

Genetic linkage map construction and QTL mapping for panicle-related traits in foxtail millet (*Setaria italica* (L.) P. Beauv.)

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Abstract

Panicle-related traits are important factors affecting foxtail millet yield and detecting the underlying genetic basis is imperative. In this study, we constructed a genetic linkage map using the F₂ population crossed by Changsheng07 and Donggu218, two foxtail millet materials with significant differences in panicle-related traits. The genetic linkage map included 159 SSR markers and 37 Indel markers, covering 1545.5 cM, with average distance of 7.89 cM between adjacent markers. According to the phenotypes of nine panicle-related traits in F_{2:3} families, a total of 22 quantitative trait loci (QTL) were identified, which could explain the phenotypic variation of 0.06–50.14%. Among them, the increased effect allele of 16 QTL were derived from the parent Changsheng07, and seven QTL (*qMPL3.1*, *qMPL5*, *qMPW2*, *qSD5*, *qTGW5.1*, *qTGW5.2* and *qGL5*) were major QTL, which explained the phenotypic variation of 11.41, 12.78, 13.75, 34.81, 50.14, 40.28 and 11.32, respectively. Moreover, we found that a class II formin *Seita.5G078800* may be the candidate gene in the mapping intervals of *qMPL5*, *qSD5*, *qTGW5.2* and *qGL5*. These results will lay a foundation for cloning and molecular mechanism analysis of candidate genes, and provide a theoretical basis for molecular marker-assisted selection breeding for high yield in foxtail millet.

Introduction

Foxtail millet (*Setaria italica*) was one of the world's earliest domesticated crops, with a cultivation history in China that dated back approximately 10,000 years ago (Mamidi et al. 2020; Lu et al. 2009). Foxtail millet has long been planted in arid and semi-arid regions, particularly in China and India (Bettinger et al. 2010). During the domestication of foxtail millet from its wild ancestor green foxtail, strong artificial selection has led to various phenotypic alterations, such as increased plant height, decreased vegetative branching, enhanced branching of inflorescences and larger seeds (Li et al. 2021a; Doust and Kellogg 2006). Moreover, it exhibits numerous properties, for instance, self-fertility, short generation times, small diploid genome, C₄ photosynthesis and small stature and so on, which make it an ideal model plant for functional genomics research in the Panicoid crops (Diao et al. 2014; Lata et al. 2013; Li and Brutnell 2011; Doust et al. 2009).

The improvement of yield and related traits has always been an important goal in foxtail millet breeding. Grain yield is a comprehensive feature, which depends on the panicle number per unit area, grain number per panicle and thousand grain weight. The panicle-related traits, such as main panicle length (MPL), main panicle diameter (MPD), main panicle weight (MPW), spikelet length (SL), spikelet number (SN), spikelet density (SD), thousand grain weight (TGW) and so on, directly influence the production of foxtail millet (Pang et al. 2020). At present, cultivating foxtail millet varieties with large panicles, long primary branches, many grains per panicle and large grains is an important breeding direction (Zhi et al. 2021). Nevertheless, the current foxtail millet breeding techniques lag far behind major crop such as maize, wheat and rice, and the yield potential is relatively low (Diao et al. 2014). Therefore, detecting the genetic foundation of panicle-related traits will lay the basis for molecular breeding and accelerate the genetic improvement of foxtail millet.

QTL mapping based on genetic linkage map is an efficient way to elucidate the genetic determinants of complex quantitative traits. Up to date, many QTL/genes associated with foxtail millet panicle-related traits have been identified using parental separation population. For example, Fang et al. (2016) constructed a high-density genetic linkage map using the F₂ population of Yugu 1 × Longgu 7 with 1,013 SSR markers, and 29 QTL were detected for 11 traits, which could explain 7.0–14.3% of the phenotypic variation. Wang et al. (2019) constructed a genetic linkage map from F₂ populations of Aininghuang and Jingu 21, and 57 QTL of 11 agronomic traits were located, which accounted for 0.38–30.52% of phenotypic variation. A genetic map was constructed using the RIL population of Longgu 7 and Yugu 1 and a total of 47 QTL were identified for four traits (panicle weight, straw weight, thousand grain weight and grain weight per plant) (Liu et al. 2020). Using the RIL populations of Ai 88 and Liaogu 1 as material, 159 QTL were detected for nine agronomic traits and accounted for phenotypic variation ranging from 0.29–25.55% (Zhi et al. 2021). A linkage map was established using Yugu 1 × Longgu 7 RIL population, resulting in the identification of 221 QTL associated with 17 traits in multiple environments, accounting for 5.5–36% of phenotypic variation (Liu et al. 2022a).

Over the past few years, with the advancement of DNA sequencing technology, genome-wide association study (GWAS) of important agronomic traits using natural populations has been widely applied for QTL mapping, which can quickly study genomic variation of foxtail millet. GWAS was performed using genotype data from 916 foxtail millet materials and phenotypic data from five environments, and 512 loci associated with 47 traits were identified (Jia et al. 2013). The 10K SNP developed by GBS-ddRAD method was used for genotyping of 142 foxtail millet varieties, and 81 MTAs (marker-trait associations) of 10 important agronomic traits were identified by GWAS (Jaiswal et al. 2019). Furthermore, GWAS of six panicle-related traits was performed using over 400 foxtail millet materials, and 87 QTL were detected in three environments (Liu et al. 2022b). In addition, 11 QTL for seven agronomic traits were identified under three different environments using 408 foxtail millet materials by two GWAS models (Dai et al. 2024).

In this study, we used foxtail millet varieties Changsheng07 (CS07) and Donggu218 (DG218) as materials to construct an F₂ mapping population, and 196 SSR and Indel markers were used to genotype F₂ individuals and construct the genetic linkage map. Phenotypes of nine panicle-related traits in F_{2:3} families were analyzed. Composite interval mapping (CIM) was used to detect QTL associated with these nine panicle-related traits and 22 QTL were identified, which explained the phenotypic variation explained (PVE) of 0.06–50.14%. The results will

provide a basis for fine mapping and cloning of candidate genes, studying of molecular mechanism research and marker-assisted selection in foxtail millet.

