




Impact of wheat-Agropyron germplasm on the improvement of grain weight and size: a comprehensive QTL analysis

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Abstract

Grain weight and size significantly influence wheat yield and quality. This study aimed to dissect the genetic basis of quantitative trait loci (QTL) associated with grain weight and size in hexaploid wheat (*Triticum aestivum* L.) using the novel wheat-Agropyron germplasm Pubing3228. A population of 210 F₈ recombinant inbred lines derived from Pubing3228 and Jing4839 was evaluated across multiple environments for grain length, width, thickness and thousand-grain weight (TGW). Genotyping was conducted using the Illumina Infinium iSelect 55 K SNP chip, followed by QTL mapping with QTL IciMapping software. A total of 114 QTLs were identified across 20 wheat chromosomes, highlighting major QTLs for grain length, width/thickness and TGW. Candidate gene analysis revealed 22 genes expressed in grains, including those specifically associated with TGW, grain width/thickness and grain length. These findings enhance our understanding of the genetic mechanisms underlying wheat grain traits, providing insights for marker-assisted selection in wheat breeding programmes.

Introduction

Wheat, one of the most vital cereal crops globally, is crucial to human food security (Kumar *et al.*, 2023; Zhang *et al.*, 2024). The demand for wheat yield and quality is increasing with the global population's continuous growth and climate change uncertainties (Mao *et al.*, 2023; Patwa and Penning, 2023; Sun *et al.*, 2023). The yield and quality of wheat result from the combined effects of multiple factors, including agronomic traits such as grain weight and size, which directly impact the crop's overall value and market competitiveness (Tong *et al.*, 2021; Jin *et al.*, 2022). Additionally, genetic factors play a significant role in wheat improvement. Selecting and breeding wheat varieties with superior genetic characteristics can enhance yield and quality (Lopes *et al.*, 2015; Gao *et al.*, 2021; Satyavathi *et al.*, 2021).

Genetic improvement of wheat is a long and complex process, encompassing a wide range of approaches from traditional breeding to modern molecular breeding techniques (Zhang *et al.*, 2021; Raj and Nadarajah, 2022). Historically, wheat breeding relied on natural variation and artificial selection, a time-consuming and inefficient method (Yu *et al.*, 2022a; Guo *et al.*, 2023). With technological advancements, molecular breeding methods, such as marker-assisted selection (MAS) and gene-editing technologies, have been applied in wheat breeding, significantly accelerating the development of superior varieties (Xu *et al.*, 2021; Wang *et al.*, 2022). However, challenges remain in wheat genetic improvement, including limitations in genetic resources, the complex genetic background of traits and environmental uncertainties (Reynolds *et al.*, 2021; Zhou *et al.*, 2021; Adhikari *et al.*, 2022).

In wheat genetics research, quantitative trait locus (QTL) analysis is essential for uncovering the genetic mechanisms that govern complex traits (Rosewarne *et al.*, 2013; Tong *et al.*, 2020; Muellner *et al.*, 2021). Researchers can identify critical genes or genomic regions by mapping QTLs associated with key agronomic traits, providing a basis for marker-assisted breeding (Li *et al.*, 2020; Yang *et al.*, 2021; Wei *et al.*, 2022). QTL analysis not only deepens our understanding of the genetic foundation of wheat but also offers new strategies for breeding, enhancing the precision and efficiency of breeding programmes.

This study aims to conduct an in-depth genetic analysis of grain traits in hexaploid wheat using the novel wheat-Agropyron germplasm Pubing3228. By performing QTL analysis on Pubing3228 and its progeny, we anticipate identifying QTLs and candidate genes closely associated with grain weight and size. These findings provide crucial genetic information for advancing wheat breeding techniques and variety improvement, especially in increasing wheat yield and enhancing quality. Furthermore, the innovation of this study lies in utilizing

the wheat-Agropyron hybrid germplasm for QTL analysis, which not only helps to expand the genetic foundation of wheat but also has the potential to uncover new genetic resources that could improve wheat adaptability and yield. Therefore, this research holds significant scientific value and immense potential for practical application, especially for enhancing crop genetic diversity and global food security.

Materials and methods

Construction of the recombinant inbred line (RIL) population

A population of 210 F₈-generation RILs derived from a cross between Pubing3228 and Jing4839 was established as the material for this study. Pubing3228, developed by Researcher Li from the Chinese Academy of Agricultural Sciences over many years, is a novel wheat germplasm originating from the progeny of common wheat 'Fukuhokomugi' and *Agropyron cristatum* accession Z55 (*A. cristatum* accession Z55) (Xu *et al.*, 2023). It exhibits stable genetic characteristics and multiple superior agronomic traits, particularly in yield. In the early 1990s, a hybrid between 'Fukuhokomugi' and *A. cristatum* accession Z55 was successfully obtained, leading to the selection of progeny lineage 4844 with prominent spike characteristics. Through multiple generations of selection of the wheat-Agropyron chromosomal addition line 4844-12 ($2n = 44$), the genetically stable derivative Pubing3228 was developed. Jing4839 is a cultivated variety known for its high grain weight. The two parental lines exhibit significant differences in agronomic traits, including yield.

Field trials and phenotypic identification

The Pubing3228/Jing4839 RIL population and its parental lines underwent field trials over three growing seasons in 2018/2019, 2019/2020 and 2020/2021 in Pingdingshan, Yangling and Xianyang under different environmental conditions. The trials were laid out in a randomized complete block design with three replicates per treatment. All materials were sown with 2.5 m between rows and 25 cm between plants. Field management followed local conventional practices. At maturity, five representative plants were randomly selected from the centre of each row for manual threshing. Subsequently, ten grains from each experimental unit were randomly selected to measure their length, width and thickness. Each measurement was repeated three times, with each repetition involving a different selection of ten grains. Similarly, for the thousand-grain weight (TGW) measurement, each replicate weighed a different set of 1000 grains. This approach was designed to capture the natural variability among grains from different tillers and florets. The replicated measurements were included in the statistical analysis to ensure the sample size was representative and the results robust.

DNA extraction and genotyping

DNA was extracted from the Pubing3228/Jing4839 RIL population using the SDS method. After assessing the quality and concentration, high-quality DNA samples were dissolved in TE buffer and sent for genotyping analysis using the Illumina Infinium iSelect 55 K SNP chip array at Beijing Boao Company. During data analysis, markers with more than 10% missing data, a minor allele frequency less than 5% or a heterozygosity rate higher than 20% were filtered out to ensure the quality of

data for subsequent linkage map construction and QTL mapping analysis.

Linkage map construction and QTL mapping

The genetic linkage map of the Pubing3228/Jing4839 RIL population was constructed, and QTLs were mapped using QTL IciMapping software V4.2 (<http://www.isbreeding.net>). Initially, all SNP markers were preprocessed using the BIN function within the software to remove distorted, missing and redundant markers. Subsequently, high-quality polymorphic SNP markers were selected to construct the framework of the genetic linkage map through the MAP function's Grouping, Ordering and Rippling processes. The order and distance between markers were determined based on the maximum likelihood principle and the Kosambi mapping function. The genetic linkage map was further refined by removing SNP markers with a linkage distance of over 50 cm. This refined genetic linkage map was then used for QTL mapping analysis. The additive-complete composite interval mapping method (ICIM-ADD) was employed for QTL TGW, grain length, grain width and grain thickness analysis. A significant QTL LOD threshold was set at 2.5. QTL analysis was performed for each trait across different environments, and only QTLs detected in more than two environments were considered stable QTLs.

Identification of candidate genes

The physical regions of the chromosomes corresponding to the QTL confidence intervals were determined based on the sequences of the SNP markers flanking the QTLs. Subsequently, candidate genes within these physical regions were identified by accessing the JBrowse website (<https://urgi.versailles.inra.fr/jbrowseiwgsc>), which is based on the latest version of the Chinese Spring wheat genome sequence (IWGSC RefSeq v2.0) released by the International Wheat Genome Sequencing Consortium (IWGSC). Annotation information for these candidate genes was obtained from the wheat genome database website (<https://wheat-urgi.versailles.inra.fr/Seq-Repository/Annotations>). The expression patterns of these candidate genes across different tissues were compared using the expVIP website (<http://wheat-expression.com>) to select candidate genes associated with grain traits.

