High-Resolution Detection of Quantitative Trait Loci for Seven Important Yield Components in Wheat (*Triticum aestivum* L.) using a High-Density SALF-Seq Genetic Map

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Research Article

Keywords: wheat, yield, yield components, specific-locus amplified fragment (SALF), linkage analysis.

Posted Date: August 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-783221/v1>

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Abstract

Background: Yield-related traits including thousand grain weight (TGW), grain number per spike (GNS), grain width (GW), grain length (GL), plant height (PH), spike length (SL), and spikelet number per spike (SNS) are greatly associated with wheat (*Triticum aestivum* L.) grain yield. To detect quantitative trait loci (QTL) associated with them, 193 recombinant inbred lines derived from two elite winter wheat varieties Chuanmai42 and Chuanmai39 were employed to perform QTL mapping in six or eight environments.

Results: A total of 30 QTLs on chromosomes 1A, 1B, 1D, 2A, 2B, 2D, 3A, 4A, 5A, 5B, 6A, 6D, 7A, 7B and 7D were identified. Among them, six major QTLs *QTgw.cib-6A.1*, *QTgw.cib-6A.2*, *QGw.cib-6A*, *QGL.cib-3A*, *QGI.cib-6A*, and *QSL.cib-2D* explaining 5.96–23.75% of the phenotypic variance were detected in multiple environments and showed strong and stable effects on corresponding traits. Three QTL clusters on chromosomes 2D and 6A containing 10 QTLs were also detected, which showed significant pleiotropic effects on multiple traits. Additionally, three Kompetitive Allele Specific PCR (KASP) markers linked to five of these major QTLs were developed. Candidate genes of *QTgw.cib-6A.1/QGI.cib-6A* and *QGL.cib-3A* were analyzed based on the spatiotemporal expression patterns, gene annotation, and orthologous search.

Conclusions: Six major QTLs for TGW, GL, GW and SL were detected. Three KASP markers linked with five of these major QTLs were developed. These QTLs and KASP markers will be useful for elucidating the genetic architecture of grain yield and developing new wheat varieties with high and stable yield in wheat.

Background

Common wheat (*Triticum aestivum* L.) is one of the three major crops worldwide and provides approximately 30% of global grain production and 20% of the calories consumed for humans [1]. Due to ongoing decrease of the global arable cultivated land area and increase of the population, the current rate of wheat yield increase will be insufficient to meet the future demand. The development of high-yield varieties, one of the important targets of modern wheat breeding programs worldwide, thus must be accelerated to fulfill future global food and nutrition security [2].

Wheat yield is a complex quantitative trait controlled by multiple genes and significantly influenced by interacting genetic and environmental factors [3, 4]. By contrast, yield components including thousand grain weight (TGW), grain number per spike (GNS), grain width (GW), grain length (GL), plant height (PH), spike length (SL) and spikelet number per spike (SNS) typically show higher heritability than that of the yield [5–7]. Therefore, targeting these traits and identifying the related genes or quantitative trait loci (QTL) is an important approach to improve grain yield potential in wheat.

To date, several genes associated with wheat yield have been mapped and cloned in wheat. For example, the application of semi-dwarfing genes *Rht-B1b* and *Rht-D1b* not only effectively improved the lodging resistance but also improved the harvest index, resulting in increased yields since the 1970s [8–10]. The vernalization insensitive alleles of *Vrn-1* (*Vrn-A1*, *Vrn-B1*, and *Vrn-D1*) shorten both the vegetative and the reproductive stages and have considerable impact on spike morphological traits [11, 12]. *TaGW2* homologous to rice *GW2* was located on chromosome 6A and could alter grain width and weight and to lesser extend grain length owing to changes in the width of the spikelet hull [13, 14]. The grain-shape gene *TasgD1* encoding a Ser/Thr protein kinase glycogen synthase kinase3 and independently control semispherical grain trait [15]. A jasmonic acid synthetic gene keto-acyl thiolase 2B was cloned in a TGW mutant and showed significant effect on TGW and GW [16].

Before the gene cloning, quantitative trait loci (QTL) mapping provides an effective approach to depict the genetic architecture of complex quantitative traits. Over the past decades, numerous QTLs associated with yield or yield components have been identified on all wheat chromosomes [3, 4, 11, 17–23]. For example, *Rht8* located on chromosome 2DS was closely linked with marker *xfdc53* and reduced plant height by 10% [24]; *Rht25* on wheat chromosome arm 6AS showed pleiotropic effects on coleoptile length, heading date, SL, SNS and grain weight [25]. Two major QTLs for grain size and weight were detected on chromosome 4B, which together explained 46.3% of the phenotypic variance [26, 27]. Five stable QTLs for PH, SL and HD on chromosomes 1A, 2A, 2D and 6A were detected in an introgression line population [28]. Twelve major genomic regions with stable QTL controlling yield-related traits were detected on chromosomes 1B, 2A, 2B, 2D, 3A, 4A, 4B, 4D, 5A, 6A, and 7A [1]. However, among those QTLs reported previously, few of them were stably detected in multienvironments, which greatly restricts their potential utilization in marker-assisted selection (MAS) in breeding programs. Additionally, the lower density of genetic maps also limits the resolution and accuracy of the QTL intervals reported previously.

In the present study, a high-resolution genetic map constructed through specific-locus amplified fragment (SALF) sequencing was adopted for detection of yield component QTLs in a recombinant inbred line (RIL) population derived from two elite winter wheat varieties Chuanmai42 (CM42) and Chuanmai39 (CM39) [29]. Seven traits including TGW, GW, GL, PH, GNS, SL and SNS were assessed in multiple environments to detect potential major and stable QTL, which will lay out a foundation for further study on fine mapping and cloning of the underlying key genes for yield.

