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QTL mapping for pre-harvest sprouting in a recombinant inbred line population of elite wheat varieties Zhongmai 578 and Jimai 22

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ABSTRACT

Pre-harvest sprouting (PHS) is one of the serious global issues in wheat production. Identification of quantitative trait loci (QTL) and closely-linked markers is greatly helpful for wheat improvement. In the present study, a recombinant inbred line (RIL) population derived from the cross of Zhongmai 578 (ZM578)/Jimai 22 (JM22) and parents were phenotyped in five environments and genotyped by the wheat 50 K single-nucleotide polymorphism (SNP) array. Two QTL of germination index (GI), *QGI.caas-3A* and *QGI.caas-5A*, were detected, explaining 4.33%–5.58% and 4.43%–8.02% of the phenotypic variances, respectively. The resistant effect of *QGI.caas-3A* was contributed by JM22, whereas that of *QGI.caas.5A* was from ZM578. The two QTL did not correspond to any previously identified genes or genetic loci for PHS-related traits according to their locations in the Chinese Spring reference genome, indicating that they are likely to be new loci for PHS resistance. Four kompetitive allele-specific PCR (KASP) markers K_AX-109605367and K_AX-179559687 flanking *QGI.caas-3A*, and K_AX-111258240 and K_AX-109402944 flanking *QGI.caas-5A*, were developed and validated in a natural population of 100 wheat cultivars. The distribution frequency of resistance alleles at *Qphs.caas-3A* and *Qphs.caas-5A* loci were 82.7% and 57.1%, respectively, in the natural population. These findings provide new QTL and tightly linked KASP markers for improvement of PHS resistance in wheat.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple food crops and is cultivated globally, right after rice (*Oryza sativa*) and maize (*Zea mays*) [1]. It serves as a source of carbohydrate, protein, vitamins and mineral elements, accounting for about 20% of the total protein and 21% of the calories worldwide [2]. Pre-harvest sprouting (PHS) is a precarious issue affecting numerous wheat production areas, frequently occurring globally in many principal wheat-producing areas including China [3–5]. It was induced by summer rainfall and high humidity [6–8], and early seed dormancy (SD) breakage under wet or humid conditions before harvest [9], leading to significant losses in the grain yield and quality of end-use products [10]. The annual economic loss caused by PHS on wheat production has been estimated to be 1.2 billion US dollars worldwide [5]. Developing wheat varieties with PHS resistance is the most effective way to reduce the loss caused by PHS [11], and identifying genetic loci of PHS resistance is very helpful for breeding resistant varieties through marker-assisted selection (MAS).

With the advent of high-throughput genotyping platforms, such as next generation sequencing (NGS) and chip-based genotyping technologies, many QTL for PHS-related traits have been identified on almost all 21 wheat chromosomes [5,9,10,12–17], among which the region on chromosomes 3A, 3B, 3D, and 4A are considered to be most important for SD and PHS [5,7,18–20]. Moreover, several candidate genes have been isolated by homolog-based cloning, such as *TaSdr* on chromosome 2B [21,22], *TaVp-1* on chromosome 3B [23–25], *TaQSd1* on chromosomes 5A, 5B and 5D [26,27], *TaDOG1L4* on chromosome 3A [28–30], and *TaABI5* on chromosome 3D [31]. Several genes for PHS resistance also were isolated by map-based cloning, including mitogen-activated protein kinase kinase 3 (*TaMKK3*) [32], *TaPHS1* [33,34], *PM19A1-A2* [11,35] and *Tamyb10* [36,37].

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Zhongmai 578 (ZM578) and Jimai 22 (JM22) are two elite white winter wheat cultivars in the Yellow and Huai River Valleys Winter Wheat region, the largest zone of wheat production in China. ZM578 is characterized with good PHS resistance, whereas JM22 has relatively poor resistance. Therefore, a recombinant inbred lines (RIL) population generated from a cross between ZM578 and JM22 was used in this study, with the aims to: 1) identify genetic loci for PHS resistance, and 2) develop breeder-friendly efficient markers for MAS in breeding on improvement of PHS resistance in white winter wheat.

