

Identification of QTL for flag leaf length in common wheat and their pleiotropic effects

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Abstract Leaf size is an important factor contributing to the photosynthetic capability of wheat plants. It also significantly affects various agronomic traits. In particular, the flag leaves contribute significantly to grain yield in wheat. A recombinant inbred line (RIL) population developed between varieties with significant differences in flag leaf traits was used to map quantitative trait loci (QTL) of flag leaf length (FLL) and to evaluate its pleiotropic effects on five yield-related traits, including spike length (SL), spikelet number per spike (SPN), kernel number per spike (KN), kernel length (KL), and thousand-kernel weight (TKW). Two additional RIL

populations were used to validate the detected QTL and reveal the relationships in different genetic backgrounds. Using the diversity arrays technology (DARt) genetic linkage map, three major QTL for FLL were detected, with single QTL in different environments explaining 8.6–23.3% of the phenotypic variation. All the QTL were detected in at least four environments, and validated in two related populations based on the designed primers. These QTL and the newly developed primers are expected to be valuable for fine mapping and marker-assisted selection in wheat breeding programs.

Yaxi Liu and Yang Tao contributed equally to this work.

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Introduction

Common wheat (*Triticum aestivum* L., $2n = 6 \times = 42$, AABBDD genomes) is the third most highly produced cereal crop after maize and rice, and it is a leading source of plant-based protein in human nutrition. Leaf size is a major factor contributing to the photosynthetic capability of wheat plants, which also significantly affects important agronomic traits such as yield and stress responses (Sourdille et al. 2002; Pérez-Pérez et al. 2010).

In cereals, the three uppermost leaves, especially the flag leaf, have been identified as the primary sources of photo-assimilates accumulated in the grain (Foyer 1987; Hirota et al. 1990; Li et al. 1998). The flag leaf has been found to contribute approximately 50% of the total photosynthetic activity and approximately 41–43% of the carbohydrates needed for grain filling (Xu and Zhao 1995; Sharma et al. 2003). Several studies have shown that flag leaf size is positively related with thousand-kernel weight, kernel number per spike, and grain yield per plant (Cui et al. 2003; Mei et al. 2003; Khaliq et al. 2008; Wang et al. 2011). Therefore, the morphology of the flag leaf, especially its length, is one of the most important determinants of grain yield potential in cereal crops (Sakamoto et al. 2006). Optimal flag leaf length (FLL) might improve photosynthesis and grain yield and consequently, it can be considered an important objective in the breeding programs of various crop species (Wang et al. 2016a; Tian et al. 2011; Xue et al. 2008).

FLL has been found to be quantitatively inherited and significantly influenced by environmental factors (Keller et al. 1999; Quarrie et al. 2006; Mason et al. 2011; Jia et al. 2013; Edae et al. 2014; Fan et al. 2015). The number of quantitative trait loci (QTL) controlling this trait varies among different genotypes; for instance, Jia et al. (2013) reported three chromosomal regions associated with FLL; Fan et al. (2015) reported 11 regions on six different chromosomes that explained 5.65–14.03% of the phenotypic variation and could be only detected in one or two different environments from all of the eight environments; Wu et al. (2016) found seven FLL QTL in four environments, but only one of them was repeatedly expressed in more than two environments; and Yang et al. (2016) detected 12

additive FLL QTL, of which only two were stably found in three of the four tested environments.

In this study, a recombinant inbred line (RIL) population was developed between varieties with significant differences in the flag leaf traits, phenotyped for FLL, and genotyped with diversity arrays technology (DArT) markers (Jaccoud et al. 2001) to identify QTL for FLL. Two additional RIL populations were used to validate the detected QTL and reveal the relationships in different genetic backgrounds.

Materials and methods

Plant materials

Three RIL populations, H461 × CM107 (200 F₉ lines), H461 × CN16 (249 F₁₀ lines), and H461 × MM37 (142 F₇ lines), were used in this study. The FLL of the common parent H461 (Xie et al. 2006; Wang et al. 2016b) was significantly longer than that of the other parents. H461 × CM107 was used for QTL analysis, whereas the other two populations were used for validating the effects of the putative QTL identified in the mapping population. These populations were generated by single-seed descent at Sichuan Agricultural University, Wenjiang District (103° 51' E, 30° 43' N), Sichuan Province, China.