Results

Phenotypic variation

The measured traits of the CM42×CM39 RILs as well as the two parents are listed in Table 1. CM42 had higher trait values for TGW, GW, GL, GNS, PH and SL than those of CM39 in each of environments and the best linear unbiased prediction (BLUP) datasets. In the RIL population, all traits showed wide and significant variations in all environments and the BLUP datasets (Table 1). Of them, the TGW ranges from 20.81 to 72.7 gram (g), the GW ranged from 2.6 to 4.21 millimeter (mm), the GL ranged from 5.88 to 8.81 mm, the PH ranged from 65.08 to 148.3 centimeter (cm), the GNS ranged from 24 to 81.2, the SL ranged from 6.65 to 18.17 cm, and the SNS ranged from 15.83 to 27 (Table 1), respectively. The BLUP datasets of all traits showed normal distributions in the RIL lines, which suggests polygenic inheritance of these traits (Fig. 1A). Additionally, the TGW, GL, PH, GNS and SL showed high broad-sense heritability of

0.54, 0.6, 0.91, 0.66 and 0.88, respectively (Table 1). Significant and positive correlations ($P < 0.01$) of the measured traits among all environments and the BLUP datasets were detected, which suggested that these traits were environmentally stable and mainly controlled by genetic factors (Table S2).

Table 1

Phenotypic variation of seven yield components, including thousand grain weight (TGW), grain number per spike (GNS), grain width (GW), grain length (GL), plant height (PH), spike length (SL) and spikelet number per spike (SNS), for the parents and the CM42×CM39 RIL lines in different environments.

Traits	Environments	Prarents		The CM42×CM39 RIL lines				
		CM42	CM39	Range	Mean	SD	CV (%)	H ²
TGW	2017SHF	54	52.94	38.34-70.88	58.57	5.84	9.98	0.54
	2017SHL	50.64	41.83	20.81-68.14	43.76	9.17	20.95	
	2018SHF	54.79	53.47	40.44-72.7	54.67	5.58	10.2	
	2018SHL	53.06	51.29	37.89-67.33	54.51	5.57	10.22	
	2019SHF	52.4	42.42	32.59-66.54	51.27	5.94	11.59	
	2019SHL	51.05	47.38	23.4-62.74	46.9	6.22	13.27	
	BLUP	52.36	50.44	38.24-62.56	51.65	3.98	7.7	
GW	2017SHF	3.68	3.42	3.19-4.21	3.82	0.16	4.28	0.49
	2017SHL	3.54	3.31	2.6-4.01	3.38	0.29	8.57	
	2018SHF	3.58	3.53	3.19-4.04	3.69	0.16	4.35	
	2018SHL	3.63	3.61	3.15-3.96	3.65	0.15	4.14	
	2019SHF	3.6	3.16	3-3.9	3.5	0.18	5.21	
	2019SHL	3.56	3.49	2.84-3.99	3.49	0.19	5.37	
	BLUP	3.59	3.51	3.21-3.87	3.59	0.11	3.06	
GL	2017SHF	7.73	7.17	6.78-8.81	7.76	0.41	5.26	0.6
	2017SHL	6.95	6.53	5.94-7.89	6.86	0.37	5.39	
	2018SHF	6.87	6.72	5.89-7.92	6.95	0.37	5.3	
	2018SHL	7.64	6.55	5.88-7.81	6.85	0.37	5.45	
	2019SHF	7.32	6.43	6-7.71	6.86	0.33	4.84	
	2019SHL	7.22	6.67	6.03-7.71	6.94	0.36	5.15	
	BLUP	7.27	6.98	6.19-7.75	7.04	0.3	4.26	
PH	2016SHF	90.34	89.5	66.5-120.3	91.53	9.5	10.38	0.91
	2016SHL	89.8	87.2	76-148.3	95.97	10.49	10.93	
	2017SHF	97.67	96.33	81.33-143	103.3	10.65	10.31	
	2017SHL	99	98.8	66.63-121.2	91.39	9.73	10.65	
	2018SHF	91.7	87.08	65.08-131.9	93.9	11.82	12.59	
	2018SHL	94.61	90	70.8-135.4	95.57	11.32	11.84	
	2019SHF	90.05	85.9	69.45-126.8	98.74	9.89	10.02	
GNS	2019SHL	93.33	89.3	78.5-127.4	97.58	8.98	9.21	0.66
	BLUP	93.24	91.91	74.65-127.5	96	9.14	9.52	
	2017SHF	54	52	24-81.2	51.01	10.39	20.38	
	2017SHL	44.5	43.6	26-77	41.94	8.08	19.27	
	2018SHF	54.6	49.9	31.6-70.8	45.62	6.11	13.4	
	2018SHL	54.5	54.1	35.3-70.8	52.07	7.18	13.78	
	2019SHF	55.7	53.7	35.2-84.6	53.66	8.18	15.24	
SL	2019SHL	56.5	56.2	35.5-75.8	53.77	7.07	13.15	0.88
	BLUP	53.17	52.44	37.76-66.18	49.85	4.62	9.26	

SHF, Shifang; SHL, Shuangliu, BLUP, best linear unbiased prediction; CV, coefficient of variation; H², broad-sense heritability

Traits	Environments	Prarents		The CM42×CM39 RIL lines				
		CM42	CM39	Range	Mean	SD	CV (%)	H ²
	2016SHL	12.1	9	6.65-14	10.53	1.61	15.33	
	2017SHF	13.5	11.5	8.5-17.88	13.04	1.73	13.23	
	2017SHL	13	11.5	8.33-17.67	12.93	1.88	14.51	
	2018SHF	11.85	9.26	7.63-14.93	11.82	1.84	15.54	
	2018SHL	13.02	10.9	7.55-15.7	11.3	1.72	15.18	
	2019SHF	13.71	11.2	8.89-18.17	13.25	1.87	14.15	
	2019SHL	12.4	10.5	8.5-16.3	12.51	1.56	12.51	
	BLUP	12.71	11.6	8.45-15.69	12.31	1.5	12.22	
SNS	2017SHF	18.6	19.6	16.2-25	19.58	1.39	7.08	0.4
	2017SHL	21.2	21.2	18-27	21.4	1.63	7.63	
	2018SHF	21.9	21.5	17.7-24.5	21.66	1.13	5.2	
	2018SHL	20.9	20.7	17.9-25.2	21.02	1.22	5.81	
	2019SHF	21.7	21.2	17.9-25	21.29	1.2	5.63	
	2019SHL	17.2	18.1	15.83-21.2	18.35	1.04	5.67	
	BLUP	20.3	20.35	18.42-22.96	20.55	0.84	4.1	
SHF, Shifang; SHL, Shuangliu, BLUP, best linear unbiased prediction; CV, coefficient of variation; H ² , broad-sense heritability								