Materials and methods

Plant materials

The excellent foxtail millet varieties Changsheng 07 (CS07) and Donggu 218 (DG218) were cultivated by Shanxi Agricultural University Millet Research Institute and Liaoning Dongya Seed Industry Ltd., respectively. The genetic differences between CS07 and DG218 are large, and their genetic relationship is relatively far. CS07 is characterized by high plant height, large panicle and grain, while DG218 had short plant height and smaller panicle and grain. The F_2 population comprising 238 plants derived from the cross between CS07 (female parent) and DG218 (male parent) and $F_{2:3}$ families were used in this study.

Field experiment

Two-parent hybridization experiment was performed at the Shenfeng Experimental Field of Shanxi Agricultural University (112.58°E/37.42°N) in Taigu, Shanxi Province, in the spring of 2021. In the winter of 2021, the F_1 individuals were cultivated in the greenhouse and self-pollinated to produce the F_2 generation, and the F_2 population was sowed at the Shenfeng Experimental Field of Shanxi Agricultural University in the spring of 2022. Young leaves of single plants in the F_2 population were sampled at the seedling stage. In the spring of 2023, the $F_{2:3}$ families comprising 238 lines were obtained by self-pollination of the F_2 population and were cultivated at the Shenfeng Experimental Field of Shanxi Agricultural University. Each family was planted in two rows with a length of two meters and a width of 0.3 meters. Normal water and fertilizer management were used. All the above experiments were carried out during the optimal sowing period and protected with the anti-bird nets from filling to mature stage.

Phenotypic data collection and statistical analysis

Nine panicle-related traits of parents and $F_{2:3}$ families were investigated, including main panicle length (MPL), main panicle diameter (MPD), main panicle weight (MPW), spikelet length (SL), spikelet number (SN), spikelet density (SD), thousand grain weight (TGW), grain length (GL) and grain width (GW). The SD was the ratio of SN to MPL. The SL was the average length of three spikelets taken from the middle of the panicle. The TGW, GL and GW were measured using WSeen SC-G automatic seed analysis and thousand grain weight meter. For all the above traits, we phenotyped 15 plants per genotype or family, and the average phenotype value of the 15 plants was used as the corresponding trait value.

Microsoft Excel 2021 software was used for basic statistical analysis of parental and population phenotypic data. SPSS 25.0 software was used for correlation analysis, and Origin 2021 was used for visualization mapping.

DNA extraction

Genomic DNA was extracted from young leaves of the F_2 population and two parents using the little modified CTAB procedure (Doyle 1990). The DNA concentration was detected using ultraviolet spectrophotometer, after which the DNA solutions were diluted to 50-200 ng μL^{-1} and then stored at -20 °C.

Genotype analysis

The published 517 SSR markers located on nine chromosomes of foxtail millet were utilized to screen for polymorphism between CS07 and DG218 (Jia et al. 2009; Han et al. 2013; Pandey et al. 2013; Zhang et al. 2014; Qin et al. 2019). Markers with obvious polymorphism were selected to amplify the F_2 population. At the same time, we performed genome-wide resequencing of CS07 and DG218 in GeekGene (<https://www.geekgene.com.cn/>). According to the results of resequencing alignment, insertion-deletion (Indel) sites were found between the two parents and Indel markers with products between 100-300 bp were designed on Primer 3.0 (<https://ginkgo.zju.edu.cn/genome/tools/primer3/>) according to the 200 bp sequence upstream and downstream of the Indel restriction site. Polymorphic Indel markers between CS07 and DG218 were used to amplify individual plants in F_2 population. The amplified polymerase chain reaction (PCR) products were genotyped on 8 % denaturing polyacrylamide gels and visualized by silver staining. The primers used in the experiment were synthesized by Beijing Tianyi Huiyuan Biotechnology Co., LTD and listed in Table S4.

Genetic map construction and QTL mapping

The construction of the genetic map was performed using the program JoinMap 4.0 software (Kyzma Corporation, Wageningen, Netherlands). The Kosambi mapping function was used to convert recombination frequency to genetic map distance in centiMorgan (cM) (Kosambi 2016).

QTL analysis was conducted with QTL Cartographer 2.5 software using composite interval mapping (CIM) with forward and backward regression (Zeng 1994). The empirical logarithm of odds (LOD) threshold was determined based on 1000 permutation tests. Positive and negative additive effects of QTL showed that the allele increasing the phenotypic value was from CS07 and DG218, respectively. The nomenclature of QTL was referred to the rules of Fang et al. (2016). A QTL with phenotypic variation explained (PVE) > 10 % was defined as a major QTL (Zhou et al. 2021). The QTL graphic representation of the linkage groups was created using MapChart 2.3 (Voorrips 2002).

QTL clusters identification

QTL cluster is a dense QTL region distributed on chromosomes, including multiple QTLs related to different traits (Wang et al. 2022a). QTL associated with at least two traits with overlapping physical interval on the same chromosome were identified as a QTL cluster. The identified QTL cluster was named "Q". For instance, for the QTL cluster named "Q2.1", "Q" represents a QTL cluster, "2" represents the chromosome where the QTL cluster is located, and "1" represents the order of the QTL clusters detected on the chromosome.

Result

Phenotypic analysis of panicle-related traits

Phenotypic data of the nine panicle-related traits between CS07 and DG218, including main panicle length (MPL), main panicle diameter (MPD), main panicle weight (MPW), spikelet length (SL), spikelet number (SN), spikelet density (SD), thousand grain weight (TGW), grain length (GL) and grain width (GW), were compared and the result showed that the values of all these nine traits of the CS07 were significantly higher than those of the DG218 (Fig 1, Table S1). In addition, there were extensive variations in the phenotypes of nine traits in CS07×DG218 F_{2:3} families. Meanwhile, these traits had approximately normal distributions, showing that they were determined by multiple loci (Fig.2, Table S1).

Correlation analysis among different panicle-related traits

We analysed the correlation among the nine traits in the F_{2:3} families using the Spearman correlation coefficient (Fig 3). SD was significant negatively correlated with SL, MPL, MPD, MPW and TGW, respectively, and significant positively correlated with SN. MPL and MPW were positively correlated with other traits except SD. SL was positively correlated with MPD, TGW, GL and GW. MPD was positively correlated with SN and TGW. There were significant positive correlations between TGW and GL, TGW and GW, and GL and GW. In this study, no notable correlations were found for other panicle-related traits. The correlation coefficients between GL and GW, SD and SN, SL and MPD and MPL and MPW were 0.66, 0.56, 0.51 and 0.50, respectively, indicating a relatively strong correlation. The findings showed significant or extremely significant correlations between the majority of the nine traits, suggesting that some traits exhibiting strong correlations might be influenced by the same pleiotropic gene or closely linked loci.