Results

Phenotypic variation in grain traits of the RIL population

In this study, we conducted a comprehensive analysis of grain traits in the Pubing3228, Jing4839 and their derived 210 F₈-generation RIL population under five different environmental conditions (E1: Pingdingshan-2019, E2: Pingdingshan-2020, E3: Pingdingshan-2021, E4: Xianyang-2020, E5: Yangling-2021) (Table 1). Observations of four key traits – grain length, width, thickness and TGW – revealed that Jing4839 generally exhibited superior grain traits compared to Pubing3228.

Significant phenotypic variation was observed in the grain traits within the RIL population. Grain length varied from 5.61 to 9.21 mm across the five environments, with an average length ranging from 7.28 to 7.48 mm. The variation in grain width was even broader, ranging from 1.50 to 5.01 mm across the environments, with average widths between 3.36 and 3.49 mm. Regarding grain thickness, the variation across different environments ranged from 1.15 to 3.61 mm, with average thicknesses

Table 1. Phenotypic values of grain traits for the Pubing3228/Jing4839 recombinant inbred lines (RIL) population and their parents in different environments

Traits	Environment	Parents		RIL population					
		Pubing3228	Jing4839	Minimum	Maximum	Mean	CV	Skewness	Kurtosis
GL	E1	6.20	7.46	6.13	8.34	7.37 ± 0.46	0.0630	-0.28	0.07
GL	E2	5.85	6.68	5.61	9.21	7.48 ± 0.59	0.0800	-0.19	0.32
GL	E3	5.83	6.35	5.72	8.67	7.39 ± 0.54	0.0734	-0.36	0.08
GL	E4	6.60	7.64	5.97	8.64	7.28 ± 0.48	0.0654	0.02	0.21
GL	E5	6.15	7.43	5.99	8.71	7.29 ± 0.43	0.0593	-0.11	0.55
GW	E1	2.88	3.86	2.18	5.01	3.37 ± 0.35	0.1033	0.15	4.44
GW	E2	2.30	3.46	2.29	4.55	3.42 ± 0.32	0.0923	-0.49	1.36
GW	E3	2.84	3.17	1.50	4.43	3.36 ± 0.41	0.1210	-1.35	3.27
GW	E4	3.34	3.84	2.40	4.08	3.49 ± 0.26	0.0754	-0.84	1.59
GW	E5	3.07	3.54	2.13	3.89	3.44 ± 0.23	0.0608	-1.48	5.11
GT	E1	2.42	2.76	2.19	3.51	2.93 ± 0.26	0.0901	-0.25	-0.08
GT	E2	2.20	2.96	2.02	3.61	2.92 ± 0.27	0.0937	-0.44	0.10
GT	E3	2.29	2.81	1.90	3.59	2.84 ± 0.31	0.1095	-0.53	0.34
GT	E4	2.58	2.76	1.15	3.57	3.04 ± 0.26	0.0741	-2.10	12.35
GT	E5	2.46	3.22	2.11	3.54	3.00 ± 0.21	0.0705	-0.54	1.38
TGW	E2	29.83	54.12	15.88	74.70	46.49 ± 10.98	0.2362	-0.31	-0.11
TGW	E3	21.76	46.57	10.24	77.56	47.299 ± 12.57	0.2659	-0.39	0.04
TGW	E4	35.84	58.06	25.84	71.14	50.49 ± 9.11	0.1804	-0.46	-0.18
TGW	E5	34.81	56.60	22.58	67.29	48.84 ± 8.27	0.1694	-0.56	0.15

GL, grain length; GW, grain width; GT, grain thickness; TGW, thousand grain weight; E1, Pingdingshan-2019; E2, Pingdingshan-2020; E3, Pingdingshan-2021; E4, Xianyang-2020; E5, Yangling-2021; CV, coefficient of variation.

between 2.84 and 3.04 mm. Notably, TGW variation within the RIL population was particularly significant, ranging from 10.24 to 77.56 g across different environments, with averages between 46.49 and 50.49 g.

Analysis of the coefficient of variation (CV) indicated that TGW exhibited the greatest genetic diversity, with CVs ranging from 16.94 to 26.59% (Fig. 1-1a-d). It was followed by grain width, which had CVs between 6.08 and 12.10% (Fig. 1-1e-i). The grain thickness (Fig. 1-2a-e) and length (Fig. 1-2f-j) CVs were mainly under 10%, indicating minor and consistent variations. Furthermore, the distribution of these traits followed a continuous normal distribution, suggesting that their genetic characteristics are consistent with the inheritance patterns of quantitative traits (Fig. 1).

These results reveal significant phenotypic variation in grain length, width, thickness and TGW among Pubing3228, Jing4839 and their derived RIL population. These findings provide a rich data set for subsequent genetic analysis and QTL mapping and further confirm that the inheritance of these traits adheres to the fundamental principles of quantitative trait genetics.

Significant correlations among grain traits in the wheat RIL population

Upon conducting a comprehensive genetic analysis of the Pubing3228/Jing4839 RIL population, we further explored the correlations among grain length, width, thickness and TGW under varying planting years within the Pingdingshan environment. As indicated in Table 2, the correlation analysis results

reveal that grain length showed significant or highly significant positive correlations with other traits across three planting seasons in most instances. For grain width, the correlations with grain length, width and thickness in 2021 and with grain length in 2019 and 2020 did not reach significance; however, in all other instances, a significant positive correlation was observed.

Similarly, grain thickness did not significantly correlate in specific year comparisons but demonstrated significant correlations with other traits in most cases. Notably, except for the correlation between TGW in 2020 and grain length in 2019, TGW exhibited significant or highly significant positive correlations with all other traits, highlighting the close interrelationships between grain length, width, thickness and TGW (Fig. 2).

In summary, significant positive correlations exist among grain length, width, thickness and TGW within the wheat RIL population. These correlation analysis results reveal the interplay among wheat grain morphological traits and provide crucial insights for further investigation into the genetic mechanisms influencing wheat yield and quality.

QTL mapping analysis identifies 114 QTLs associated with grain traits

To delve into the QTL affecting wheat grain traits, a comprehensive QTL mapping analysis was conducted on the Pubing3228/Jing4839 RIL population, identifying 114 QTLs associated with grain traits. These QTLs are distributed across all wheat chromosomes except for 2B, with LOD scores ranging from 2.51 to 15.33