Correlation analyses among different traits

The BLUP datasets of each trait was employed to assess their correlations in the CM42×CM39 RIL population. TGW had significantly positive correlation with GW, GL, PH and SL, and significantly negative correlation with GNS and SNS ($P < 0.001$) (Fig. 1). GW was significantly and positively correlated with GL ($P < 0.001$), weakly and positively correlated with SL ($P < 0.05$), significantly and negatively correlated with GNS and SNS ($P < 0.001$), and not correlated with PH, respectively (Fig. 1). GL had significantly positive correlation with PH and SL ($P < 0.001$), significantly negative correlation with GNS ($P < 0.001$), and weakly negative correlation with SNS ($P < 0.05$) (Fig. 1). Moreover, significantly positive correlations between PH and SL, GNS and SNS, and SL and SNS ($P < 0.001$), weakly positive correlations between PH and SNS ($P < 0.05$), significantly negative correlations between PH and GNS ($P < 0.001$), and no correlations between GNS and SL were detected, respectively (Fig. 1).

QTL detection

Phenotypic data of all traits evaluated in each environment and the BLUP datasets were used for QTL detection, in which the BLUP datasets were treated as an additional environment. A total of 30 QTLs were identified in multiple environments and located on all chromosomes excepting 3B, 3D, 4B, 4D, 5D and 6B (Table 2).

For TGW, two QTLs were detected on chromosomes 6A. *QTgw.cib-6A.1* was detected in two environments and the BLUP datasets, explaining 9.89–16.38% of the phenotypic variance. *QTgw.cib-6A.2* was a major QTL detected in four environments and the BLUP datasets and explained 15.31–23.75% of the phenotypic variance. Alleles of CM42 for the two QTLs contributed to higher TGW (Table 2).

For GW, six QTLs were identified on chromosomes 2A, 2B, 5A, 6A and 7B. Of them, a major QTL *QGw.cib-6A* was identified in four environments and the BLUP datasets, explaining 8.6-23.31% of the GW variation. The allele of CM42 contributed positively to the GW. The rest five minor QTLs were identified in two environments and explained 5.2–9.89% of the GW variation. The favorable alleles of *QGw.cib-2A* and *QGw.cib-5A* were contributed by CM39, and that of *QGw.cib-2B.1*, *QGw.cib-2B.2* and *QGw.cib-7B* were contributed by CM42 (Table 2).

Among the six QTLs for GL, two major QTL *QGL.cib-3A* and *QGL.cib-6A* were identified in five environments and the BLUP datasets, explaining 6.55–11.86% and 5.96–13.11% of the GL variation, respectively. The positive additive effects of the two QTLs on GL were contributed by CM42. The rest four minor QTLs were identified in two or three environments on chromosomes 5A, 6D and 7D, explaining 5.17–11.34% of the GL variation. The positive alleles of *QGL.cib-5A.1*, *QGL.cib-5A.2* and *QGL.cib-7D* were derived from CM42, and that of *QGL.cib-6D* was from CM39 (Table 2).

Among the six QTLs for PH, *QPh.cib-2D* on chromosome 2D was a stable QTL and detected in five environments and the BLUP datasets, explaining 4.54–9.38% of the PH variation. The allele of CM39 contributed to higher PH. The rest five minor QTLs on chromosomes 1A, 4A, 5A, 5B and 6A were detected in two or three environments, explaining 3.8-11.37% of the PH variation. The positive alleles of *QPh.cib-1A* and *QPh.cib-5B* were from CM39, and that of *QPh.cib-4A*, *QPh.cib-5A* and *QPh.cib-6A* were from CM42 (Table 2).

Two minor QTLs for GNS on chromosomes 2D and 6A were detected in two environments and the BLUP datasets and explained 4.97–6.46% and 6.56–7.73% of the GNS variation, respectively. Alleles from CM42 and CM39 at *QGns.cib-2D* and *QGns.cib-6A*, respectively, contributed to positive effects on GNS (Table 2).

For SL, four QTLs were detected on chromosomes 2D, 5A, 5B and 6A. A major QTL *QSl.cib-2D* was detected in eight environments and the BLUP datasets, explaining 6.18–14.89% of the SL variation. *QSl.cib-5B* was a stable QTL and detected in three environments and the BLUP datasets, explaining 3.79–5.96% of the SL variation. Alleles of CM39 for the two QTLs contributed to increase of SL. Two minor QTLs *QSl.cib-5A* and *QSl.cib-6A* were detected in two or three environments, explaining 3.47–7.8% and 5.63–5.9% of the SL variation, respectively. The positive alleles of the two QTLs were contributed by CM42 (Table 2).

Four QTLs for SNS were identified on chromosomes 1B, 1D, 4A and 7A. Of them, *QSns.cib-1B* and *QSns.cib-4A* were detected in three environments and the BLUP datasets, explaining 7.47–16.18% and 2.34–10.46% of the SNS variation, respectively. *QSns.cib-1D* and *QSns.cib-7A* were detected in two environments, explaining 6.77–8.39% and 5.06–8.18% of the SNS variation, respectively. The favorable alleles of *QSns.cib-1B* and *QSns.cib-7A* were contributed by CM39, and that of *QSns.cib-1D* and *QSns.cib-4A* were contributed by CM42 (Table 2).

