Genome-wide association analysis of type II resistance to Fusarium head blight in common wheat (#74108)

First revision

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Genome-wide association analysis of type II resistance to Fusarium head blight in common wheat

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Background. *Fusarium* head blight (FHB) is a disease affecting wheat spikes caused by *Fusarium* species, which leads to cases of severe yield reduction and seed contamination. Therefore, identifying resistance genes/QTLs from various wheat germplasm is always of importance for improving the wheat FHB resistance in wheat production. **Methods.** Our study using a high-density genetic map created with 90K single nucleotide poly-morphism (SNP) arrays in a panel of 205 elite winter wheat accessions, a genome-wide association study (GWAS) with a focus on FHB was carried out in three environments. **Results.** Sixty-six significant marker–trait associations (MTAs) were identified ($P<0.001$) on fifteen chromosomes with explaining phenotypic variation ranging from 5.4 to 11.2%. Some important new MTAs in genomic regions involving FHB resistance were found on chromosomes 2A, 3B, 5B, 6A, and 7B. On chromosome 7B, 6 MTAs at 92 cM were found in two environments. Moreover, there were 11 MTAs consistently associated with diseased spikelet rate and diseased rachis rate as pleiotropic effect loci, and *D_contig74317_533* on chromosome 5D was novel for FHB resistance. Eight new candidate genes of FHB resistance were predicated in wheat. Of which, three candidate genes, *TraesCS5D02G006700*, *TraesCS6A02G013600*, and *TraesCS7B02G370700* on chromosome 5DS, 6AS, and 7BL, respectively, were perhaps important in defending against FHB by regulating intramolecular transferase activity, GTP binding, chitinase activity in wheat by blasting their functions, but this needs further validation in future. In addition, a total of five favorable alleles associated with wheat scab resistance were discovered. These results provide important genes/loci for enhancing FHB resistance in wheat breeding by marker-assisted selection.

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Abstract

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Methods. Our study using a high-density genetic map created with 90K single nucleotide polymorphism (SNP) arrays in a panel of 205 elite winter wheat accessions, a genome-wide association study (GWAS) with a focus on FHB was carried out in three environments.

Results. Sixty-six significant marker-trait associations (MTAs) were identified (P<0.001) on fifteen chromosomes with explaining phenotypic variation ranging from 5.4 to 11.2%. Some important new MTAs in genomic regions involving FHB resistance were found on chromosomes 2A, 3B, 5B, 6A, and 7B. On chromosome 7B, 6 MTAs at 92 cM were found in two environments. Moreover, there were 11 MTAs consistently associated with diseased spikelet rate and diseased rachis rate as pleiotropic effect loci, and *D_contig74317_533* on chromosome 5D was novel for FHB resistance. Eight new candidate genes of FHB resistance were predicated in wheat. Of which, three candidate genes, *TraesCS5D02G006700*, *TraesCS6A02G013600*, and *TraesCS7B02G370700* on chromosome 5DS, 6AS, and 7BL, respectively, were perhaps important in defense against FHB by regulating intramolecular transferase activity, GTP binding, chitinase activity in wheat by blasting their functions, but this needs further validation in future. In addition, a total of five favorable alleles associated with wheat scab resistance were

discovered. These results provide important genes/loci for enhancing FHB resistance in wheat breeding by marker-assisted selection.

Keywords
Wheat; Fusarium head blight; GWAS; SNP; MTA.

Introduction

Wheat (*Triticum aestivum L.*), one of the three major food crops, is grown worldwide as an important source of food and fodder. Therefore, maintaining consistent wheat production has become a frequent focus of agricultural experts worldwide. Wheat is susceptible to both biotic (diseases, insect pests, etc.) and abiotic (drought, freezing damage, etc.) stresses because of its long growing phase. *Fusarium* head blight (FHB), also known as scab, is an infection of wheat spikes mainly brought on by a Fusarium species. FHB is a quantitative trait controlled by multiple genes that is affected by both the environment and genetics (Buerstmayr et al. 2015; Liu et al. 2016b; Bai et al. 1994). This disease has become an important disease in the Yellow and Huai River Valleys of China (Zhu et al. 2018), and seriously threatens wheat production and processing.This disease not only causes severe wheat yield reduction but also contaminates wheat seeds with deoxynivalenol (DON) toxins (Bai et al. 1994). DON, also known as vomitoxin, is regards as a teratogen, neurotoxin and immunosuppressant, which could cause adverse health effects (Ennouari et al., 2013). In order to control human exposure to DON, the Food and Drug Administration (FDA, 2010) has set an advisory limit of 1 mg/kg for finished wheat products. In addition to negatively affecting wheat production in the middle and lower Yangtze River Valley region of China, FHB has become more common during the past 20 years in the Yellow and Huai River Valley regions as a result of climatic and tillage system changes. Moreover, it has become the most destructive spike disease in the world because no completely resistance varieties have been found so far, which seriously threatens food production and food security. Breeding resistant or complete resistance varieties and discovering resistant genes are the most effective ways to solve the problem of FHB.