2. Materials and methods

2.1. Plant materials and field trials

A mapping population with 262 RILs derived from a cross of white grain winter wheat ZM578 and JM22 by the single seed descent method was used in this study. ZM578 that was selected from the cross of Zhongmai 255 and JM22, is a high-yielding variety with good tolerance to PHS (Fig. S1). The field trial for the RILs and parents was conducted in five environments including Xinxiang (34°53′N, 113°23′E) in 2019–2020 and 2020–2021 in Henan province (defined as E1 and E2), Shangqiu (34°44′N, 115°65′E) in 2020–2021 (E3) in Henan province, Luoyang (34°32′N, 112°16′E) in 2020–2021 (E4) in Henan province, and Gaoyi (37°33′N, 114°26′E) in 2020–2021 (E5) in Hebei province. A randomized complete block design with three replications was used in all environments. Thirty seeds were sown in each row, with each plot comprising a 1.0 m row spaced 25.0 cm apart. A panel of 100 wheat varieties from Yellow and Huai Valley Winter Wheat region was used to validate the effectiveness of KASP markers, with the phenotypic data available in Zhang et al. [22].

2.2. Evaluation of pre-harvest sprouting

Pre-harvest sprouting resistance was evaluated based on the germination index (GI) value. Five spikes in each plot, were collected from each environment at physiological maturity by cutting the peduncle about 10 cm below the base of the spike, when a loss of green color occurred in the spikes [38,39]. Harvested spikes were air-dried for 5 d in a greenhouse at (25 ± 5) °C, hand threshed and the seeds were kept in a freezer at −20 °C to maintain dormancy until phenotyping [22,40]. All seeds were taken out from the freezer and air-dried again for an additional 2 d on greenhouse desks. One hundred healthy grains of each line were surface-sterilized with 5% NaClO for 15 min and washed three times with sterile water. The sterilized seeds were placed in a 90 mm Petri dish containing filter paper and 8 mL of distilled water, and incubated at 23 °C for 7 d. Germinated grains were counted daily and removed. The weighted germination index was calculated according to the following formula [41]:

$$\mathrm{GI} = \frac{\sum_{i=1}^{7} n_i[7-(i-1)]}{7N}$$

where n_i is the number of grains germinated in the ith day; N is the total number of grains.

2.3. Genotyping and linkage map construction

The 262 RILs and parents were genotyped using wheat 50 K Infinium iSelect SNP arrays containing 55,224 SNPs outsourced from CapitalBio Corporation (https://www.capitalbio.com). The SNP data were processed by removing monomorphic markers, SNPs with more than 20% missing data or minor allele frequency (MAF) of < 0.3. The remaining high-quality polymorphic SNPs (9354) were further analyzed to remove redundant markers using the BIN function in IciMapping v4.2 [42] (https://www.isbreeding.net/). The resulting genotypic data consisting of 1501 non-redundant markers (bin markers) were used to generate a linkage map with JoinMap v4.0 software using the regression mapping algorithm. The linkage maps were drawn by the MapChart v2.32 software [43] (https://www.wur.nl/en/show/Mapchart.htm).

2.4. Statistical analysis

The best linear unbiased estimation (BLUE) values of lines in each environment and across environments were calculated for subsequent analysis using the PROC MIXED function in SAS 9.4 software (SAS Institute Inc, Cary, NC https://www.sas.com). Descriptive statistics and correlation analyses were conducted using PROC MEANS and PROC CORR functions. Analysis of variance (ANOVA) was performed using PROC general linear model (GLM) function. Broad-sense heritability (H_b^2) was estimated according to the following equation;

$$H_b^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{e} + \frac{\sigma_\varepsilon^2}{re}}$$

where σ_g^2, σ_{ge}^2, and σ_ε^2 are the variances of genotypes, genotype by environment interaction, and residual error, respectively, and r and e are the numbers of replications and environments, respectively [44]. Bar graph was used to perform multiple comparison for combining effect of stable QTL, and their distribution was indicated by GraphPad prism Version 8.0.1 (GrapPad Software, San Diego, CA, USA).

