Dissection of novel genetic regions and superior alleles related to high grain number per spike in a new wheat-Agropyron germplasm Pubing3228

Jiansheng Wang (wjsheng1998@163.com)
Pingdingshan University
Erwei Wang
Shiping Cheng
Aichu Ma

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Abstract

Agropyron cristatum (L.) can be used as a valuable gene resource for broadening the genetic basis of common wheat in breeding programs. Pubing3228 is a new elite wheat-Agropyron hybrid germplasm that possesses many desirable agronomical traits, includingthe grain number per spike (GNS), the main yield component. Uncovering the genetic architecture of GNS in Pubing3228 is important for increasing wheat yield through molecular assisted breeding. In the present study, genetic regions and alleles associated with high GNS from Pubing3228 were analyzed using a recombination inbred line (RIL) population derived from the cross between Pubing3228 and Jing4839. In total, 75 QTLs were identified for GNS. These QTLs mapped to 21 chromosomes (i.e.,all except for 5B and 6A) and explained 3.30–17.17% of the total phenotypic variation. Five QTLs (QGns.wa-1D, QGns.wa-5A, QGns.wa-7D.1, QGns.wa-7D.2, and QGns.wa-7D.3) explained more than 10% of the phenotypic variation in at least two environments. Pubing3228 contributed 94.67% of the total number of QTLs associated with GNS. Candidate gene analysis was conducted for the stable QTLs, and 11 candidate genes were obtained for GNS. Among these 11 genes, a senescence-associated protein gene (TraesCS7D01G148000) linked with the most significant single nucleotide polymorphism (SNP) AX-108748734 on chromosome 7D was relevant to remobilizing the nutrients from the senescing tissues to the developing seeds. The findings of this study will not only provide new insights into the genetic mechanism of high GNS of Pubing3228 but willalso be useful for marker-assisted selection for yield improvement in breeding practice.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cerealcrop in the world. Although yield and totalproduction of wheat have increased significantly over the past several decades, the current level of wheat grain production will be unable to meet the future demand of anever-expanding global population. Furthermore, this challenge will become increasingly serious due to the gradual decrease in farmland. Therefore, improving wheat grain yield is necessary to guarantee global food and nutritional security. This makes increased grain yield the main objective inwheat breeding.

Wheat yield is largely determined by three components, the grain number per spike (GNS), grain weight, and spike number. Previousstudies have suggested that wheat grain yield is affected more by variationin GNS than by variation in grain weight (Reynolds et al. 2009). In wheat breeding practice, high yield has been achieved primarily through an increase in GNS rather than through increasesin grain weight (Dobrovolskaya et al. 2015; Fischer et al. 2008; Fischer 2011; Dolferus 2011). The utilization of genetic variation in GNS has become a promising approach to improving yield levels in the future. Grain number per spike isa complex quantitative trait in nature and is controlled by multiple genes. Quantitative trait locus (QTL) mapping has provided an effective way to analyze suchtraits.With the development of molecular markers, many QTL analyses have been conducted for GNS, and numerous QTLs have been identified (Li et al. 2019; Cuthbert et al. 2008; Cui et al. 2017; Jia et al. 2013; Cui et al. 2014; Li et al. 2007; Shi et al. 2017; Hai et al. 2008; Liu et al. 2014; Bhusal et al. 2017; Onyemaobi et al. 2018; Xu et al. 2017; Guan et al. 2018; Liu et al. 2018; Muhammad et al. 2020; Jung et al. 2021; Zheng et al. 2022; Zhai et al. 2018; Guo et al. 2018; Li et al. 2018; Liu et al. 2018; Börner et al. 2002; Li et al. 2015; Guo et al. 2017; Pang et al. 2020; Chen et al. 2017; Deng et al. 2017; Ding et al. 2011; Gao et al. 2015; Gerard et al. 2019; Wang et al. 2017; Wu et al. 2012; Zhou et al. 2017). These reported QTLs are distributed throughout the entire wheat genome.

Inthe QTL mapping process, saturated genetic linkage maps play a crucial role in QTL identification, as they not only provide measurements of the relative effects of loci in the mapped genetic regions but also provide useful DNA markers for marker-assisted selection (MAS) in breeding practice. However, due to the lack of molecular markers in the past, unsaturated genetic linkage maps were used, resulting in linkage markers that were genetically distant from the target QTLs. This restricts further understanding of the genetic structure of GNS and utilization of the markers in wheat breeding. With the rapid development of next-generation sequencing (NGS) technology, reference genomes of Chinese Spring and other varieties have been released (IWGSC 2018), and these can be used to remove the above restrictions. Recently, diverse SNP genotyping platforms have been developed based on NGS, and significant progress has been reported for QTL analysis of wheat GNS. At present, high-density SNP genotyping arrays such as 9K, 55K, 660K, and 820K SNP assays have been developed for wheat (Akhunov et al. 2009; Akpinar et al. 2017; Cui et al. 2017; Gao et al. 2016; Zhou et al. 2018; Ma et al. 2018), and these arrays have been widely employed in QTL analysis. For example, the 90K wheat SNP chip has been used to study the genetic control of yield-related traits in a panel of 66 elite wheat accessions derived from Xiaoyan6, and a total of 803 significant marker-trait association (MTAs) were identified that explained up to 35.0% of the phenotypic variation (Ma et al. 2018).

The limited genetic diversity of common wheat has resulted inbreeding bottlenecks; therefore, it is very important to discover new and potentially useful genetic loci controlling yield-related traits in order to broaden genetic variation and accelerate varietal improvement in future wheat breeding. Wild related species are important gene sources for improvement of common wheat. *Agropyron* is an important wild relative of wheat that could provide new gene variants for wheat improvement (Dong et al. 1992). The intergeneric hybrids of common wheat with *Agropyron cristatum* (L.) were successfully obtained for the transfer of desirable alleles from *Agropyron* spp. to wheat (Li et al. 1991; 1993; 1995; 1997; 1998). To date, many novel wheat-*Agropyron* germplasm resources with desirable agronomic traits have been produced by Li et al. Moreover, further research has been conducted on the genetic mechanism and the chromosomal segments or genes of *Agropyron* in the background of common wheat (Luan et al. 2010; Wu et al. 2006; Zhang et al. 2015; Zhang et al. 2019; Song et al. 2016a, 2016b; Zhang et al. 2015; Zhou et al. 2018; Sun et al. 2021; Wang et al. 2011; Zhang et al. 2015). For example, Zhang et al. (2019) used genomic in situ hybridization (GISH), dual-color fluorescencein situ hybridization (FISH), and molecular markers to study the wheat-*A.cristatum* translocation line Pubing2978 that has a high GNS; they found that the *A. cristatum* 6P chromosomal segment played a crucial role in increasing the GNS. Sun et al. (2021) identified an enhanced grain weight locus from *Agropyron cristatum* chromosome 7P and mapped this locus to 7PS1-2 using 158 STS markers. The mechanism of this locus on wheat grain weight was

furtherly examined, and it was found that two translocation lines (7PT-A18 and 7PT-B4) with 7P chromosomal segments could simultaneously increase grain weight, grain length, and grain width.

