A.1.2 was repeatedly detected in three environments as well as BLUP data, contributing 7.02–12.13% of the phenotypic variation and LX99 contributed the favorable allele. The physical position of QSsv-cau-1A.1.2 located on 462,634,655–492,004,197 bp by comparing flanking markers 1A36 and 1A215 to CS RefSeqv1.0 (Appels et al., 2018; Figure 2 and Supplementary Table 5).

Effects of QSsv-cau-1A.1.1 and QSsv-cau-1A.1.2 in Different Genetic Backgrounds
The flanking markers 1A248, 1A332 and 1A36, 1A215 delimiting confidence intervals of QSsv-cau-1A.1.1 and QSsv-cau-1A.1.2 (Figure 2) separately were used to detect genotypes in the DH population.
FIGURE 2 | Genetic and physical locations of QSsv.cau-1A.1.1 and QSsv.cau-1A.1.2. (A) QTL mapping for SSV in seven individual environments (E1–E7) and BLUP using a saturated genetic map of chromosome arm 1AL. (B) Saturated genetic map of chromosome arm 1AL with newly integrated InDel markers in the DH population. The black and red rectangles indicate QTLs with positive alleles from the parent ND3753 and the parent LX99, respectively. The black horizontal lines and bars above the genetic map represent the confidence interval of the two QTLs. (C) Corresponding physical positions according to the Chinese Spring IWGSC RefSeq v1.0 sequence.

and BC\textsubscript{3}F\textsubscript{2}–N populations. AA and aa represented genotypes with homozygous favorable and unfavorable alleles of QSsv.cau-1A.1.1 from ND3753, respectively, whereas BB and bb were symbols of that of QSsv.cau-1A.1.2 from LX99.

DH lines could be grouped into three genotypes which included two parental genotypes AAbb, aaBB, and one recombined genotype aabb, and each contained 51, 59, and 13 lines. The reason for the absence of genotype AABB remained unknown. The average SSV values in BLUP of genotype AAbb remained unknown. The average SSV values in BLUP of genotype AAbb, aaBB, and aabb were 21.2, 19.9, and 17.8 mL, respectively, of which AAbb was significantly higher than aaBB and both were significantly higher than that of aabb (Figure 3A). This suggested that QSsv.cau-1A.1.1 had a stronger effect on SSV than QSsv.cau-1A.1.2, which was consistent with their contribution rates of phenotypic variation and additive effects in QTL analysis.

In BC\textsubscript{3}F\textsubscript{2}–N population four allele combinations AaBB, AAbb, aaBB, and aabb were identified, which had SSV average values of 20.4, 17.5, 17.9, and 15.8 mL from 2, 23, 20, and 19 plants, respectively. Similarly, the SSV values of genotypes AaBB, AAbb, and aaBB were significantly higher than that of aabb (Figure 3B). However, although genotype AaBB had the distinct highest value, three genotypes with favorable alleles had no significant difference. This was possibly due to the too-small sample number of genotypes AaBB. Nevertheless, all results above could still prove that the favorable allele has positive effects.

This study did not find the combination type of AABB but found the type AaBB in the BC\textsubscript{3}F\textsubscript{2}–N population. In summary, the combination of two favorable loci had the high SSV, whereas recombinants with unfavorable loci had the lowest. In future research, the homozygous lines of the BC\textsubscript{3}F\textsubscript{2}–N population will be extracted to develop the corresponding NIL pairs to further verify their effects and examine their genetic effect on some wheat qualities, such as gluten content, extensograph, farinograph, alveograph, and gluten index.

Mapping of QSsv.cau-1A.1.1

In order to further verify and narrow down the confidence interval of QSsv.cau-1A.1.1, the BC\textsubscript{3}F\textsubscript{1} population in the
FIGURE 3 | The phenotypic effect of QSsv.cau-1A.1.1 and QSsv.cau-1A.1.2 in the DH and BC3F2-N populations according to the BLUP value for SSV and the means of SSVs of different types, respectively. (A) DH population; (B) BC3F2-N population; *, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels, respectively (Student’s t-test). aabb: QSsv.cau-1A.1.1 carrying homozygous alleles from LX99 and QSsv.cau-1A.1.2 carrying homozygous alleles from ND3753; aaBB: QSsv.cau-1A.1.1 carrying homozygous alleles from LX99 and QSsv.cau-1A.1.2 carrying homozygous alleles from LX99; AAbb: QSsv.cau-1A.1.1 carrying homozygous alleles from ND3753 and QSsv.cau-1A.1.2 carrying homozygous alleles from ND3753; AaBB: QSsv.cau-1A.1.1 carrying heterozygous alleles and QSsv.cau-1A.1.2 carrying homozygous alleles from LX99. The numbers in parentheses indicate sample size.