Table 2

Quantitative trait loci (QTLs) for thousand grain weight (TGW), grain number per spike (GNS), grain width (GW), grain length (GL), plant height (PH), s

Trait	QTL	Environment	Chromosome	Interval (cM)	Flanking Markers	LOD
TGW	<i>QTgw.cib-6A.1</i>	18SHF/18SHL/BLUP	6A	41.3-42.46	Marker87546- Marker87736	6.17/8.03/4.44
	<i>QTgw.cib-6A.2</i>	17SHF/18SHL/19SHF/19SHL/BLUP	6A	52.98-59.52	Marker90290- Marker91587	9.48/7.95/7.27/1
GW	<i>QGw.cib-2A</i>	18SHF/19SHF	2A	14.86-17.08	Marker26336- Marker26958	5.48/2.76
	<i>QGw.cib-2B.1</i>	19SHF/19SHL	2B	39.6-43.07	Marker29502- Marker29525	2.66/4.09
	<i>QGw.cib-2B.2</i>	17SHF/BLUP	2B	121.67-121.93	Marker34419- Marker34417	3.83/3.7
	<i>QGw.cib-5A</i>	17SHF/BLUP	5A	27.76-27.97	Marker70243- Marker70216	3.91/4.73
	<i>QGw.cib-6A</i>	17SHF/17SHL/18SHL/19SHF/19SHL/BLUP	6A	49.98-58.87	Marker90210- Marker91133	13.09/4.95/8.93,
	<i>QGw.cib-7B</i>	18SHF/19SHL	7B	179.93-180.13	Marker111000- Marker110965	5.36/5.98
	<i>QGL.cib-3A</i>	17SHF/17SHL/18SHL/19SHF/19SHL/BLUP	3A	64.7-66.41	Marker40793- Marker40901	5.31/2.97/6.1/5.
GL	<i>QGL.cib-5A.1</i>	17SHL/BLUP	5A	3.46-7.55	Marker69377- Marker69395	2.83/3.66
	<i>QGL.cib-5A.2</i>	18SHL/19SHF	5A	86.87-87.49	Marker71923- Marker71919	3.79/3.82
	<i>QGL.cib-6A</i>	17SHF/18SHF/18SHL/19SHF/19SHL/BLUP	6A	42.36-43.4	Marker87807- Marker87738	5.32/4.62/7.72/3
	<i>QGL.cib-6D</i>	18SHL/19SHF/BLUP	6D	76.06-83.69	Marker99119- Marker99140	4.17/5/3.17
	<i>QGL.cib-7D</i>	17SHF/17SHL/BLUP	7D	32.68-38.76	Marker111521- Marker111597	3/4.64/6.19
	<i>QPh.cib-1A</i>	16SHF/17SHL/19SHF	1A	28.34-30.95	Marker5758- Marker6328	5.58/6.07/3.64
	<i>QPh.cib-2D</i>	16SHF/17SHL/18SHF/18SHL/19SHF/BLUP	2D	1.48-5.16	Marker35344- Marker35422	3.42/4.31/3/4.7/
PH	<i>QPh.cib-4A</i>	16SHF/17SHL	4A	82.78-83.05	Marker57956- Marker57959	4.76/5.71
	<i>QPh.cib-5A</i>	17SHF/19SHL/BLUP	5A	126.27-126.52	Marker72631- Marker72950	3.02/2.91/2.52
	<i>QPh.cib-5B</i>	16SHF/17SHL	5B	134.43-134.74	Marker83905- Marker83879	3.07/3.18
	<i>QPh.cib-6A</i>	16SHF/17SHL/19SHF	6A	54.61-54.76	Marker90459- Marker90388	6.13/8.86/5.6
	<i>QGns.cib-2D</i>	18SHF/19SHF/BLUP	2D	0-5.16	Marker35164- Marker35422	2.57/2.63/4.73
	<i>QGns.cib-6A</i>	18SHL/19SHF/BLUP	6A	56.45-59.52	Marker90628- Marker91587	3.35/3.83/4.91
SL	<i>QSl.cib-2D</i>	16SHF/16SHL/17SHF/17SHL/18SHF/18SHL/19SHF/19SHL/BLUP	2D	1.48-5.16	Marker35344- Marker35422	3.42/6.86/6.82/8
	<i>QSl.cib-5A</i>	18SHL/19SHF/BLUP	5A	17.71-21.48	Marker69427- Marker69525	2.59/6/2.61
	<i>QSl.cib-5B</i>	16SHF/16SHL/17SHF/BLUP	5B	40.07-40.38	Marker81580- Marker81513	2.99/3.06/2.87/2
	<i>QSl.cib-6A</i>	19SHF/BLUP	6A	58.87-64.37	Marker91133- Marker91933	4.62/3

PVE, mean of phenotypic variation explained; LOD, logarithm of the odd; Add, mean of additive effect (Positive values indicate that alleles from CM39 are in prediction.

Trait	QTL	Environment	Chromosome	Interval (cM)	Flanking Markers	LOD
SNS	<i>QSnS.cib-1B</i>	17SHF/18SHL/19SHF/BLUP	1B	28.8-33.3	Marker15740- Marker17413	16.86/4.13/6.11,
	<i>QSnS.cib-1D</i>	17SHL/BLUP	1D	146.67-148.82	Marker23471- Marker23475	5.65/4.49
	<i>QSnS.cib-4A</i>	17SHF/18SHL/19SHF/BLUP	4A	72.98-81.71	Marker57882- Marker57915	2.51/4.94/5.3/5.
	<i>QSnS.cib-7A</i>	19SHF/19SHL	7A	81.76-85.42	Marker103527- Marker103903	3.25/4.47
<i>PVE</i> , mean of phenotypic variation explained; <i>LOD</i> , logarithm of the odd; Add, mean of additive effect (Positive values indicate that alleles from CM39 are in prediction.						

Effects of major QTL in mapping populations

Six major QTLs *QSl.cib-2D*, *QGl.cib-3A*, *QTgw.cib-6A.1*, *QTgw.cib-6A.2*, *QGw.cib-6A*, and *QGl.cib-6A* were stably identified in multiple environments and the BLUP datasets (Table 2). We further analyzed their effect on corresponding traits based on the flanking markers in the CM42×CM39 RIL population. As expected, lines possessing the positive alleles of the six loci showed significantly better performance on the corresponding traits compared with those have alleles showing negative effects in all environments and the BLUP datasets (Fig. 2). Additionally, three Kompetitive Allele Specific PCR (KASP) markers linked with *QTgw.cib-6A.1*, *QTgw.cib-6A.2*, *QGw.cib-6A*, *QGl.cib-6A*, and *QSl.cib-2D* were developed in the present study, which could be utilized in MAS for wheat yield improvement (Table S3, Figure S1).