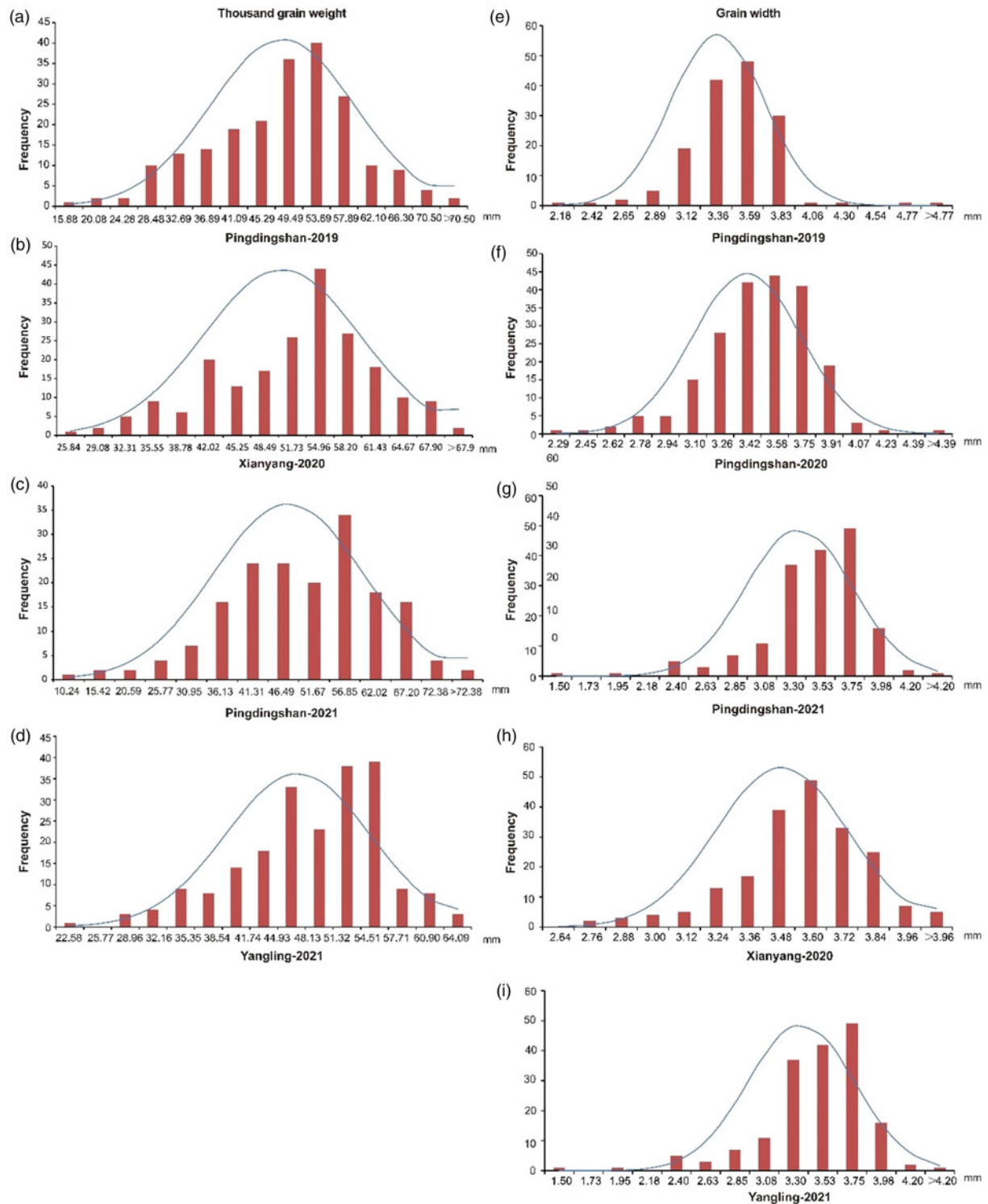


Figure 1. Frequency distribution of grain traits in the Pubing3228/Jing4839 recombinant inbred lines (RIL) population under various environmental conditions. Note: Figure 1-1. Thousand-grain weight and grain width of the Pubing3228/Jing4839 RIL population in five different environments (Pingdingshan-2019, Xianyang-2020, Pingdingshan-2021 and Yangling-2021); (a–d) represent thousand-grain weight (TGW) in four different environments (Pingdingshan-2019, Xianyang-2020, Pingdingshan-2021 and Yangling-2021); (e–i) represent grain width in the same five environments (Pingdingshan-2019, Pingdingshan-2020, Pingdingshan-2021, Xianyang-2020 and Yangling-2021); Figure 1-2. Grain thickness and length of the Pubing3228/Jing4839 RIL population in the same five environments; (a–e) represent grain thickness in five different environments (Pingdingshan-2019, Pingdingshan-2020, Pingdingshan-2021, Xianyang-2020 and Yangling-2021); (f–j) represent grain length in the five environments (Pingdingshan-2019, Pingdingshan-2020, Pingdingshan-2021, Xianyang-2020 and Yangling-2021).

and phenotypic variance explained (PVE) from 0.20 to 15.99%. Detailed information on these QTLs is provided in Table S1 and illustrated in Fig. S1.

For grain length, 36 QTLs were identified across chromosomes 1A, 1D, 2D, 3A, 3D, 4A, 4D, 5A, 5B, 5D, 7A, 7B and 7D, with PVEs ranging from 3.33 to 14.19%. Eleven of these QTLs, with

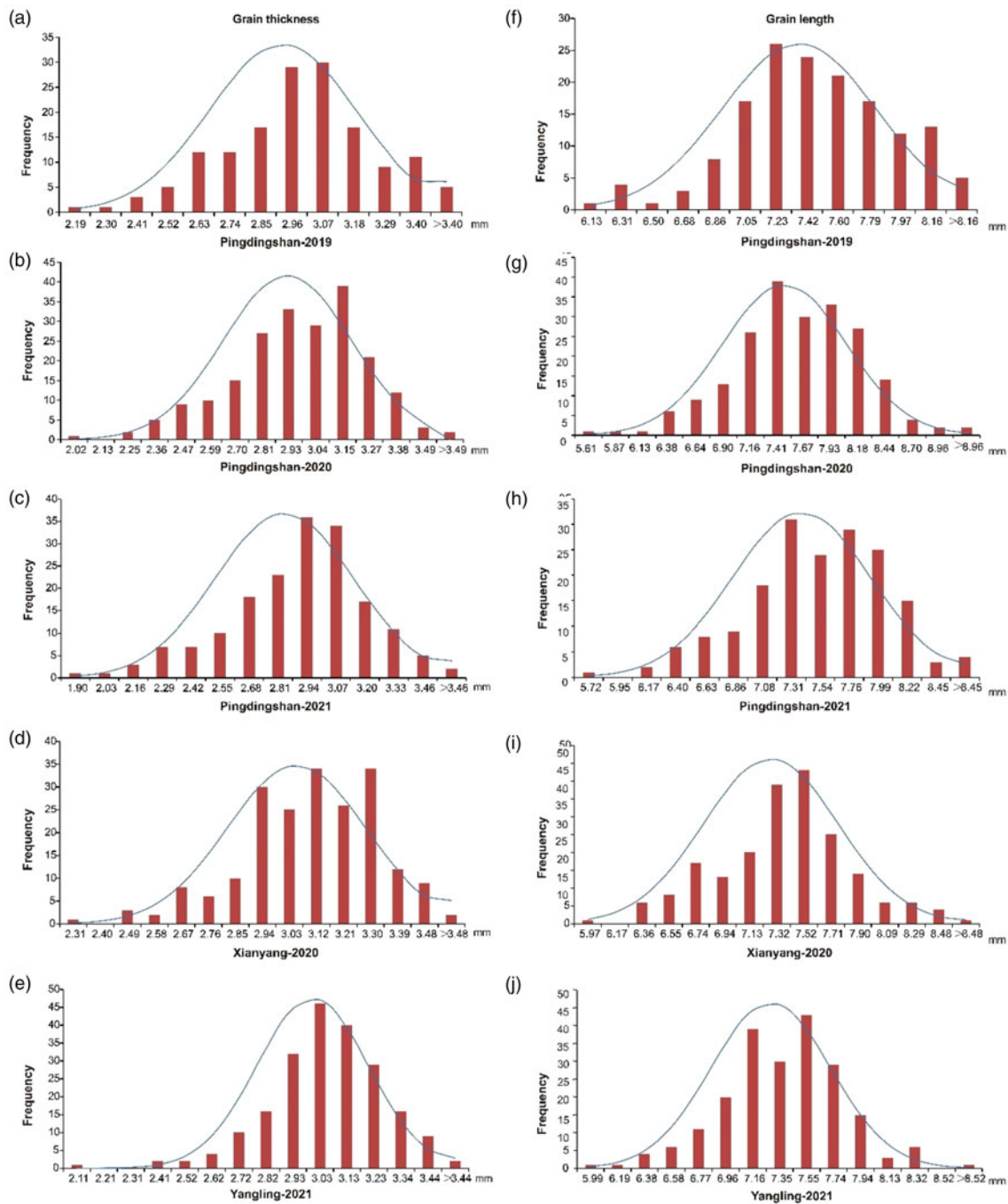


Figure 1. Continued.

PVEs exceeding 10%, were considered major QTLs, with QGL.wa-7B.e4, located between AX-108795893 and AX-109871179, having the highest PVE. Notably, chromosomes 7A and 7B each harboured three QTLs, indicating these chromosomes as focal points for grain-length QTL distribution.

Thirteen QTLs were identified for grain width, predominantly on chromosomes 2D, 4A, 4B, 4D, 5D, 6B, 7A and 7B, with chromosome 5D hosting the most grain width QTLs (four). Twenty-four QTLs were found for grain thickness, distributed across chromosomes 1B, 2A, 2D, 3B, 4A, 4B, 4D, 5A, 5D, 6A, 6D, 7A, 7B, 7Da and 7Db, with chromosome 4D identified as a significant locus for grain thickness traits.