Although many previous studies have been conducted and numerous QTLs have been identified for GNS, most of these studies were carried out using common wheat. Pubing3228 is a new wheat germplasm that was selected from the progeny of common wheat and *Agropyron*. It possesses many elite agronomic traits, especially yield traits (Wang et al. 2009). To date, QTL analysis of the new wheat-*Agropyron* germplasm is still in the early stages, and the molecular mechanism controlling GNS in Pubing3228 isstill largely unknown. In this study, we developed a recombination line (RIL) population derived from the cross of Pubing3228 and Jing4839 that exhibited large differencesin GNS. Based on the newly developed wheat 55K genotyping assay, our main objectives were to identify novel genetic regions and superior alleles related to GNS and to provide a foundation for gene fine mapping and exploration of functional genes for GNS.

Materials and Methods

Materials

A RIL population consisting of 210 families derived from the cross of the new germplasm Pubing3228 and Jing4839 was used for QTL analysis. This population was constructed using the single seed descent method. Pubing3228 was a genetically stable derivative selected from the progeny of a wheat-*A.cristatum* chromosome addition line 4844-12 (2n=44). This cross is an elite wheat germplasm with desirable yield traits, including along spike, a large number of spikelets per spike, and a high GNS. This wheat germplasm was developed by Li et al. of the Chinese Academy of Agricultural Sciences over the past several decades. Additionally, Jing4839 is a cultivar with higher grain weight but fewer grains per spike. The two parents had large differences in several yield traits, especially the GNS.

Field experiment and phenotype

The 210 recombinant inbred lines of Pubing3228/Jing4839 along with their parents were grown in Pingdingshan (PDS) during three crop years (2018, 2019, and 2020), in Yangling (YL) during two crop years (2019 and 2020), and in Xianyang (XY) in 2020. In each field environment, the materials were planted in randomized complete block designs with three replications. Each block contained three rows, each 2 m long,witha 30 cm distance between rows. All field management practices followed the local standards. When plants reached physiological maturity, 10 representative plants per genotype from each replication were harvested and manually threshed. The GNS was counted directly.

DNA extraction and genotyping

Genomic DNA of the RIL population and their parents was extracted using the SDS method. DNA quality was checked by 0.8% agarose gel electrophoresis, and DNA concentration was determined using amicroplate reader. The DNA was then sent to Beijing Capital Bio-Corporation (Beijing, China) for genotyping using an Illumina Infinium iSelect 55K SNP array. After removing the markers with a minimum allele frequency (MAF) of less than 5%, markers with more than 10% missing data,and markers with more than 20% heterozygosity, a final total of 3334 good-quality SNP markers were obtained and used for QTL analysis.

Linkage group construction and QTL mapping

The polymorphic SNP markers between Pubing3228 and Jing 4839 were used to construct a genetic linkage map using QTL IciMapping software V4.2 (Wang 2009) (http://www.isbreeding.net). First, the BIN function was performed for all SNP markers to filter markers with segregation distortion, missing data, and redundancy. Then, the remaining SNP markers were used to construct the frame of the genetic linkage map by using the MAP function. In addition, the order and distance between markers were determined by maximum likelihood using Kosambi's function. Finally, the genetic linkage markers were used for QTL mapping via IciMapping software V4.2. We chose the model of composite interval mapping (ICIM) to search for QTLs involved with GNS. A significant QTL was declared if its LOD score was greater than 2.5. QTL analysis was performed separately for each environment, and QTLs detectedin more than two environments simultaneously were considered to be stable.

Identification of candidate genes

SNP markers flanking the QTL confidence intervals were used to determine the physical intervals of QTLs on chromosomes. A gene located in a physical interval of a QTL would be considered as a candidate gene for GNS. The candidate genes were obtained from the JBrowse website (https://urgi.versailles.inra.fr/jbrowseiwgsc). The expression profiles of candidate genes in the different tissues were obtained from expVIP (http://wheat-expression.com). The candidate genes associated with GNS were obtained based on comparisons of the expression of the preliminary candidate genes.

Results

Phenotypic analysis

The two wheat cultivars Pubing3228 and Jing4839 exhibited remarkable differences in GNS when grown in the same environment (Table1). Compared to Jing4839, Pubing3228 had a significantly greater GNS. Therefore, a RIL population was generated by crossing Pubing3228 and Jing4839 and was then used for QTL mapping of GNS. In the RIL population, GNS exhibited approximately continuous variation in the three environments during 2019–2021 (Table 1, Figure 1), indicating that the variation patterns were typical of quantitative traits and were fit for QTL mapping. The highest GNS was observed in the PDS environment in 2021 (~140 grains), while the lowest GNS was observed in YL in 2021 (~19.67grains) (Table 1). The mean GNS varied from 47.81 to 59.69 among the three environments during 2019–2021. Additionally, the absolute values of skewness and kurtosis were <1, except for 2020 PDS and 2021 YL, indicating that the phenotypic data were approximately normally distributed in this population.

Linkage group construction

Based on the 52,478 SNPs produced from the 55 K iSelect SNP array, a total of 3334 SNP markers were polymorphic between Pubing3228 and Jing4839 and were used for linkage map construction when the markers that either co-segregated at the same position or that had missing values in more than 10% of the lines were excluded. A total of 21 linkage groups were constructed, representing each of the hexaploid wheat chromosomes, and each chromosome was represented by one linkage group (Figure 2, Table 2). The map covered 9494.36cM in length, with an average locus spacing of 2.90cM. The number of loci varied from 76 on chromosome 4D to 262 on chromosome7D, whereas the spacing of an average locus varied from 0.89cM on chromosome 1A to 9.03cM on chromosome 4D. The map of the A genome had 1166 loci (34.97%) with a total length of 2077.44cM and an average spacing of 1.76 per locus. The map of the B genome had 1017 loci (30.50%) with a total length of 1871.35cM and an average spacing of 1.86cM per locus. The map of the D genome included 1151 loci (34.53%) with a total length of 5545.57cM and an average spacing of 5.08 per locus. The number of loci in genome A was approximately the same as the number of loci in genomes B and D, and the B genome had the lowest marker coverage (Table 2).