QTL clusters on chromosome 2D and 6A

The QTL cluster on 2D, including three QTLs *QSl.cib-2D*, *QPh.cib-2D* and *QGns.cib-2D*, was co-located between Marker35164 and Marker35422 (Table 2). The CM39 alleles at the locus contributed to longer spike and higher plant, but less GNS. Two QTL clusters were identified on chromosome 6A (Table 2). One comprised two QTLs, *QTgw.cib-6A.1* and *QGl.cib-6A*, was located between Marker87546 and Marker87738. The alleles of CM42 at the locus contributed to increased grain length and weight (Table 2). The other one contained five QTLs, *QTgw.cib-6A.2*, *QGw.cib-6A*, *QPh.cib-6A*, *QGns.cib-6A* and *QSl.cib-6A*, was located between Marker90210 and Marker91587. The allele of CM39 at the locus contributed to higher TGW, GW, PH and SL, but less GNS (Table 2).

Discussion

QTL analysis and comparison with previous studies

Wheat yield components are significantly associated with yield and typically show higher heritability than the yield itself, and thus, mining the genes or QTLs related to yield components will be help for elucidating the genetic basis of wheat yield and facilitating the genetic improvement of varieties with high yield [5–7]. In the present study, a RIL population derived from two elite winter wheat varieties were used to dissect the genetic basis of variation for seven yield components, including TGW, GNS, GW, GL, PH, SL and SNS. A total of 30 QTLs were identified in multiple environments, explaining 2.34–23.75% of the phenotypic variance.

Fourteen QTLs were identified for grain size and weight, including two for TGW, six for GW and six for GL. Among them, *QTgw.cib-6A.1* and *QGl.cib-6A* were co-located on chromosome arm 6AS, which was near to *QTKw-6A.1* and *QTgw.cau-6A.4* [1, 30]. *QTgw.cib-6A.2* was located on chromosome arm 6AL and near to *QTKW.caas-6AL* and *QTKW-6A.1* [31, 32]. The QTL *QGw.cib-6A* for GW was located in a large interval on chromosome 6A. This interval near to a known gene *TaGW2* controlling TGW and GW [33, 34]. *QGw.cib-2A* on chromosome 2A was overlapped with *QGwt.crc-2A* detected by McCartney et al [35]. *QGw.cib-2B.1* on chromosome 2B was overlapped with *qKW2B-1* detected by Xin et al [23]. *QGw.cib-7B* on chromosome 7B was located near to a QTL for TGW *QTgw.wa-7BL* [6]. Two QTLs for GL *QGl.cib-3A* and *QGl.cib-5A.1* on chromosomes 3A and 5A, respectively, were overlapped with two QTLs for GL detected by Mohler et al [36]. *QGl.cib-5A.2* was near to a QTL for TGW *QTKW.ndsu.5A.1* reported previously [34]. *QGl.cib-7D* was overlapped with *QGl.cau-7D* detected by Yan et al [37]. For the rest three QTLs *QGw.cib-2B.2*, *QGw.cib-5A* and *QGl.cib-6D*, no stable QTL for grain size reported previously was overlapped with them, indicating they were likely novel QTL (Table. 3).

PH and SL are important traits related to plant architecture and yield potential in wheat [12, 38]. In the present study, six and four QTLs for PH and SL were identified, respectively. Among them, *QPh.cib-2D* and *QSl.cib-2D* were co-located in the same interval on chromosome arm 2DS, which was overlapped with the dwarfing gene *Rht8* [24, 39]. *QPh.cib-4A* and *QPh.cib-5A* were located near to two loci for PH reported by Luján Basile et al [40]. *QPh.cib-6A* on chromosome 6A was overlapped with the dwarfing gene *Rht18* [41]. *QSl.cib-5A* on chromosome 5A was located near to *QSL.5A.3* detected by Liu et al [42]. For the rest four QTLs *QPh.cib-1A*, *QPh.cib-5B*, *QSl.cib-5B* and *QSl.cib-6A*, no stable QTL for PH and SL reported previously was overlapped with them, indicating they were likely novel (Table. 3).

Additionally, two QTLs for GNS and four QTLs for SNS were identified in the present study. Of them, *QGns.cib-2D* were co-located with *QPh.cib-2D* and *QSl.cib-2D* on chromosome 2D and overlapped with the dwarfing gene *Rht8* [24, 39]. *QGns.cib-6A* was co-located with *QTgw.cib-6A.2* and near to two QTLs for TGW *QTKW.caas-6AL* and *QTKW-6A.1* [31, 32]. *QSnS.cib-1B* for SNS on chromosome 1B was overlapped with the *QSn.sau-1BL* reported recently [5]. *QSnS.cib-7A* for SNS on chromosome 7A was overlapped with *QSn-7A.2* detected by Cao et al [43]. For the rest two QTLs *QSnS.cib-1D* and *QSnS.cib-4A*, no stable QTL for SNS reported previously was overlapped with them, indicating they were likely novel (Table. 3).

Table 3
The physical interval of QTLs detected in the present study and comparison with QTLs previously reported.