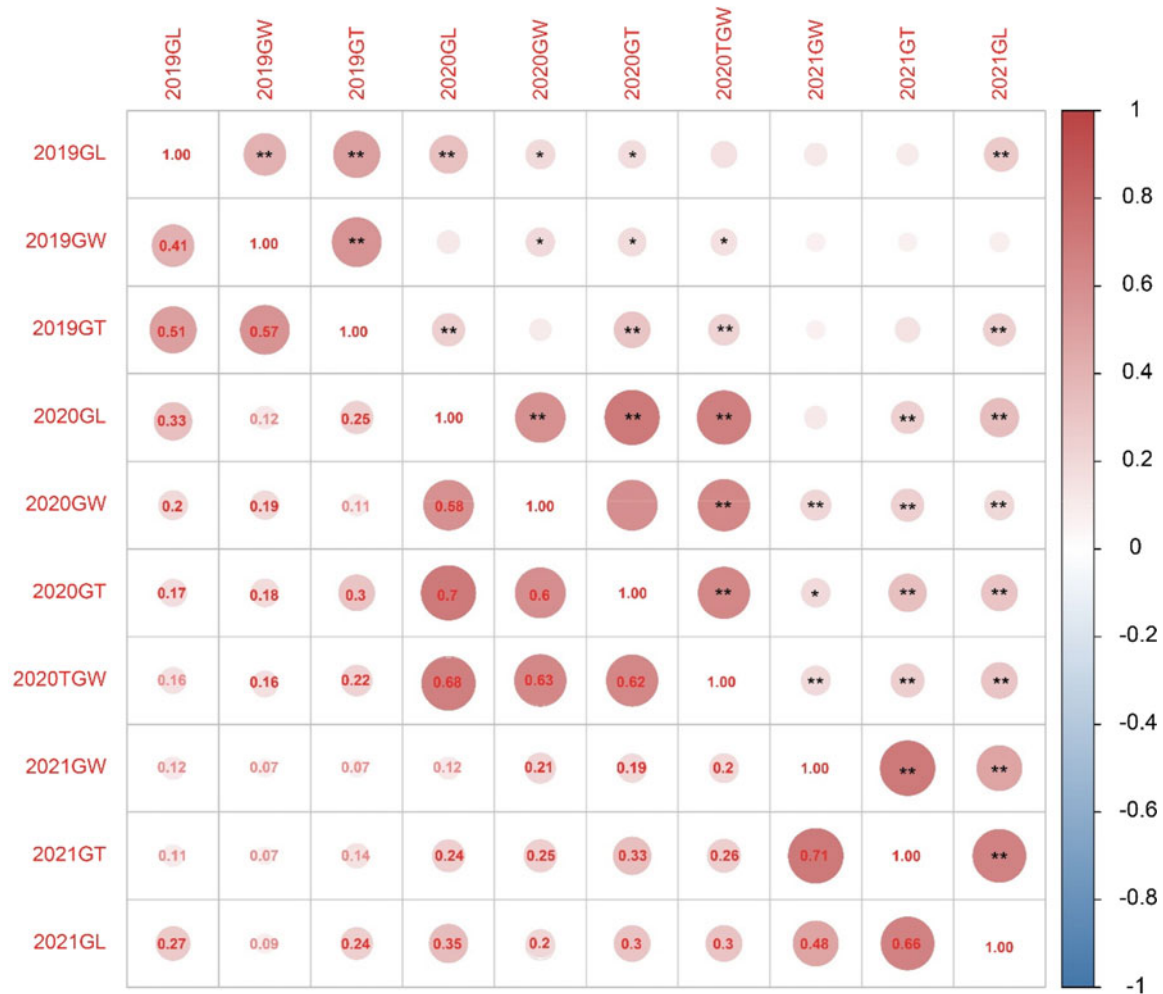
Forty-one QTLs related to TGW were distributed across chromosomes 1A, 1D, 2A, 2D, 3A, 3B, 3D, 4A, 4B, 4D, 5A, 5B, 5D, 6B, 6D, 7A, 7B and 7Da, with PVEs ranging from 2.65 to 15.99%. Five major QTLs, explaining over 10% of the variance, underscore their significant role in controlling TGW traits.

These results successfully identify multiple QTLs influencing wheat grain traits, enriching the genetic foundation knowledge of wheat grain characteristics and providing critical molecular markers for future wheat quality improvement and molecular breeding. Notably, the discovery of significant QTLs lays a solid foundation for further genetic mechanism studies of these traits and trait improvement through MAS.

Table 2. Correlation analysis for wheat grain traits in Pingdingshan environment for 2019, 2020 and 2021

	2019GL	2019GW	2019GT	2020GL	2020GW	2020GT	2020TGW	2021GW	2021GT	2021GL
2019GL	1	0.412**	0.507**	0.334**	0.198*	0.175*	0.158	0.119	0.106	0.270**
2019GW		1	0.567**	0.119	0.190*	0.181*	0.159*	0.075	0.073	0.086
2019GT			1	0.247**	0.109	0.302**	0.222**	0.073	0.141	0.240**
2020GL				1	0.585**	0.702**	0.677**	0.117	0.244**	0.349**
2020GW					1	0.595**	0.628**	0.214**	0.247**	0.201**
2020GT						1	0.625**	0.190*	0.332**	0.304**
2020TGW							1	0.199**	0.258**	0.303**
2021GW								1	0.708**	0.479**
2021GT									1	0.663**
2021GL										1

GL, grain length; GW, grain width; GT, grain thickness; TGW, thousand grain weight.
Significant correlations marked by asterisks (* $P < 0.05$, ** $P < 0.05$).

**Figure 2.** Correlation analysis of grain traits in wheat for 2019, 2020 and 2021.

Note: The grain traits analysed include grain length (GL), grain width (GW), grain thickness (GT) and thousand grain weight (TGW). GL, GW and GT are measured in millimetres (mm), while TGW is measured in grams (g). Significant correlations marked by asterisks (* $P < 0.05$, ** $P < 0.01$).

Expression analysis and identification of candidate genes associated with grain traits

Building on the QTL mapping analysis of grain traits in the Pubing3228/Jing4839 RIL population, a detailed expression analysis was conducted on the initially screened candidate genes. By comparing the expression patterns of these candidate genes in wheat roots, stems, leaves and grains, 22 candidate genes expressed explicitly in grains were identified. These include six associated with TGW, nine with grain width/thickness and seven with grain length (Table 3, Fig. 3).

Identifying these specifically expressed candidate genes provides new insights into the genetic regulatory mechanisms underlying wheat grain traits and offers potential targets for future wheat quality improvement through gene editing technologies.

Discussion

As one of the world's principal cereal crops, improving wheat yield and quality has always been a focal point of agricultural research (Senatore *et al.*, 2021; Qiu *et al.*, 2022). Grain weight and size are direct determinants of wheat yield and processing

quality, making the genetic foundation of these traits a subject of significant theoretical and practical interest (Park *et al.*, 2021; Liu *et al.*, 2022; Yu *et al.*, 2022b). Previous studies have primarily focused on the genetic analysis of common wheat germplasm. However, this study introduces the novel wheat-Agropyron germplasm Pubing3228, expanding the scope of genetic diversity research and offering a fresh perspective on the complex genetic mechanisms of wheat grain traits.

Creating an F₈ generation RIL population through Pubing 3228 crossing with Jing4839 exceeds the scale of most similar studies in quantity, providing ample genetic variation for research. The design of field trials across three different environments accounts for the impact of environmental factors on trait expression, enriching the study's depth and breadth. Moreover, using the Illumina Infinium iSelect 55 K SNP chip and QTL IciMapping software demonstrates the study's advancement and efficiency in genetic analysis methods.

