Mapping of QTL for total spikelet number per spike on chromosome 2D in wheat using a high-density genetic map

Mei Deng¹*, Fangkun Wu¹*, Wanlin Zhou¹, Jing Li¹, Haoran Shi¹, Zhiqiang Wang¹, Yu Lin¹, Xilan Yang¹, Yuming Wei¹, Youliang Zheng¹ and Yaxi Liu¹

¹Triticeae Research Institute, Sichuan Agricultural University, Wenjiang, Chengdu 611130, China

Send correspondence to Yaxi Liu. Triticeae Research Institute, Sichuan Agricultural University, Wenjiang, Chengdu 611130, China. E-mail: liuyaxi@sicau.edu.cn

*Mei Deng and Fangkun Wu contributed equally to this work
Abstract

Total spikelet number per spike (TSS) is one of the key components of grain yield in wheat. Chromosome (chr.) 2D contains numerous genes that control TSS. In this study, we evaluated 138 F₈ recombinant inbred lines (RILs) derived from an F₂ population of a synthetic hexaploid wheat line (SHW-L1) and a common wheat cultivar (Chuanmai 32) for TSS in six different environments. To identify quantitative trait loci (QTL) for TSS, we constructed an integrated high-density genetic map of chr. 2D containing two simple sequence repeats, 35 diversity array technology markers, and 143 single nucleotide polymorphisms. We identified three stable QTL for TSS that individually explained 9.7–19.2% of the phenotypic variation and predicted 23 putative candidate genes within the QTL mapping interval. Overall, our results provide insight into the genetic basis of TSS in synthetic hexaploid wheat that may be useful in breeding high-yielding wheat cultivars.

Keywords: recombinant inbred line, synthetic hexaploid wheat, quantitative trait locus, total spikelet number per spike

Received: May 9, 2018; Accepted: December 4, 2018.

Introduction

To feed the ever-growing population, improving the yield of wheat, one of the most important food crops globally, is becoming increasingly important (Rajaram 2001; Charles et al. 2010; Reynolds et al. 1996). Among the factors determining wheat yield, total spikelet number per spike (TSS) is considered one of the key factors, and previous studies have shown that spikelet number is closely related to grain number (Rawson 1970) and determines where spikelets can set (Slafer and Andrade 1993). As the basal units of inflorescences, spikelets are crucial for reproductive success and final yield (Cai et al. 2014).

TSS, as an important quantitative agronomic trait, is controlled by polygenes and influenced by the environment (Zhou et al. 2017). Understanding the genetic factors underlying variations in TSS without environmental interference is essential for the genetic improvement of wheat (Mackay 2001; Wurschum 2012). Previous genetic studies have revealed that chromosome (chr.) 2D is rich in genes that control spikelet number per spike in common wheat, and many quantitative trait loci (QTL), such as QSsn.cau-2D.2 and QSpn.nau-2D, have been discovered on this chromosome (Ma et al. 2007;
Zhai et al. 2016). However, information about TSS-QTL on chr. 2D is still limited for synthetic hexaploid wheat (SHW), which contains a combination of genes from Aegilops tauschii and common wheat (Triticum aestivum) as well as novel functional genes (Mares and Mrva 2008). Yu et al. (2014) identified a stable QTL (in the region wPt-6133-gpw4473) for TSS on chr. 2D in a population developed from a cross between SHW (SHW-L1) and the common wheat variety Chuanmai 32, using a genetic map containing simple sequence repeats (SSRs) and diversity arrays technology (DArT) markers. To accurately parse this QTL, we integrated the markers reported by Yu et al. (2014) with novel SNP markers into a new chr. 2D high-density genetic map and identified QTL for TSS. Our data might help to better understand the genetic basis of TSS in SHW and accelerate the development of new high-yielding wheat cultivars.

Materials and methods

Plant material
A total of 138 F8 recombinant inbred lines (RILs) derived from an F2 SHW-L1/Chuanmai 32 population were used to construct an integrated linkage map for chr. 2D and detect QTL for TSS. SHW-L1 is an SHW derived from a cross between Triticum turgidum ssp. turgidum AS2255 (AABB) and A. tauschii ssp. tauschii AS60 (DD) (Zhang et al. 2004), whereas Chuanmai 32 is a commercial hexaploid wheat cultivar grown in the southwest winter-wheat areas of China. Transgressing segregations for TSS have been previously observed in SHW-L1/Chuanmai RILs, and a total of 68 SSRs and 1794 DArT markers for important agronomic traits have been mapped (Yu et al. 2014).