Genetic linkage map

In this study, the published 517 SSR markers were used to detect the polymorphism between parents, and 159 pairs of SSR primers showing polymorphism between CS07 and DG218 and 37 Indel markers designed based on parental resequencing results were used for genotyping of the CS07×DG218 F₂ population. The genetic linkage map was constructed using Joinmap 4.0, which covered the genetic length of nine chromosomes of foxtail millet about 1545.5 cM, with average distance of 7.89 cM between adjacent markers. The Chr.9 with the longest genetic distance spanned 240.0 cM and the chromosome with the shortest genetic distance is Chr.8, spanning 88.2 cM. The number of markers on chromosomes ranged from 9 to 31, with a maximum of 31 markers on Chr.2 and a minimum of nine markers on Chr.4. In addition, these 196 markers covered a total of 377.48 Mb physical length on nine chromosomes of foxtail millet, with each chromosome ranging from 27.75 Mb to 56.21 Mb (Fig. 4, Table 1).

QTL mapping

In the F_{2:3} families, 22 QTL were identified for seven of the nine traits (no QTL were found for SN and GL), which distributed on five linkage groups, and the LOD values were between 3.54 and 14.59. These QTL explained 0.06-50.14 % of the phenotypic variation, of which seven QTL with PVE > 10 % were major QTL. Among the 22 QTL, allele with increased phenotypic value of 16 QTL was derived from the parent cultivar CS07 and that of the six remaining QTL was from the parent cultivar DG218 (Table 2).

QTL of main panicle length

Five QTL for MPL were detected on four linkage groups and explained 0.67-12.78 % of the phenotypic variance. Two QTL (*qMPL3.1* and *qMPL3.2*) were located on Chr.3 and the rest on Chr.2 (*qMPL2*), Chr.5 (*qMPL5*) and Chr.7 (*qMPL7*), respectively. The *qMPL3.1* and *qMPL5.1* were two major QTL and explained 11.41 % and 12.78 % of the phenotypic variance, respectively. The allele with increased phenotypic value of four QTL except *qMPL7* was contributed by the CS07 (Fig. 4, Table 2).

QTL of main panicle diameter

Four QTL for MPD (*qMPD2.1*, *qMPD2.2*, *qMPD7* and *qMPD9*) were mapped on Chr.2, Chr.7 and Chr.9, explaining 2.59-8.42 % of the phenotypic variance. The allele that increased MPD for these four QTL was came from CS07 (Fig. 4, Table 2).

QTL of main panicle weight

On Chr.2 and Chr.9, two QTL controlling MPW with the LOD of 6.58 and 3.64 were detected. The *qMPW2* with 13.75 % of PVE was one major QTL. The CS07 allele increased MPW for *qMPW2*, on the contrary, the DG218 allele increased MPW for *qMPW9* (Fig. 4, Table 2).

QTL of spikelet length, spikelet number and spikelet density

For SL, two QTL were located on Chr.2 and Chr.5 and explained 8.67 % and 6.09 % of the variance observed, the allele with increased phenotypic value for which was contributed by the CS07. In our study, no QTL were detected for SN. Two QTL for SD (*qSD5* and *qSD7*) were detected on two linkage groups and *qSD5* was one major QTL with a PVE of 34.81 %. The increased effect allele of *qSD5* was contributed by the DG218, while the increased effect allele of *qSD7* was contributed by the CS07 (Fig. 4, Table 2).

QTL of thousand grain weight

For TGW, five QTL (*qTGW2.1*, *qTGW2.2*, *qTGW2.3*, *qTGW5.1* and *qTGW5.2*) were detected in two linked groups, explaining 0.06-50.14 % of the phenotypic variance. The allele increasing TGW for the three QTL on Chr.2 was contributed by DG218, while that for the two QTL on Chr.5 was contributed by CS07. The two QTL *qTGW5.1* and *qTGW5.2* were major QTL, which explained 50.14 % and 40.28 % of the phenotypic variance, with LOD values of 6.27 and 14.15, respectively (Fig. 4, Table 2).

QTL of grain length and grain width

For GL, there were two QTL detected on Chr.3 and Chr.5, and the increased effect allele of these two QTL was derived from CS07. The *qGL5* is one major QTL with a LOD value of 4.78, which explains 11.32 % of the phenotypic variance. The LOD value of *qGL3* was 3.72, which explained 0.29 % of the phenotypic variance. No QTL were detected for GW (Fig. 4, Table 2).

Identification and analysis of QTL clusters

Phenotypically correlated traits are often localized to similar genomic regions (Zhang et al. 2017). We detected the overlapping QTL for multiple traits and four QTL clusters were identified, which located on Chr.2, Chr.5 and Chr.7. The *Q5* (*qMPL5/qSL5/SD5/qTGW5.2/qGL5*) was one QTL cluster of five traits and *Q2.2* (*qMPL2/qMPD2.2/qMPW2/qSL2*) was one QTL cluster of four traits, while *Q2.1* (*qTGW2.1/qMPD2.1*) and *Q7* (*qMPD7/qSD7*) were two QTL clusters of two traits. Interestingly, the quintuple co-located QTL of *Q5* had large effects, explaining 6.09 % (*qSL5*) to 40.28 % (*qTGW5.2*) of phenotypic variation. In addition, the effects of the quadruple colocated QTL *Q2.2* were also large, explaining 2.28 % (*qMPL2*) to 13.75 % (*qMPW2*) (Table S2).