DISCUSSION

Correlation of Sodium Dodecyl Sulfate-Sedimentation Volume and Farinograph Parameters

Sodium dodecyl sulfate (SDS)-sedimentation volume (SSV) is a comprehensive indicator for indirectly testing wheat quality and one of the important tests to evaluate the gluten strength of flour and is closely related to the processing and baking quality of flour (Axford et al., 1978; Peña-Bautista, 2002; He et al., 2004). SSV is well correlated with other quality traits, such as grain protein content, gluten index, wet gluten content, bread volume, and farinograph parameters (Cubadda et al., 1992).

Our results showed that SSV was significantly positively correlated with stability time, formation time, and water absorption of dough, which is consistent with previous studies (Table 2; Cubadda et al., 1992). This confirms that SSV can be used as a suitable substitute for farinograph indicators that require a great number of samples to evaluate the rheological properties of wheat dough, thereby indirectly measuring the baking and processing quality of wheat flour.
Novel Quantitative Trait Locus for Sodium Dodecyl Sulfate-Sedimentation Volume

We compared the physical locations of QTLs for SSV reported in previous studies and those revealed in this study (Li et al., 2009; Reif et al., 2011; Deng et al., 2015; Würschum et al., 2016; Yang et al., 2020). The physical position of QSsv.cau-1A.2 was agreed with that of a previously reported QTL controlling SSV (Yang et al., 2020). SSV was found to be affected by allelic variations at Glu-A1 (508,726,618–508,725,448 bp, RefSeqv1.0) and Glu-A3 (4,203,001–4,202,275 bp, RefSeqv1.0) loci in several previous studies (Li et al., 2009; Reif et al., 2011; Deng et al., 2015; Würschum et al., 2016). Some QTLs associated with SSV was reported on chromosome 1A. For instance, Yang et al. (2020) identified a QTL (540,660,000–544,610,000 bp, RefSeqv1.0) for SSV that is located on chromosome 1A by genome-wide association study (GWAS). However, the physical position of QSsv.cau-1A.1.1 (371,573,909–386,426,688 bp, RefSeqv1.0) and QSsv.cau-1A.1.2 (419,490,584–492,004,197 bp, RefSeqv1.0) did not overlap with those of the above-mentioned QTLs/genes, suggesting these two QTLs may be novel. Li et al. (2009) identified a QTL (470,230,000–570,420,000 bp, RefSeqv1.0) for SSV on chromosome 2D using a recombinant inbred line population. Four QTLs (16,340,000, 59,102,000, 615,470,000, 646,600,000 bp, RefSeqv1.0) controlling SSV were reported to be located on chromosome 2D by multi-locus GWAS (Yang et al., 2020). However, the physical position of these QTLs and that of QSsv.cau-2D (140,759,212–467,689,413 bp, RefSeqv1.0) were not consistent, indicating that QSsv.cau-2D may also be a novel QTL. Li et al. (2009) identified a QTL for SSV on chromosome 5DS. SSV was found to be affected by the allelic variation at the Pinb-D1 locus on chromosome 5DS in some previous studies (Li et al., 2009; Reif et al., 2011; Deng et al., 2015; Würschum et al., 2016). However, the physical locations of these QTLs/genes and QSsv.cau-5D.1 does not match, implying that QSsv.cau-5D.1 on chromosome 5DL may be a novel QTL.