QTL detection

In the present study, a total of 75 QTLs controlling GNS were detected across three environments during 2019–2021 (Table 3).These QTLs were distributed on 21 chromosomes (all except 5B and 6A).The largest number of QTLs (14 QTLs) was detected on chromosome 7D, while only one QTL was found on chromosomes 1B, 2A, 3B, 4D, and 7A. The identified QTLs had an LOD score range of 2.52–33.21 and explained 3.30–17.17% of the phenotypic variation. Among the QTLs, Q*Gns.wa-1A* had the highest amount of phenotypic variance explanation (PVE) (17.17%) and was mapped to the distance between AX-94465571 and AX-109859174 on chromosome 1A. The next highest in order were Q*Gns.wa-7D* between AX-110826147 and AX-108748734 and Q*Gns.wa-1D* between AX-109994213 and AX-89491149, contributing 15.12% and 14.83% PVE, respectively. Regarding the stable QTLs,11 were detected simultaneously in different environments. Among these, five stable QTLs were found on chromosome 7D; two stable QTLs were detected on chromosome 5A; and one reliable QTL was identified on 1D, 3D, 4A, and5D. It is noteworthy that five QTLs (Q*Gns.wa-1D*, Q*Gns.wa-5A*, Q*Gns.wa-7D.1*, Q*Gns.wa-7D.2*, and Q*Gns.wa-7D.3*) were identified in three environments. Moreover, the five stable QTLs, except for Q*Gns.wa-7D.1*, had higher PVEs. Q*Gns.wa-1D* between AX-109994213 and AX-89491149 on chromosome 1D was found in 2019E1, 2020E1, and 2020E3, contributing 9.89–14.83% of the phenotypic variation. Q*Gns.wa-5A* was detected between AX-108905072 and AX-109108075 on chromosome 5A in 2020E1, 2020E2, and 2020E3, explaining 9.095–11.13% of the phenotypic variation. The other three stable QTLs, Q*Gns.wa-7D.1*, Q*Gns.wa-7D.2*, and Q*Gns.wa-7D.3*, were detected on chromosome 7D and explained 3.49–5.45%, 8.90–13.82%, and 4.92–15.12% of the phenotypic variation, respectively. In addition, all 11 stable QTLs, with the exception of Q*Gns.wa-7D.1*, were from Pubing3228. The GNS QTLs were unevenly distributed among the three sub-genomes, with 39 QTLs on the D sub-genome, 24 QTLs on the A sub-genome, and 12 QTLs on the B sub-genome. Notably, all ofthe favorable alleles of QTLs, except the four QTLs Q*Gns.wa-7D.e1.1* detected in 2019, Q*Gns.wa-7D.e1.1* detected in 2020, Q*Gns.wa-3A.e3.1*, and Q*Gns.wa-7D.e3.1*,were contributed by Pubing3228, suggesting that Pubing3228 could harbor elite high-GNS features that should be further explored.

Prediction of candidate genes

To understand the molecular mechanisms of GNS in wheat, candidate gene analysis was conducted for several stable QTLs affecting GNS. A total of 1265 genes were obtained. An expression heat map was constructed for these genes using the public database of Wheat Expression Browser (http://www.wheat expression.com), and genes that were only specifically expressed in grains were identified as candidate genes for GNS. Finally, 11 candidate genes were identified for GNS in wheat (Table 4). These candidate genes weremostly distributed on chromosomes 4A, 5D, and 7D, and the largest number of candidate genes was on chromosome 7D. On chromosome 4A, only one candidate gene (*TraesCS4A01G601800*) was found, and it showed potential relevance to 4-hydroxybenzoate octaprenyltransferase in wheat. Three candidate genes (*TraesCS5D01G044000*, *TraesCS5D01G051000*, and *TraesCS5D01-G033700*) were identified on chromosome 5D. *TraesCS5D01G044000* probably encoded for 60S ribosomal protein l28, *TraesCS5D01G051000* was probably relevant to a senescence-associated protein, and *TraesCS5D01G033700* possibly encoded a YABBY transcription factor. Seven candidate genes, namely *TraesCS7D01G056100*, *TraesCS7D01G060100*, *TraesCS7D01G064300*, *TraesCS7D01G109200*, *TraesCS7D01G148000LC*, *TraesCS7D01G117-600*, and *TraesCS7D01G117100*, were identified on chromosome 7D. Interestingly, each of theseven candidate genes hada distinct biological function. For example, *TraesCS7D01G056100* possibly encoded an S-adenosylmethionine decarboxylase proenzyme; *TraesCS7D01G060100* possibly encoded acysteine protease; *TraesCS7D01G064300* possiblyencoded a starch synthase; *TraesCS7D01G109200* possibly encoded a GDSL esterase/lipase; *TraesCS7D01G148000* possibly encoded a senescence-associated

protein; *TraesCS7D01G117600* possibly encoded an ethylene-responsive transcription factor; and *TraesCS7D01G117100* possibly encoded a Histone H3. Further work on these genes is necessary to identify the genes involved in the regulation of GNS.

Discussion

Grain number per spike is the main yield component of wheat. Several previous studies have demonstrated that the progress in wheat yield was primarily attributed to an increase in GNS (Dobrovolskaya et al. 2015; Fischer et al. 2008, 2011). Therefore, GNS has become an important selection objective in wheat breeding practice. In China, although significant progress in GNS has been achieved, and this has contributed substantially to yield increases in wheat breeding over the past several decades, there has been no significant development in wheat yield improvement in recent years. This has likely been due to the slow progress in identification and utilization of new genetic resources related to GNS. Therefore, it is necessary to further analyze the genetic mechanism of high grain number in Pubing3228.

Pubing3228 can be used as a valuable genetic resource

The material of the present study, Pubing3228, is a new wheat germplasm obtained from the distant hybridization progeny of common wheat and *Agropyron.* This germplasm possesses many desirable agronomical traits, especially traits related to GNS (Wang et al. 2009). The results of previous studies have shown that the GNS of Pubing3228 not only has a desirable phenotype (the mean of GNS reaches that of 90) but also performs very stably in different environments (Wang et al. 2009). Except for the high GNS, Pubing3228 also performs better in spike-related traits such as spike length, high spikelet number per spike, and high grain number per spikelet. To explore the utility of Pubing3228 in improving wheat spike traits, hybrid populations between Pubing3228 and the five key varieties from Huanghuai winter wheat regions of China (Xiaoyan6, Lumai14, Gaocheng8901, Yumai18, and Shan7859) were developed, and their spike-related traits were analyzed. The results showed that Pubing3228 had a better effect on improving the spike-related traits for these five Chinese wheat varieties (Wang et al. 2010). In addition to Pubing3228, a series of new wheat germplasm resources derived from the hybrid progeny of common wheat and *Agropyron* were developed by Li et al. (Luan et al. 2010; Wu et al. 2006; Zhang et al. 2015; Zhang et al. 2019; Song et al. 2016a, 2016b; Zhang et al. 2015; Zhou et al. 2018; Sun et al. 2021; Wang et al. 2011; Zhang et al. 2015). The results of previous studies have shown that these new wheat germplasms all harbored many novel chromosome segments or gene resources controlling desirable agronomic traits such as high yield and disease resistance. Furthermore, some new wheat germplasms have been applied to wheat breeding, and several elite wheat varieties have been successfully developed (Ding et al. 2020). These results suggested that wheat-*Agropyron* germplasms, including Pubing3228, could be used as valuable resources for broadening the genetic basis of common wheat in breeding programs.