The interactions between genotype and environment have a substantial impact on FHB, a complex trait with a quantitative nature. Previous research has demonstrated that FHB resistance is influenced by plant height, heading date, blooming period, another extrusion, etc. The weather (sunny or wet) during blossoming is crucial for the development of this illness. Genetic linkage analysis has been used to study FHB resistance extensively in wheat, and numerous QTL (Quantitative Trait Loci) (more than 400 scattered on 21 chromosomes) related to FHB resistance have been reported (Ma et al. 2020). Five categories of FHB resistance exist at the moment: type I resistance to initial spike infection, type II resistance to spread spike infection, type III resistance to accumulation of mycotoxins, type IV resistance to kernel infection, and type V resistance to yield reduction (Mesterházy 1995). Type I resistance and type II resistance were distinguished in the seminal study by Schroeder and Christensen (1963). Almost all reports on FHB resistance have been type II. Recently, numerous small-effect Type II FHB QTLs are reviewed by Buerstmayr et al. (2019). Seven genes (*Fhb1* to *Fhb7*) for FHB resistance have been

found, and *Fhb1*, *Fhb2*, *Fhb4*, and *Fhb5* were on chromosomes 3BS, 6BS, 4BL, and 5A, respectively in common wheat; however, the remaining genes, *Fhb3*, *Fhb6,* and *Fhb7*, were derived from wheat relative species (Cainong et al., 2015; Jia et al., 2018; Li et al., 2019; Qi et al., 2008; Su et al., 2019; Wang at al., 2020; Xue et al., 2010; Xue et al., 2011). The *Fhb1* gene has been widely dissected and sequenced to find a pore-forming toxin-like (*PFT*) gene that is responsible for FHB resistance (Rawat et al. 2016). Later, another new gene was discovered for *Fhb1*, encoding a putative histidine-rich calcium-binding protein (*His* or *TaHRC*) that was adjacent to *PFT* (Li et al. 2019; Su et al. 2019). These research have led to the development of *Fhb1* function markers that are being employed in molecular breeding to improve scab resistance. However, it appears that the mechanisms by which *His* and *TaHRC* impart resistance are distinct. Because of this, more research on this gene is still required to understand its molecular mechanisms (Li et al. 2019; Su et al. 2019). Recently the candidate gene for *Fhb7* was determined and cloned, which revealed that it encoded a glutathione (GST) that can detoxify trichothecene toxins (Wang et al. 2020). Its resistance depends on a reduction of pathogen growth in spikes, which is different from the resistance of *Fhb1*. The remaining five FHB genes, however, have not yet been cloned.

Some significant loci for resistance have been discovered in addition to these seven FHB genes. For instance, *QFhb.mgb-2A* was identified as a *WAK2* gene (Giancaspro et al. 2016), and the function of *WAK2* for FHB resistance was validated (Gadaleta et al. 2019). Another important locus on chromosome 2DL was considered to be transcription factor *TaWRKY70*, which regulates the expression of metabolite biosynthetic genes including *TaACT*, *TaDGK*, and *TaGLI* to influence FHB resistance (Kage et al. 2017a, b). Using two Recombinant Inbred Lines (RILs) populations with one common parent, named AC Barrie, from Canadian spring wheat, *QFhb.mcb-3B*, *QFhb.mcb-6B*, and *QFhb.mcb-5A.1* were mapped to the expected location of *Fhb1*, *Fhb2*, and *Fhb5*, respectively (Thambugala et al. 2020). On chromosome 5B, the prominent resistance gene, *QFhb.mbr-5B* was found to explain up to 36% of the phenotypic variation (Thambugala et al. 2020).

With the development of genomics, growing research has been done on the use of genome-wide association study (GWAS) to analyze wheat FHB resistance. One study (Zhu et al. 2020) used a mixed linear model (MLM) to consistently identify five quantitative trait loci (QTL) related with FHB on chromosome arms 1AS, 2DL, 5AS, 5AL, and 7DS. These QTLs accounted for 5.6, 10.3, 5.7, 5.4, and 5.6% of the variation in phenotype, respectively. Tessmann et al. (2019) used GWAS (based on 2-yr entry means) identified 16 significant ($p < 0.001$) single nucleotide polymorphisms (SNPs) associated with disease traits on multiple chromosomes. Single nucleotide polymorphism association ranged from −2.14 to 4.01% of the mean of a given trait. Another GWAS identified 26 loci (88 marker-trait associations), which explained 6.65–14.18% of the phenotypic variances. The associated loci distributed across all chromosomes except 2D, 6A, 6D and 7D (Hu et al. 2020). In addition to this there have been numerous studies using GWAS in FHB resistance recently (Verges et al. 2021; Gaire et al. 2021; Ghimire et al.

2022; Wu et al. 2019). It appears that using GWAS to investigate wheat FHB is a very promising endeavor.

Therefore, our study using a high-density genetic map created with 90K single nucleotide polymorphism (SNP) arrays in a panel of 205 elite winter wheat accessions, a genome-wide association study (GWAS) with a focus on FHB was carried out in three environments. The objective of this study was to identify some novel genomic regions associated with the type II resistance of wheat in different environments, and to predict candidate genes for loci associated with these traits, which could improve wheat FHB resistance by molecular breeding in the future.

Materials & Methods

Plant materials

The association mapping panel of 205 wheat accessions for GWAS comprised 77 released cultivars, 55 founder parents including 2 lines from Mexico and France, and 73 breeding lines from 10 provinces that represent the major winter wheat production regions in China (Chen et al. 2015).

Sumai 3 was selected as an FHB high resistant check, Yangmai158 as a moderately resistant check, and Ningmaizi 22 as a moderately susceptible check.