Field experiment and phenotyping
All RILs and their parents were evaluated in a completely randomized block design with two replicates, at the experimental stations of Dujiang Weir (31°01’N and 103°32’W) in 2008, 2009, and 2010 (environments E1, E2, and E3, respectively), Guanghan (30°99’N and 104°25’W) in 2009 and 2010 (environments E4 and E5), and Wenjiang (30°36’N and 103°41’W) in 2011 (environment E6). Plants were sown in single 1.5-m rows with a 30-cm space between rows and a 10-cm space between individuals. Data for TSS were manually counted from 10 randomly selected guarded main spikes from each line in each environment (Yu et al. 2014).

Statistical analysis
To estimate random effects, a best linear unbiased prediction (BLUP) mixed model was used to obtain BLUP-TSS values (Piepho et al. 2008). The BLUP for the phenotypic value of plant $Y_i$ was calculated as follows: $Y_i = X_i f + a_i + e_i$, where $f$ is a vector of fixed effects, $X_i$ is an incidence vector, $e_i$ is the environmental deviation, and $a_i$ is the phenotypic value (Goddard 1992). An analysis of variance (ANOVA) was performed using SAS 9.1.3 (SAS Institute, Cary, NC, USA) to estimate the effects of genotype on TSS. The estimated broad-sense heritability of TSS was calculated as follows: $h = \frac{\sigma^2 G}{\sigma^2 G + \sigma^2 e/r}$, where $\sigma^2 G$ is the genetic variance, $\sigma^2 e$ is the residual variance, and $r$ is the number of replicates per genotype.

**Construction of a genetic map for chr. 2D**

A total of 13 SSRs, 93 DArT markers, and 2306 SNPs reported in previous studies (Yu et al. 2014; Yang 2016) were used to construct a genetic map for chr. 2D. After the removal of redundant markers that were located on the same loci (Yang 2016), the genetic map consisted of 13 SSRs, 86 DArT markers, and 244 SNPs. The remaining markers were assigned to linkage groups using Joinmap 4.0 (Van Ooijen 2006) with a recombination frequency of 0.25–0.05. The final genetic distances were obtained using the Kosambi mapping function (Kosambi 2016).

**QTL mapping**

QTL screening was conducted using interval mapping (IM) in MapQTL 6.0 (Van Ooijen 2009). Logarithm of odds (LOD) threshold values for IM were determined based on 1000 permutations to declare significant QTL at $p < 0.05$, whereas QTL with LOD values < 3.0 were excluded to ensure the authenticity and reliability of the reported QTL. QTL that explained more than 10% of variation in TSS were considered as major QTL.

**Prediction of candidate genes**

To predict candidate/flanking genes, the interval flanking marker sequence was aligned via a BLAST search against the International Wheat Genome Sequencing Consortium (https://urgi.versailles.inra.fr/blast_iwgscblast.php) and EnsemblPlants (http://plants.ensembl.org/hmmer/index.html) databases to determine the position with the highest identity and detect genes within the closed interval. To predict the function of the candidate genes,
we conducted gene ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis at $p < 0.05$, using *Arabidopsis thaliana* as a background species, in KOBAS 3.0 (http://kobas.cbi.pku.edu.cn/).

**Results**

**TSS variation in RILs**

The results of the mean phenotypic performance and BLUP values for the TSS of RILs and their parents in the six environments are presented in **Table 1**; the ANOVA and heritability ($h^2$) values are presented in **Table 2**. Variation among the RILs was high, with a coefficient of variation ranging from 9.53 % in E1 to 14.39 % in E6. Distributions were continuous across all environments (**Fig. 1**), and, thus, the RILs were suitable for analyzing QTL for TSS.

**Construction of genetic linkage map for chr. 2D**

Different types of molecular markers were used to construct a genetic map for chr. 2D. At a maximum recombination frequency score of 0.4 and a minimum LOD score of 1.00, 180 markers were assigned to two different linkage groups (LG) that covered 207.33 cM, with a mean interval distance of 1.15 cM between the markers; however, the other 163 markers remained unassigned. LG 1 consisted of two SSRs, 35 DArT markers, and 90 SNPs, whereas LG 2 consisted of 53 SNPs.