Phenotypic evaluation for FLL and yield-related traits

H461 × CM107 was evaluated in five different environments: WenJiang (103° 51' E, 30° 43' N) in 2014 and 2015 (2014WJ and 2015WJ), ChongZhou (103° 38' E, 30° 32' N) in 2015 and 2016 (2015CZ and 2016CZ), and Ya'An (103°0'E, 29°58' N) in 2015 (2015YA). H461 × CN16 was evaluated in six different environments (2013WJ, 2014WJ, 2015WJ, 2015CZ, 2015YA, and 2016CZ), whereas H461 × MM37 was evaluated in two different environments (2015WJ and 2016CZ). The field designs were randomized complete blocks with three replications. Each plot comprised three rows with a length of 1.5 m and a spacing of 30 cm between rows; the sowing density was 15 seeds per row. Field management was the same as that commonly practiced in wheat production. Ten representative primary tillers in the middle of a row for each plot were selected at maturity for measuring FLL using a ruler. In addition, the flag leaf width (FLW), flag leaf area (FLA) (= FLL ×

FLW \times 0.83), and the weather data for each environment were also evaluated (Fig. S2).

Five yield-related traits were also evaluated in this study: the spike length (SL), spikelet number per spike (SPN), kernel number per spike (KN), kernel length (KL), and thousand-kernel weight (TKW). SL, SPN, and KN were investigated according to Fan et al. (2015) by choosing the main shoots of five plants in the center of each row randomly. To measure KL, 100 kernels from each plot were collected after harvesting and scanned as pictures using an Epson Perfection V700 photo scanner. Subsequently, digital images were analyzed using Winseedle Pro 2012a software (Regent Instruments, Sainte-Foy, QC, Canada). In addition, TKW was measured using an electronic balance according to Ramya et al. (2010).

Analysis of variance (ANOVA) for FLL and other traits in each trial and calculation of Pearson correlations among locations were performed using SPSS 22 (IBM SPSS, Armonk, NY, USA). The broad-sense heritability (h^2) was calculated across environments according to Smith et al. (1998) and Qin et al. (2016). The best linear unbiased predictions (BLUPs), which were used for QTL detection and correlation analyses, were calculated for all the traits using SAS V8.0 (SAS Institute, Cary, North Carolina).

Genotyping

Genomic DNA from each line of the H461 \times CM107 population was extracted from the leaves of 30-day-old plants using the cetyl trimethyl ammonium bromide method (Murray and Thompson 1980) and sent to Diversity Arrays Technology Pty Ltd. (Canberra, Australia, <http://www.DiversityArrays.com/>) for genotyping H461 \times CM107 using 22,174 random DArT markers. The presence or absence of each marker was determined by labeling signals and image analysis. DArT marker data were binary (1, present; 0, absent) as described by Akbari et al. (2006). Moreover, published markers for *Ppd-D1* (Xgwm484, Hanocq et al. 2007), *Vrn-B1* (TaVrn-B1_5400, Guedira et al. 2016), and *Qfl.acs-4D* (Xwmc473 and Xwmc622, Yang et al. 2016) loci were evaluated in the population (Table S7).

Map construction and QTL mapping

The genetic map of H461 \times CM107 was constructed using IciMapping 4.1 (Meng et al. 2015) and JoinMap

4 (Van Ooijen 2006). Markers were binned if the correlation coefficient between them was “one” using the BIN function in IciMapping 4.1 according to the method reported by Winfield et al. (2016). Logarithm of the odds (LOD) threshold values ranging from 3.0 to 10.0 were tested until the optimum number of markers for maintaining the linkage order and distance was obtained. Known chromosome information on the DArT markers was used for assigning linkage groups to specific chromosomes based on wheat consensus map version 3.0 (<http://www.diversityarrays.com/sequence-maps>). QTL were analyzed using MapQTL 6.0 (Van Ooijen 2009). The missing phenotypic data were replaced by the “*” symbol. Interval mapping was then used to identify tightly linked markers. After automatic cofactor selection to detect significantly associated markers, we used the composite interval mapping model (CIM) to identify major QTL (<https://www.kyazma.nl/index.php/mc.MapQTL/>). For each trial, a test of 1000 permutations was performed to identify the LOD threshold that corresponds to a genome-wide false discovery rate of 5% ($p < 0.05$). We deleted QTL with LOD values lower than the permutation test results to increase the authenticity and reliability of the reported QTL.