2.5. QTL mapping

QTL analysis was performed by the Inclusive composite interval mapping (ICIM) method with additive effect, using IciMapping v4.1 software [42]. The logarithm of odds (LOD) threshold for declaring significant QTL was 2.5 based on 1000 permutations with type 1 error (α = 0.05). The physical positions of mapped SNPs in the region of detected QTL were identified by blasting the SNP flanking sequences against reference genome sequences of the Chinese Spring (RefSeq 1.0, IWGSC, International Wheat Genome Sequencing Consortium, https://urgi.versailles.inra.fr/blast_iwgsc/). The QTL was named following the International Rules of Genetic Nomenclature (https://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm). QTL detected in two or more environments were regarded as stable.

2.6. KASP marker development and validation

Sequences of SNPs flanking stable QTL for the pre-harvest sprouting resistance were converted to KASP markers. The KASP primers were designed using PolyMarker (https://www.polymarker.info/) following Li et al. [17]. The 384-well optically clear plates were read on PHERAstarplus SNP (BMG Labtech GmbH, Ortenberg, Germany) to detect the fluorescence, and data analysis was carried out using KlusterCaller (LGC, Hoddesdon, UK). KASP markers were validated using a panel of 100 wheat varieties.

2.7. Analysis of the additive effect of stable QTL for PHS resistance

The additive effect of stable QTL was determined by categorizing the RIL or cultivars in the natural population into four different genotypic groups (1^R, 2^R, 1^S and 2^S) based on the flanking markers of *QGI.caas-3A* and *QGI.caas-5A*. The two QTL were temporarily designated as loci *1* and *2* respectively, where *R* and *S* represent resistance and susceptibility alleles, respectively. The GI values of the

four genotypic groups were compared in all environments and BLUE, analyzed in SAS 9.2 software (SAS Institute Inc, Cary, NC, USA) using PROC MIXED model by treating group as fixed effect, while line or variety nested in each group as random effect.

3. Results

3.1. Phenotypic performance

The RILs and parents were evaluated for PHS using GI values across five environments. The mean GI value of ZM578 had a significantly lower value (3.7%) than that of JM22 (44.73%) at $P < 0.001$ and continuous distribution of GI in the RIL population was observed, indicating a typical polygenic inheritance (Fig. S2; Table S1). ANOVA revealed that GI was significantly influenced by the effects of genotype, environment, and genotype × environment interactions ($P < 0.01$) (Table 1), and broad-sense heritabilities ranged from 0.91 to 0.98 among the five environments, with a high mean value of 0.91 (Table S1). Also, among-environments, correlations of GI were significant and positive, with the values ranging from 0.52 to 0.76 ($P < 0.001$) (Table S2).

3.2. QTL detection

A high-density genetic linkage map of the ZM578/JM22 population was constructed with 1501 bin markers on all chromosomes except 6B, spanning 2384.95 cM with an average distance of 1.59 cM per bin marker. The maximum number of markers was recorded in A genome (562), followed by B (545) and D (394) genomes (Tables S3, S4). In addition, SNPs were unevenly distributed among chromosomes, such as 7A (133) and 2B (132) having the highest number, while chromosome 6A had the lowest number of SNPs (11). No SNP was mapped on Chromosome 6B, suggesting that this chromosome might have a large similarity between the two parents. Six QTL for PHS resistance were detected on chromosomes 1D, 2B, 3A, 3B, 4A, and 5A, designated as *QGI.caas-1D*, *QGI.caas-2B*, *QGI.caas-3A*, *QGI.caas-3B*, *QGI.caas-4A* and *QGI.caas-5A*, respectively, explaining 29.84% of the total phenotypic variance, with LOD values ranging from 2.57 to 7.17. Among these, two were stably detected in at least two environments on chromosomes 3A (*QGI.caas-3A*) and 5A (*QGI.caas-5A)*, respectively (Fig. 1; Table 2). *QGI.caas-5A* was detected in four environments and the BLUE value, explaining 4.43%–8.02% of the phenotypic variances, with the resistance allele contributed by ZM578. *QGI.caas-3A* was identified in two environments and the BLUE value, contributing for 4.33%–5.58% of the phenotypic variances, with the resistance allele derived from JM22.