Growth conditions

The materials were grown in the field and greenhouse of Shandong Agricultural University (117°16′ E, 36°17′ N) during the 2015–2016 and 2016–2017 cropping seasons, hereafter referred to as 2016 and 2017, respectively. The terms E1, E2, and E3 represent the experimental field of Shandong Agricultural University in 2017, the greenhouse of Shandong Agricultural University in 2017, and the experimental field of Shandong Agricultural University in 2016, respectively. In the field, the randomized block design was used, with two replications. All lines were planted in 2 m plots with three rows uniformly spaced at 25 cm intervals. Each row contained 70 seeds evenly distributed. The local recommended field crop management practices were followed and no pests or diseases were found in the field. But in the greenhouse, the seeds of materials were germinated and vernalized for an additional 4 weeks (4℃, 12 h light/dark regime) before being transferred to the greenhouse. Plants were potted in a mixture of compost, sand chalk and common soil. Each plant was planted in an individual pot (the diameter is 30cm and the depth is 25cm, with four seedlings) and in three replications (pots). The temperatures of greenhouse were gradually increased from 15℃/13℃ during day/night to 20℃/18℃, and a 16 h/day photoperiod at the time of anthesis. The growing conditions in the greenhouse have been described in our previous article (Zhao et al. 2023).

Inoculum preparation and Inoculation

In this study, the conidiospore suspension of 7136, F301, F609, and F15 virulent strains of *F. graminearum* used, was obtained with the courtesy of Nanjing Agricultural University. The pathogen was propagated in a mung bean medium (Buerstmayr et al. 2000) and incubated on a shaker at 150 rpm under 25 ℃ for 4–5 days. After culturing and filtering, the mass of conidia was examined under a microscope, and then, the four pathogen strains were mixed equally and

stored at 4℃ for later use. The preparation of *F. graminearum* was also the same as our previous study (Zhao et al. 2023).

Wheat was inoculated with 10 μl of the *F. graminearum* conidia suspension (50,000 spores/mL) applied to a pair of florets in the middle of the spikeduring flowering (Guo et al. 2015). Ten spikes were inoculated per line from each replication. The whole wheat spike was then covered with a self-sealing bag to retain moisture, and had the self-sealing bag removed after 3 days. The disease symptoms were investigated on day 21 after inoculation, and the diseased spikelet rate (DSR), diseased spike rachis rate, and disease index (DI) were calculated. Both the field nursery and greenhouse point adopt this method. All spikes were classified into five classes of disease severity according to the diseased spikelet rate (DSR): 0% (class 0), 1–25% (class 1), 26–50% (class 2), 51–75% (class 3), and 76–100% (class 4) (Lu et al. 2001). The disease index (DI) was calculated based on the rules for monitoring and forecasting wheat head blight (Chinese Standard: GB/T 15796-2011).

$$Diseased\ spikelets\ rate = \frac{Number\ of\ \ infected\ spikelets}{Total\ spikelets\ per\ spike} \times 100\%$$

$$Diseased\ spike\ rachis\ rate = \frac{Number\ of\ \ infected\ rachis}{Total\ rachis\ per\ spike} \times 100\%$$

$$DI(\%) = \frac{\sum h_i \times i}{H \times 4} \times 100\%$$

where i is the class of disease severity, hi is the number of wheat spike in each class, and H is the number of all investigated wheat spikes.

The standard of wheat FHB resistance was as follows: Immune (DI=0), High resistant (DI< Sumai 3), Moderately resistant (Sumai 3< DI< Yangmai 158), Moderately susceptible (Yangmai 158< DI< Ningmaizi 22), High susceptible (DI> Ningmaizi 22).

Genome-wide association analysis

SNP markers, genotyping, and the population structure of the samples have been previously reported (Chen et al. 2015; Chen et al. 2016; Chen et al. 2017). A total of 24,355 mapped SNPs was used for MTA analysis. According to Chen et al (2017), the association population was divided into four categories using STRUCTURE's maximum membership probability (comprised of 43, 32, 105 and 25 varieties, respectively). Chen et al. (2016) also reported LD values for different chromosomes.

Based on this information, significant marker-trait associations (MTAs) were identified using a mixed linear model (MLM) in TASSEL3.0. The P-value was used to determine whether a QTL was associated with a marker, while the phenotypic variation explained (PVE) was used to evaluate the magnitude of the MTA effects. SNPs with P≤10-3 were considered to be significantly associated with phenotypic traits, SNPs with P≤10-4 were considered to be extremely significantly associated with phenotypic traits. Further, when the marker was detected in two or more environments at the same time, it was considered a stable MTA.

Statistical analysis

Analysis of variance (ANOVA) and correlations among phenotypic traits were carried out using the statistical software SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Forecasting candidate genes for FHB resistance

A BLAST (Basic Local Alignment Search Tool) search was performed on the International Wheat Genome Sequencing Consortium database (RefSeq v1.0; https://urgi.versailles.inra.fr/blast/) using the sequence of the significant SNP markers identified by GWAS. When an SNP marker sequence from the IWGSC was 100% identical to any wheat contig, the sequence was extended by 2Mb for each marker using the IWGSC BLAST results. Afterwards, the extended sequence was used to run a BLAST search on the National Center for Biotechnology Information (NCBI) database (http: //www.ncbi.nlm.nih.gov) and on Ensembl Plants (http://plants.ensembl.org/Triticum_aestivum/ Tools/Blast) to confirm possible candidate genes and functions.

Analysis of marker haplotype

The effect of resistance genes was calculated by using the average diseased spikelet rate of various gene combinations. Effect of resistance genes=(Average diseased spikelet rate of materials carrying resistance gene - Average diseased spikelet rate of materials without resistance gene)/Average diseased spikelet rate of materials without resistance gene.