Marker development and assay

DArT markers flanking QTL were converted into high-resolution melt (HRM) or simple sequence repeat (SSR) markers. Sequences of DArT markers were used for BLAST against the available wheat sequences at the International Wheat Genome Sequencing Consortium (IWGSC; <ftp://ftpmips.helmholtz-muenchen.de/plants/wheat/IWGSC/>) database. Primers were designed based on extended sequences of 1000–1500 bp using the NCBI primer designing tool (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/index>). Subsequently, we compared the sequences from H461 with those from CM107 and searched their single-nucleotide polymorphisms (SNPs) using DNAMAN 6.0 (Lynnon Biosoft, Quebec, Canada). The identified SNPs were then converted into HRM markers for tracking QTL using quantitative real-time PCR. Single-base differences were identified by HRM analysis (Wittwer et al. 2003; Wang et al. 2016b; Zhou et al. 2016). Markers were designed using Beacon Designer 7.9 and evaluated using Oligo 6.0 (Zhang and Gao 2004). The parameters were set as follows: inner product size

of 60–100 bp, melting temperature of 55 ± 5 °C, primer length of 20 ± 3 bp, and 3'-end stability to avoid self-complementarity and primer dimer formation using Primer Premier (Premier Biosoft International, Palo Alto, CA, USA). The other unidentified SNPs in DaRT markers were converted into SSR markers. The software SSR Hunter 1.3.0 (Qiang Li, Nanjing Agricultural University, Nanjing, China) was used to perform a general screen on the scaffold to detect SSRs (Li and Wan 2005).

For the SSR markers, amplification reactions were conducted using the following conditions (10 μ l total volume): 100 ng of template DNA, 5 μ l of $2 \times$ Taq PCR Master Mix, 5 pmol of each forward and reverse primer, and DNase/RNase-free water to make up the final volume. PCR conditions were as follows: denaturation at 94 °C for 5 min; amplification for 35 cycles at 94 °C for 30 s, annealing at 54 to 60 °C (depending on the marker) for 30 s, extension at 72 °C for 1 min; and termination at 72 °C for 10 min. For the HRM primers, slightly different conditions were used. For a 10- μ l reaction, 100 ng of template DNA, 5 μ l of SsoFast EvaGreen mixture, 5 pmol of each forward and reverse primer, and DNase/RNase-free water to make up the final volume. PCR conditions were adjusted according to primer sets as follows: 4 min at 94 °C, 50 cycles of 15 s at 94 °C and 30 s at 55 °C. The amplicon DNA was then precisely heated from 65 to 95 °C. The melting temperature of the amplicon is reached, and the two strands of DNA separate or “melt” apart at some point during this process (Han et al. 2012).

Parental lines and some extreme phenotypes were used to validate the polymorphic markers, and the specificity and sensitivity of all tested markers were determined according to Wang et al. (2016b). Based on the HRM or SSR analysis of marker alleles in the parental lines and their progeny, validation lines were grouped into two classes: lines with homozygous alleles from H461 (designated as aa, bb, and cc) and lines with homozygous alleles from non-H461 (designated as AA, BB, and CC).

QTL validation

The FLL and five yield-related traits of all homozygous lines in each of the three populations were recorded. On the basis of marker profiles, individuals in each population were grouped into two classes as described above, and the difference in the average FLL between these

classes was used for evaluating the QTL effects within each population. Student's *t* test ($p < 0.05$) was used to calculate the differences in FLL between these two classes of alleles and to evaluate the QTL effects within the population. Additionally, effects of the FLL QTL for yield-related traits were estimated with the aid of the corresponding markers in the same manner. All the phenotype data from different environments were calculated as the BLUP value.

Results

Phenotypic variation

The FLL of H461 \times CM107 and the parental lines were evaluated in different environments, with the BLUP data of FLW and FLA (Table 1). The FLL of H461 ranged from 28.7 to 31.76 cm, that of CM107 ranged from 18.56 to 22.70 cm, and that of H461 \times CM107 ranged from 15.00 to 35.12 cm. H461 showed longer FLL than CM107 in each of the five environments tested (Table 1; Fig. 1). The FLL of H461 \times CM107 showed continuous variation and fitted a normal distribution (Fig. S1). The BLUP FLL of H461 was 48.97% longer ($p < 0.01$) than that of CM107. The broad-sense heritability of FLL was 73%. Correlation coefficients (*r*) among the different environments were all significant, ranging from 0.326 to 0.686 ($p < 0.01$) (Table S1), and ANOVA showed that FLL was significantly affected by genotype, environment, and G \times E interactions (Table S2). FLL was also significantly correlated to the five yield-related traits (SL, SPN, KN, KL, and TKW) based on the BLUP value of all environments (Table S3).