Sodium dodecyl sulfate (SDS)-sedimentation volume (SSV) is a quantitative trait affected by both environmental and genetic factors; thus, some QTLs can only be detected in specific environments (Supplementary Table 2). We found 11 such QTLs located on chromosomes 1B, 1D, 2A, 4B, 4D, 5A, 5D, 6B, and 6D, which is consistent with previous results (Li et al., 2009; Kerfal et al., 2010; Reif et al., 2011; Deng et al., 2015; Würschum et al., 2016; Liu et al., 2017a; Mir Drikvand et al., 2018; Goel et al., 2019; Guo et al., 2020; Yang et al., 2020). The Glu-D1 gene, which is located in the interval of QSsv.cau-1D, may be a candidate gene for QSsv.cau-1D. QSsv.cau-4B.1 and QSsv.cau-4D was located next to the dwarf genes Rht-B1 and Rht-D1, respectively. Previous studies have also revealed QTL-enrichment areas near Rht-B1 and Rht-D1, which are associated with kernel size, kernel hardness, kernel protein, pasting properties, and mixing properties (Shanhong et al., 2001; Li et al., 2006; Wang et al., 2012, 2017; Patil et al., 2013; Zhang et al., 2013; Jin et al., 2016; Liu et al., 2017b).

However, there is not enough evidence to support the correlation between Rht-B1/D1 and SSV, and the gene that controls SSV near Rht-B1 and Rht-D1 has not been cloned. We hypothesized: (1) there may be other genes affecting quality traits...
Genetic Effects and Putative Annotated Genes of the Major Quantitative Trait Locus

In recent years, a large number of QTLs for SSV have been identified and characterized through GWAS and linkage analysis, and some of these QTLs are related to allelic variants of Glu-1, Glu-A3, Glu-B3, Glu-B1, and Pina-D1 (Ahn et al., 2014; Deng et al., 2015; Guo et al., 2020). However, most of the other QTLs have not been further verified or fine mapped. SSV is a typical quantitative trait with a complex genetic mechanism. The lack of information on the authenticity and genetic effects of these QTLs for SSV not only hinders the exploration of their genetic and molecular mechanisms but also fails to provide breeders with sufficient new high-quality genetic resources for wheat quality improvement. In this study, we verified the effects of QSsv.cau-1A.1.1 and QSsv.cau-1A.1.2 on SSV and the interaction between the two QTLs in the DH and BC3F2-N populations. Therefore, the InDel markers are closely linked to QSsv.cau-1A.1.1 and QSsv.cau-1A.1.2 developed in this study can be used by breeders to aggregate high-quality genes for wheat quality improvement.

In particular, QSsv.cau-1A.1.1 was delimited to an approximate 14.9 Mb between markers 1A1 and 1A366 (Figure 4). We conduct an orthologous analysis for the candidate region to predict HC genes in QSsv.cau-1A.1.1, but no ones are associated with SSV in Oryza sativa and Arabidopsis thaliana (Supplementary Table 6). This may be because Oryza sativa and Arabidopsis thaliana do not have gluten, and SSV is related to gluten strength in the common wheat.

DATA AVAILABILITY STATEMENT

The raw data of Nongda3753 and Liangxing99 presented in the study are deposited in the NCBI Sequence Read Archive repository, accession number PRJNA722149.

AUTHOR CONTRIBUTIONS

MY conceived the project. ST performed the research, constructed the linkage map, developed an InDel marker of the QTL region of interest, and developed the BC3F1 and BC3F2 populations. JL, MZ, and SW participated in the field trials. ST, CB, HZ, CW, ZC, JY, and XS performed the phenotypic analysis. YZ, CX, BL, RL, and QS assisted in revising the manuscript. ST and YZ analyzed the experimental results. ST and MY wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.747775/full#supplementary-material

REFERENCES

Addo, K., Pomeranz, Y., Huang, M. L., Rubenthaler, G. L., and Jeffers, H. C. (1991). Steamed Bread. II. Role of protein content and strength. Cereal Chem. 68, 39–42.

Ahn, J. H., Kang, C. S., Jeung, J. U., Baik, B. K., and Park, C. S. (2014). Effect of loci to predict HC genes in QSsv.cau-1A.1.1, but no ones are associated with SSV in Oryza sativa and Arabidopsis thaliana (Supplementary Table 6). This may be because Oryza sativa and Arabidopsis thaliana do not have gluten, and SSV is related to gluten strength in the common wheat.