3.3. QTL validation

Four KASP markers, *K-AX-109605367* and *K-AX-179559687* for *QGI.caas-3A* and *K-AX111258240* and *K-AX-109402944* for *QGI.caas-5A,* were successfully developed, and their genetic effects were verified in a panel of the natural population with 100 cultivars (Fig. S3; Tables 3, S5, S6). Both of the genotypes with resistance alleles at both *QGI.caas-3A* and *QGI.caas-5A* have significantly lower GI values than those with both susceptible alleles in the natural population of 100 cultivars, with 82.7% of germplasm possessing the resistance allele of *QGI.caas-3A* and 57.1% having the resistance allele of *QGI.caas-5A*, respectively (Table 3).

Table 1
Analysis of variance of germination index (GI) in the RIL population of Zhongmai 578/Jimai 22

Source of variation[a]	*df*	Sum of square	*F* value
Gen	261	170606.34	126.19 ***
Env	4	3162.78	203.53 ***
Gen × Env	1044	48102.4	11.86 ***
Rep (Env)	8	241.03	5.82 **
Error	2600	9924.73	–

Gen, genotype; Env, environment; Rep (Env), replication nested within environment. **, $P < 0.01$; ***, $P < 0.001$.

3.4. Additive effect of QTL for PHS resistance

Using the flanking markers *AX-111055367* and *AX-179559687* of *QGI.caas-3A*, and *AX-111258240* and *AX-109402944* of *QGI.caas-5A*, 262 RILs were divided into four groups (1^R, 2^R, 1^S and 2^S). The results indicated that the genotypic combination with both resistance alleles (1^R2^R) at *QGI.caas-3A* and *QGI.caas-5A* had a significantly lower GI value in all environments, as well as the BLUE value in comparison with the other three groups, whereas those with both susceptible alleles (1^S2^S) had a significantly higher GI value in all environments, as well as the BLUE value than the other three groups. (Fig. 2).

Moreover, in the natural population of 100 cultivars, the genotypic combination with both resistance alleles (1^R2^R) at *QGI.caas-3A* and *QGI.caas-5A* showed a significantly lower GI value than the other three groups, whereas those with both susceptibility alleles (1^S2^S) had a significantly higher GI value than the other three groups (Table S7). Therefore, pyramiding the resistance alleles of *QGI.caas-3A* and *QGI.caas-5A* can effectively improve PHS resistance.

4. Discussion

4.1. Comparisons of the QTL with previous studies

PHS affects end-use quality and wheat production in many regions across the world; therefore, developing cultivars with good resistance is an effective way to minimize economic losses, and markers tightly linked to the identified resistance genes are helpful in accelerating the development of new cultivars. The current study detected two stable QTL, *QGI.caas-3A* and *QGI.caas-5A*, for PHS resistance. *QGI.caas-3A* was detected on chromosome 3A at 568.7–573.9 Mb based on the flanking markers *AX-111055367* and *AX-179559687*. Liu et al. [45] and Liu and Bai [46] identified *QPhs.pseru-3AS* with flanking markers *Xbarc12* and *Xbarc321* located at 11.7–15.5 Mb. Shao et al. [5] detected two QTL at 11.7 Mb. Kulwal et al. [38] identified a major QTL for PHS linked with marker *Xgwm155* at 703 Mb. Osa et al. [19], Nakamura et al. [33], and Mori et al. [47] reported a QTL *QPhs.ocs-3A.1* for grain dormancy between markers *Xbarc310* and *Xbarc 321* spanning 7.1–11.7 Mb. Miao et al. [48] identified *QPhs.caas-3AS.1* for PHS flanked by markers *Xbarc294* and *Xbarc57* at 7.9–10.3 Mb. Fofana et al. [49] reported a QTL for GI linked with marker *Xcfa2* at 690.7 Mb. Himi et al. [36] found a QTL for PHS associated with a red grain color gene *Tamyb10-A1* at 703.9 Mb, which is more than 100 Mb away from the *QGI.caas-3A*. Therefore, *QGI.caas-3A* appears to have a different position from these previously identified QTL on chromosome 3A (Table S8), indicating that it is probably a new genetic locus for PHS resistance. The reason for relatively low mapping resolution of QTL on chromosome 3A may lie in that the susceptible parent JM22 is one of the parents of ZM578. Therefore, the RILs of ZM578/JM22 used in this study have high genetic similarities, which make little polymorphisms in the genetic background, and the KASP markers developed in the study should be effective in testing the diversity panel.