Linkage map construction

In total, 4345 bin markers were selected and used for constructing the genetic map of H461 \times CM107, including Xwmc473, Xwmc622, Xgwm484, and TaVrn-B1_5400. The map consisted of 21 linkage groups, with a total length of 2820.97 cM and an average interval distance of 0.65 cM. The maps of the A, B, and D genomes had lengths of 984.8, 922.6, and 913.6 cM, with a density of 0.65, 0.52, and 0.87 cM/marker, respectively (Table S4; Supplementary Material 3). In

Table 1 Phenotypic variation of the mapping population H461 × CM107 and parental lines in different environments

Trait	Environment	Parents		H461 × CM107			
		H461	CM107	Min–Max	Mean	SD	h^2
FLL (cm)	2014WJ	31.10	22.70	15.00–34.10	25.09	3.18	
	2015WJ	31.76	20.98	15.90–34.10	24.51	3.58	
	2015YA	28.70	19.20	16.40–32.93	24.95	3.65	
	2015CZ	30.36	18.56	16.68–35.12	24.57	4.18	
	2016CZ	30.42	20.82	19.24–32.70	25.61	2.87	
	BLUP	30.47	20.45	17.95–31.11	24.99	2.50	0.73
FLW (cm)	BLUP	2.47	2.34	2.13–3.05	2.50	0.16	0.44
FLA (cm ²)	BLUP	62.58	40.32	38.39–76.45	52.34	7.20	0.79
HD (d)	BLUP	137.67	140.30	134.49–143.45	138.91	1.92	0.65
PH (cm)	BLUP	82.30	84.41	65.41–106.20	90.00	6.84	0.80

SD, standard deviation; h^2 , broad-sense heritability; BLUP, phenotype values based on BLUP

addition, 201 loci were mapped on Chr. 2D, with an average distance of 0.65 cM between markers; 93 loci were mapped on Chr. 4D, with an average distance of 1.38 cM between markers; and 227 loci were mapped on Chr. 5B, with an average distance of 0.73 cM between markers.

Identification of putative QTL for FLL in H461 × CM107

To identify QTL for FLL in H461 × CM107, the population was evaluated in five different environments (Table 2). Permutation tests resulted in LOD values between 2.5 and 3.0. On the basis of the

corresponding LOD score, three major QTL located on Chr. 2D, 4D, and 5B were detected. The QTL on the long arm of Chr. 2D (designated as *QFll.sicau-2D*, where “Fll” represents “flag leaf length” and “sicau” represents “Sichuan Agriculture University”; Fig. 2) was located between the markers MK6882 and MK7069. This locus was detected in four of the five environments, and it explained 13.1–23.3% of the phenotypic variation. The QTL on Chr. 4D (*QFll.sicau-4D*; Fig. S3) was flanked by markers MK3365 and MK10165. This locus was present in all five environments and explained 17.7% of the phenotypic variation in the BLUP value. The QTL on the long arm of

Fig. 1 Morphological features of the flag leaf (a), kernel (b), and spike (c) in H461, CM107, CN16, and MM37