Comparison with our previous preliminary QTL analysis for Pubing3228

To explore the genetic mechanism of GNS in Pubing3228, the $F_{2:3}$ population between Pubing3228 and Jing4839 was developed and was used to carried out a preliminary QTL analysis for GNS as in the previous experiment (Wang et al. 2011). A total of 12 QTLs were found for GNS, and these QTLs were distributed on chromosomes 1A, 4A, 5A, 7A, 3B, 5B, 4B, 7B, and 2D. Compared with the previous experiment, more QTLs were identified for GNS, and a total of 75 QTLs were identified. These QTLs were mostly distributed on18 chromosomes, with none found on 5B or 6A. Interestingly, QTLs were also detected on the same chromosomes, except for 5B where the previous QTLs were located. To accurately compare the results of the two experiments, the physical positions of QTLs in the previous experiment were obtained by the BLAST method. Notably, QTLs were identified within or near the mapping intervals of the previous QTLs. For example, one QTL (Q*Gns.wa-5BL.e3*) was detected in a 671.39–698.18Mb interval on chromosome 5A in the previous study. Within the same chromosomeregion, we also identified one QTL (Q*Gns.wa-5A.e3.1*) in the 671.35–678.80Mb interval. The two QTLs were possibly the same QTL, and the comparison allowed the mapping interval to be shortened by 20Mb. On chromosome 7A, we found one QTL (Q*Gns.wa-7A.e3*) in the interval 552.68-572.18 Mb, while one QTL (Q*Gns.wa-7AL.e2*) was mapped to the interval 67.52-681.39 Mb in the previous study. In addition, we also found one QTL (Q*Gns.wa-7B.e1.2*) in the interval 608.41–645.39Mb on chromosome 7B, where one QTL was identified in the genetic interval 172.35-729.06 Mb in the previous study. From the above comparisons, we can conclude that the results of this study were basically consistent with those of the previous study. Furthermore, most QTLs of the previous study were confirmed by the present study. However, some differences were found between this study and the previous study. These differences were possibly attributed to the following two factors. The first was that the two studies used different type mapping populations. The permanent genetic population (the RIL population) was used in this study, but a temporary genetic population (the $F_{2:3}$ population) was used in the previous experiment. The second reason was possibly related to the different genetic linkage maps that were constructed by the two studies. In this study, we constructed a more high-density genetic linkage map with 3334 SNP markers, while only 179 SSR markers were used to construct the genetic linkage map in the previous study (Wang et al. 2011).

Comparison with previous studies

Elucidating the genetic basis of GNS is very important for rapidly improving wheat yield and for identifying QTLs. QTL analysis has become an effective method that can not only provide elite gene resources but also can yield useful information for MAS in wheat breeding practice. Numerous studies have been conducted on GNS, and several QTLs havebeen identified for this trait (Li et al. 2019; Cuthbert et al. 2008; Cui et al. 2017; Jia et al. 2013; Cui et al. 2014; Li et al. 2007; Shi et al. 2017; Hai et al. 2008; Liu et al. 2014; Bhusal et al. 2017; Onyemaobi et al. 2018; Xu et al. 2017; Guan et al. 2018; Liu et al. 2018; Muhammad et al. 2020; Jung et al. 2021; Zheng et al. 2022; Zhai et al. 2018; Guo et al. 2018; Li et al. 2018; Liu et al. 2018;

Börner et al. 2002; Li et al. 2015; Guo et al. 2017; Pang et al. 2020; Chen et al. 2017; Deng et al. 2017; Ding et al. 2011; Gao et al. 2015; Gerard et al. 2019; Wang et al. 2017; Wu et al. 2012; Zhou et al. 2017). We retrieved the major QTLs for GNS identified in previous studies, and a total of 169 GNS QTLs were obtained that were distributed among all 21 chromosomes. To effectively compare the GNS QTLs of this study with those identified in previous studies, the physical genetic positions of the previously reported GNS QTLs were determined, and finally, the physical positions of 153 QTLs were successfully obtained. Significant differences were observed when comparing the GNS QTLs of this study with those of previous studies. We found that the positions of several QTLs (21.33% of the total number of QTLs) from this study approximately coincided with those of previous studies. For example, previous studies conducted by Li et al. (2019) and Ding et al. (2011) reported one GNS QTL near the 498Mb position on chromosome 1A, while one QTL (Q*Gns.wa-1A.e1.1*) in the 498-499Mb position was also identified by us. Using a RIL population derived from a hybrid between NongDa3331 and Zang 1817, Liu et al. (2014) identified one QTL (Q*Gns-7B*) for GNS near the 30.7–723.2Mb position on chromosome 7B, whereas in this genetic region, three GNS QTLs (Q*Gns.wa-7B.e1.1*, Q*Gns.wa-7B.e1.2*, andQ*Gns.wa-7B.e2*) were found near the 58.73–59.74Mb, 71.15–72.51Mb, and 608.41–645.39Mb positions, respectively, in the present study. Muhammad et al. (2020) reported single QTLs for GNS on chromosomes 5A and 6B. In the corresponding mapping positions, two GNS QTLs (Q*Gns.wa-5A.e1.2* and Q*Gns.wa-6B.e1*) were also identified in this study. Cui et al. (2017), Guan et al. (2018), and our group all found one GNS QTL near position 680Mb on chromosome 4A. In addition, the locations of some GNS QTLs from this study were also found in previous studies, suggesting that the results of this study were consistent with those of previous studies. Notably, in the present study, three major QTLs (Q*Gns.wa-1A*, Q*Gns.wa-7D*, and Q*Gns.wa-1D*) were mapped to the region between AX-94465571 and AX-109859174 on chromosome 1A, between AX-110826147 and AX-108748734 on chromosome 7D, and between AX-109994213 and AX-89491149 on chromosome 1D, respectively. These had the highest PVE values and were stable among the three environments. Among these QTLs, only the locus near the position of the GNS QTL on chromosome 1D was reported by Li et al. (2007), while the other two major GNS QTLs were previously unreported. Compared with previous studies, we detected more QTLs for GNS, possibly due to Pubing3228 having been used in this study as a new germplasm obtained from the hybrid progeny of common wheat and *Agropyron*. In addition, to help detect more GNS QTLs, a high-density linkage map was constructed using a large number of gene-based SNP markers. Although no GNS QTLs were found on 5B or 6A in our study, QTLs controlling GNS were identified on those chromosomes in other studies (Li et al. 2019; Zhai et al. 2018; Guo et al. 2018; Li et al. 2015; Zhou et al. 2017).