**Stable QTL for TSS**

Three QTL for TSS (**QTSS.sicau-2D.1**, **QTSS.sicau-2D.2**, and **QTSS.sicau-2D.3**) with significant effects in at least four environments were identified on chr. 2D. The existence and stability of the QTL were confirmed by BLUP values. The three major QTL individually explained 9.7–19.2 % of the phenotypic variation (**Table 3, Fig. 2**), and the additive effects of QTL showed that the positive alleles (i.e., those related to a high number of TSS) on chr. 2D originated from SHW-L1. Of these, **QTSS.sicau-2D.1** was detected in all environments, except E5, whereas **QTSS.sicau-2D.2** and **QTSS.sicau-2D.3** were found in four environments (E1–3 and E6).

**Putative candidate genes in QTL intervals**

A total of 23 putative candidate genes associated with TSS were identified; two genes were predicted
in the QTSS.sicau-2D.1 interval (IWGSC_ref_V1_chr2D|chr2D:9346330-9579108), 19 in the QTSS.sicau-2D.2 interval (IWGSC_ref_V1_chr2D|chr2D:38222754-43976070), and two in the QTSS.sicau-2D.3 interval (IWGSC_ref_V1_chr2D|chr2D:77381440-78089285). Of these, five genes, LECRK42, AT2G34930, PME21, COBL7, and PIP5K4, regulate flower development, and three, CRK8, RPPL1, and AT4G29780, are related to spikelet number differentiation. KEGG pathway enrichment analysis showed that PIP5K4 is involved in inositol phosphate metabolism, phosphatidylinositol signaling system, and endocytosis; PME21 and PME53 are involved in starch and sucrose metabolism and pentose and glucoronate interconversions; and AT2G07689 and ATP1 are involved in oxidative phosphorylation.

Discussion

In the present study, by using an integrated high-density genetic map, three major QTL for TSS were detected on chr. 2DS (short arm of chr. 2D). Among them, QTSS.sicau-2D.2 was located in the marker interval wPt6133–gpw4473, which might correspond to that reported by Yu et al. (2014) in the same marker interval (Fig. 3). Notably, using the integrated high-density genetic map for chr. 2D, we managed to decrease the marker interval range from 15.6 cM to 1.04 cM, which is a substantial improvement over that obtained in previous studies, and two additional QTL were detected. Similarly, by high-density consensus map, Marone et al. (2012) increased the map density from 11.8 cM per marker (as obtained by Nachit et al.; 2001) to 1.6 cM per marker, and Sourdille et al. (2003) confirmed previously detected QTL and identified three novel ones, suggesting that good coverage of chromosome is important for QTL detection. Therefore, this study provides a strategy for identifying QTL, which combines new molecular data with phenotypic data and enables possible detection of previously overlooked QTL.

For chr. 2D, previous studies have identified numerous putative QTL (Li et al. 2002; Sourdille et al. 2003; Quarrie et al. 2006; Cui et al. 2012; Liu et al. 2014; Zhai et al. 2016; Zhou et al. 2017). Ma et al. (2007) reported two QTL for TSS in the marker intervals Xwmc181.1–Xaf12d (near IWGSC_ref_V1_chr2D|chr2D:593738612-593738636) and Xaf12–Xcdf239 (near IWGSC_ref_V1_chr2D|chr2D:647432804-647432824); Cui et al. (2012) also reported two QTL for TSS in the marker intervals Xcdf267–Xmag3596 (near IWGSC_ref_V1_chr2D|chr2D:608198901-608198921) and Xbarc228–Xwmc181.1 (near IWGSC_ref_V1_chr2D|chr2D:593738612-
Zhou et al. (2017) also reported a QTL for TSS, named \textit{QTsn.czm-2D.3} (near IWGSC\_ref\_V1\_chr2D|chr2D:480324893-480325330). Comparison with data in the IWGSC database revealed that these above-mentioned QTL were found on chr.2DL (long arm of chr.2D). The three QTL we detected were located on chr. 2DS; so, we paid more attention to the QTL previously detected on chr. 2DS. Li et al. (2002) reported a QTL for TSS in the marker interval \textit{Xbcd611–Xgwm484} (IWGSC\_ref\_V1\_chr2D|chr2D: 34894502-48174395) on chr. 2DS; by comparison, this marker interval is different from those of \textit{QTSS.sicau-2D.1} (IWGSC\_ref\_V1\_chr2D|chr2D:9346330-9579108) and \textit{QTSS.sicau-2D.3} (IWGSC\_ref\_V1\_chr2D|chr2D:77381440-78089285), but contains \textit{QTSS.sicau-2D.2} (IWGSC\_ref\_V1\_chr2D|chr2D:38222754-43976070). Zhou et al. (2017) also reported two QTL for TSS on chr. 2DS: \textit{QTsn.czm-2D.2} was located in the marker interval \textit{XPpd_D1–2DS_5382880_5243} (IWGSC\_ref\_V1\_chr2D|chr2D:29716047-67557838) that contains \textit{QTSS.sicau-2D.2}, while the other QTL, named \textit{QTsn.czm-2D.1} (IWGSC\_ref\_V1\_chr2D|chr2D:19623154-29716165), was different from \textit{QTSS.sicau-2D.1} and \textit{QTSS.sicau-2D.3}. Therefore, \textit{QTSS.sicau-2D.1} and \textit{QTSS.sicau-2D.3} are probably novel QTL that can be used for further fine mapping and genetic analysis.