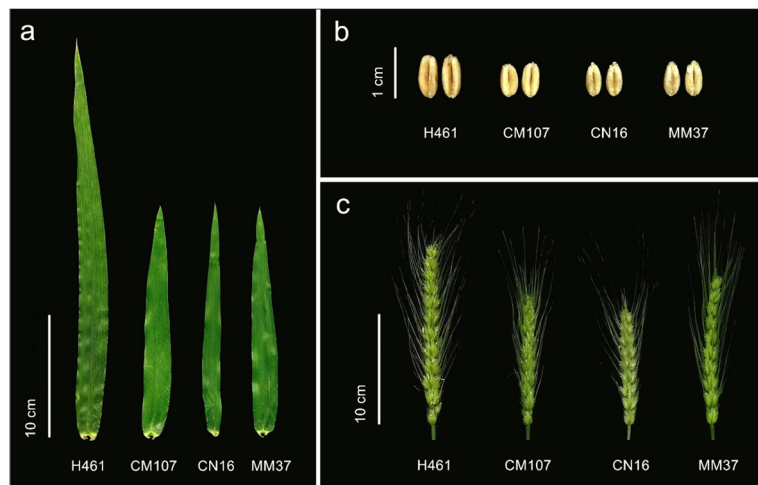


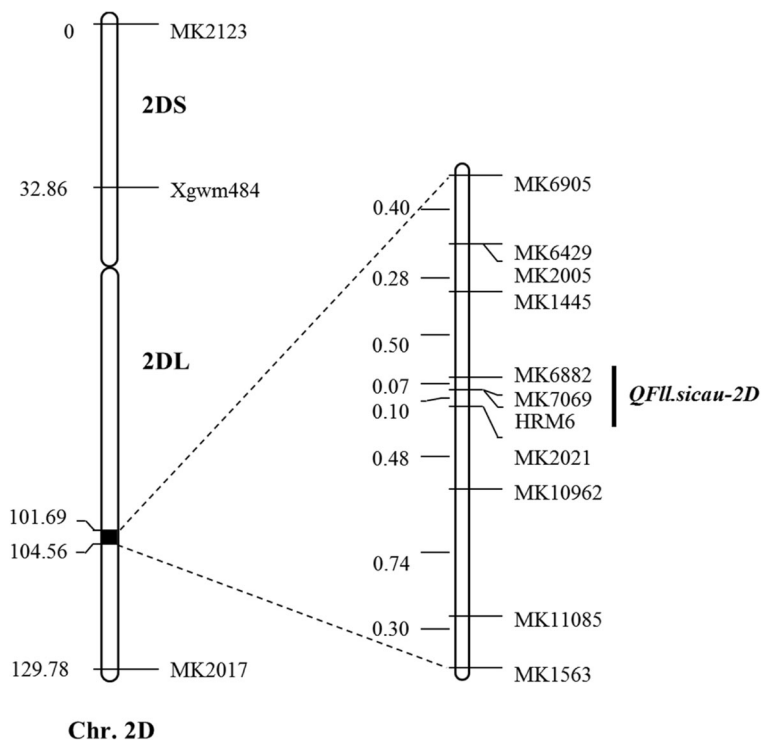
Table 2 Quantitative trait loci for flag leaf length identified in the mapping population H461 × CM107 evaluated in different trials

QTL	Trial	Group	Interval	Flanking marker	LOD	LOD threshold	PVE (%)
<i>QFl.sicau-2D</i>	2014WJ	6	102.87~102.94	MK6882 and MK7069	9.4	2.8	23.3
	2015WJ	6	102.87~102.94	MK6882 and MK7069	5.4	3.0	14.1
	2015YA	6	102.87~102.94	MK6882 and MK7069	5.1	2.5	13.1
	2015CZ	6	102.87~102.94	MK6882 and MK7069	5.4	3.0	14.0
	BLUP	6	102.87~102.94	MK6882 and MK7069	8.8	4.0	21.8
<i>QFl.sicau-4D</i>	2014WJ	12	67.84~68.07	MK3365 and MK10165	3.8	2.7	10.3
	2015WJ	12	67.84~68.07	MK3365 and MK10165	5.4	3.0	14.0
	2015YA	12	67.84~68.07	MK3365 and MK10165	6.5	3.0	16.7
	2015CZ	12	67.84~68.07	MK3365 and MK10165	3.6	2.5	9.7
	2016CZ	12	67.84~68.07	MK3365 and MK10165	3.3	2.5	10.6
	BLUP	12	67.84~68.07	MK3365 and MK10165	7.0	2.8	17.7
<i>QFl.sicau-5B</i>	2014WJ	14	116.66~117.32	MK3747 and MK676	3.2	2.5	8.6
	2015WJ	14	116.66~117.32	MK3747 and MK676	4.4	3.0	11.4
	2015YA	14	116.66~117.32	MK3747 and MK676	3.3	2.5	8.7
	2015CZ	14	116.66~117.32	MK3747 and MK676	4.8	3.0	12.5
	2016CZ	14	116.66~117.32	MK3747 and MK676	2.9	2.5	9.3
	BLUP	14	116.66~117.32	MK3747 and MK676	5.6	2.7	14.4

PVE, phenotypic variance estimated from marker regression against phenotype

Chr. 5B (*QFl.sicau-5B*; Fig. S4) was flanked by markers MK3747 and MK676. This locus was also detected in all five environments and explained 8.6–14.4% of the phenotypic variation.