Results

Phenotypic variation analysis of wheat FHB resistance

The variation coefficient of diseased wheat spikelet rate (DSR) was the highest in E2 (52.96%), followed by that in E3 (44.30%), and E1 (36.55%) (Table 1), indicating that DSR genetic variation was abundant. The variance analysis of FHB resistance of the spikelet and spike rachis indicated that significant differences were present between cultivars and environments, and their interactions (Table 2). This illustrated that the FHB resistance was a quantitative trait affected not only by genotype but also by the environment. Furthermore, there were significant positive correlation coefficients between spikelet and spike rachis in the three environments, indicating that the resistance trend of FHB was consistent between spikelet and spike rachis (Table 3).

Marker–trait associations (MTAs) of FHB resistance

Sixty-six MTAs associated with FHB resistance were distributed on chromosomes 1A, 1B, 2A, 2B, 2D, 3B, 3D, 4A, 5A, 5B, 5D, 6A, 6B, 7A, and 7B (Table S1; Table S2; Figure 1). The phenotypic variation explained (PVE) of MTA loci to FHB resistance ranged from 5.45% to 11.20%, of which, 11 MTA loci were detected in both spikelet and spike rachis. On chromosome 7B, a novel genomic region from genetic position 92 to 103, significantly associated with FHB resistance, was detected in all three environments. Moreover, there was one major locus at genetic position 92 of chromosome 7B accounting for 11.20% of the phenotypic variation in the spikelets, namely locus *BS00025286_51*, which was also detected for the spike rachis, explaining 7.07% of its phenotypic variation. In E3, four loci on chromosome 7B were found to be associated with both diseased spikelet rate and diseased spike rachis rate, but these MTAs are

located in the same region and may represent one QTL (Table 4; Table S2). In addition, there were some genomic regions associated with FHB resistance on chromosomes 5B, 6A, 2A, and 3B, but they were found only in a single environment. The other six loci, including *D_contig74317_533* on chromosome 5D, *Kukri_c14239_1995* on chromosome 6A, *Kukri_c7087_896* on chromosome 3B, *RAC875_c35801_905* on chromosome 3D, *BS00099729_51* on chromosome 5B, and *RAC875_c68525_284* on chromosome 6B, were also identified to be associated with both the diseased spikelet rate and the diseased spike rachis rate. The remaining MTA loci were detected only for a single trait in a single environment.

The allelic variation analysis of MTAs loci

According to the PVE of MTA loci (Table S2), we selected 10 of them and analyzed their allelic variation (Table 4).. Alleles T and C of the marker, *Kukri_c14239_1995* on chromosome 6A were associated with the largest phenotypic difference (0.2297). Specifically, the phenotypic value of the diseased spikelet rate associated with *Kukri_c14239_1995-T* was significantly higher than that associated with *Kukri_c14239_1995-C*, indicating that *Kukri_c14239_1995-C* was better than *Kukri_c14239_1995-T* for FHB resistance (Table 4). Furthermore, because allele C of *D_contig74317_533* showed a significantly higher diseased spikelet rate than *D_contig74317_533-T*, allele T was deemed to be better for improving FHB resistance. On chromosome 7B, allele C of *BS00025286_51* had a higher diseased spikelet rate than allele T; thus, allele T for this locus was favorable for FHB resistance. Nevertheless, for the other four loci on this chromosome, significant differences between the two alleles for the diseased spikelet rate seemed to be at 5%. The least difference for diseased spikelet resistance was observed between *Kukri_c7087_896-G* and *Kukri_c7087_896-A*, which indicated that this locus affected FHB resistance to a smaller degree. Moreover, on chromosome 3D, *RAC875_c35801_905-G* yielded better results than *RAC875_c35801_905-A* for FHB resistance.

Prediction of candidate genes for some important loci

Eight important candidate genes were screened for important loci significantly associated with diseased spikelet rate and diseased spike rachis rate in wheat (Table S3). Of which, one candidate gene, *TraesCS3D02G326700* located on chromosome 3D is found associated with actin-binding in wheat. The candidate gene, *TraesCS5D02G006700* of the marker, *D_contig74317_533* on 5DS was predicated in wheat, whose function was intra-molecular transferase activity. Two candidate genes *TraesCS6A02G013700* and *TraesCS6A02G013800*, predicated by *IAAV9150*, participate in the ubiquitin-dependent ER-associated degradation (ERAD) pathway in wheat. The candidate gene, *TraesCS6A02G013600* of *Excalibur_c20597_509* has the function of GTP binding in wheat. On chromosome 7BL, *TraesCS7B02G370700* of *BS00025286_51* is involved in the biological process of defense response to fungi. There is one candidate gene, *TraesCS7B02G340200*, for the three loci, *RAC875_c18043_369*, *RAC875_c18043_411*, and *Kukri_c4143_1055*, on chromosome 7BL identified because of being in the same physical location. The candidate gene, *TraesCS7B02G340100* of *RAC875_c5646_774* is associated with the carbohydrate metabolic process in *Triticum aestivum*. By analyzing the homologous genes of these candidate genes, we

found the functions or biological processes of most homologous genes in other crops, such as *Japonica rice*, *Hordeum vulgare*, *Oryza sativa Indica*, *Oryza sativa Japonica*, *Arabidopsis thaliana*, etc., are involved in the defense response of fungi, which indicate that these candidate genes perhaps relate to the FHB resistance, but this needs further verification in wheat.