Detecting 114 QTLs associated with grain traits, both in quantity and distribution, marks a significant departure from previous studies. Identifying these QTLs, particularly the major-effect QTLs, presents new targets for the genetic improvement of wheat grain traits. Compared to existing research, these results

Table 3. The candidate genes and their information for the related grain traits identified in this study

Traits	No ^a	Chromosome	Identified loci in current study	Position (bp) ^b	Candidate genes (closest/nearby)	Annotation
TGW	1	3A	AX-111824379	8927912	TraesCS3A01G027200LC	6-phosphogluconate dehydrogenase family protein
	2	3A	AX-111824379	8927912	TraesCS3A01G048500LC	LIM domain-containing protein 1
	3	3A	AX-111824379	8927912	TraesCS3A01G021600LC	Zinc finger MYM-type protein 1
	4	5A	AX-110417422	656939685	TraesCS5A01G644100LC	AT hook motif-containing protein
	5	5A	AX-110417422	656939685	TraesCS5A01G481700	Plant invertase/pectin methylesterase inhibitor superfamily protein
	6	5A	AX-110417422	656939685	TraesCS5A01G485000	Protein NRT1/PTR FAMILY 5.5
GW/GT	1	4D	AX-111054538	49474918	TraesCS4D01G049900LC	Ribosomal protein S19
	2	4D	AX-111054538	49474918	TraesCS4D01G032500	Germin-like protein 1-1
	3	4D	AX-111054538	49474918	TraesCS4D01G023400LC	Oleosin
	4	4D	AX-94578696	57075250	TraesCS4D01G044800LC	Bifunctional protein GlmU
	5	4D	AX-94578696	57075250	TraesCS4D01G063800LC	LUC7 N terminus domain-containing protein
	6	4D	AX-94578696	57075250	TraesCS4D01G025600	Cysteine proteinase inhibitor
	7	4D	AX-94578696	57075250	TraesCS4D01G032500	Germin-like protein 1-1
	8	4D	AX-94578696	57075250	TraesCS4D01G076400	Gibberellin-regulated protein 1
	9	4D	AX-94578696	57075250	TraesCS4D01G074800	
GL	1	5A	AX-109466244	458663036	TraesCS5A01G244500	Carboxypeptidase
	2	7A	AX-109418049	124409029	TraesCS7A01G170400	Glycosyltransferase
	3	7A	AX-109418049	124409029	TraesCS7A01G179200	Translation initiation factor IF-2
	4	7A	AX-109418049	124409029	TraesCS7A01G183800	Heat-shock protein
	5	7A	AX-109418049	124409029	TraesCS7A01G185300	Transcription factor protein
	6	7A	AX-109418049	124409029	TraesCS7A01G244800LC	60S ribosomal protein L11
	7	7B	AX-109871179	72510553	TraesCS7B01G066800	F-box protein

GL, grain length; GW, grain width; GT, grain thickness; TGW, thousand grain weight.

^aThe number of candidate genes for wheat grain traits.

^bPhysical position of the SNP as reported in the IWGSC Chinese Spring reference genome RefSeq v2.0.

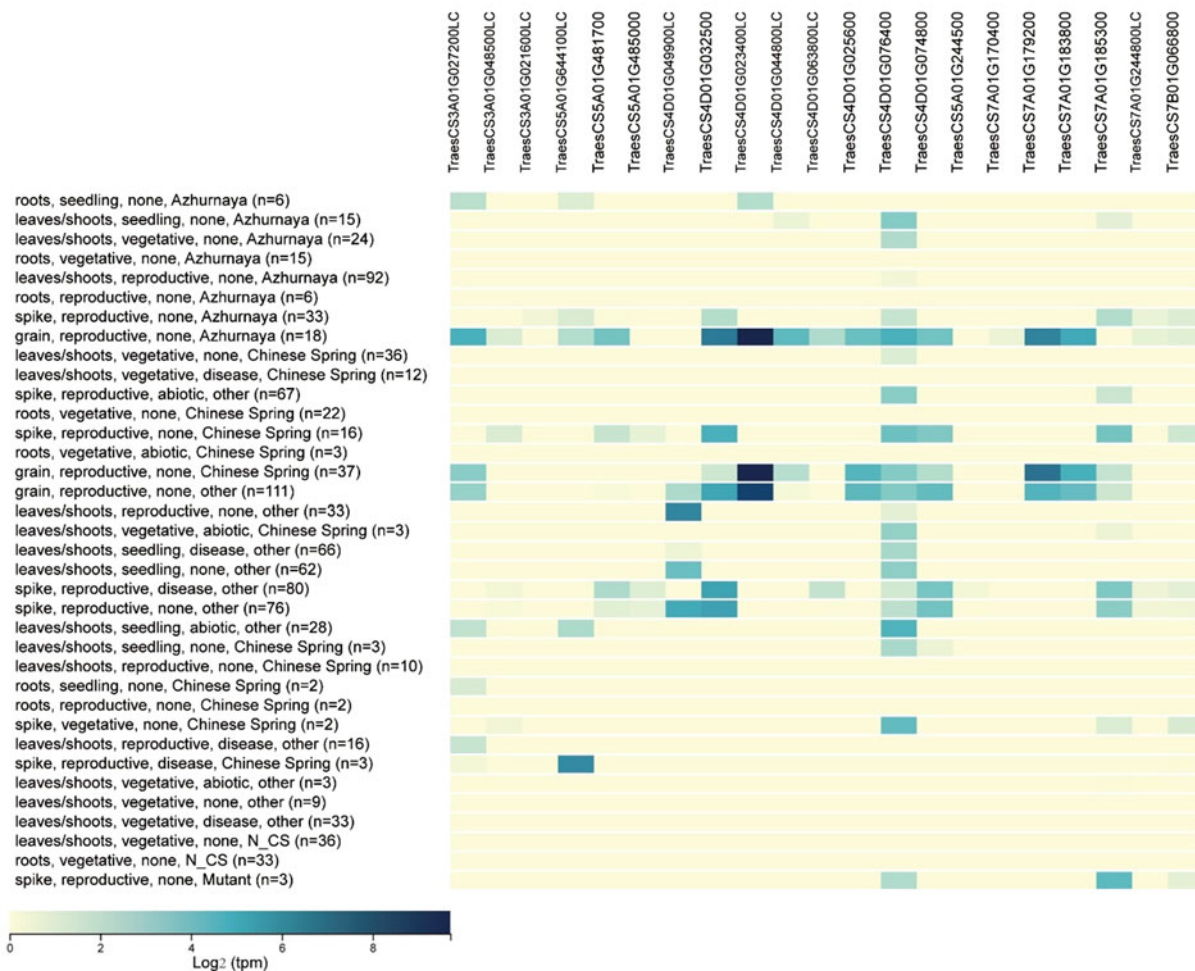


Figure 3. Expression profile analysis of grain trait-related candidate genes in different tissues.

Note: *n* indicates the sample size, Azhurnaya and Chinese Spring represent the wheat varieties used in the study.

underscore the significant increase in genetic diversity and improvement potential facilitated using the Pubing3228 germplasm (Dong *et al.*, 2022; Qu *et al.*, 2022; Sharma *et al.*, 2023).

The discovery of 22 candidate genes, especially those associated with grain length, width/thickness and TGW, lays a foundation for further functional validation and molecular breeding. Compared to prior studies, the specific expression patterns of these genes and their association with traits enrich the theoretical basis for the genetic control of wheat grain traits and provide new tools for marker-assisted breeding.

Among the TGW-associated candidate genes, for instance, TraesCS3A01G027200LC is believed to encode a 6-phosphogluconate dehydrogenase family protein involved in various abiotic stress responses, potentially affecting plant growth (Corpas *et al.*, 2016; Li *et al.*, 2021). TraesCS3A01G048500LC likely encodes a protein with an LIM domain, which is involved in regulating the actin cytoskeleton necessary for pollen development and acts as an actin-binding protein to modulate the cytoskeletal structure and actin network, significantly impacting seed yield (Lopez *et al.*, 1996; Papuga *et al.*, 2010; Li *et al.*, 2015).

For genes associated with grain width/thickness, TraesCS4D01G049900LC may encode ribosomal protein S19, an essential component of the protein synthesis machinery that affects plant growth and development (Fallahi *et al.*, 2005).

TraesCS4D01G032500 could encode a Germin-like protein that plays multiple roles in plant development (He *et al.*, 2021; Anum *et al.*, 2022; To *et al.*, 2022).