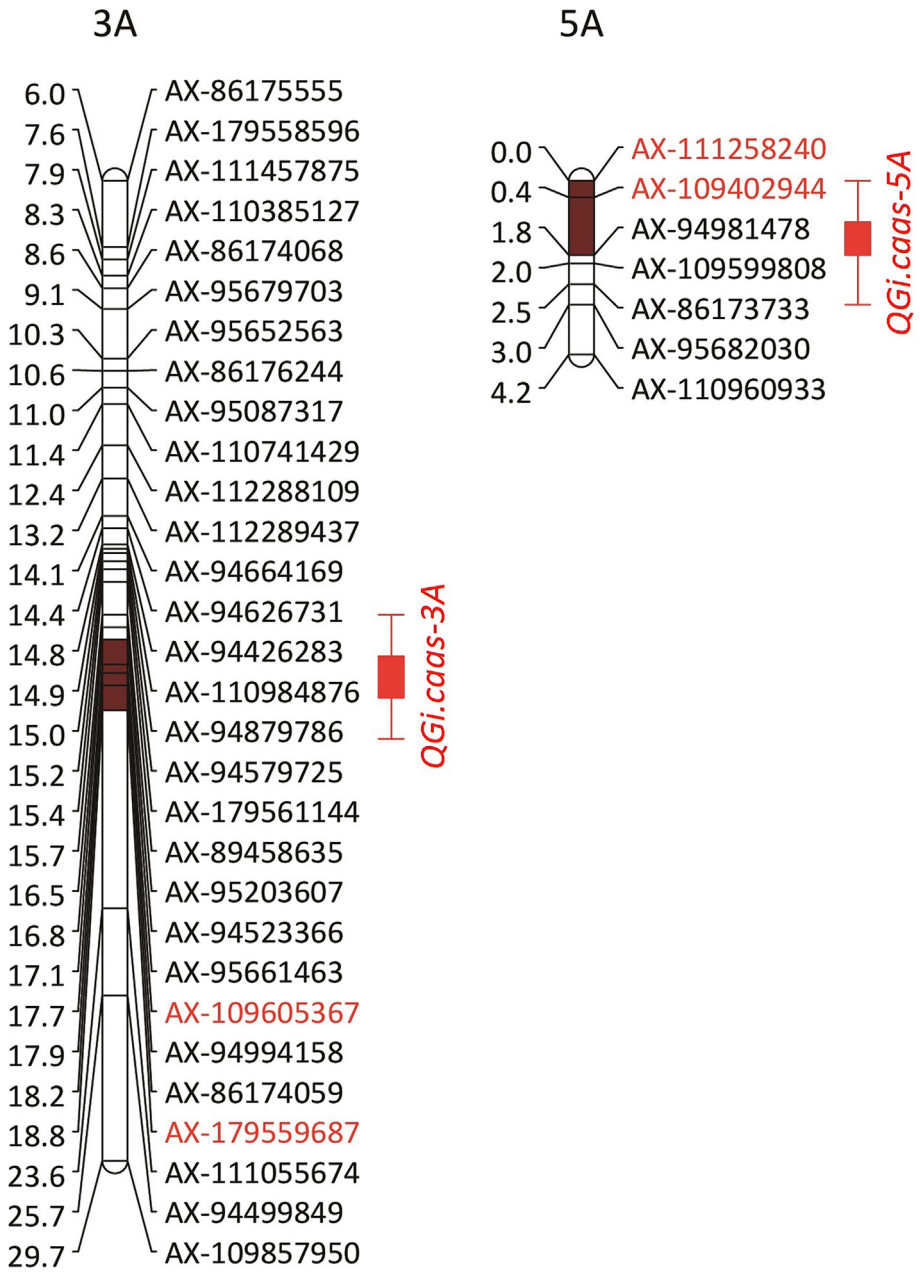

Fig. 1. Genetic mapping of *QGI.caas-3A* and *QGI.caas-5A* in the recombinant inbred line (RIL) population of Zhongmai 578 /Jimai 22 for pre-harvest sprouting resistance. Target regions of the quantitative trait loci (QTL) are indicated with brown bars, and flanking markers are shown in red color.

QGI.caas-5A was mapped at 17.1–17.2 Mb on chromosome 5A, flanked by SNP markers *AX-111258240* and *AX-109402944*. Several previous studies have reported QTL associated with PHS and SD on the long arm of chromosome 5A [12,50–54]. Borner et al. [12] identified a QTL for PHS linked with markers *Xgwm186* and *P7560-439*, located at 471.7 Mb for the closest marker. Groos et al. [55] reported a QTL for PHS and grain color on the short arm of chromosome 5A flanked by markers *Xbcd1871-Xgwm304*, with the closest marker located at 105.5 Mb. Fakthongphan et al. [50] detected a QTL linked with marker *Barc10* at 2.1 Mb using three RIL populations. Zhu et al. [51] reported a QTL linked with marker *Xbarc 360* at 458.3 Mb using 260 wheat varieties and advanced lines. Martinez et al. [52] detected a QTL linked with marker *IWB10250* at 540 Mb using a panel of white winter wheat cultivars and elite breeding lines. Zuo et al. [53] identified a QTL for PHS resistance linked with marker *BS00036907_51* at 438.1 Mb, explaining up to 4.2% of the phenotypic variance. Lin et al. [54] reported two QTL linked with marker *Bobwhite_4004_61* at 33.2 Mb and *Excalibur_c54774_408* at 593.0 Mb, using 260 wheat varieties and advanced lines. Nakamura et al. [56] identified an ABA signaling gene *TmERA3* on the centromere region of chromosome 5A at 343 Mb by *in silico* analysis. The position of *QGI.caas-5A* is located differently from all the reported QTL (Table S8), based on the wheat reference genome (IWGSC RefSeq 1.0), indicating that it is likely to be a new QTL for PHS resistance.