Analysis of marker haplotype and resistance

Among the alleles at the marker-trait associations, the alleles with decreasing diseased spikelet rate were assumed to be the resistance alleles at this site. In this study, five important SNP loci were selected to evaluate the effect of aggregation of their favorable alleles on decreasing diseased spikelet rate (Table 5). In general, with the increase in the number of favorable alleles, the effect of reducing the rate of diseased spikelets showed more obvious, which could improve the FHB resistance. By gene combination analysis (Table 6), two samples, B202 and B34, had four favorable alleles with TCTAC and TTTGC haplotypes, respectively. The haplotype TCTAC showed high resistant to FHB (Table 6). Six haplotypes with three favorable alleles were found. Of which, the materials with the haplotypes TCCGA and TTTAC showed high resistant to FHB, including B70, B72 and B179. The residue haplotypes showed moderate resistant or moderate susceptible to FHB. This result indicated that multiple haplotype of the materials played an important role in the screening of anti-scab materials. In addition, by comparing the reported GWAS results for plant height using the same panel materials (Chen et al. 2015), these five loci had no significant effect on plant height (Table S4, Table S5).

Discussion

The majority of earlier researchers focused on finding the significant genes/loci of FHB via QTL mapping or association mapping in order to improve the resistance of FHB in wheat cultivars (Loeffler et al. 2009; Li et al. 2011; Venske et al. 2019). The previous findings suggested that practically all of wheat's chromosomes were implicated (Yu et al. 2007; Ma et al. 2020), but the chromosomes 3B, 4B, 5A, and 6B seemed to be important because of the *Fhb1*, *Fhb2*, *Fhb4*, and *Fhb5* genes (Loeffler et al. 2009; Zhang et al. 2018). Five QTLs were discovered by GWAS analysis on chromosome arms 1AS, 2DL, 5AS, 5AL, and 7DS (Zhu et al. 2020). Of which, the locus on chromosome 5B for decreasing the amount of deoxynivalenol may be novel. However, in our study, fifteen chromosomes were involved in the MTA loci, and some important genomic regions involving FHB resistance were found on chromosomes 2A, 3B, 5B, 6A, and 7B. The significance of chromosome 3B for FHB resistance was further supported by this result. Six MTAs at 92 cM were also discovered on chromosome 7B in two different contexts. Of this, the BS00025286 51 locus could explain 11.20% of the phenotypic variation and five MTAs consistently associated with diseased spikelet rate and diseased rachis rate. It appeared that FHB resistance was relevant in this region. What's more intriguing is that five SNP markers (*Kukri_c14239_1995* on chromosome 6A, *Kukri_c7087_896* on chromosome 3B, *RAC875_c35801_905* on chromosome 3D, *BS00099729_51* on chromosome 5B, and *RAC875_c68525_284* on chromosome 6B) in this study have appeared in previous reports

(Venske et al. 2019), which proved the reliability of our results. There was one novel locus *D_contig74317_533* on chromosome 5D found for FHB resistance.

According to previous research, plant height had an impact on FHB resistance under field conditions. QTL mapping showed that approximately 40% of the QTL for plant height overlapped with the QTL for FHB resistance on 14 chromosomes (Buerstmayr et al. 2019). Five QTLs for FHB resistance were discovered by Zhu et al. (2020), among which *QFhb.hbaas-5AS* had a significant correlation with plant height. 38 MTAs loci for plant height were discovered on chromosomes 1B, 2A, 2B, 3A, 3B, 3D, 4A, 4B, 5A, and 6D by Chen et al. (2015) using a panel of 205 wheat accessions for the GWAS analysis of plant height. Of which, there were 11 loci detected on chromosome 6D in two or more environments (Chen et al. 2015). But in this study, there were five MTAs loci for FHB resistance showed no significant relationship with plant height, that is, *D_contig74317_533* on chromosome 5D, *Kukri_c14239_1995* on 6A, *RAC875_c35801_905* on 3D, *Kukri_c4143_1055* and *BS00025286_51* on 7B, which indicated these loci can be flexible use in breeding. Additionally, wheat materials with the aforementioned loci were screened, and by using molecular markers-assisted selection, they can be incorporated into Chinese major planted varieties without compromising plant height.

In fact, plant disease resistance is a complex molecular process controlled by genes (Ma et al. 2020). Only a few significant genes were found, despite the fact that researchers have discovered hundreds of QTLs scattered across wheat, including 21 chromosomes from common wheat varieties or related species (Buerstmayr et al. 2009; Liu et al. 2009). Nevertheless, it has gotten simpler to find additional genes as a result of the advancement of molecular technology and the wheat genome sequence (both major and minor). Moreover, the isolation and functional verification of FHB resistance genes are beneficial to understanding the pathogenesis and resistance mechanism of wheat FHB at the molecular level (Liu et al. 2016b). Previous research has demonstrated that the mechanism of the genes/loci identified in FHB resistance could involve a complicated signal transduction pathway and be associated with the synergistic effect of many protein factors (Zhang et al. 2018; Liu et al. 2016a; Dweba et al. 2017). For example, the genes encoding a 12-oxophytodienoate reductase-like protein identified in the region of *QFh.hbaas-1AS* may be related to the biosynthesis or metabolism of signaling molecules, oxylipins, such as jasmonic acid (JA) (Ding et al. 2011; Qi et al. 2016). These genes were discovered to encode several different proteins, including receptor-like kinase, UDP-glycosyltransferase, pathogenesis-related protein 1, and glucan endo-1,3-beta-glucosidase (PR2). (Anand et al. 2003; Pan et al. 2018; Ma et al. 2020).