Regarding grain length, TraesCS5A01G244500 might encode a carboxypeptidase, potentially influencing cell elongation (Fu *et al.*, 2023). TraesCS7A01G170400 is thought to encode a glycosyltransferase, an enzyme essential for average plant growth and development (Lao *et al.*, 2014; Li *et al.*, 2017, 2018).

Overall, this study's findings stand out in identifying QTLs and candidate genes, illustrating the uniqueness brought about by the genetic materials used, experimental design and molecular marker technologies. These discoveries emphasize the importance of exploring genetic diversity and employing high-throughput genetic analysis techniques in unveiling the genetic foundation of crop traits.

This study delves into the novel wheat-Agropryron germplasm Pubing3228, uncovering QTL and candidate genes associated with hexaploid wheat (*Triticum aestivum* L.) grain weight and size. It provides a vital theoretical foundation and technological pathways for genetic improvement and quality enhancement in wheat (Fig. 4). Utilizing high-density SNP chip analysis and advanced genetic analysis methods identified 114 QTLs related to grain traits. Further analysis led to the identification of 22 genes with specific expression patterns, expanding our understanding of wheat genome functions and laying the groundwork

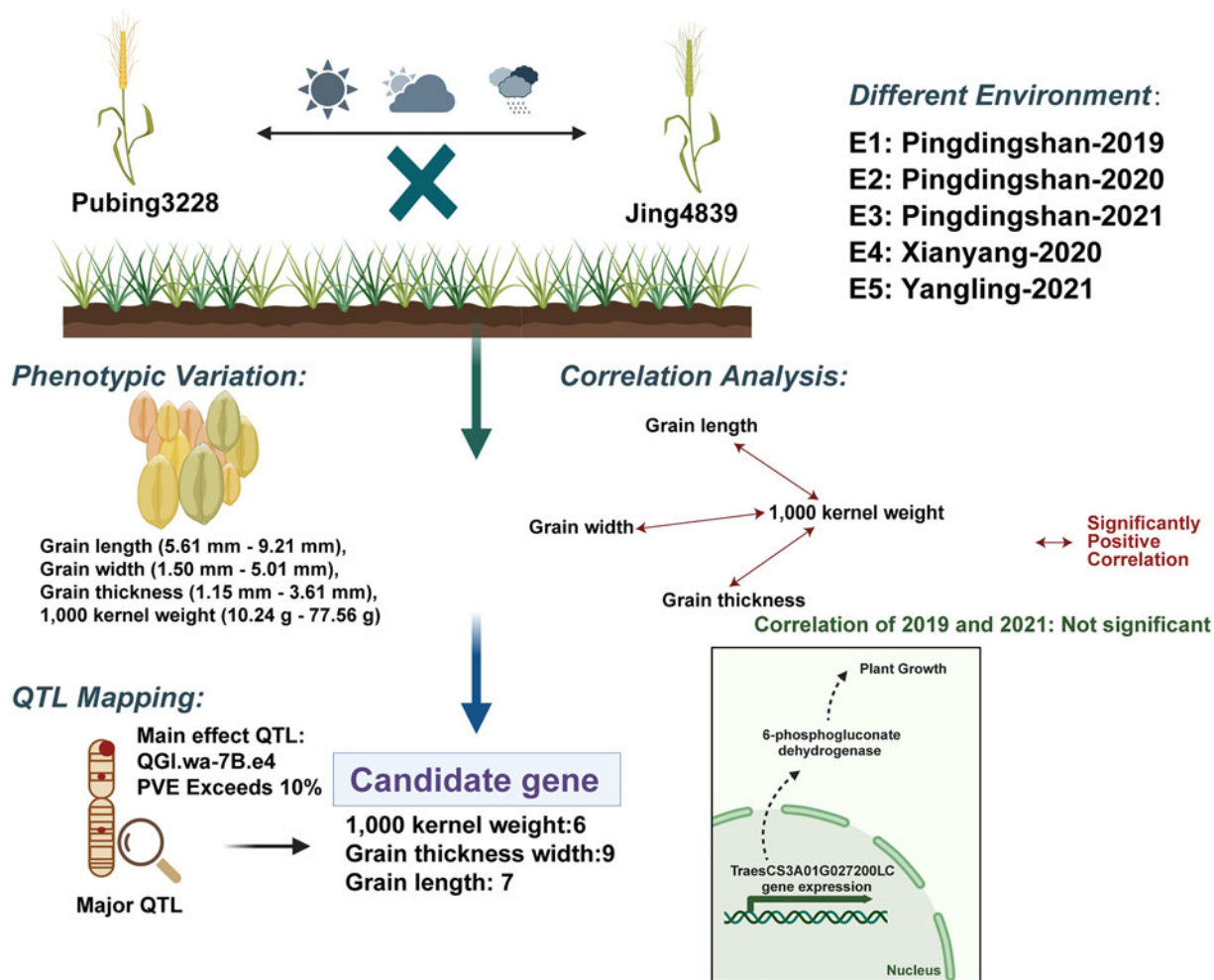


Figure 4. Genetic analysis of quantitative trait loci (QTL) for grain weight and size in the Pubing3228/Jing4839 recombinant inbred lines (RIL) population.

for investigating the molecular regulatory mechanisms of these traits. The scientific value of these achievements lies in furnishing crucial resources for basic genetic research, while their practical value emerges in accelerating wheat variety improvement through MAS, promoting precision agriculture, and ultimately enhancing wheat yield and quality to meet the growing global demand for food security and nutrition. This research advances wheat scientific studies and contributes to global food security and sustainable agricultural development, with long-term impacts on wheat breeding and production practices.

Despite significant progress, the study's limitations must be acknowledged. The identified QTLs and candidate genes require further validation and functional studies to confirm their roles in wheat grain trait development. Additionally, the research, conducted under specific environmental conditions and genetic backgrounds, lacks comprehensive testing across diverse environments and genetic backgrounds for universality and stability. Moreover, grain trait formation is complex and influenced by multiple genes and environmental factors, and the study's analysis of these intricate interactions still needs to be completed. Thus, while the findings offer guidance, they necessitate cautious evaluation and interpretation in practical applications.

Future research directions should include validating the identified QTLs' stability and universality under broader environmental conditions and diverse genetic backgrounds to ensure their practical value in wheat breeding. Advanced molecular biology techniques and bioinformatics methods should be employed to explore the functions and mechanisms of these candidate genes, understanding their specific roles in grain morphology and quality formation. Additionally, leveraging the latest gene-editing technologies, such as the CRISPR/Cas9 system, for precise modifications of critical genes can provide a more efficient means for developing new wheat varieties. Finally, through interdisciplinary collaboration, integrating computational biology, genetics, breeding, and molecular biology, a more comprehensive and in-depth genetic regulatory network model for wheat grain traits can be constructed, contributing to global food security and sustainable agricultural development.

Conclusion

This study provides a comprehensive genetic analysis of wheat grain traits, identifying 114 QTLs and 22 candidate genes associated with grain weight and size. The findings contribute

valuable insights into the genetic mechanisms underlying these traits, offering new avenues for MAS in wheat breeding. However, further research is needed to validate these QTLs and candidate genes across diverse environments and genetic backgrounds to ensure their practical application in improving wheat yield and quality.