Chen, K., Zhang, P., Deng, G., and Zou, C. (2011). Electromagnetic correlation attacks and the minimum number of attack traces analyzing. J. Huazhong Univ. Sci. Technol. 39:1009.

Clarke, J. M., Clarke, F. R., Ames, N. P., McCaig, T. N., and Knox, R. E. (2000). Evaluation of predictors of quality for use in early generation selection. Options Méditerr. Sér. A Sémin. Méditerranéens 40, 439–446.

Cubadda, R., Caracea, M., and Pasqui, L. A. (1992). Suitability of the Gluten Index method for assessing gluten strength in durum-wheat and semolina. Cereal. Foods World 37, 866–869.

Curtis, T., and Halford, N. G. (2014). Food security: the challenge of increasing wheat yield and the importance of not compromising food safety. Ann. Appl. Biol. 164, 354–372. doi: 10.1111/aab.12108

De Buysur, J., and Henry, Y. (1980). Induction of haploid and diploid plants though in vitro anther culture of haploid wheat (n=3x=21). Theor. Appl. Genet. 57, 57–58. doi: 10.1007/BF00475029

Deng, Z., Tian, J., Chen, F., Li, W., Zheng, F., Chen, J., et al. (2015). Genetic dissection on wheat flour quality traits in two related populations. Euphytica 203, 221–235. doi: 10.1007/s10681-014-1318-7

Doerge, R. W. (2002). Mapping and analysis of quantitative trait loci online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.747775/full#supplementary-material

Chen, K., Zhang, P., Deng, G., and Zou, C. (2011). Electromagnetic correlation attacks and the minimum number of attack traces analyzing. J. Huazhong Univ. Sci. Technol. 39:1009.

Clarke, J. M., Clarke, F. R., Ames, N. P., McCaig, T. N., and Knox, R. E. (2000). Evaluation of predictors of quality for use in early generation selection. Options Méditerr. Sér. A Sémin. Méditerranéens 40, 439–446.

Cubadda, R., Caracea, M., and Pasqui, L. A. (1992). Suitability of the Gluten Index method for assessing gluten strength in durum-wheat and semolina. Cereal. Foods World 37, 866–869.

Curtis, T., and Halford, N. G. (2014). Food security: the challenge of increasing wheat yield and the importance of not compromising food safety. Ann. Appl. Biol. 164, 354–372. doi: 10.1111/aab.12108

De Buysur, J., and Henry, Y. (1980). Induction of haploid and diploid plants though in vitro anther culture of haploid wheat (n=3x=21). Theor. Appl. Genet. 57, 57–58. doi: 10.1007/BF00475029

Deng, Z., Tian, J., Chen, F., Li, W., Zheng, F., Chen, J., et al. (2015). Genetic dissection on wheat flour quality traits in two related populations. Euphytica 203, 221–235. doi: 10.1007/s10681-014-1318-7

Doerge, R. W. (2002). Mapping and analysis of quantitative trait loci in experimental populations. Nat. Rev. Genet. 3, 43–52. doi: 10.1038/ nrg703
Elangovan, M., Rai, R., Dholakia, B. B., Lagu, M. D., Tiwari, R., Gupta, R. K., et al. (2008). Molecular genetic mapping of quantitative trait loci associated with loaf volume in hexaploid wheat (Triticum aestivum). J. Cereal Sci. 47, 587–598. doi: 10.1016/j.csc.2007.07.003

Gianinelli, M. C., Larroque, O. R., MacRitchie, F., and Wrigley, C. W. (2001). Biochemical, genetic, and molecular characterization of wheat glutenin and its component subunits. Cereal Chem. 78, 635–646. doi: 10.1094/CCHEM.2001.78.6.635

Goel, S., Singh, K., Singh, B., Grewal, S., Dwivedi, N., Alqarawi, A. A., et al. (2019). Analysis of genetic control and QTL mapping of essential wheat grain quality traits in a recombinant inbred population. PLoS One 14:e0200669. doi: 10.1371/journal.pone.0200669

Guo, Y., Zhang, G., Guo, B., Qu, C., Zhang, M., Kong, F., et al. (2020). QTL mapping for quality traits using a high-density genetic map of wheat. PLoS One 15:e0236001. doi: 10.1371/journal.pone.0236001