Traits	QTLs	Chromosomes	Physical position (Mb)	Nearby known locus	References
TGW	<i>QTgw.cib-6A.1</i>	6A	73.08-82.67	<i>QTKw-6A.1, QTgw.cau-6A.4</i>	[1, 30]
	<i>QTgw.cib-6A.2</i>	6A	442.82-554.21	<i>QTKW.caas-6AL, QTKW-6A.1</i>	[31, 32]
GW	<i>QGw.cib-2A</i>	2A	517.02-581.44	<i>QGwt.crc-2A</i>	[35]
	<i>QGw.cib-2B.1</i>	2B	150.75-151.74	<i>qKW2B-1</i>	[23]
	<i>QGw.cib-2B.2</i>	2B	734.72-734.72		
	<i>QGw.cib-5A</i>	5A	202.92-212.92		
	<i>QGw.cib-6A</i>	6A	422.35-537.67	<i>TaGW2</i>	[33, 34]
	<i>QGw.cib-7B</i>	7B	735.93-740.06	<i>QTgw.wa-7BL</i>	[6]
GL	<i>QGL.cib-3A</i>	3A	659.71-668.09	<i>IWA4298-IWB11347</i>	[36]
	<i>QGL.cib-5A.1</i>	5A	26.14-29.28	<i>IWA4871-IWB34408</i>	[36]
	<i>QGL.cib-5A.2</i>	5A	453.5-453.6	<i>QTKW.ndsu.5A.1</i>	[34]
	<i>QGL.cib-6A</i>	6A	79.99-82.67		
	<i>QGL.cib-6D</i>	6D	75.08-83.92		
	<i>QGL.cib-7D</i>	7D	66.19-107.61	<i>QGL.cau-7D</i>	[37]
PH	<i>QPh.cib-1A</i>	1A	345.37-443.28		
	<i>QPh.cib-2D</i>	2D	20.68-29.35	<i>Rht8, QPLH-2D</i>	[24, 39, 55]
	<i>QPh.cib-4A</i>	4A	704.53-704.58	<i>Chr4A-B57-Hap6</i>	[40]
	<i>QPh.cib-5A</i>	5A	501.62-523.22	<i>Chr5A-B54-Hap3</i>	[40]
	<i>QPh.cib-5B</i>	5B	607.07-608.06		
	<i>QPh.cib-6A</i>	6A	447.77-451.27	<i>Rht18</i>	[41]
GNS	<i>QGns.cib-2D</i>	2D	8.4-29.35	<i>Rht8</i>	[24, 39]
	<i>QGns.cib-6A</i>	6A	471.16-554.21	<i>QTKW.caas-6AL, QTKW-6A.1</i>	[31, 32]
SL	<i>QSL.cib-2D</i>	2D	20.68-29.35	<i>Rht8, QPLH-2D</i>	[24, 39, 55]
	<i>QSL.cib-5A</i>	5A	35.84-45.91	<i>QSL5A.3</i>	[42]
	<i>QSL.cib-5B</i>	5B	404.42-406.31		
	<i>QSL.cib-6A</i>	6A	537.67-584		
SNS	<i>QSns.cib-1B</i>	1B	381.92-439.8	<i>QSn.sau-1BL</i>	[5]
	<i>QSns.cib-1D</i>	1D	482.32-485.76		
	<i>QSns.cib-4A</i>	4A	691.53-703.17		
	<i>QSns.cib-7A</i>	7A	524.95-562.63	<i>QSn-7A.2</i>	[43]

QTL cluster on chromosomes 2D and 6A

Numerous co-located QTLs associated with multiple traits have been reported in previous studies [2, 5, 17, 44, 45], which are beneficial to improve breeding efficiency for multiple elite traits, and thus is favorable for pyramiding breeding or MAS. In the present study, three QTLs *QSL.cib-2D*, *QPh.cib-2D* and *QGns.cib-2D* were co-located in the interval of 8.4-29.35 Mb on chromosome arm 2DS (Table 3). The allele of CM42 at the locus decreases SL and PH while increasing GNS. Additionally, the locus was overlapped with the dwarfing gene *Rht8*, which has been reported to associated with QTLs for PH, SL, SNS, GNS, spikelet compactness, TGW, and grain yield [12, 39, 46–48]. Interestingly, no QTL for grain size and weight detected in the present study was overlapped with the locus, indicating it had no effect on grain size and weight. Given CM42 was bred by utilizing synthetic wheat germplasm [49], further studies, such as fine-mapping and map-based cloning are needed to future reveal the relationship between the locus and *Rht8*. However, the results in this study showed that the locus could be utilized in optimization PH with no penalty for grain size and weight in MAS.

Two QTL clusters were detected on chromosome 6A in the present study. One harbored two QTLs for TGW and GL with overlapped intervals on chromosome arm 6AS (Table 2), indicating the locus increases TGW by mainly increasing GL. The other one comprised five QTLs for TGW, GW, GNS, PH and SL overlapped on chromosome arm 6AL (Table 2), indicating the locus increases TGW by mainly increasing GW. However, interval of the QTL cluster on chromosome 6AL were large and near to *TaGW2* and *Rht18* [41, 50, 51]. Therefore, additional populations may be needed to dissect the relationships of them.

Potential applications of these major QTLs for yield improvement

To date, numerous QTL for wheat yield and yield components have been identified on all wheat chromosomes [3, 21, 52–54]. However, due to the negative correlation between GNS and TGW, a challenge must be faced during increasing yield is how to optimize the trade-off between GNS and TGW by choosing suitable QTLs in breeding practice [2]. In the present study, three major QTLs, *QPh/Sl.cib-2D*, *QGl.cib-3A* and *QTgw.cib-6A.2* were selected to explore the balance of TGW and GNS. As showed in the Table 4, lines possessing the allele form CM42 at the three loci have relatively higher TGW and GNS, which might partly explain the high yield of CM42. Additionally, lines possessing the allele from CM42 at *QPh/Sl.cib-2D* and *QTgw.cib-6A.2* and the allele from CM39 at *QGl.cib-3A* also have relatively higher TGW and GNS. However, for the other combination schemes, either the higher TGW but lower GNS, or higher GNS but lower TGW, or both lower TGW and GNS were harvested. Overall, the QTLs and KASP markers in this study will be useful for elucidating the genetic architecture of grain yield and developing new wheat varieties with high and stable yield in wheat.

Table 4
Haplotype analysis for three major QTLs *QPh/Sl.cib-2D*, *QGl.cib-3A* and *QTgw.cib-6A.2* in the CM42×CM39 RILs.