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References

- Adhikari L, Raupp J, Wu S, Wilson D, Evers B, Koo DH, Singh N, Friebe B and Poland J (2022) Genetic characterization and curation of diploid A-genome wheat species. *Plant Physiology* **188**, 2101–2114. <https://doi.org/10.1093/plphys/kiac006>
- Anum J, O'Shea C, Zeeshan Hyder M, Farrukh S, Skriver K, Malik SI and Yasmin T (2022) Germin like protein genes exhibit modular expression during salt and drought stress in elite rice cultivars. *Molecular Biology Reports* **49**, 293–302. <https://doi.org/10.1007/s11033-021-06871-3>
- Corpas FJ, Aguayo-Trinidad S, Ogawa T, Yoshimura K and Shigeoka S (2016) Activation of NADPH-recycling systems in leaves and roots of *Arabidopsis thaliana* under arsenic-induced stress conditions is accelerated by knock-out of Nudix hydrolase 19 (AtNUDX19) gene. *Journal of Plant Physiology* **192**, 81–89. <https://doi.org/10.1016/j.jplph.2016.01.010>
- Dong Y, Xu D, Xu X, Ren Y, Gao F, Song J, Jia A, Hao Y, He Z and Xia X (2022) Fine mapping of QPm.caas-3BS, a stable QTL for adult-plant resistance to powdery mildew in wheat (*Triticum aestivum* L.). TAG. *Theoretical and Applied Genetics. Theoretische und Angewandte Genetik* **135**, 1083–1099. <https://doi.org/10.1007/s00122-021-04019-2>
- Fallahi M, Crosthwait J, Calixte S and Bonen L (2005) Fate of mitochondrially located S19 ribosomal protein genes after transfer of a functional copy to the nucleus in cereals. *Molecular Genetics and Genomics* **273**, 76–83. <https://doi.org/10.1007/s00438-004-1102-9>
- Fu G, Chen B, Pei X, Wang X, Wang X, Nazir MF, Wang J, Zhang X, Xing A, Pan Z, Lin Z, Peng Z, He S and Du X (2023) Genome-wide analysis of the serine carboxypeptidase-like protein family reveals Ga09G1039 is involved in fiber elongation in cotton. *Plant Physiology and Biochemistry* **201**, 107759. <https://doi.org/10.1016/j.plaphy.2023.107759>
- Gao Y, An K, Guo W, Chen Y, Zhang R, Zhang X, Chang S, Rossi V, Jin F, Cao X, Xin M, Peng H, Hu Z, Guo W, Du J, Ni Z, Sun Q and Yao Y (2021) The endosperm-specific transcription factor TaNAC019 regulates glutenin and starch accumulation and its elite allele improves wheat grain quality. *The Plant Cell* **33**, 603–622. <https://doi.org/10.1093/plcell/koaa040>
- Guo X, Huang Y, Wang J, Fu S, Wang C, Wang M, Zhou C, Hu X, Wang T, Yang W and Han F (2023) Development and cytological characterization of wheat-Thinopyrum intermedium translocation lines with novel stripe rust resistance gene. *Frontiers in Plant Science* **14**, 1135321. <https://doi.org/10.3389/fpls.2023.1135321>
- He ZD, Tao ML, Leung DWM, Yan XY, Chen L, Peng XX and Liu EE (2021) The rice Germin-like protein OsGLP1 participates in acclimation to UV-B radiation. *Plant Physiology* **186**, 1254–1268. <https://doi.org/10.1093/plphys/kiab125>
- Jin HY, Yue JQ, Yan YQ, Zhang DQ, Yang C, Li XD, Shao YH and Fang BT (2022) [Soil bacterial communities of different crop rotations and yield of succeeding wheat.]. *Ying Yong Sheng Tai Xue Bao = The Journal of Applied Ecology* **33**, 2954–2962. <https://doi.org/10.13287/j.1001-9332.202211.013>
- Kumar V, Singh V, Flessner ML, Haymaker J, Reiter MS and Mirsky SB (2023) Cover crop termination options and application of remote sensing for evaluating termination efficiency. *PLoS ONE* **18**, e0284529. <https://doi.org/10.1371/journal.pone.0284529>
- Lao J, Oikawa A, Bromley JR, McInerney P, Suttangkakul A, Smith-Moritz AM, Plahar H, Chiu TY, González Fernández-Niño SM, Ebert B, Yang F, Christiansen KM, Hansen SF, Stonebloom S, Adams PD, Ronald PC, Hillson NJ, Hadi MZ, Vega-Sánchez ME, Loqué D, Scheller HV and Heazlewood JL (2014) The plant glycosyltransferase clone collection for functional genomics. *The Plant Journal: for Cell and Molecular Biology* **79**, 517–529. <https://doi.org/10.1111/tpj.12577>
- Li L, Li Y, Wang NN, Li Y, Lu R and Li XB (2015) Cotton LIM domain-containing protein GhPLIM1 is specifically expressed in anthers and participates in modulating F-actin. *Plant Biology* **17**, 528–534. <https://doi.org/10.1111/plb.12243>
- Li P, Li YJ, Wang B, Yu HM, Li Q and Hou BK (2017) The Arabidopsis UGT87A2, a stress-inducible family 1 glycosyltransferase, is involved in the plant adaptation to abiotic stresses. *Physiologia Plantarum* **159**, 416–432. <https://doi.org/10.1111/ppl.12520>
- Li Q, Yu HM, Meng XF, Lin JS, Li YJ and Hou BK (2018) Ectopic expression of glycosyltransferase UGT76E11 increases flavonoid accumulation and enhances abiotic stress tolerance in Arabidopsis. *Plant Biology* **20**, 10–19. <https://doi.org/10.1111/plb.12627>
- Li Y, Xiong H, Guo H, Zhou C, Xie Y, Zhao L, Gu J, Zhao S, Ding Y and Liu L (2020) Identification of the vernalization gene VRN-B1 responsible for heading date variation by QTL mapping using a RIL population in wheat. *BMC Plant Biology* **20**, 331. <https://doi.org/10.1186/s12870-020-02539-5>
- Li C, Li K, Zheng M, Liu X, Ding X, Gai J and Yang S (2021) Gm6PGDH1, a cytosolic 6-phosphogluconate dehydrogenase, enhanced tolerance to phosphate starvation by improving root system development and modifying the antioxidant system in soybean. *Frontiers in Plant Science* **12**, 704983. <https://doi.org/10.3389/fpls.2021.704983>
- Liu K, Wang X, Liu H, Wu J, Liang F, Li S, Zhang J and Peng X (2022) OsAT1, an anion transporter, negatively regulates grain size and yield in rice. *Physiologia Plantarum* **174**, e13692. <https://doi.org/10.1111/ppl.13692>
- Lopes MS, El-Basyoni I, Baenziger PS, Singh S, Royo C, Ozbek K, Aktas H, Ozer E, Ozdemir F, Manickavelu A, Ban T and Vikram P (2015) Exploiting genetic diversity from landraces in wheat breeding for adaptation to climate change. *Journal of Experimental Botany* **66**, 3477–3486. <https://doi.org/10.1093/jxb/erv122>
- Lopez I, Anthony RG, Maciver SK, Jiang CJ, Khan S, Weeds AG and Hussey PJ (1996) Pollen specific expression of maize genes encoding actin depolymerizing factor-like proteins. *Proceedings of the National Academy of Sciences of the USA* **93**, 7415–7420. <https://doi.org/10.1073/pnas.93.14.7415>
- Mao H, Jiang C, Tang C, Nie X, Du L, Liu Y, Cheng P, Wu Y, Liu H, Kang Z and Wang X (2023) Wheat adaptation to environmental stresses under climate change: molecular basis and genetic improvement. *Molecular Plant* **16**, 1564–1589. <https://doi.org/10.1016/j.molp.2023.09.001>
- Muellner AE, Buerstmayr M, Eshonkulov B, Hole D, Michel S, Hagenguth JF, Pachler B, Pernold R and Buerstmayr H (2021) Comparative mapping and validation of multiple disease resistance QTL for simultaneously controlling common and dwarf bunt in bread wheat. *Theoretical and Applied Genetics. Theoretische und Angewandte Genetik* **134**, 489–503. <https://doi.org/10.1007/s00122-020-03708-8>