Fig. 2 Location of *QFl.sicau-2D* on linkage map chromosome 2D



Validation of FLL QTL in different genetic backgrounds

The flanking DArT markers (MK7069, MK10165, and MK676) and three pairs of new designed primers (HRM6, HRM18, and SSR5, Table S7) were used to track each of the three major QTL in the mapping population and two validation populations, respectively. For *QFll.sicau-2D*, the FLL of the homozygous “aa” lines ranged from 23.09 to 26.63 cm, whereas that of the homozygous “AA” lines ranged from 22.20 cm to 24.09 cm (Table 3). The average FLL of lines with homozygous alleles from H461 was significantly higher ($p < 0.05$) than that of lines with homozygous alleles from the non-H461 parents, with an average effect of 7.44% among the three populations. For *QFll.sicau-4D*, the FLL of the homozygous “bb” lines ranged from 23.16 to 27.57 cm, whereas that of homozygous “BB” lines ranged from 22.22 to 26.64 cm. The effect of the

two alleles was significantly different ($p < 0.05$) in BLUP value (Table 3), with an average difference of 5.78% among populations. For *QFll.sicau-5B*, the FLL of the homozygous “cc” lines ranged from 23.28 to 28.99 cm, whereas that of the homozygous “CC” lines ranged from 22.12 to 27.03 cm (Table 3). The FLL of lines with homozygous alleles from H461 was also significantly longer ($p < 0.05$) than that of lines with homozygous alleles from non-H461 parents, with an average difference of 6.84% between populations.

Based on the corresponding markers, the effects of FLL QTL for five yield-related traits in the three populations were also estimated (Table 3). For SL, the effect of *QFll.sicau-2D* was extremely significant ($p < 0.01$) among the three populations; *QFll.sicau-4D* had an extremely effect on H461 × CM107; *QFll.sicau-5B* did not show a significant effect. For SPN, *QFll.sicau-5B* had a significant effect on all the studied populations,

Table 3 Effects of QTL among three populations evaluated according to BLUP value

Population	Trait	<i>QFll.sicau-2D</i>			<i>QFll.sicau-4D</i>			<i>QFll.sicau-5B</i>		
		aa ^a	AA ^b	Difference ^c (%)	bb ^a	BB ^b	Difference (%)	cc ^a	CC ^b	Difference (%)
H461 × CM107	FLL	26.63	24.09	10.52 **	26.27	23.96	9.62 **	26.18	24.23	8.04 **
	SL	13.60	12.02	13.16 **	13.18	12.17	8.29 **	12.83	12.46	3.03
	SPN	24.71	23.46	5.34 **	24.20	23.77	1.83 *	23.40	24.22	-3.4 **
	KN	66.03	59.28	11.39 **	61.87	61.96	-0.15	61.25	62.51	-2.01
	KL	7.20	7.03	2.43 **	7.18	7.03	2.21 **	7.19	7.05	2.01 **
	TKW	46.29	45.55	1.62	46.41	45.32	2.4 *	45.70	45.91	-0.46
H461 × CN16	FLL	23.09	22.20	4.02 **	23.16	22.22	4.24 **	23.28	22.12	5.23 **
	SL	12.19	11.85	2.92 **	12.10	11.96	1.17	12.14	11.95	1.62
	SPN	22.54	22.23	1.41 *	22.41	22.41	0	22.22	22.55	-1.51 *
	KN	50.87	48.08	5.82 **	49.53	49.94	-0.83	49.98	49.55	0.88
	KL	7.27	7.18	1.15 *	7.21	7.26	-0.73	7.28	7.17	1.56 **
	TKW	51.58	48.83	5.63 **	49.33	51.39	-4.17 *	50.50	49.44	2.14
H461 × MM37	FLL	25.41	23.57	7.77 **	27.57	26.64	3.49 **	28.99	27.03	7.26 *
	SL	14.76	13.98	5.58 **	15.03	14.90	0.88	15.26	14.69	3.89
	SPN	22.04	22.01	0.13	21.41	19.97	7.24 *	22.00	22.06	-0.29 *
	KN	59.45	58.39	1.81 **	58.90	58.83	0.11	58.93	58.63	0.52
	KL	7.15	7.03	1.74 *	7.07	7.14	-1.08	7.13	7.03	1.41
	TKW	47.13	42.79	10.14 **	44.41	45.04	-1.43	44.33	45.17	-1.88