Guzman, C., Peña, R. J., Singh, R., Autrique, E., Dreisigacker, S., Crossa, J., et al. (2016). Wheat quality improvement at CIMMYT and the use of genomic selection on it. Appl. Transl. Genom. 11, 3–8. doi: 10.1016/j.atg.2016.10.004

He, Z. H., Yang, J., Zhang, Y., Quail, K. J., and Peña, R. J. (2004). Pan bread and dry white Chinese noodle quality in Chinese winter wheats. Euphytica 139, 257–267. doi: 10.1007/s10681-004-3283-z

Huang, X. Q., Cloutier, S., Lycar, L., Rodavonovic, N., Humphreys, D. G., Noll, J. S., et al. (2006). Molecular detection of QTLs for agronomic and quality traits in a doubled haploid population derived from two Canadian wheats (Triticum aestivum L.). Theor. Appl. Genet. 113, 753–766. doi: 10.1007/s00122-006-0346-7

ICC (1996). Standard Methods of The International Association for Cereal Science and Technology, Vienna: ICC.

Jin, H., Wen, W., Liu, J., Zhai, S., Zhang, Y., Yan, J., et al. (2016). Genome-wide QTL mapping for wheat processing quality parameters in a Gaosheng 8901/Zhoumai 16 recombinant inbred line population. Front. Plant Sci. 7:1032. doi: 10.3389/fpls.2016.01032

Kerfal, S., Giraldo, P., Rodriguez-Quijano, M., Vázquez, J. F., Adams, K., Lukow, G., et al. (2001). Mapping some seed quality traits in bread wheat (Triticum aestivum L.) by association mapping using six markers. J. Appl. Biotechnol. Rep. 5, 92–99. doi: 10.29252/JABR.05.03.02

Koskel, S., Tiftik, B., and Koksel, H. (2008). Predicting the cookie quality of flours by using Mixolab®. Eur. Food Res. Technol. 227, 1549–1554. doi: 10.1007/s00217-008-0879-x

Park, C. S., Kang, C. S., Cheong, Y. K., Jung, W., and Woo, S. H. (2010). Influence of puroindoline genotypes on grain characteristics, physico-chemical properties of flour and end-use quality of Korean wheats. Breed. Sci. 60, 233–242. doi: 10.1270/bibs.60.233

Park, C.-S., Kim, H.-S., Kim, D.-H., Hyun, J.-N., and Kang, C.-S. (2012). Environmental impacts of Korean and CIMMYT wheat lines on protein characteristics and bread making quality. Korean J. Crop Sci. 57, 60–70. doi: 10.7740/kjcs.2012.57.1.060

Patil, R. M., Tamhankar, S. A., Oak, M. D., Raut, A. L., Honrao, B. K., Rao, V. S., et al. (2013). Mapping of QTL for agronomic traits and kernel characters in durum wheat (Triticum durum Desf.). Euphytica 190, 117–129. doi: 10.1007/s10681-012-0785-y

Payne, P. I., Holt, L. M., Jackson, E. A., Law, C. N., and Damania, A. B. (1984). Wheat storage proteins: their genetics, and their potential for manipulation by plant breeding. Philos. Trans. R. Soc. Lond. B Biol. Sci. 304, 359–371. doi: 10.1098/rstb.1984.0031

Payne, P. I., and Lawrence, G. J. (1983). Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1, and Glu-D1 which code for high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res. Commun. 11, 29–35. doi: 10.1016/j.jcres.2014.01.020

Peña-Batista, R. J. (2002). “Wheat for bread and other foods,” in Bread Wheat: Improvement and Production, eds B. C. Curtis, S. Rajaram, and H. G. Macpherson (Rome: FAO Plant Production and Protection Series).