In wheat, the development of polymorphism markers based on QTL is an effective method for molecular-assisted breeding (Roussel et al. 2005); so, the three QTL for TSS identified in this study may be used for the breeding of high yield wheat varieties. Furthermore, the results revealed that SHW-L1 contributed positively to all the three major loci; so, future breeding programs can use the QTL-associated markers to fully exploit the genetic potential of QTL in increasing SHW-L1 production.

A total of 23 \textit{A. thaliana} gene homologs were predicted in the three QTL intervals. The results of GO annotation suggest that seven candidate genes deserve our attention; these are: \textit{LECRK42}, \textit{AT2G34930}, \textit{PME21}, \textit{COBL7}, \textit{PIP5K4} (located in the \textit{QTSS.sicau-2D.2} intervals), \textit{CRK8} (located in the \textit{QTSS.sicau-2D.3} intervals), and \textit{RPPL1} (located in the \textit{QTSS.sicau-2D.1} intervals). Among them, \textit{LECRK42}, \textit{PME21}, and \textit{PIP5K4} play critical roles in pollen and pollen tube development (Sousa et al. 2008; Wan et al. 2008; Oo et al. 2014); \textit{AT2G34930} encodes cell wall proteins in the apoplastic fluids of rosettes (Boudart et al. 2005); and \textit{COBL7} influences the development and function of the gynoecium (Scutt et al. 2003). Pollen and flower development is closely related to flowering time, and flowering time genes affect ear differentiation, including TSS (Jiang et al. 1982). Moreover,
differentiation of TSS indicates a switch from vegetative to reproductive growth (Li 1976). Interestingly, $CRK8$ is involved in reproductive signal transduction (Zhao et al. 2011), and $RPPL1$, which interacts with $GRF2$, plays crucial roles in controlling growth and development in plants (Gökirmak et al. 2015; Ghorbel et al. 2017). For all these reasons, the seven candidate genes located in the three QTL intervals were considered to be closely related to TSS, which validates the accuracy of our results, provides reference for future map-based cloning experiments, and helps to better understand the genetic mechanism of spikelet growth and development in wheat.

**Conclusions**

In this study, we provided a strategy of identifying QTL by combining new molecular data with phenotypic data, and identified two novel QTL for TSS. A total of seven candidate genes associated with TSS were predicted. Overall, our data provides insight into the genetic basis of TSS, which might accelerate the development of high-yielding wheat cultivars.

**Acknowledgments**

This study was supported by the National Natural Science Foundation of China (31771794), the outstanding Youth Foundation of the Department of Science and Technology of Sichuan Province (2016JQ0040), the Key Technology Research and Development Program of the Department of Science and Technology of Sichuan Province (2016NZ0057), and the International Science & Technology Cooperation Program of the Bureau of Science and Technology of Chengdu China (No. 2015DFA306002015-GH03-00008-HZ).

**Conflict of interest**

The authors declare that they have no conflict of interest.