^a“aa”, “bb”, and “cc” represents homozygous alleles from H461

^b“AA”, “BB”, and “CC” represents homozygous alleles from the non-H461 parents

^cStudent’s *t* test ($p < 0.05$) was used to identify differences between the parental lines

** , significant at $p < 0.01$; * , significant at $p < 0.05$

with an average difference of 1.73%. For KN, *QFll.sicau-2D* increased KN by 11.39, 5.82, and 1.81% in the three populations. For KL, a significant effect of *QFll.sicau-5B* were observed in both H461 × CM107 and H461 × CN16 populations, and *QFll.sicau-2D* showed significant effect in all the populations. For the last traits, the TKW of homozygous “aa” lines were significantly higher ($p < 0.05$) than that of homozygous “AA” lines, with a maximum of 10.14% in two populations.

Discussion

FLL is a major factor that influences leaf morphology, plant architecture, and yield potential in wheat. The improvement of FLL has been an important objective in many cereal breeding programs. H461, the common parent of all three populations in this study, was an ideal material for investigation, with long FLL, large panicle, long KL, and high TKW (Xie et al. 2006). The construction of three RIL populations between varieties with significant differences in FLL would help to detect QTL and reveal the relationships between FLL and other yield-related traits in different genetic backgrounds.

Two novel QTL for FLL

Two putative QTL for FLL have been reported on Chr. 2D, including *QFll.cau-2D* (Wu et al. 2016) and *Qfll.acs-2D* (Yang et al. 2016). However, neither of them seems to be the same as the *QFll.sicau-2D* detected in the present study. BLAST results showed that the sequences of the flanking markers for *QFll.cau-2D* (wsnp_JD_c69_109951 and wsnp_Ex_c1944_3664205) were located on the contigs 2DS_5321656 and 2DS_5343186, whereas the flanking markers for QTL detected in the present study (MK2117 and MK11176) were located on the contigs 2DL_9908762 and 2DL_8817443 (<https://urgi.versailles.inra.fr/blast>). *Qfll.acs-2D* might also be different from the *QFll.sicau-2D* detected in this study. Marker analysis showed that the flanking markers for *Qfll.acs-2D* (Xmag1280 and Xgwm157, Table S7) were not polymorphic in H461 × CM107 population. Moreover, *Qfll.acs-2D* was detected in only one of the four tested environments, and it explained only 2.93% of the phenotypic variance.

A previous study reported four potential QTL for FLL on Chr. 4D, i.e., *Qfll.acs-4D.1*, *Qfll.acs-4D.2*, *Qfll.acs-4D.3*, and *Qfll.acs-4D.4* (Yang et al. 2016), but only *Qfll.acs-4D.1* was identified in three of the four environments, and it explained a maximum of 6.59% of the phenotypic variation. The first three QTL, *Qfll.acs-4D.1*, *Qfll.acs-4D.2*, and *Qfll.acs-4D.3*, were tightly linked to the markers Xwmc473, Xwmc489, and Xgdm61, respectively, and were closely linked over a genetic distance of less than 1 cM. Consequently, *Qfll.acs-4D.1*, *Qfll.acs-4D.2*, and *Qfll.acs-4D.3* could be considered as a single locus. Integrating Xwmc473 and Xwmc622 in our genetic linkage map, we observed that they were 9.72 and 34.41 cM away from *QFll.sicau-4D*, respectively (Fig. S3). These data were discordant with those for *QFll.sicau-4D*, which was detected in all five tested environments.

A single QTL for FLL has been reported on chromosome arm 5BL between the SNP markers wsnp_Ra_c5131_9153817 and wsnp_Ra_c26091_35652620, close to *Vrn-B1* (Wu et al. 2016). *QFll.sicau-5B* was 5.17 cM away from the STS marker (TaVrn-B1_5400) for *Vrn-B1* (Guedira et al. 2016) in our linkage map (Fig. S4). Therefore, *QFll.cau-5B* and *QFll.sicau-5B* might be the same locus.