- Papuga J, Hoffmann C, Dieterle M, Moes D, Moreau F, Tholl S, Steinmetz A and Thomas C (2010) Arabidopsis LIM proteins: a family of actin bundlers with distinct expression patterns and modes of regulation. *The Plant Cell* **22**, 3034–3052. <https://doi.org/10.1105/tpc.110.075960>
- Park JR, Resolus D and Kim KM (2021) OsBRKq1, related grain size mapping, and identification of grain shape based on QTL mapping in rice. *International Journal of Molecular Sciences* **22**, 2289. <https://doi.org/10.3390/ijms22052289>
- Patwa N and Penning BW (2023) Genetics of a diverse soft winter wheat population for pre-harvest sprouting, agronomic, and flour quality traits. *Frontiers in Plant Science* **14**, 1137808. <https://doi.org/10.3389/fpls.2023.1137808>
- Qiu B, Hu X, Chen C, Tang Z, Yang P, Zhu X, Yan C and Jian Z (2022) Maps of cropping patterns in China during 2015–2021. *Scientific Data* **9**, 479. <https://doi.org/10.1038/s41597-022-01589-8>
- Qu X, Li C, Liu H, Liu J, Luo W, Xu Q, Tang H, Mu Y, Deng M, Pu Z, Ma J, Jiang Q, Chen G, Qi P, Jiang Y, Wei Y, Zheng Y, Lan X and Ma J (2022) Quick mapping and characterization of a co-located kernel length and thousand-kernel weight-related QTL in wheat. *Theoretical and Applied Genetics*. *Theoretische und Angewandte Genetik* **135**, 2849–2860. <https://doi.org/10.1007/s00122-022-04154-4>
- Raj SRG and Nadarajah K (2022) QTL and candidate genes: techniques and advancement in abiotic stress resistance breeding of major cereals. *International Journal of Molecular Sciences* **24**, 6. <https://doi.org/10.3390/ijms24010006>
- Reynolds MP, Lewis JM, Ammar K, Basnet BR, Crespo-Herrera L, Crossa J, Dhugga KS, Dreisigacker S, Juliana P, Karwat H, Kishii M, Krause MR, Langridge P, Lashkari A, Mondal S, Payne T, Pequeno D, Pinto F, Sansaloni C, Schultness U, Singh RP, Sonder K, Sukumaran S, Xiong W and Braun HJ (2021) Harnessing translational research in wheat for climate resilience. *Journal of Experimental Botany* **72**, 5134–5157. <https://doi.org/10.1093/jxb/erab256>
- Rosewarne GM, Herrera-Foessel SA, Singh RP, Huerta-Espino J, Lan CX and He ZH (2013) Quantitative trait loci of stripe rust resistance in wheat. *Theoretical and Applied Genetics*. *Theoretische und Angewandte Genetik* **126**, 2427–2449. <https://doi.org/10.1007/s00122-013-2159-9>
- Satyavathi CT, Ambawat S, Khandelwal V and Srivastava RK (2021) Pearl millet: a climate-resilient nutriceal for mitigating hidden hunger and provide nutritional security. *Frontiers in Plant Science* **12**, 659938. <https://doi.org/10.3389/fpls.2021.659938>
- Senatore MT, Ward TJ, Cappelletti E, Beccari G, McCormick SP, Busman M, Laraba I, O'Donnell K and Prodi A (2021) Species diversity and mycotoxin production by members of the *Fusarium tricin* species complex associated with Fusarium head blight of wheat and barley in Italy. *International Journal of Food Microbiology* **358**, 109298. <https://doi.org/10.1016/j.ijfoodmicro.2021.109298>
- Sharma D, Kumari A, Sharma P, Singh A, Sharma A, Mir ZA, Kumar U, Jan S, Parthiban M, Mir RR, Bhati P, Pradhan AK, Yadav A, Mishra DC, Budhlakoti N, Yadav MC, Gaikwad KB, Singh AK, Singh GP and Kumar S (2023) Meta-QTL analysis in wheat: progress, challenges and opportunities. *Theoretical and Applied Genetics*. *Theoretische und Angewandte Genetik* **136**, 247. <https://doi.org/10.1007/s00122-023-04490-z>
- Sun H, Ma J and Wang L (2023) Changes in per capita wheat production in China in the context of climate change and population growth. *Food Security* **15**, 597–612. <https://doi.org/10.1007/s12571-023-01351-x>
- To HTM, Pham DT, Le Thi VA, Nguyen TT, Tran TA, Ta AS, Chu HH and Do PT (2022) The Germin-like protein OsGER4 is involved in promoting crown root development under exogenous Jasmonic acid treatment in rice. *The Plant Journal: for Cell and Molecular Biology* **112**, 860–874. <https://doi.org/10.1111/tpj.15987>
- Tong J, Sun M, Wang Y, Zhang Y, Rasheed A, Li M, Xia X, He Z and Hao Y (2020) Dissection of molecular processes and genetic architecture underlying iron and zinc homeostasis for biofortification: from model plants to common wheat. *International Journal of Molecular Sciences* **21**, 9280. <https://doi.org/10.3390/ijms21239280>
- Tong HW, Zhu ZW, Liu YK, Chen L, Zou J, Zhang YQ, Yang Y and Gao CB (2021) Effects of three fertilizer application patterns on wheat sowed by machinery in slope cropland of Western Hubei, China. *Ying Yong Sheng Tai Xue Bao = The Journal of Applied Ecology* **32**, 3969–3976. <https://doi.org/10.13287/j.1001-9332.202111.027>
- Wang K, Shi L, Liang X, Zhao P, Wang W, Liu J, Chang Y, Hiei Y, Yanagihara C, Du L, Ishida Y and Ye X (2022) The gene TaWOX5 overcomes genotype dependency in wheat genetic transformation. *Nature Plants* **8**, 110–117. <https://doi.org/10.1038/s41477-021-01085-8>
- Wei J, Fang Y, Jiang H, Wu XT, Zuo JH, Xia XC, Li JQ, Stich B, Cao H and Liu YX (2022) Combining QTL mapping and gene co-expression network analysis for prediction of candidate genes and molecular network related to yield in wheat. *BMC Plant Biology* **22**, 288. <https://doi.org/10.1186/s12870-022-03677-8>
- Xu J, Hu P, Tao Y, Song P, Gao H and Guan Y (2021) Genome-wide identification and characterization of the lateral organ boundaries domain (LBD) gene family in polyploid wheat and related species. *PeerJ* **9**, e11811. <https://doi.org/10.7717/peerj.11811>
- Xu YF, Ma FF, Zhang JP, Liu H, Li LH and An DG (2023) Unraveling the genetic basis of grain number-related traits in a wheat-*Agropyron cristatum* introgressed line through high-resolution linkage mapping. *BMC Plant Biology* **23**, 563. <https://doi.org/10.1186/s12870-023-04547-7>
- Yang G, Boshoff WHP, Li H, Pretorius ZA, Luo Q, Li B, Li Z and Zheng Q (2021) Chromosomal composition analysis and molecular marker development for the novel Ug99-resistant wheat-*Thinopyrum ponticum* translocation line WTT34. *Theoretical and Applied Genetics*. *Theoretische und Angewandte Genetik* **134**, 1587–1599. <https://doi.org/10.1007/s00122-021-03796-0>
- Yu H, Hao Y, Li M, Dong L, Che N, Wang L, Song S, Liu Y, Kong L and Shi S (2022a) Genetic architecture and candidate gene identification for grain size in bread wheat by GWAS. *Frontiers in Plant Science* **13**, 1072904. <https://doi.org/10.3389/fpls.2022.1072904>
- Yu M, Yu Y, Guo S, Zhang M, Li N, Zhang S, Zhou H, Wei F, Song T, Cheng J, Fan Q, Shi C, Feng W, Wang Y, Xiang J and Zhang X (2022b) Identification of TaBADH-A1 allele for improving drought resistance and salt tolerance in wheat (*Triticum aestivum* L.). *Frontiers in Plant Science* **13**, 942359. <https://doi.org/10.3389/fpls.2022.942359>
- Zhang Y, Li A, Zhu S, Li L, He X, Sun Z and Li T (2021) Basal rachis internode injection: a novel inoculation method to evaluate wheat resistance to fusarium head blight. *Phytopathology* **111**, 1670–1674. <https://doi.org/10.1094/PHYTO-11-20-0488-R>
- Zhang C, Xi Y, Zhang Y, He P, Su X, Fan F, Wu M, Kong X and Shi Y (2024) Genetic association analysis of dietary intake and idiopathic pulmonary fibrosis: a two-sample Mendelian randomization study. *BMC Pulmonary Medicine* **24**, 15. <https://doi.org/10.1186/s12890-023-02831-8>
- Zhou J, Li C, You J, Tang H, Mu Y, Jiang Q, Liu Y, Chen G, Wang J, Qi P, Ma J, Gao Y, Habib A, Wei Y, Zheng Y, Lan X and Ma J (2021) Genetic identification and characterization of chromosomal regions for kernel length and width increase from tetraploid wheat. *BMC Genomics* **22**, 706. <https://doi.org/10.1186/s12864-021-08024-z>