Discussion

Foxtail millet is regarded as a model plant for functional gene research of gramineae and C₄ crops (Diao et al. 2014). The panicle of foxtail millet is composed of a large number of codes formed by branches at all levels. The size of the panicle varies greatly due to different varieties and environments, and it can directly affect the grain yield of foxtail millet. Therefore, detecting the genetic basis of panicle-related traits is crucial for the genetic improvement of foxtail millet. QTL mapping using parental separation population is an effective method to elucidate complex traits. In this study, an F₂ mapping population was constructed using foxtail millet varieties CS07 and DG218 and used to detect QTL for panicle-related traits. Then, a total of 22 QTL were identified by using the genotype of F₂ population and the phenotype of nine panicle-related traits of F_{2:3} families, among which the alleles increasing the phenotype value of 16 QTL were from CS07. In addition, seven major QTL were detected, namely *qMPL3.1*, *qMPL5*, *qMPW2*, *qSD5*, *qTGW5.1*, *qTGW5.2* and *qGL5* (Fig.4, Table 2).

Parents, F₂ and F_{2:3} mapping population

The selection of parents of the mapping population is very important for QTL mapping. People generally choose parents with significant phenotypic differences, for which may lead to the wider trait variation in the subsequent generations. In this study, CS07 and DG218 with significant differences in panicle characters were selected to construct the mapping population and a wide range for nine panicle-related traits were observed in the F_{2:3} families (Fig.1, Fig.2, Table 1). F₂ population is a mapping population commonly used for initial QTL localization, and its construction time is relatively short (Lander et al. 1987). Yet, each individual in the F₂ population has a unique genotype, which also means that it has only one phenotype and no biological duplication. In addition, QTL mapping accuracy is generally low for polygenic traits with weak heritability in the F₂ population. These problems can be solved by the strategy of F_{2:3} families. In the data analysis, the average phenotype of

F_{2:3} families can be used to represent the phenotype of the F₂ generation (Zhang and Xu 2004). In this study, the F₂ populations of CS07 and DG218 were genotyped and the genetic linkage map was constructed. We used the average phenotype of the F_{2:3} family to represent the phenotype of the F₂ generation, and the QTL were detected.

Comparison the QTL identified in this study with previous researches

A total of 22 QTL were detected in this study, with PVE ranging from 0.06 % to 50.14 %, and the increased effect alleles for 16 QTL were from CS07 (Fig.4, Table 2). We compared the genomic locations of the QTL detected in this study with those previously identified in bi-parental and natural populations and found that 12 QTL overlapped with those found in other studies and the remaining 10 QTL might be novel (Table S3).

For main panicle length, five QTL (*qMPL2*, *qMPL3.1*, *qMPL3.2*, *qMPL5* and *qMPL7*) on Chr.2, Chr3, Chr.5 and Chr.7 were detected. Among them, *qMPL5* might be a new QTL. The *qMPL2* (39.14-44.71 Mb) was overlapped with *qMPL2.1* (Chr2:36524427-39883917 bp) and *qMPL2.2* (Chr2:44023148-45068576 bp) mapped by Liu et al. (2022a), *qPL02.19-qPL02.22* identified by Dai et al. (2024) and *qMPL2.1* (Chr2-GSA02220:39536906-39537056 bp) mapped by Fang et al. (2016). The *qMPL3.1* was overlapped with QTL identified by Zhi et al. (2021), Dai et al. (2024) and Jia et al. (2013). The *qMPL3.2* (47.02-48.18 Mb) in this study was in accordance with *qPL03.13* (Chr3:LDB_3_47070176) discovered by Dai et al. (2024). Moreover, *qMPL7* (22.34-29.18 Mb) identified in our study was overlapped with *qPL7.2* (Chr7:21055940-26580634 bp) and *qPL7.3* (Chr7:28099425-28456882 bp) discovered by Zhi et al. (2021) and *qPL07.11* (Chr7:LDB_7_26882815) discovered by Dai et al. (2024) (Table S3).

In our study, four QTL (*qMPD2.1*, *qMPD2.2*, *qMPD7* and *qMPD9*) controlling main panicle diameter were detected, of which *qMPD2.2* and *qMPD9* might be two new QTL. The *qMPD2.1* (6.52-31.53 Mb) was overlapped with *qPD2.2* (Chr2:28932236-32347878 bp) by Zhi et al. (2021), *qPD02.5-qPD02.13* by Dai et al. (2024) and *qpd2* (Chr2-bin392:31510348-32450713 bp) under a long-day photoperiod by Zhang et al. (2017). In addition, *qMPD7* have been detected by Liu et al. (2022a) and Dai et al. (2024) (Table S3).

For main panicle weight, two QTL (*qMPW2* and *qMPW9*) were detected in our study. The *qMPW2* (37.48-40.53 Mb) and *qMPW9* (52.91-58.05 Mb) in our study were overlapped with *qPWP2.2* (Chr2:38474641-41662480 bp) and *qPWP9.2* (Chr9:55978627-57486191 bp) mapped by Liu et al. (2022a), respectively. In addition, *qMPW2* have been detected by Zhi et al. (2021) and *qMPW9* have been mapped by Liu et al. (2020) and Dai et al. (2024) (Table S3).

There were five QTL (*qTGW2.1*, *qTGW2.2*, *qTGW2.3*, *qTGW5.1* and *qTGW5.2*) located on Chr2 and Chr5 affecting thousand grain weight in this study and *qTGW5.2* might be a new QTL. The *qTGW2.1* (6.52-31.53 Mb) was in accordance with *qTGW2.1* (Chr2:28215787-28932236 bp) by Zhi et al. (2021) and *qTGW02.5-qTGW02.7* by Dai et al. (2024). The *qTGW2.2* (31.53-32.76 Mb) was overlapped with *qTGW2.2* (Chr2:32093363-32838266 bp) identified by Zhi et al. (2021). The *qTGW2.3* (33.71-37.34 Mb) and *qTGW5.1* (1.85-3.49 Mb) were overlapped with *qTGW02.8* (Chr2:LDB_2_35148511_35163319) and *qTGW05.1* (Chr5:LDB_5_1927553_1928145) detected by Dai et al. (2024), respectively (Table S3). For spikelet length, spikelet density and grain length, only two QTL for each trait were detected in this study, and all of them have been not detected by the previous researches and may be new QTL (Table S3).