Haplotypes	Lines	TGW(g) **	GNS *
aabbcc	20	46.97 ± 2.85 ^a	52.25 ± 5.25 ^{cd}
AABBcc	14	47.63 ± 3.29 ^a	49.78 ± 5.12 ^{abc}
AAbbcc	7	49.75 ± 3.76 ^{ab}	55.2 ± 5.51 ^d
aaBBcc	16	51.53 ± 2.42 ^{bc}	50.58 ± 3.48 ^{bc}
aabbCC	20	53.13 ± 3.49 ^{cd}	47.74 ± 4.1 ^a
AabbCC	18	53.17 ± 2.17 ^{cd}	50.58 ± 4 ^{bc}
aaBBCC	21	54.25 ± 2.94 ^d	48.84 ± 3.47 ^{ab}
AABBCC	31	53.32 ± 2.88 ^d	50.07 ± 2.86 ^{bc}

aa, bb and *cc*, alleles from CM39 at *QPh/Sl.cib-2D*, *QGl.cib-3A* and *QTgw.cib-6A.2*, respectively; *AA, BB* and *CC*, alleles from CM42 at *QPh/Sl.cib-2D*, *QGl.cib-3A* and *QTgw.cib-6A.2*, respectively; *Lines*, number of different haplotypes; *TGW*, thousand grain weight; *GNS*, grain number per spike; * and ** represent significance at $P < 0.05$ and $P < 0.01$, respectively; The superscript letter indicates significant difference among groups.

Potential candidate genes for QTgw.cib-6A.1/QGl.cib-6A and QGl.cib-3A

Among these major QTL, *QSl.cib-2D* was likely allele with *Rht8*. In the previous study, TraesCS2D01G055700 was reported by Chai et al [55] as a possible candidate gene for *Rht8*. *QTgw.cib-6A.2* and *QGw.cib-6A* needed additional populations to narrow their intervals. Therefore, we mainly analyzed possible candidates for *QTgw.cib-6A.1/QGl.cib-6A* and *QGl.cib-3A* in the present study.

QTgw.cib-6A.1 and *QGl.cib-6A* were co-located between 73.08 and 82.67 Mb on CS chromosome 6A, and *QGl.cib-3A* was located between 659.71 and 668.09 Mb on CS chromosome 3A (Table 3). In the interval of *QTgw.cib-6A.1/QGl.cib-6A* and *QGl.cib-3A*, there were 81 and 85 predicted genes in the CS genome, respectively. Expression pattern analyses showed that 45 and 57 genes in the interval of *QTgw.cib-6A.1/QGl.cib-6A* and *QGl.cib-3A* expressed in various tissue, respectively (Fig. 3, Table S4, Table S5) [56, 57]. Among them, several were abundantly expressed in grain, indicating they are likely associated with grain growth and development (Fig. 3). For example, TraesCS6A02G107800 is an ortholog of the rice *RGG2* and encodes a guanine nucleotide-binding protein subunit gamma 2 (Table S4). Miao et al [58] previously reported that *RGG2* played a negative role in plant growth and yield production and that manipulation of *RGG2* can increase the plant biomass, grain weight, length and yield in rice. TraesCS6A02G112400 and TraesCS3A02G424000 encode polyubiquitin and small ubiquitin-related modifier, respectively (Table S4, Table S5). TraesCS3A02G421900 encodes a 26S proteasome regulatory subunit (Table S5), which participates in the ubiquitin/26S proteasome pathway and mediate the degradation of the complex of ubiquitin receptor and polyubiquitinated protein [59, 60]. Previous studies revealed that the ubiquitin pathway play a important role in regulation grain size and weight in rice [61, 62]. These results indicated that the four genes were likely closely related to grain size and weight in wheat and they were useful in our following work of fine mapping and cloning of *QTgw.cib-6A.1/QGl.cib-6A* and *QGl.cib-3A*.

Conclusion

In this study, a total of 30 QTLs for TGW, GNS, GW, GL, PH, SL, and SNS were identified, explaining 2.34–23.75% of the phenotypic variance. Among them, six major QTLs *QTgw.cib-6A.1*, *QTgw.cib-6A.2*, *QGw.cib-6A*, *QGl.cib-3A*, *QGl.cib-6A*, and *QSl.cib-2D* showed strong and stable effects on corresponding traits in different environments and the BLUP datasets. Three KASP markers linked with five of these major QTLs were developed. These QTLs and KASP markers will be useful for elucidating the genetic architecture of grain yield and developing new wheat varieties with high and stable yield in wheat. Additionally, candidate genes of *QTgw.cib-6A.1/QGl.cib-6A* and *QGl.cib-3A* were preliminary analyzed.

Methods

Plant materials and field trials

A RIL population (F_{10}) comprising 193 lines derived from a cross CM42 and CM39 was used for QTL detection in the present study, which were developed by our laboratory in Chengdu Institute of Biology (CIB). CM42 is the first wheat elite variety in the world bred by using synthetic hexaploid wheat (*Triticum turgidum*×*Aegilops tauschii*) germplasm, and showed high yield potential in Sichuan and the Yangzi River region [49], while CM39 is a elite winter wheat

variety with different genetic background to that of CM42. CM42 and CM39 seeds were obtained from Sichuan Academy of Agricultural Sciences (SAAS). The RIL population and their parents were evaluated at two experimental sites in Sichuan province of China, Shuangliu (SHL, 103° 52'E, 30°34'N) and Shifang (SHF, 104°11'E, 31°6'N), during four growing seasons from 2015–2016 to 2018–2019. Randomized block design was adopted for all of the trials. Each line was planted in a one-row plot with 50 seeds per row, a row length of 2.0 m, and a row spacing of 0.3 m. Five replicates were performed under each environment. Nitrogen and superphosphate fertilizers were applied at a rate of 80 and 100 kg/ha, respectively, at sowing. Crop management and disease control were performed according to local cultivation practices.