**Authors’ contributions**

MD conducted data analysis and drafted the manuscript; FW performed the analysis of genotyping data and partially drafted the manuscript; WZ, JL, YL and XY performed the phenotypic evaluation and helped with data analysis; HS and ZW performed part of the population genotyping; YW participated in the design of the study; YZ coordinated the study and helped to draft the manuscript;
YL designed and coordinated this study and revised the manuscript; All authors have read and approved the final manuscript.
References


Yang J (2016) QTL mapping for pre-harvest sprouting resistance and molecular characterization of six grain germination-related genes in synthetic wheat. Triticeae Research Institute, Sichuan Agricultural University 23-25. (in Chinese with English abstract)


**Internet Resources**


Figure 1. Frequency distribution of TSS in the SHW-L1/Chuanmai 32 recombinant inbred line (RIL) population under 5 environments. The horizontal axis indicates TSS value, the ordinate axis indicates frequency.
Figure 2. Chromosomal locations of quantitative trait loci for TSS and associated markers in the SHW-L1/Chuanmai 32 recombinant inbred line (RIL) population under 5 environments. The black bar points to the LOD peak of QTL.
Figure 3. A comparison of stable putative QTL for TSS between a former study (Yu et al. 2014) and our result. The left side shows the results of previous studies, and the right side shows the results of our studies.
Table 1. The mean phenotypic performance for TSS of the recombinant inbred lines (RILs) and their parents in six environments.

<table>
<thead>
<tr>
<th>Environments</th>
<th>Parent</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHW-L1</td>
<td>Chuanmai 32</td>
</tr>
<tr>
<td>E1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>20.4</td>
<td>21</td>
</tr>
<tr>
<td>E3</td>
<td>21.6</td>
<td>20.5</td>
</tr>
<tr>
<td>E4</td>
<td>20.8</td>
<td>25</td>
</tr>
<tr>
<td>E5</td>
<td>21.2</td>
<td>22.4</td>
</tr>
<tr>
<td>E6</td>
<td>19.6</td>
<td>19.6</td>
</tr>
<tr>
<td>BLUP</td>
<td>20.9</td>
<td>21.8</td>
</tr>
</tbody>
</table>

Table 2. Analysis of variance (ANOVA) and the heritability (h²) values.

<table>
<thead>
<tr>
<th>Degrees of freedom (DF)</th>
<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Significance</th>
<th>Heritability (h²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E G E*G</td>
<td>E G E*G</td>
<td>E G *E</td>
<td>E E E</td>
<td>E  G  G</td>
<td>0.77</td>
</tr>
<tr>
<td>2 139 278</td>
<td>5.3 6.1 0.0</td>
<td>2.6 .0 3</td>
<td>3.5 4 4</td>
<td>5 1 6</td>
<td></td>
</tr>
</tbody>
</table>

E, environment; G, genotype. **Significant at P < 0.01 level, ***Significant at P < 0.001 level.
Table 3. Quantitative trait loci (QTL) for TSS identified in the SHW-L1/Chuanmai 32 recombinant inbred line (RIL) population under 5 environments.

<table>
<thead>
<tr>
<th>QTL</th>
<th>Environments</th>
<th>Marker interval</th>
<th>Nearest flanking marker</th>
<th>Max LOD</th>
<th>Combined LOD</th>
<th>% Expl.</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>QTSS.sicau-2D.1</td>
<td>E1, E2, E3, E4, E6, BLUP</td>
<td>AX94814133-AX110571866</td>
<td>AX110571866</td>
<td>3.31-6.48</td>
<td>25.17</td>
<td>9.7–19.2</td>
<td>SHW-L1</td>
</tr>
<tr>
<td>QTSS.sicau-2D.2</td>
<td>E1, E2, E3, E6, BLUP</td>
<td>gpw4473-wPt740855</td>
<td>wPt740855</td>
<td>4.17-6.22</td>
<td>23.67</td>
<td>12.8–18.5</td>
<td>SHW-L1</td>
</tr>
<tr>
<td>QTSS.sicau-2D.3</td>
<td>E1, E2, E3, E6, BLUP</td>
<td>AX110089401-AX94499721</td>
<td>AX94499721</td>
<td>3.21-4.69</td>
<td>17.78</td>
<td>10.2–14.3</td>
<td>SHW-L1</td>
</tr>
</tbody>
</table>