QTL clustering for multiple traits

QTL clustering is a common phenomenon in preliminary QTL mapping studies. Here, we detected four QTL clusters located on Chr.2, Chr.5 and Chr.7, each associated with two or more traits related to panicle (Table S2). The QTL cluster *Q2.2* harbored four QTL associated with MPL, MPD, MPW and SL, respectively, and the QTL cluster *Q5* contained five QTL related to MPL, SL, SD, TGW and GL, respectively. These two QTL clusters had the large PVE and might contain major genes controlling panicle related traits. QTL clusters may represent pleiotropic effects of a single gene/QTL within the same genomic region or linkage of genes/QTL. These QTL clusters laid a foundation for further exploration of target genes controlling panicle-related traits.

Seita.5G078800 might be the candidate gene of *qMPL5*, *qSD5*, *qTGW5.2* and *qGL5*

At present, there are few reports on genes related to panicle traits in foxtail millet. For instance, a single G-to-A transition in the fifth intron of *Loose Panicle1* (*LP1*), encoding a novel WRKY transcription factor, led to three disorganized splicing events in mutant plants, resulting in a loose panicle phenotype (Xiang et al. 2017). A single G-to-A transition occurred in the fifth intron of *SiMADS34* in the mutant, resulting in a variable splicing event and premature termination, and ultimately leading to an abnormal panicle morphology (Hussin et al. 2021). *SiBOR1* encoded a membrane-localized boron efflux transporter, and its loss-of-function mutant *sibor1* had fewer primary branches and necrosis was observed at the apices of peduncles (Wang et al. 2022b). *SiAUX1* encoded an auxin influx transporter and loss-of-function plants displayed fewer primary branches and a sparsely branched panicle. Furthermore, *SiAUX1* overexpressing plants had more lateral roots than wild type plants (Tang et al. 2022). Tang et al. (2023) identified an important RING-type E3 ligase SGD1 and its E2 partner SiUBC32 responsible for grain weight, grain size, and panicle size.

By contrast, many panicle-related genes in rice (*Oryza sativa* L.) have been identified in the past decades. According to the core regulatory factors, it could be divided into six regulatory pathways: GRAIN NUMBER1a (Gn1a), IDEAL PLANT ARCHITECTURE1 (IPA1), LAX PANICLE1 (LAX1), FRIZZY PANICLE (FZP), MONOCULM 1 (MOC1) and TAWAWA1 (TAW1) (Komatsu et al. 2003; Li et al. 2003; Ashikari et al. 2005; Jiao et al. 2010; Yoshida et al. 2013; Huang et al. 2018; Li et al. 2021b). The actin cytoskeleton, including actin filaments and microtubules, relies on essential cytoskeleton-binding proteins and plays a crucial role in plant morphogenesis. Formins are one of the most important kinds of cytoskeleton-binding proteins, and *Oryza sativa* formin homolog 3 (OsFH3) and OsFH5 are two of the five known type II formation proteins in rice, which played a crucial role in rice morphogenesis. The loss-of-function mutant *osfh3* is semi-dwarf, and has fewer primary branches per panicle and smaller grain length (Chang et al. 2021). *BUI1* encodes the class II formin FH5 and the *bui1* mutant showed pleiotropic phenotypes, including dwarfing, more tillers, smaller and looser panicles, and smaller grains (Yang et al. 2011). Interestingly, based on gene function annotation and homologous gene comparison, we found that *Seita.5G078800* (Chr5:6718817-6732492 bp), a homologous gene of *OsFH3*, might be the candidate gene in the mapping intervals of *qMPL5* (Chr5: 6.21-7.90 Mb), *qSD5* (Chr5: 6.21-10.56 Mb), *qTGW5.2* (Chr5: 6.21-8.39 Mb) and *qGL5* (Chr5: 5.24-26.92 Mb), which explained the phenotypic variation rates of 12.78 %, 34.81 %, 40.28 % and 11.32 %, respectively. However, this needs to be further proved by experiments such as parental gene sequence alignment and transgenic function verification and so on.

Declarations

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Contributions

LLG, GHY and JGW designed the experiments. LLG, GHY, HL and QXZ performed experiments. SYW, JHF, TGW, LJY, YQZ, YXM and LC collected the phenotype. LLG and LH wrote the manuscript. XRL, SQD, XQC and XYY helped revise the manuscript. All authors reviewed and approved the final manuscript.

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Data availability

The datasets generated and/or analysed during the current study are available in the NCBI SRA database (BioProject accession number: PRJNA1177802).

Ethics declarations

Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1 Number of SSR and Indel markers on the chromosomes

Chr.	Marker number	Genetic distance (cM)	Average interval (cM)	Cover length (Mb)
1	25	228.2	9.13	41.78
2	31	188.4	6.08	44.58
3	27	195.0	7.22	50.06
4	9	134.3	14.92	38.88
5	30	145.1	4.84	44.30
6	14	168.5	12.04	35.76
7	15	157.8	10.52	27.75
8	15	88.2	5.88	38.16
9	30	240.0	8.00	56.21
total	196	1545.5	7.89	377.48