In this study, the candidate genes on chromosome 3D encoded UDP-glycosyltransferase activity and were related to the defense response to biotic stimulus (Li et al. 2017). This indicated that this gene may enhance resistance to FHB because this protein could detoxify both DON and NIV produced by *F. graminearum* (Poppenberger et al. 2003; Zhu et al. 2020). By performing homologous gene detection on the *D_contig74317_533* locus of chromosome 5D, the genes were found to have homologous in nucleic acid binding and defense function in barley, *Arabidopsis*, Indica rice, Japonica rice, and wild rice. Of these, the *AT2G39510* gene is related to

the activity of glutamine transmembrane transporter protein. Previous studies have shown that the glutamine-gated ion channel is related to the function of the *Fhb5* gene, which can control Ca^{2+} influx (Dennison et al. 2000; Kugler et al. 2013). It was also found that Ca^{2+} was involved in early signaling defense to FHB (Ding et al. 2011). Recent research has revealed that wall-associated kinase (WAK) is a kind of receptor-like protein kinase, which is involved in signal transduction and the defense response of plants (Zhang et al. 2006; Hu et al. 2010). In this study, it was shown that the gene *Traescs6A02G013600* contains homologous genes in Arabidopsis and Japonica rice, some of which encoded members of the receptor-like cytoplasmic kinase (RLCK) and wall-associated kinase (WAK) families. Therefore, it is possible that this gene contributes to wheat FHB resistance, but more research is needed. Additionally, earlier research has demonstrated that the pathogenesis-related protein (PR) chitinase participates in the plant's fundamental defense mechanism and starts to accumulate during pathogen infection (Ma et al. 2020). Fortunately, the gene, *TraesCS7B02G370700* of *BS00025286_51* on the 7BL chromosome was also found to be associated with the chitinase activity and the defense response for fungi in our study. Meanwhile, the eight candidate genes identified were associated with either calcium ion binding or GTP binding, which has been shown to be involved in the early response of wheat to *F. graminearum* infection by salicylic acid (SA) and Ca^{2+} signals (Ding et al. 2011). Given that Ca^{2+} signal transduction was discovered to be crucial for the transcriptional reprogramming of innate plant immunity (Boudsocq et al. 2010), it is possible that these candidate genes associated with Ca^{2+} signals will be crucial in protecting against FHB in wheat.

Conclusions

In this study, sixty-six significant marker-trait associations (MTAs) were identified (P<0.001) on fifteen chromosomes with explaining phenotypic variation ranging from 5.4% to 11.2%. Some genomic regions involving FHB resistance were found on chromosomes 2A, 3B, 5B, 6A and 7B. There were eleven MTAs consistently associated with disease spikelet rate and disease rachis rate as pleiotropic effect locus. Eight new candidate genes of FHB resistance were predicated in wheat. Of which, three genes *TraesCS5D02G006700*, *TraesCS6A02G013600 and TraesCS7B02G370700* on chromosome 5DS, 6AS and 7BL, respectively were important to defend FHB by regulating intramolecular transferase activity, GTP binding, chitinase activity in wheat. In addition, a total of five favorable alleles associated with wheat scab resistance were discovered in this study. In the materials with multiple favorable alleles, the resistance was mostly moderately resistant or moderately susceptible.

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References

Anand A, Zhou T, Trick HN, Gill BS, Bockus WW, Muthukrishnan S (2003) Greenhouse and field testing of transgenic wheat plants stably expressing genes for thaumatin-like protein chitinase and glucanase against Fusarium graminearum. J Exp Bot 54(384): 1101-1111. https://doi.org/10.1093/jxb/erg110

Bai G, ShanerG (1994) Scab of Wheat: Prospects For Control. Plant Disease 78(8):760. https://doi.org/10.1094/PD-78-0760

Boudsocq M, Willmann MR, McCormack M, Lee H, Shan L, He P, Bush J, Cheng SH, Sheen J (2010) Differential innate immune signaling via $Ca2^{+}$ sensor protein kinases. Nature 464: 418-422. https://doi.org/10.1038/nature08794

Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: A review. Plant Breeding 128(1): 1-26. https://doi.org/10.1111/j.1439-0523.2008.01550.x

Buerstmayr H, Steiner B, Lemmens M, Ruckenbauer P (2000) Resistance to fusarium head blight in winter wheat: Heritability and trait associations. Crop Sci. 40(4):1012-1018.

Buerstmayr M, Buerstmayr H (2015) Comparative mapping of quantitative trait loci for Fusarium head blight resistance and anther retention in the winter wheat population Capo×Arina. Theor Appl Genet. 128: 1519-1530. https://doi.org/10.1007/s00122-015-2527-8

Buerstmayr M, Steiner B, and Buerstmayr H. (2019). Breeding for Fusarium head blight resistance in wheat — Progress and challenges. Plant Breed. https://doi.org/10.1111/pbr.12797

Cainong JC, Bockus WW, Feng YG, Chen PD, Qi LL, Sehgal SK, Danilova TV, Koo DH, Friebe B, Gill BS. (2015). Chromosome engineering, mapping, and transferring of resistance to Fusarium head blight disease from Elymus tsukushiensis into wheat. Theor. Appl. Genet. 128, 1019–1027. https://doi.org/10.1007/S00122-015-2485-1

Chen GF, Zhang H, Deng ZY, Wu RG, Li DM, Wang MY, Tian JC (2016) Genome-wide association study for kernel weight-related traits using SNPs in a Chinese winter wheat population. Euphytica 212:173–185. https://doi.org/10.1007/s10681-016-1750-y

Chen GF, Wu RG, Li DM, Yu HX, Deng ZY, Tian JC (2017) Genome-wide association study for seedling emergence and tiller number using SNP markers in an elite winter wheat population. J. Genet. 96:177–186. https://doi.org/10.1007/s12041-016-0731-1

Dennison KL (2000) Glutamate-Gated Calcium Fluxes in Arabidopsis. Plant Physiol 124(4): 1511-1514. https://doi.org/10.1104/pp.124.4.1511