Phenotyping and statistical analysis

At maturity, ten representative plants from middle row of each line were randomly selected to investigate agronomic traits including TGW, GL, GW, GNS, PH, SL and SNS. SL was measured as the length from the base of the rachis to the tip of the terminal spikelet, excluding the awns. SNS was determined by counting the number of spikelets in main spikes; PH was measured from the soil surface to the tip of the spike, excluding the awns. Subsequently, the main spike of all selected plants were harvested and manually threshed for evaluating GNS, TGW, GW and GL using SC-G software (Wseen Co., Ltd, China). PH and SL were evaluated in eight environments, and the rest traits were evaluated in six environments.

Basic phenotypic statistical analyses, frequency distribution, correlation analyses and student's t tests were performed with SPSS version 20.0 (Chicago, IL, USA). The phenotype distribution graph was drawn using the plugin "CorrPlot" in Tbttools [63]. The relationships among measured traits were visualized using the R package "qgraph". The BLUP dataset across evaluated environments was calculated using the "lmer" function implemented in R package "lme4". The broad sense heritability (H^2) was estimated using the PROC GLM procedure in SAS (SAS Institute Inc., North Carolina, USA) based on the following equation; where σ^2_g is the variance of genotypes, σ^2_{ge} is the variance of genotype by environmental effect, σ^2_r is the residual variance, n is the number of environments and r is the number of replicates [64].

Linkage map construction and QTL detection

A whole-genome genetic map constructed previously was adopted for QTL mapping [29]. The genetic map was constructed using the CM42×CM39 RIL population with SALF markers. A total of 4996 Bin SLAFs were distributed in 21 linkage groups and covered a total genetic distance of 2,859.94 cM with an average interval of 0.57 cM between adjacent Bin marker (Table S1) [29].

QTL analysis was conducted using the inclusive composite interval mapping (ICIM) function of IciMapping 4.1 (<https://www.isbreeding.net>) with the minimal LOD score was set at 2.5. The missing phenotype was deleted in QTL analysis. QTL was named according to the provision of Genetic Nomenclature (<http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm>), where 'CIB' represents Chengdu Institute of Biology.

Prediction of candidate gene

Flanking markers for each QTL were blasted against the Chinese Spring (CS) reference genome sequence (RefSeq v1.0) published by the International Wheat Genome Sequencing Consortium (IWGSC; <https://wheat-urgi.versailles.inra.fr/>) to confirm their physical positions [65, 66]. Genes between flanking markers were extracted from IWGSC RefSeq v1.1 annotation for CS (<https://wheat-urgi.versailles.inra.fr/>). The annotations and functions of a given gene were analyzed using UniProt (<https://www.uniprot.org/>). The expression pattern analysis was performed by using Wheat Expression Browser (<http://www.wheat-expression.com/>), and the circle graph of expression values was drawn using Tbttools [63]. The orthologous gene analysis between wheat and rice was conducted using the Triticeae-Gene Tribe (<http://wheat.cau.edu.cn/TGT/>) [67].

List of abbreviations

CM42: Chuanmai42; CM39: Chuanmai39; SHF: Shifang; SHL: Shuangliu; TGW: Thousand grain weight; GNS: Grain number per spike; GW: Grain width; GL: Grain length; PH: Plant height; SL: Spike length; SNS: Spikelet number per spike; QTL: Quantitative trait loci; SALF: Specific-locus amplified fragment; MAS: Marker-assisted selection; KASP: Kompetitive Allele Specific PCR; BLUP: Best linear unbiased prediction

Declarations

Funding

This work is supported by National Key R&D Program of China (2016YFD0100102), Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDA08020205), Science and Technology Support Project of Sichuan Province, China (2016NZ0103), Key Project of Crop Breeding of Sichuan Province (2016NYZ0030), and Science and technology projects of Sichuan Province (2020YFSY0049).

Conflict of interest

All authors declare that they have no conflict of interest.

Ethics approval

The authors declare that this research has no human and animal participants and that the experiments comply with the current laws of China.

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All data used in this study was present in the manuscript and supporting materials.

Code availability

Not applicable.

Author contributions statement

TL undertook the field trials and subsequent analysis of all available data including the phenotyping and population genotyping, and drafted this manuscript. QL and ZP undertook the genetic map constructed. JW, ZY, YT, YS, JZ, XQ and XP participated in phenotyping. ZL, WY and JL provided us the CM42 and CM39 seeds. MY, JL and HZ discussed results. HL, GD and YW designed the experiments, guided the entire study, participated in data analysis, discussed results and revised the manuscript. All authors have read and approved the manuscript.

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68. Supplementary file

Figures

Figure 1

Phenotypic performances, distribution, and correlation coefficients of thousand grain weight (TGW), grain number per spike (GNS), grain width (GW), grain length (GL), plant height (PH), spike length (SL) and spikelet number per spike (SNS) in the CM42×CM39 RIL lines based on the BLUP datasets (A). B Visualization of correlations among investigated traits; Darkred and darkgreen lines represent positive and negative correlation, respectively; The line weight represent the size of correlation coefficient; *, ** and *** represent significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Figure 2

Genetic map of six major QTLs, and their effects on corresponding traits in the CM42×CM39 RIL population. Six major QTLs including QSL.cib-2D (A), QGL.cib-3A (B), QGL.cib-6A (C), QTgw.cib-6A.1 (C), QGw.cib-6A (C) and QTgw.cib-6A.2 (C). The lines drawn on the bar (chromosome) indicate the mapped markers; cM, centimorgans; CM42 and CM39 indicate the lines with the alleles from CM42 and CM39, respectively; ** and *** represent significance at $P < 0.01$ and $P < 0.001$, respectively.

Figure 3

Expression pattern of genes within the QTgw.cib-6A.1/QGI.cib-6A and QGI.cib-3A intervals. 1, 2, 3 and 4 marked by the arrow represent TraesCS6A02G107800, TraesCS6A02G112400, TraesCS3A02G421900 and TraesCS3A02G424000, respectively; A represents the physical interval of QTgw.cib-6A.1/QGI.cib-6A and QGI.cib-3A on chromosome 6A and 3A; B, C, D and E represent root, leaf/shoot, spike and, grain, respectively.

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