Table 2 QTL analysis of panicle-related traits in foxtail millet

Trait	QTL name	Chr.	Nearest maker	QTL Peak Position (cM)	Marker interval		Position (Mb)	Additive	Dominant	LOD score	PVE (%)
MPL	<i>qMPL2</i>	2	SICAAS2046	166.6	SICAAS2046	b169	39.14-44.71	0.79	0.71	6.51	2.28
	<i>qMPL3.1</i>	3	S220	143.4	S220	SICAAS3043	42.73-47.02	0.84	-0.39	5.20	11.41
	<i>qMPL3.2</i>	3	b163	155.4	SICAAS3043	b198	47.02-48.18	0.78	-0.22	4.91	8.56
	<i>qMPL5</i>	5	LiB5005	69.8	LiB5105	LiB5110	6.21-7.90	1.03	-0.14	7.67	12.78
	<i>qMPL7</i>	7	SICAAS7028	88.2	SICAAS7057	SICAAS7038	22.34-29.18	-0.52	-0.81	3.57	0.67
MPD	<i>qMPD2.1</i>	2	b242	104.2	b157	b115	6.52-31.53	0.97	-0.45	4.57	8.41
	<i>qMPD2.2</i>	2	LiB2107	157.1	LiB2107	SICAAS2015	37.48-40.53	1.08	-0.20	5.06	8.42
	<i>qMPD7</i>	7	SICAAS7057	65.8	SICAAS7026	SICAAS7028	21.68-23.27	0.96	0.00	4.62	6.01
	<i>qMPD9</i>	9	SICAAS9023	36.6	SICAAS9071	SICAAS9031	4.45-7.33	0.86	0.46	4.56	2.59
MPW	<i>qMPW2</i>	2	LiB2107	155.1	LiB2107	SICAAS2015	37.48-40.53	1.48	-0.29	6.58	13.75
	<i>qMPW9</i>	9	b105	202.0	b105	SICAAS9067	52.91-58.05	-1.06	0.61	3.64	9.17
SL	<i>qSL2</i>	2	SICAAS2016	178.1	SICAAS2046	b169	39.14-44.71	0.41	-0.07	4.83	8.67
	<i>qSL5</i>	5	LiB5005	69.8	LiB5105	LiB5106	6.21-6.67	0.45	0.12	6.20	6.09
SD	<i>qSD5</i>	5	LiB5006	71.0	LiB5105	b188	6.21-10.56	-0.45	0.15	14.59	34.81
	<i>qSD7</i>	7	SICAAS7057	68.8	SICAAS7057	SICAAS7028	22.34-23.27	0.26	0.02	4.13	6.62
TGW	<i>qTGW2.1</i>	2	b242	103.2	b157	b115	6.52-31.53	-0.09	-0.08	3.54	0.06
	<i>qTGW2.2</i>	2	SICAAS2030	120.3	b115	SICAAS2043	31.53-32.76	-0.11	-0.04	4.76	3.66
	<i>qTGW2.3</i>	2	LiB2103	138.9	LiB2101	LiB2106	33.71-37.34	-0.10	-0.01	4.68	6.59
	<i>qTGW5.1</i>	5	LiB5102	44.0	LiB5102	S262	1.85-3.49	0.01	-0.18	6.27	50.14
	<i>qTGW5.2</i>	5	LiB5005	69.8	LiB5105	SICAAS5009	6.21-8.39	0.18	-0.10	14.15	40.28
GL	<i>qGL3</i>	3	SICAAS3069	0.0	SICAAS3069	SICAAS3004	0.14-1.00	0.03	0.04	3.72	0.29
	<i>qGL5</i>	5	LiB5107	70.7	LiB5104	S278	5.24-26.92	0.05	-0.01	4.78	11.32

Figures

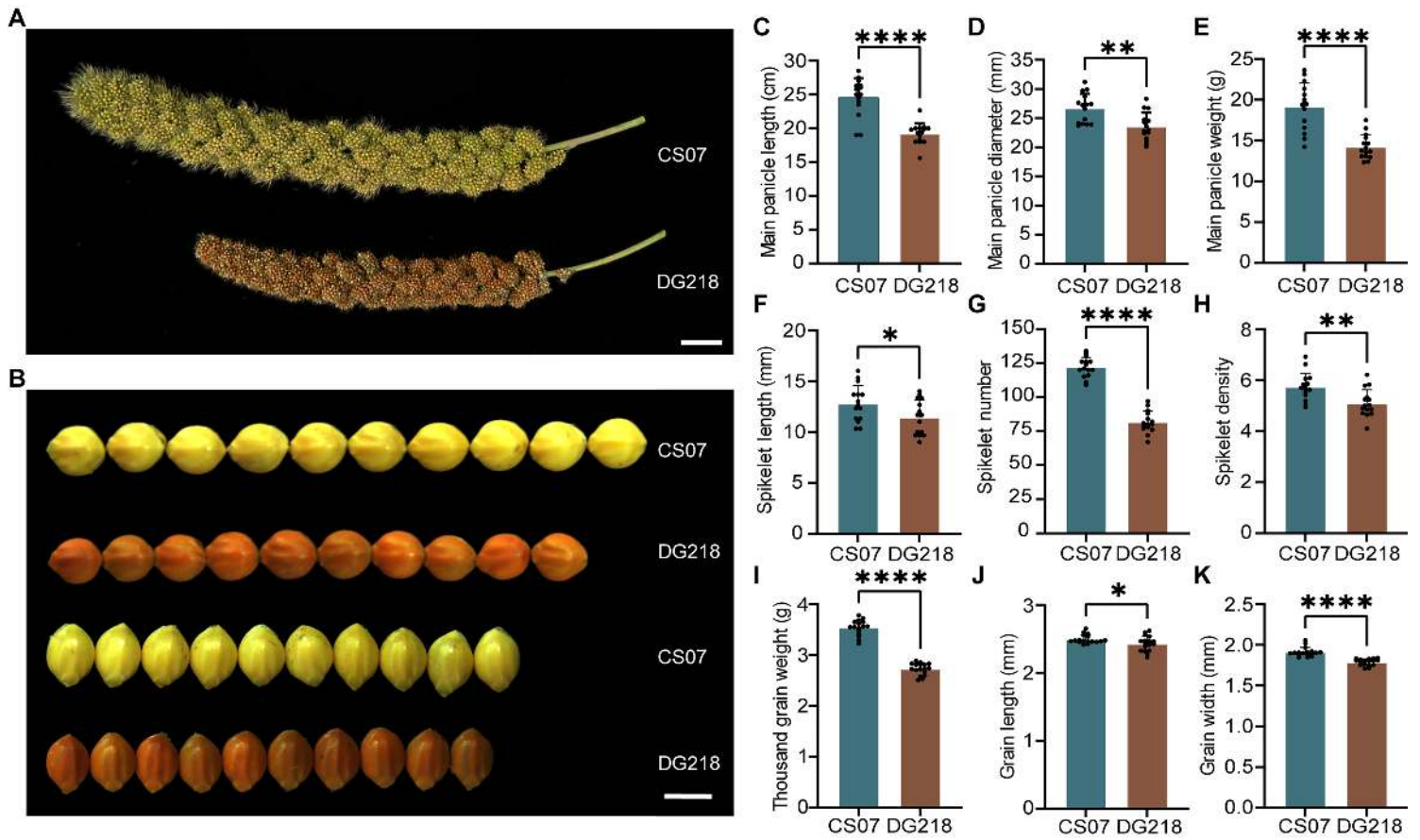


Figure 1

Phenotypes of CS07 and DG218. (A) The panicle morphology of CS07 and DG218 (bar = 2 cm). (B) The grain morphology of CS07 and DG218 (bar = 2 mm). The main panicle length (C), the main panicle diameter (D), the main panicle weight (E), the spikelet length (F), the spikelet number (G), the spikelet density (H), the thousand grain weight (I), the grain length (J) and the grain width (K) of CS07 and DG218. Differences between genotypes were assessed with Student's *t* test. **p* < 0.05, ***p* < 0.01, *****p* < 0.0001