Several important chromosomes/regions for GNS QTLs

In the present study, QTLs for GNS were distributed unevenly among the chromosomes. Most GNS QTLs were highly concentrated on a small set of chromosomes, including 1A, 1D, 3A, 3D, 5A, 5D, and 7D. For example, five QTLs for GNS were detected near the positions between 126cM and 129cM on chromosome 1D. An additional five QTLs were found between the positions 326cM and 338cM on chromosome 5A. Although only four QTLs were found for GNS on chromosome 3A, these QTLs were concentrated within are latively short interval (from 203cM to 258cM). Other important areas were the region between 809cM and 835cM on chromosome 5D and the region between 165cM and 168cM on chromosome 1A, where four GNS QTLs were identified within each interval. Although more QTLs for GNS were also found on chromosomes 3D and 7D, these QTLs were distributed separately on the two chromosomes. These important regions or chromosomes should be emphasized in future studies. Some similar regions/chromosomes for GNS have also been found in other studies. Although many previous studies have been conducted on wheat yield-related traits, few were specifically carried out for GNS (Cui et al. 2017; Shi et al. 2017; Onyemaobi et al. 2018). Onyemaobi et al. (2018) identified and validated the major regions on chromosomes 5A and 2A for high GNS using the RILs developed from a cross between a synthetic hexaploid wheat ‘W7984’ and a spring wheat variety ‘Opata M85’. Especially noteworthy are the two GNS QTLs on chromosome 6D. Pubing3228 was derived from the hybrid progeny of common wheat and *Agropyron*. Wu et al. (2006) showed that genetic control of increased numbers of florets and kernels per spike of the wheat–*A.cristatum* chromosome addition line 4844-12(2n=44) was attributed to a pair of *A.cristatum* chromosomes (6P) that replaced wheat chromosome 6D, and Pubing3228 was selected from the progeny of 4844-12 across several generations. Zhang et al. (2019) also found that an *A. cristatum* 6P chromosomal segment had a crucial role in increasing the GNS in the wheat-*A.cristatum* translocation line Pubing2978 that showed a high GNS. Therefore, further analysis is needed to determine whether the two QTLs on chromosome 6D originated from *Agropyron*.

Candidate genes predicted for grain number per spike of Pubing3228

Although many QTLs have been identified for GNS in wheat, candidate genes for GNS have seldom been reported. To date, the genetic mechanisms of GNS of wheat remain unclear. In the present study, seven stable QTLs were identified for GNS in at least two environments. Thus, candidate gene analysis was conducted for these QTL. Based on both the gene functional annotation and their relative expression levels, 11 genes were predicted as potential candidate genes for GNS. Three candidate genes were found that had the most significant SNP, AX-108748734 onchromosome7D, with the highest average PVE (12.62%). The first gene was *TraesCS7D01G148000* that probably encoded a senescence-associated protein. Senescence is a highly coordinated developmental program in wheat that has a significant impact on grain yield (Sultana et al. 2021; Sebastian et al. 2014). Several previous studies have shown that the nutrients from the senescing tissues are remobilized to the developing seeds if senescence has been initiated, suggestingthat this gene related to senescence possibly affects GNS to some extent (Distelfeld et al. 2014; Sultana et al. 2021; Liang et al. 2017). The second gene, *TraesCS7D01G117600*, possibly encodes an ethylene-responsive transcription factor. Ethylene response factor (ERF) belonging to the AP2/ERF family (Djemal et al. 2015; Makhloufi et al. 2014; Xu et al. 2007, 2011) is known to play a crucial role in the expression of genes related to stress responses, reproduction, defense, and hormone secretion. Shi et al. (2011) demonstrated that ERF also plays an important role in controlling floral development. The third gene, *TraesCS7D01G117100*, was possibly associated with histone H3. Several studies (Diallo et al. 2012; Nakayama et al. 1993) have indicated that the flowering transition induced by vernalization in winter wheat is partly mediated by histone

methylation of the chromatin region. The histone H3 promoter also exhibits independentcell division activity demonstrated in the anther wall and the pistil (Diallo et al. 2012). The other four candidate genes, i.e., two genes (*TraesCS7D01G056100, TraesCS7D01G060100*) for SNP AX-108759691, one gene (*TraesCS7D01G064300*) for SNP AX-110520245, and one gene (*TraesCS7D01G109200*) for SNP AX-110826147, were also identified on chromosome 7D. The candidate gene *TraesCS7D01G056100* encoding an S-adenosylmethionine decarboxylase proenzyme may play an important role in polyamine biosynthesis (Jangpromma et al. 2010). Results from some studies (Li et al. 2000; Tian et al. 2004) suggest that polyamines such as spermidine and spermine and their precursor putrescine are possibly related to cell division, embryogenesis, and floral and fruit development. The candidate gene *TraesCS7D01G060100* probably encodes a cysteine protease. Cysteine proteases are a large class of proteases in plants. They are involved in a number of processes, including digestion and post-translational modification of storage proteins, development, senescence, and programmed cell death as well as antibiotic responses (Vaseva et al. 2016; Kiyosakia et al. 2009; Simova-Stoilova et al. 2010; Watanabe et al. 2009). The candidate genes *TraesCS7D01G064300* and *TraesCS7D01G109200* possibly encode a starch synthase and a GDSL esterase/lipase, respectively. Starch synthase is the key enzyme involved in starch synthesis in wheat endosperm. Starch in the endosperm is the major form of storage reserves among proteins, lipids, and carbohydrates and thus directly affects the final yield of wheat (Jiang et al. 2003, 2011). GDSL esterases/lipases play important roles in the regulation of plant development and morphogenesis (Su et al. 2020; Yuan et al. 2020). An et al. (2019) reported an endoplasmic reticulum-localized GDSL lipase, ZmMs30, that was specifically expressed in maize anthers and was required for anther cuticle and pollen exine development. A GDSL-type esterase/lipase gene, GELP77, was identified and found toregulate pollen wall characteristics in *Arabidopsis* (Tsugama et al. 2020). Three candidate genes (*TraesCS5D01G044000, TraesCS5D01G051000*, and *TraesCS5D01G033700*) were identified on chromosome 5D. The two genes *TraesCS5D01G044000* and *TraesCS5D01G051000* possibly encode a 60S ribosomal protein l28 and a senescence-associated protein, respectively. The 60S ribosomal protein l28 is possibly associated with translational activity. This gene could affect translation in plant mitochondria and other activities in plant cells (Handa et al. 2001; Cuming 1984). The candidate gene *TraesCS5D01G033700* encodes a YABBY transcription factor. Some YABBY transcription factors have been identified in wheat flowers, and they were considered to regulate the development of pistils and stamens (Liao et al. 2022). In addition, high expression levels of some TaYABBY-genes were also observed during the grain development process (Buttar et al. 2020). The significant SNP AX-108908317 on chromosome 4A was associated with a gene encoding a 4-hydroxybenzoate octaprenyltransferase. The results of previous studies showed that 4-hydroxybenzoate octaprenyltransferase was a key enzyme for ubiquinone biosynthesis in rice (Ohara et al. 2006). Ubiquinone is a lipid-soluble electron carrier required for the respiratory chain in the mitochondria of eukaryotic cells (Grunler et al. 1994). Arrested embryonic development was found inaubiquinone-deficient *Arabidopsis thaliana* mutant at an early stage of embryogenesis (Okada et al. 2004).