It should be indicated that only two of the six QTL mapped in this study were found to be stable, and the phenotypic variance explained was not high, which may be attributed to the following two reasons: 1) PHS is a complex quantitative trait and controlled by numerous minor genes, and it is highly influenced by environments; 2) JM22 is one of the parents of ZM578, and theoretically near 75% of the genetic background of the RILs is originated from

Table 2
QTL detected for germination index (GI) in the RIL population of Zhongmai 578/Jimai 22.

QTL name	Env[a]	Physical position[b] (Mb)	Flanking marker		LOD score	PVE[c] (%)	Add[d]
QGi.caas-1D	E2	203.0–206.0	*AX-86172873*	*AX-86179801*	3.29	4.93	−1.66
QGi.caas-1D	BLUE	203.0–206.0	*AX-86172873*	*AX-86179801*	4.56	4.58	−2.32
QGi.caas-2B	E1	146.0–148.0	*AX-86163497*	*AX-112289330*	3.73	8.01	−2.73
QGi.caas-2B	BLUE	146.0–148.0	*AX-86163497*	*AX-112289330*	3.50	5.57	−1.73
QGi.caas-3A	E2	568.7–573.9	*AX-10605367*	*AX-179559687*	3.85	4.33	2.25
QGi.caas-3A	E3	568.7–573.9	*AX-10605367*	*AX-179559687*	3.11	5.58	1.71
QGi.caas-3A	BLUE	568.7–573.9	*AX-10605367*	*AX-179559687*	3.47	5.35	1.66
QGi.caas-3B	E3	746.0–751.0	*AX-179559693*	*AX-110463744*	2.99	5.32	1.66
QGi.caas-4A	E2	711.0–713.0	*AX-179477416*	*AX-94907472*	2.65	2.96	−1.86
QGi.caas-5A	E1	17.1–17.2	*AX-111258240*	*AX-109402944*	5.06	7.45	−2.67
QGi.caas-5A	E2	17.1–17.2	*AX-111258240*	*AX-109402944*	7.17	8.02	−3.06
QGi.caas-5A	E5	17.1–17.2	*AX-111258240*	*AX-109402944*	2.52	4.43	−1.82
QGi.caas-5A	E4	17.1–17.2	*AX-111258240*	*AX-109402944*	3.23	6.53	−1.61
QGi.caas-5A	BLUE	17.1–17.2	*AX-111258240*	*AX-109402944*	4.00	6.06	−1.77