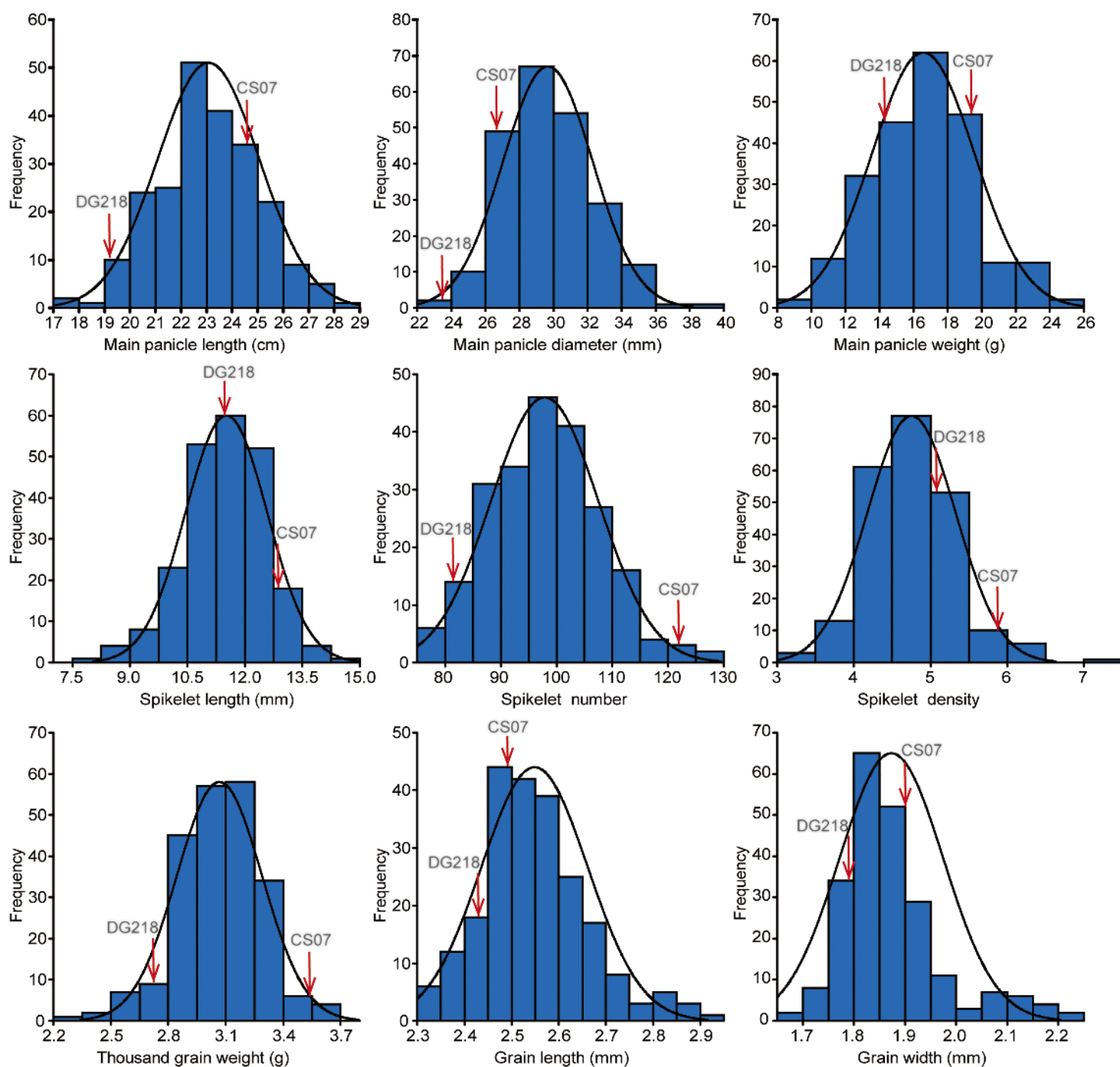


Figure 2

Phenotypic frequency distribution of the nine panicle-related traits in CS07 × DG218 $F_{2:3}$ families.