Preston, K. R., March, P. R., and Tipple, K. H. (1982). An assessment of the SDS-sedimentation test for the prediction of canadian bread wheat quality. Can. J. Plant Sci. 62, 545–553. doi: 10.4141/cps82-083

Rasheed, A., Xia, Y., Yan, Y., Appel, R., Mahmood, T., and He, Z. (2014). Wheat seed storage proteins: advances in molecular genetics, diversity and breeding applications. J. Cereal Sci. 60, 11–24. doi: 10.1016/j.jcs.2014.01.020

Reif, J. C., Gowda, M., Maurer, H. P., Longin, C. F. H., Korzun, V., Elbeyr, E., et al. (2011). Association mapping for quality traits in soft winter wheat. Theor. Appl. Genet. 122, 961–970. doi: 10.1007/s00122-010-1502-7

Rubenthaler, G. L., Huang, M. L., and Pomeranz, Y. (1990). Steamed bread. I. Chinese steamed bread formulation and interactions. Cereal Chem. 67, 471–475.

Shanhong, W., Fanhua, M., Li, Y., and Binghua, L. (2001). The effect of dwarf genes on agronomic characters in wheat. J. Triticeae Crop. 21, 5–9.

Shewry, P. R., Halford, N. G., and Lafandra, D. (2003). Genetics of wheat gluten proteins. Adv. Genet. 49, 111–184. doi: 10.1016/S0005-2660(03)01003-4

Slade, L., and Levine, H. (1994). Structure-function relationships of cookie and cracker ingredients. Sci. Cookie Cracker Prod. 9, 23–141.

Tiao, T. J., Simsek, S., Ohm, J. B., Harelend, G. A., Chao, S., and Anderson, J. A. (2011). Quantitative trait loci influencing endosperm texture, dough-mixing
strength, and bread-making properties of the hard red spring wheat breeding lines. *Genome* 54:460470. doi: 10.1139/g11-012

Van Der Borght, A., Goesaert, H., Veraverbeke, W. S., and Delcour, J. A. (2005). Fractionation of wheat and wheat flour into starch and gluten: overview of the main processes and the factors involved. *J. Cereal Sci.* 41, 221–237. doi: 10.1016/j.jcs.2004.09.008

Veraverbeke, W. S., and Delcour, J. A. (2002). Wheat protein composition and properties of wheat glutenin in relation to breadmaking functionality. *Crit. Rev. Food Sci. Nutr.* 42, 179–208. doi: 10.1080/10408690290825510

Voorrips, R. E. (2002). MapChart: software for the graphical presentation of linkage maps and QTLs. *J. Hered.* 93, 77–78. doi: 10.1093/jhered/93.1.77

Wang, F., Liu, T., Li, Q., An, Y., Xie, C., Sun, X., et al. (2017). QTL mapping of the pasting properties of wheat flour treated by papain digestion. *Starch Stärke* 69:1600077. doi: 10.1002/star.201600077

Wang, L., Cui, F., Wang, J., Jun, L., Ding, A., Zhao, C., et al. (2012). Conditional QTL mapping of protein content in wheat with respect to grain yield and its components. *J. Genet.* 91, 303–312. doi: 10.1007/s12041-012-0190-2

Würschum, T., Leiser, W. L., Kazman, E., and Longin, C. F. H. (2016). Genetic control of protein content and sedimentation volume in European winter wheat cultivars. *Theor. Appl. Genet.* 129, 1685–1696. doi: 10.1007/s00122-016-2732-0

Yang, Y., Chai, Y., Zhang, X., Lu, S., Zhao, Z., Wei, D., et al. (2020). Multi-Locus GWAS of quality traits in bread wheat: mining more candidate genes and possible regulatory network. *Front. Plant Sci.* 11:1091. doi: 10.3389/fpls.2020.01091

Yang, Y., Li, S., Zhang, K., Dong, Z., Li, Y., An, X., et al. (2014). Efficient isolation of ion beam-induced mutants for homoeologous loci in common wheat and comparison of the contributions of Glu-1 loci to gluten functionality. *Theor. Appl. Genet.* 127, 359–372. doi: 10.1007/s00122-013-2224-4

Zhang, J., Dell, B., Biddulph, B., Drake-Brockman, F., Walker, E., Khan, N., et al. (2013). Wild-type alleles of Rht-B1 and Rht-D1 as independent determinants of thousand-grain weight and kernel number per spike in wheat. *Mol. Breed.* 32, 771–783. doi: 10.1007/s11032-013-9905-1

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