[a] E1, Xinxiang 2019 – 2020; E2, Xinxiang 2020 – 2021; E3, Shangqiu 2020 – 2021; E4, Luoyang 2020–2021; E5, Gaoyi 2020–2021.
[b] Physical positions of markers are based on the Chinese Spring reference genome in IWGSC (RefSeq v1.0, https://www.wheatgenome.org/; IWGSC, 2018).
[c] PVE means the phenotypic variance explained by the QTL.
[d] Estimated additive effect of QTL, positive and negative additive values indicate resistance alleles contributed by Jimai 22 and Zhongmai 578, respectively.

Table 3
Effects of *QGI.caas-3A* and *QGI.caas-5A* on germination index (GI) in the natural population.

QTL	Marker name	Genotype	Number of lines	[b]GI	*P*-value
QGi.caas-3A	*K_AX-109605367*	GG[a]	15	46.75	0.014 **
		TT[b]	77	32.84	
	K_AX-179559687	TT[c]	16	47.27	0.007 ***
		GG[d]	76	32.55	
QGi.caas-5A	*K_AX-111258240*	TT[e]	51	30.71	0.018 **
		CC[f]	41	40.59	
	K_AX-109402944	GG[g]	55	30.99	0.016 **
		CC[h]	37	41.23	

[a] GG, [c]TT, [e]TT and [g]GG are Zhongmai 578 genotypes; [b]TT, [d]GG, [f]CC and [h]CC are Jimai 22 genotypes. **, $P < 0.01$; ***, $P < 0.001$.