QTL relationships with *Ppd*, *Rht*, and *Vrn* genes

Photoperiodic response (*Ppd*) gene, vernalization requirement (*Vrn*) gene, and reduced height (*Rht*) gene are three major genes that have important effects on wheat growth. In this study, the three major QTL for FLL were related to those genes. To test for any possible effects of heading date (HD) and plant height (PH) on FLL, BLUP data from the two traits were used for covariance analysis against the BLUP data from the FLL (Table S5).

Ppd genes play an important role in determining the climatic adaptability of wheat varieties, affecting the flowering time and adaptation, and are associated with flag leaf size and grain yield (Kirby 1992; Snape et al. 2001; Foulkes et al. 2004). *Ppd-D1* is the most potent gene for photoperiod insensitivity (Scarath and Law 1984). *Ppd-D1* homologous loci are near Xgwm484 (Hanocq et al. 2007). Our genetic map showed that Xgwm484 locus is located on chromosome arm 2DS and is 72.196 cM away from *QFll.sicau-2D*

(Fig. 2). Therefore, it can be inferred that *QFll.sicau-2D* is different from *Ppd-D1* gene.

Vrn genes are major regulators of the transition from the vegetative to reproductive growth phase, ensuring that flowering occurs when temperatures are favorable and that water is abundant to support growth (Worland 1996; Cockram et al. 2007; Guedira et al. 2016). In the present study, we analyzed the STS marker (TaVrn-B1_5400) for *Vrn-B1*. The results showed that H461 has *vrn-B1_non-del* allele, whereas CM107 has *vrn-B1_del* allele. TaVrn-B1_5400 locus in our linkage map was close to *QFll.sicau-5B* (Fig. S4). Moreover, covariance analysis showed that its LOD was significantly decreased when the effect of HD was accounted for (Table S5). Therefore, *QFll.sicau-5B* and *Vrn-B1* might be the same locus.

GA-insensitive dwarfing genes such as *Rht-D1* have been widely used to reduce plant height, increase yield, and improve harvest index (Worland et al. 1994; Miralles and Slafer 1995; Beharav et al. 1998; Ellis et al. 2002, 2004). To eliminate the interference of PH, we regarded the BLUP data of PH as a covariate and performed QTL mapping with a covariance analysis. The results showed that there was no significant variation for the effect of PH (Table S5). Moreover, the putative gene *Rht-D1* was located on chromosome arm 4DS, different from the *QFll.sicau-4D* detected in this study.

Effects of *QFll.sicau-2D*, *QFll.sicau-4D*, and *QFll.sicau-5B*

In the present study, FLL was significantly correlated to yield-related traits. The three QTL, *QFll.sicau-2D*, *QFll.sicau-4D*, and *QFll.sicau-5B*, were all stably detected and explained 8.6–23.3% of the phenotypic variation. The effects of the QTL on FLL and five yield-related traits were also validated in two additional genetic backgrounds using the BLUP value. *QFll.sicau-2D* was the most potent QTL, having a significant effect on all the traits, and the traits of homozygous alleles from H461 were all significantly higher ($p < 0.05$) than those of homozygous alleles from the non-H461 parents. It can be inferred that *QFll.sicau-2D* has the potential to increase grain yield, but whether it is a pleiotropic QTL or a QTL cluster needs to be further investigated. Moreover, the homozygous “bb” alleles of *QFll.sicau-4D* increased SPN in both H461 × CM107 and H461 × MM37, and increased TKW only in H461 ×

CM107, whereas the homozygous “cc” alleles of *QFll.sicau-5B* decreased SPN in all three populations. Both *QFll.sicau-4D* and *QFll.sicau-5B* showed increased FLL in the two validation populations. Future studies should investigate the interaction effects among FLL and yield-related traits of the three QTL and improve FLL and SPN, FLL and KL, FLL and TKW or any other yield-related traits.

Authors’ contributions YL designed and coordinated this study and revised the manuscript.

YT conducted data analysis and drafted the manuscript.

ZW performed the analysis of genotyping data.

QG, FW, XY, and MD performed the phenotypic evaluation and helped with data analysis.

JM helped to draft the manuscript.

GC performed part of the population genotyping.

YW participated in the design of the study.

YZ coordinated the study and helped to draft the manuscript.

All authors have read and approved the final manuscript.

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Compliance with ethical standards

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

Ethical standard The experiments comply with the ethical standards in the country in which they were performed.

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