Abbreviations

GNS:Grain number per spike; RIL: Recombination inbred line; SNP:Single nucleotide polymorphism; QTL:Quantitative trait locus; MAS:Marker-assisted selection; NGS: Next-generation sequencing; MTA:Marker-trait association; GISH:Genomic in situ hybridization; FISH:Fluorescence in situ hybridization; PDS:Pingdingshan; YL: Yangling; XY: Xianyang; PVE: Phenotypic variance explanation.

Declarations

Availability of data and materials

The main datasets and materials supporting the conclusions of this article are included within the article and its additional files.

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Authors' contributions

Jiansheng Wang designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables; Erwei Wang performed the experiments; Shiping Cheng performed the experiments; Aichu Ma performed the experiments; Jinpeng Zhang conceived and designed the experiments; Lihui Li conceived, designed and directed the experiments. All authors authored or reviewed drafts of the manuscript, and approved the final draft

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Ethics approval and consent to participate

All Experimental research and field studies on plants complied with relevant institutional, national, and international guidelines and legislation. All materials including Pubing3228 are obtained from the corresponding author on his permission.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no competing interests.

Author details

[1] College of Chemistry and Environment Engineering, Pingdingshan University, Pingdingshan 467000, China. China.[2] Pingdingshan Academy of Agricultural Science, Pingdingshan 467001, China. [3] Henan Key Laboratory of Germplasm Innovation and Utilization of Eco-economic Woody Plant, Pingdingshan, Henan, China

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Tables

Table 1 Phenotypic values of grain number per spike for RIL population in different environments

Environment	Years	Parents		RIL population						
		Pubing3228	Jing4839	Maximum	Minimum	Mean±SD	Range	CV(%)	Skewness	Kurtosis
E_1	2019	88.00	32.50	95.67	29.50	55.28±12.63	66.17	22.84	0.79	0.62
E_1	2020	87.25	24.00	113.00	28.00	59.69±14.76	85.00	24.73	0.85	1.39
E_2	2020	91.15	26.28	84.67	25.00	53.78±10.30	59.67	19.16	0.28	0.29
E_3	2020	89.34	29.61	97.00	24.00	58.13±13.09	73.00	22.53	0.35	-0.08
E_1	2021	92.31	36.07	140.00	22.00	73.75±22.79	118.00	31.72	0.57	0.12
E_2	2021	90.06	25.18	112.00	19.67	47.81±12.72	92.33	26.61	0.88	2.59

Note: E_1, E_2, E_3 refer to Pingdingshan, Yangling,Xianyang, respectively.

Table 2 The high-density linkage map for RIL population

Chromosome	Primary SNPs	Loci	Length(cM)	Max spacing(cM)	Min spacing(cM)	Ave. spacing(cM)
1A	2640	190	168.99	8.61	0.2439	0.89
1B	2634	104	250.19	39.74	0.2427	2.41
1D	2558	144	586.92	47.64	0.2427	4.08
2A	2634	123	217.25	30.09	0.2451	1.77
2B	2646	128	232.97	40.99	0.2415	1.82
2D	2649	172	684.04	45.56	0.2404	3.98
3A	2208	217	362.14	35.05	0.2415	1.67
3B	2647	154	225.22	18.19	0.2451	1.46
3D	2111	160	709.68	39.76	0.2415	4.44
4A	2639	145	184.83	16.35	0.2439	1.27
4B	2638	133	154.07	18.08	0.2404	1.16
4D	1613	76	685.98	46.67	0.2439	9.03
5A	2653	165	497.04	49.94	0.2415	3.01
5B	2658	187	283.98	12.77	0.2415	1.52
5D	2200	211	1060.59	52.76	0.2415	5.03
6A	2649	108	160.39	17.83	0.2415	1.49
6B	2667	125	295.18	47.89	0.2404	2.36
6D	2112	126	498.13	39.02	0.2427	3.95
7A	2654	218	486.81	41.49	0.2392	2.23
7B	2596	186	429.73	46.41	0.2392	2.31
7D	2653	262	1320.22	45.66	0.2415	5.04
Genome A	18077	1166	2077.44	49.94	0.2392	1.76
Genome B	18486	1017	1871.35	47.89	0.2392	1.86
Genome D	15896	1151	5545.57	52.76	0.2404	5.08
Overall	52459	3334	9494.36	52.76	0.2392	2.90

Table 3 Significant QTLs for GNS identified in the RIL populations under different environments

Environment	Years	QTL[a]	Marker interval	Position(cM)	LOD	PVE(%)	A[b]
E_1	2019	QGns.wa-1D.e1.1	AX-109994213 AX-89491149	127	5.76	12.78	4.19
E_1	2019	QGns.wa-1D.e1.2	AX-111090826 AX-108775918	439	2.98	6.46	2.96
E_1	2019	QGns.wa-2B.e1	AX-111502586 AX-110024591	74	2.8	6.95	3.09
E_1	2019	QGns.wa-3D.e1	AX-111579109 AX-111676471	576	3.04	8.76	4.71
E_1	2019	QGns.wa-4A.e1	AX-108908317 AX-109987309	131	3.44	4.6	3.44
E_1	2019	QGns.wa-4B.e1	AX-109526283 AX-109469073	15	4.54	8.63	3.63
E_1	2019	QGns.wa-5A.e1.1	AX-109285497 AX-110430928	164	3.3	6.49	3.15
E_1	2019	QGns.wa-5A.e1.2	AX-109959220 AX-108905072	334	6.39	12.75	4.36
E_1	2019	QGns.wa-5D.e1.1	AX-110478685 AX-109292429	409	3.77	10.61	4.83
E_1	2019	QGns.wa-5D.e1.2	AX-89633041 AX-89700472	809	3.37	4.92	3.27
E_1	2019	QGns.wa-6B.e1	AX-111038900 AX-111601539	188	3.23	7.31	3.16
E_1	2019	QGns.wa-6D.e1	AX-108805031-AX-111480830	244	3.99	9.17	3.54
E_1	2019	QGns.wa-7B.e1.1	AX-94770114-AX-110935237	50	4.16	4.69	3.13
E_1	2019	QGns.wa-7B.e1.2	AX-111171468 AX-111044632	422	3.56	5.73	3.47
E_1	2019	QGns.wa-7Da.e1.1	AX-108759691 AX-110520245	64	2.94	3.64	-2.77
E_1	2019	QGns.wa-7Da.e1.2	AX-111061288 AX-110826147	106	9.4	11.21	4.85
E_1	2019	QGns.wa-7Da.e1.3	AX-111638626 AX-109833182	343	4.55	13.82	5.38
E_1	2020	QGns.wa-1A.e1.1	AX-109351333 AX-110421994	78	2.73	6.55	3.38
E_1	2020	QGns.wa-1A.e1.2	AX-110669146 AX-110584864	168	4.17	10.25	4.21
E_1	2020	QGns.wa-1D.e1.1	AX-109994213 AX-89491149	128	4.41	9.89	4.48
E_1	2020	QGns.wa-1D.e1.2	AX-110168974 AX-110580127	206	2.59	5.65	3.38
E_1	2020	QGns.wa-3A.e1	AX-109340041 AX-108852672	258	2.79	6.11	3.62
E_1	2020	QGns.wa-3D.e1	AX-111579109 AX-111676471	570	4.39	9.96	6.54
E_1	2020	QGns.wa-5A.e1	AX-108905072 AX-109108075	338	4.7	9.15	5.16
E_1	2020	QGns.wa-5D.e1	AX-108803037 AX-110591185	835	3.15	6.67	4.17
E_1	2020	QGns.wa-6B.e1	AX-111525440 AX-109598916	182	2.85	6.12	3.72
E_1	2020	QGns.wa-7Da.e1.1	AX-108759691 AX-110520245	64	3.45	5.45	-3.87
E_1	2020	QGns.wa-7Da.e1.2	AX-111061288 AX-110826147	106	8.77	13.22	6.01
E_1	2020	QGns.wa-7Da.e1.3	AX-111638626 AX-109833182	339	2.96	8.9	4.93
E_1	2021	QGns.wa-1A.e1.1	AX-109290544 AX-110657398	84	2.75	7.41	5.77
E_1	2021	QGns.wa-1A.e1.2	AX-94465571 AX-109859174	167	3.01	8.2	6.08
E_1	2021	QGns.wa-1D.e1	AX-94777221 AX-108768444	126	3.05	7.94	6.14
E_1	2021	QGns.wa-2A.e1	AX-111570923 AX-109368419	209	2.66	7.13	5.9
E_1	2021	QGns.wa-2B.e1	AX-110932072 AX-111722030	232	3.52	8.92	6.64
E_1	2021	QGns.wa-2D.e1	AX-109913269 AX-111678720	362	3.09	7.69	6.02