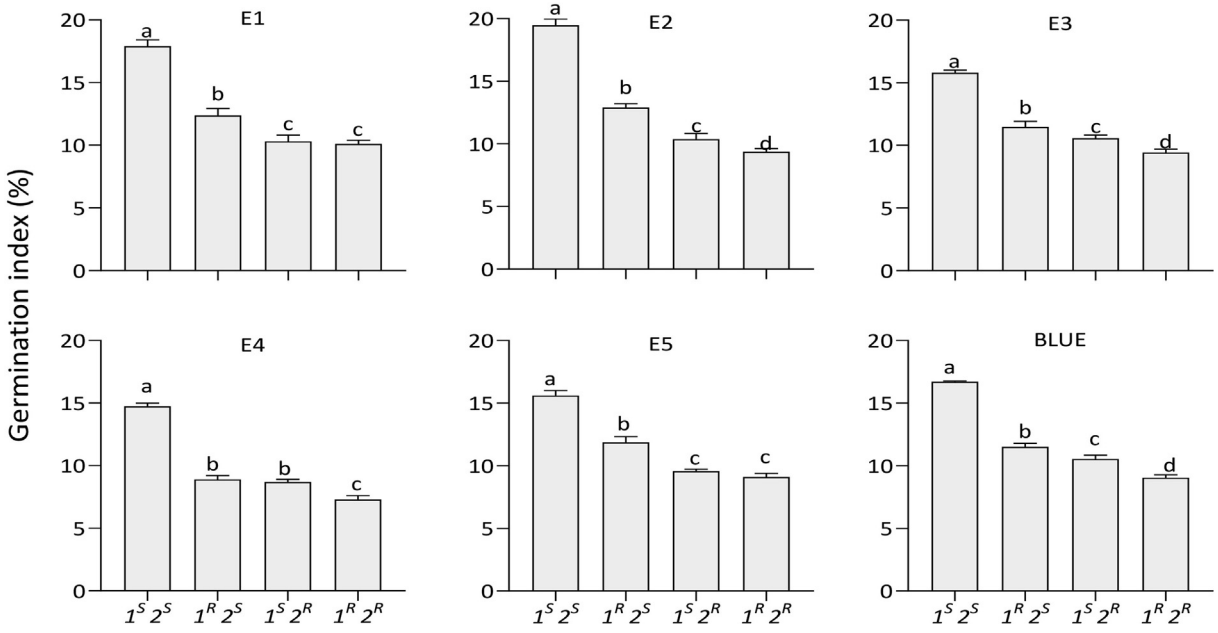

Fig. 2. Distributions of germination index (GI) among four genotypic combinations of *Qphs.caas-3A* and *Qphs.caas-5A* grown in five environments. The *x*-axis shows the genotypic groups, and the *y*-axis indicates GI (%). The numbers 1 and 2 represent *Qphs.caas-5A* and *Qphs.caas-3A*, respectively; the superscript letters R and S represent resistance and susceptibility alleles, respectively. Genotypes with different letters indicate significant differences ($P < 0.05$) in GI, and those with the same letters show no significant differences (P greater than 0.05).

JM22, thus it is not possible to identify common PHS resistance genes in this RIL population. Therefore, it worth conducting further research using a population of ZM578 with some other cultivars rather than JM22, to find more PHS-related genes in ZM578. Moreover, we believe, from breeding point of view, many genes detected previously act as minor genes in the background of modern leading

wheat cultivars like JM22 and ZM578, which have combined with many favorable alleles of genes underling good agronomic parameters. It is much worthy doing QTL mapping or fine mapping in modern wheat cultivars, and the favorable alleles of QTL mapped can easily be used in wheat breeding program.

4.2. Potential value of the QTL for wheat breeding

The two stable QTL *QGI.caas-3A* and *QGI.caas-5A* identified in this study showed an additive effect on PHS resistance when combined, suggesting the genetic complex systems in regulating wheat PHS and the importance of pyramiding a specific combination of QTL. Zhongmai 578 contributed the resistance allele of *QGI.caas-5A*, and the resistance for PHS of *QGI.caas-3A* was contributed by Jimai 22. Therefore, tracing the pedigree for the favorable alleles will provide important information for future MAS and the developed KASP marker *K-AX-111258240* (Table S6) tightly linked with *QGI.caas-5A* was successfully used to trace the origination. The result indicated that the favorable allele of *QGI.caas-5A* was inherited from Zhengzhou 761 through Yumai 49 to Zhongmai 255 (Fig. S4; Table S9), providing the marker for MAS on PHS when using Zhongmai 578 as a core parent in the future, as well as other cultivars in the pedigree such as Yumai 49 and Zhongmai 255 with excellent pan bread quality. It should be indicated that some favorable alleles in Zhongmai 578 inherited from Jimai 22 could not be detected in this RIL population because Jimai 22 is one of the parents of Zhongmai 578.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Rabiu Sani Shawai: Conceptualization, Methodology, Visualization, Investigation, Formal analysis, Validation, Resources, Writing – original draft, Writing – review & editing. **Dan Liu:** Investigation, Validation, Resources. **Lingli Li:** Investigation, Validation, Resources. **Tiantian Chen:** Investigation. **Ming Li:** Validation, Resources. **Shuanghe Cao:** Methodology, Validation. **Xianchun Xia:** Methodology, Validation. **Jindong Liu:** Methodology, Formal analysis, Validation, Resources. **Zhonghu He:** Conceptualization, Validation, Supervision, Project administration, Funding acquisition. **Yong Zhang:** Conceptualization, Methodology, Visualization, Investigation, Formal analysis, Validation, Resources, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

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