Ding LM, Xu HB, Yi HY, Yang LM, Kong ZX, Zhang LX, Xue SL, Jia HY, Ma ZQ (2011) Resistance to hemi-biotrophic F graminearum infection is associated with coordinated and ordered expression of diverse defense signaling pathways. PLoS ONE 6: e19008. https://doi.org/10.1371/journal.pone.0019008

Dweba CC, Figlan S, Shimelis HA, Motaung TE, Sydenham S, Mwadzingeni L, Tsilo TJ (2017) Fusarium head blight of wheat: Pathogenesis and control strategies. Crop Prot 91: 114-122. https://doi.org/10.1016/j.cropro.2016.10.002

Ennouari, A., Sanchis, V., Marı´n, S., Rahouti, M. & Zinedine, A. (2013). Occurrence of deoxynivalenol in durum wheat from Morocco. Food Control, 32, 115–118.

Gadaleta A, Colasuonno P, Giove SL, Blanco A, Giancaspro A (2019) Map-based cloning of QFhbmgb-2A identifies a WAK2 gene responsible for Fusarium Head Blight resistance in wheat. Sci Rep 9(1):6929. https://doi.org/10.1038/s41598-019-43334-z

Gaire, R., Brown-Guedira, G., Dong, Y., Ohm, H., & Mohammadi, M. (2021). Genome-wide association studies for Fusarium head blight resistance and its trade-off with grain yield in soft red winter wheat. Plant disease, 105(9), 2435-2444.

Ghimire, B., Mergoum, M., Martinez‐Espinoza, A. D., Sapkota, S., Pradhan, S., Babar, M. A., ... & Buck, J. W. (2022). Genetics of Fusarium head blight resistance in soft red winter wheat using a genome‐wide association study. The Plant Genome, e20222.

Giancaspro A, Giove SL, Zito D, Blanco A, Gadaleta A (2016) Mapping QTLs for Fusarium Head Blight Resistance in an Interspecific Wheat Population. Front Plant Sci 7:1381. https://doi.org/10.3389/fpls.2016.01381

Guidance for industry and FDA. (2010). Advisory levels for Deoxynivalenol (DON) in finished wheat products for human consumption and grains and grain by-products used for animal feed. U.S. Department of Health and Human Services, Food and Drug Administration.

Guo, J., Zhang, X., Hou, Y., Cai, J., Shen, X., Zhou, T., ... & Kong, L. (2015). High-density mapping of the major FHB resistance gene Fhb7 derived from Thinopyrum ponticum and its pyramiding with Fhb1 by marker-assisted selection. Theoretical and Applied Genetics, 128, 2301-2316. https://doi.org/10.1007/s00122-015-2586-x

Hu LF, Lo YG (2010) Role of WAK gene family in signal rransduction and plant resistance. National Symposium on chemical ecology (in Chinese)

Hu, W., Gao, D., Wu, H., Liu, J., Zhang, C., Wang, J., ... & Lu, C. (2020). Genome-wide association mapping revealed syntenic loci QFhb-4AL and QFhb-5DL for Fusarium head blight resistance in common wheat (Triticum aestivum L.). BMC plant biology, 20(1), 1-13.

Jia HY, Zhou JY, Xue SL, Li GQ, Yan HS, Ran CF, Zhang YD, Shi JX, Jia L, Wang X, Luo J, Ma ZQ (2018). A journey to understand wheat Fusarium head blight resistance in the Chinese wheat landrace Wangshuibai. Crop Journal. 6, 48–59. doi:10.1016/J.CJ.2017.09.006

Kage U, Karre S, Kushalappa AC, McCartney C (2017) Identifcation and characterization of a Fusarium head blight resistance gene TaACT in wheat QTL-2DL. Plant Biotechnol J 15(4): 447-457. https://doi.org/10.1111/pbi.12641

Kage U, Yogendra K N, Kushalappa AC (2017) TaWRKY70 transcription factor in wheat QTL-2DL regulates downstream metabolite biosynthetic genes to resist Fusarium graminearum infection spread with in spike. Sci Rep 7:42596. https://doi.org/10.1038/srep42596

Kugler KG, Siegwart G, Nussbaumer T, Ametz C, Spannagl M, Steiner B, Lemmens M, Mayer KF, Buerstmayr H, Schweiger W (2013) Quantitative trait loci-dependent analysis of a gene co-expression network associated with Fusarium head blight resistance in bread wheat (Triticum aestivum L.). BMC Genom 14(1):728. https://doi.org/10.1186/1471-2164-14-728

Li G, Zhou J, Jia H, Gao Z, Fan M, Luo Y, Zhao P, Xue S, Li N, Yuan Y, Ma S, Kong Z, Jia L, An X, Jiang G, Liu W, Cao W, Zhang R,Fan J, Xu X, Liu Y, Kong Q, Zheng S, Wang Y, Qin B, Cao S, Ding Y, Shi J, Yan H, Wang X, Ran C, Ma Z (2019) Mutation of a histidine-rich

calcium-binding-protein gene in wheat confers resistance to Fusarium head blight. Nat Genet 51: 1106-1112. https://doi.org/10.1038/s41588-019-0426-7

Li T, Bai GH, Wu SY, Gu SL (2011) Quantitative trait loci for resistance to Fusarium head blight in a Chinese wheat landrace Haiyanzhong. Theor Appl Genet 122(8): 1497-1502. https://doi.org/10.1007/s00122-011-1549-0