E_1	2021	QGns.wa-3A.e1	AX-109465246	AX-111129296	235	2.76	6.93	5.84
E_1	2021	QGns.wa-3D.e1	AX-111138348	AX-111149842	210	2.69	6.89	6.12
E_1	2021	QGns.wa-5A.e1.1	AX-109861283	AX-109931609	265	3.77	7.2	6.77
E_1	2021	QGns.wa-5A.e1.2	AX-109959220	AX-108905072	326	2.78	10.15	8.04
E_1	2021	QGns.wa-5D.e1	AX-110986471	AX-94458300	519	4.01	10.05	7.25
E_1	2021	QGns.wa-7Da.e1	AX-110826147	AX-108748734	110	4.09	10.11	7.97
E_2	2020	QGns.wa-1A.e2	AX-94465571	AX-109859174	166	5.61	11.8	3.58
E_2	2020	QGns.wa-1B.e2	AX-109384977	AX-110688811	71	2.78	5.38	2.39
E_2	2020	QGns.wa-1D.e2	AX-108768444	AX-109994213	127	5.84	11.71	3.5
E_2	2020	QGns.wa-2B.e2	AX-111639840	AX-108839131	67	2.9	5.57	2.56
E_2	2020	QGns.wa-2D.e2	AX-110412287	AX-110574726	525	3.18	7.08	2.91
E_2	2020	QGns.wa-3B.e2	AX-110525210	AX-110931375	51	3.08	7.56	2.58
E_2	2020	QGns.wa-3D.e2	AX-108850890	AX-109499958	445	2.52	6.07	2.34
E_2	2020	QGns.wa-4D.e2	AX-111002463	AX-89617545	83	3.24	6.76	2.73
E_2	2020	QGns.wa-5A.e2	AX-108905072	AX-109108075	335	4.71	9.09	3.17
E_2	2020	QGns.wa-5D.e2	AX-108803037	AX-110591185	834	3.99	8.14	3.32
E_2	2020	QGns.wa-7B.e2	AX-108795893	AX-109871179	56	3.65	5.45	2.74
E_2	2020	QGns.wa-7Da.e2.1	AX-110826147	AX-108748734	109	3	4.92	2.6
E_2	2020	QGns.wa-7Da.e2.2	AX-110538984	AX-110625335	415	4.76	9.81	3.68
E_3	2020	QGns.wa-1A.e3	AX-94465571	AX-109859174	165	8.39	17.17	5.43
E_3	2020	QGns.wa-1D.e3	AX-109994213	AX-89491149	129	7.36	14.83	5.12
E_3	2020	QGns.wa-1D.e3	AX-89543409	AX-110332164	361	2.69	5	2.96
E_3	2020	QGns.wa-3A.e3.1	AX-109478387	AX-109956153	203	33.21	13.61	-12.02
E_3	2020	QGns.wa-3A.e3.2	AX-111451084	AX-111531488	208	23.89	8.74	9.84
E_3	2020	QGns.wa-3D.e3	AX-110525165	AX-109416738	433	4.65	8.59	3.76
E_3	2020	QGns.wa-4A.e3.1	AX-108908317	AX-109987309	132	3.36	6.28	3.21
E_3	2020	QGns.wa-4A.e3.2	AX-108877111	AX-109936672	184	3.72	6.85	3.36
E_3	2020	QGns.wa-4B.e3	AX-109385774	AX-112287589	32	4.9	9.69	3.73
E_3	2020	QGns.wa-5A.e3.1	AX-108813895	AX-111120428	14	2.53	4.86	2.64
E_3	2020	QGns.wa-5A.e3.2	AX-109369427	AX-110020985	146	3.69	7.19	3.26
E_3	2020	QGns.wa-5A.e3.3	AX-108905072	AX-109108075	336	5.48	11.13	4.02
E_3	2020	QGns.wa-5D.e3.1	AX-109510714	AX-94468261	5	3.99	7.98	3.91
E_3	2020	QGns.wa-5D.e3.2	AX-110421468	AX-108803037	826	4.95	9.91	4.35
E_3	2020	QGns.wa-6D.e3	AX-108747095	AX-111029656	92	2.68	6.06	3.09
E_3	2020	QGns.wa-7A.e3	AX-108807618	AX-110391898	132	2.95	6.76	3.21
E_3	2020	QGns.wa-7Da.e3.1	AX-108759691	AX-110520245	64	2.71	3.49	-2.71

E_3	2020	QGns.wa-7Da.e3.2	AX-110826147	AX-108748734	107	11.42	15.12	5.64
E_3	2020	QGns.wa-7Da.e3.3	AX-111638626	AX-109833182	348	4.78	12.86	5.19
E_3	2020	QGns.wa-7Da.e3.4	AX-110538984	AX-110625335	421	2.72	3.3	2.63
E_3	2020	QGns.wa-7Db.e3.5	AX-110504662	AX-111707163	379	3.53	4.25	2.99

Note: E_1, E_2, E_3 refer to Pingdingshan,: Yangling,:Xianyang, respectively.