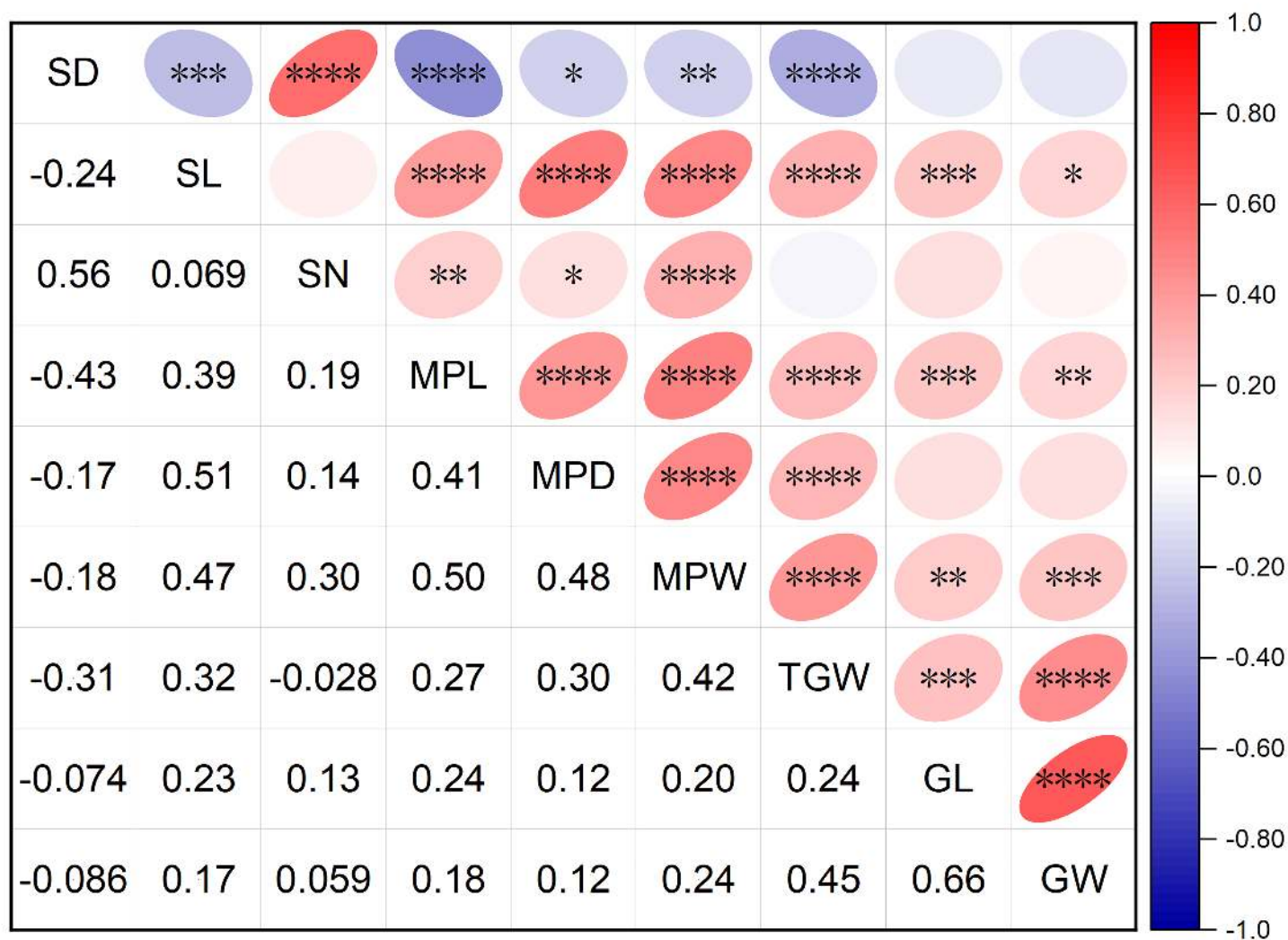


Figure 3

Correlation coefficient of the nine panicle-related traits in the $F_{2:3}$ families. *, **, *** and **** represent significance at the 0.05, 0.01, 0.001 and 0.0001 probability levels

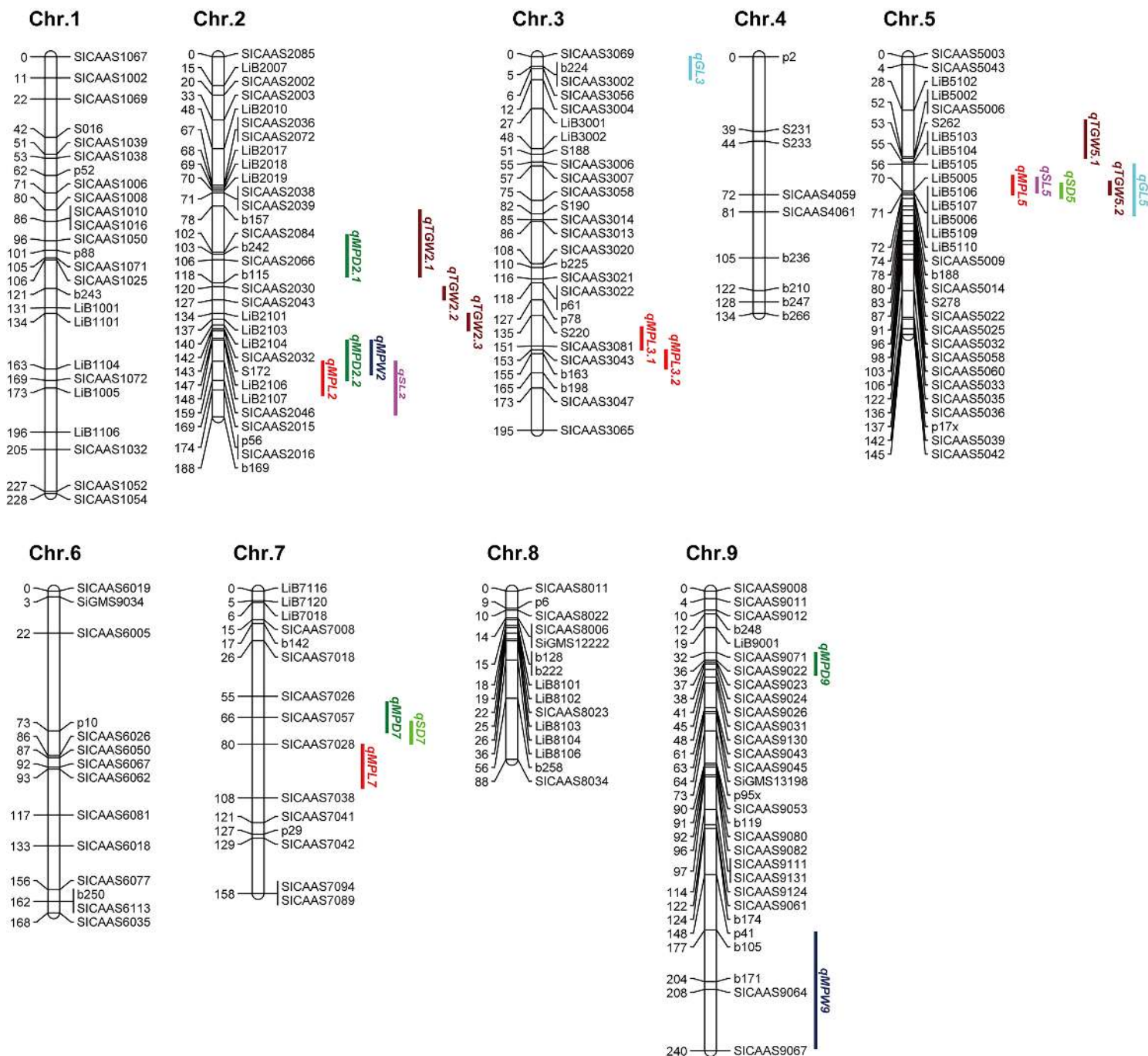


Figure 4

Genetic map and QTL for nine panicle-related traits. The marker name and the genetic location (cM) were shown on the right and on the left of the chromosome, respectively.

Supplementary Files

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