[a] Nomenclature for QTL in wheat: "Q" refers to QTL, followed by a trait designator, "wa" for the laboratory, and chromosome

[b] Positive additive effects indicate increased effects from Pubing3228, and negative additive effects indicate increased effects from Jing 4839

Table 4 The candidate genes and their information for GNS identified in this study

No[a]	Chromosome	Identified loci in current study	Position (bp)[b]	Candidate genes (closest/nearby)	Annotation
1	4A	AX-108908317	679194458	TraesCS4A01G601800	4-hydroxybenzoate octaprenyltransferase
2	5D	AX-108803037	27452078	TraesCS5D01G044000	60S ribosomal protein l28
3	5D	AX-108803037	31475580	TraesCS5D01G051000	Senescence-associated protein
4	5D	AX-110591185	32303148	TraesCS5D01G033700	YABBY transcription factor
5	7D	AX-108759691	29882930	TraesCS7D01G056100	S-adenosylmethionine decarboxylase proenzyme
6	7D	AX-108759691	32673051	TraesCS7D01G060100	Cysteine protease
7	7D	AX-110520245	35599225	TraesCS7D01G064300	Starch synthase
8	7D	AX-110826147	65750064	TraesCS7D01G109200	GDSL esterase/lipase
9	7D	AX-108748734	74057072	TraesCS7D01G148000	Senescence-associated protein
10	7D	AX-108748734	72522918	TraesCS7D01G117600	Ethylene-responsive transcription factor
11	7D	AX-108748734	72216910	TraesCS7D01G117100	Histone H3

Note:

[a] The number of candidate genes for wheat grain Fe concentration.

[b] Physical position of the SNP as reported in the IWGSC Chinese Spring reference genome RefSeq v2.0

Figures

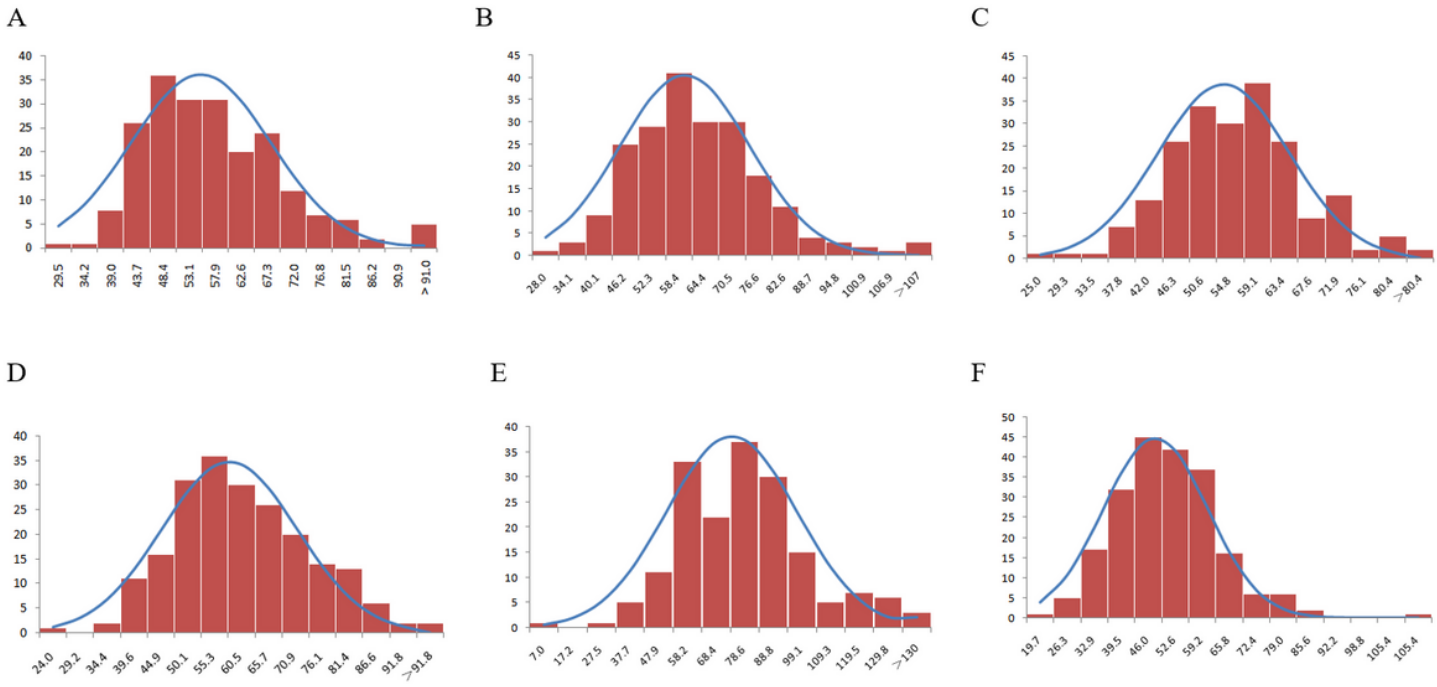

Figure 1

Distribution of grain number per spike in RILpopulations derived from Pubing3228 and Jing4839 in three locations and three years

(A-F) Distribution of grain number per spike for RIL population in Pingdingshan in 2019 (PDS-2019), Pingdingshan in 2020 (PDS-2020), Yangling in 2020 (YL-2020), Xianyang in 2020 (XY-2020), Pingdingshan in 2021 (PDS-2021), Yangling in 2021 (YL-2021) environment, respectively.

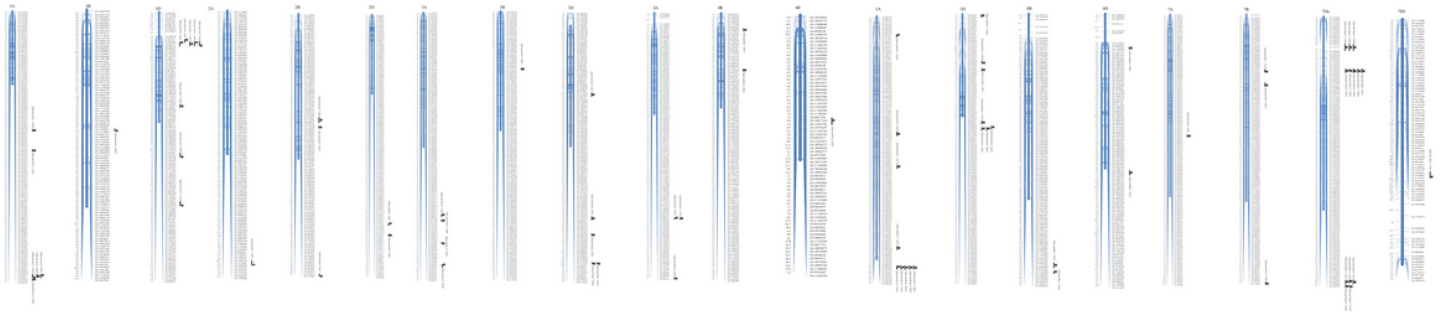

Figure 2

The genetic map of 19 linkage groups and QTL analysis for GNS in the RIL population

Supplementary Files

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- SupplementalTableS2.pdf
- SupplementalTableS3.pdf
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