Li X, Michlmayr H, Schweiger W, Malachova A, Shin S, Huang Y, Dong Y, Wiesenberger G, McCormick S, Lemmens M, Fruhmann P, Hametner C, Berthiller F, Adam G, Muehlbauer GJ (2017) A barley UDP-glucosyltransferase inactivates nivalenol and provides Fusarium head blight resistance in transgenic wheat. J Exp Bot 68: 2187-2197. https://doi.org/10.1093/jxb/erx109

Liu SY, Hall MD, Griffey CA, McKendry AL (2009) Meta-analysis of QTL associated with Fusarium head blight resistance in wheat. Crop Sci 49: 1955-1968. https://doi.org/10.2135/cropsci2009.03.0115

Liu YK, Tong HW, Zhu ZW, Chen L, Zou J, Zhang YQ, Gao CB (2016) Progress in Research on Mechanism of Resistance to Fusarium Head Blight in Wheat. Sci Agric Sin 49(08): 1476-1488 (in Chinese). https://doi.org/10.3864/j.issn.0578-1752.2016.08.005

Liu YK, Tong HW, Zhu ZW, Chen L, Zou J, Zhang YQ, Jiao CH, Gao CB (2016) Review on Improvement of Fusarium Head Blight Resistance in Wheat. J Triticeae Crops 36(1): 51-57 (in Chinese). https://doi.org/10.7606/j.issn.1009-1041.

Löffler M, Schön CC, Miedaner T (2009) Revealing the genetic architecture of FHB resistance in hexaploid wheat (Triticum aestivum L.) by QTL meta-analysis. Mol Breeding 23: 473-488. https://doi.org/10.1007/s11032-008-9250-y

Lu WZ, Cheng SH, Wang YZ (2001) Research on wheat Fusarium head blight Science Press:Beijing China (in Chinese)

Ma ZQ, Xie Q, Li GQ, Jia YH, Zhou JY, Kong ZX, Li N, Yuan Y (2020) Germplasms, genetics and genomics for better control of disastrous wheat Fusarium head blight. Theor Appl Genet 133: 1541-1568. https://doi.org/10.1007/s00122-019-03525-8

Mesterházy, A. (1995). Types and components of resistance to Fusarium head blight of wheat. Plant Breed. 114, 377-386. https://doi.org/10.1111/J.1439-0523.

Pan Y, Liu Z, Rocheleau H, Fauteux F, Wang Y, McCartney C, Ouellet T (2018) Transcriptome dynamics associated with resistance and susceptibility against Fusarium head blight in four wheat genotypes. BMC Genom 19: 642. https://doi.org/10.1186/s12864-018-5012-3

Poppenberger B, Berthiller F, Lucyshyn D, Sieberer T, Schuhmacher R, Krska R, Kuchler K, Glössl J, Luschnig C, Adam G (2003) Detoxifcation of the Fusarium mycotoxin deoxynivalenolby a UDP-glucosyltransferase from Arabidopsis thaliana. J Biol Chem 278(48): 47905-47914. https://doi.org/10.1074/jbc.m307552200

Qi LL, Pumphrey MO, Friebe B, Chen PD, Gill BS (2008). Molecular cytogenetic characterization of alien introgressions with gene Fhb3 for resistance to Fusarium head blight

511 disease of wheat. Theor. Appl. Genet. 117, 1155–1166. https://doi.org/10.1007/S00122-008-
512 0853-9
513 Qi P, Balcerzak M, Rocheleau H, Leung W, Wei Y, Zheng Y, Ouellet T (2016) Jasmonic
514 acid and abscisic acid play important roles in host-pathogen interaction between Fusarium
515 graminearum and wheat during the early stages of Fusarium head blight. Physiol Mol Plant P 93:
516 39-48. https://doi.org/10.1016/j.pmpp.2015.12.004
517 Rawat N, Pumphrey MO, Liu S, Zhang X, Tiwari VK, Ando K, Trick HN, Bockus WW,
518 Akhunov E, Anderson JA, Gill BS (2016) Wheat Fhb1 encodes a chimeric lectin with agglutinin
519 domains and apore-forming toxin-like domain conferring resistance to Fusarium head blight. Nat
520 Genet 48: 1576-1580. https://doi.org/10.1038/ng.3706
521 Schroeder, H. W., & Christensen, J. J. (1963). Factors affecting resistance of wheat to scab
522 caused by Gibberella zeae. Phytopathology, 53, 831–838.
523 Su Z, Bernardo A, Tian B, Chen H, Wang S, Ma H, Cai S, Liu D, Zhang D, Li T, Trick H,
524 Amand P, Yu J, Zhang Z, Bai G (2019) A deletion mutation in TaHRC confers Fhb1 resistance
525 to Fusarium head blight in wheat. Nat Genet 51: 1099-1105. https://doi.org/10.1038/s41588-019-
526 0425-8
527 Tessmann, E. W., Dong, Y., & Van Sanford, D. A. (2019). GWAS for Fusarium head blight
528 traits in a soft red winter wheat mapping panel. Crop Science, 59(5), 1823-1837.
529 Thambugala D, Brûlé-Babel AL, Blackwell BA, Fedak G, Foster AJ, MacEachern D,
530 Gilbert J, Henriquez MA, Martin RA, McCallum BD, Spaner D, Iqbal M, Pozniak CJ, N'Diaye
531 A, McCartney CA (2020) Genetic analyses of native Fusarium head blight resistance in two
532 spring wheat populations identifies QTL near the B1, Ppd-D1, Rht-1, Vrn-1, Fhb1, Fhb2 and
533 Fhb5 loci. Theor Appl Genet 133: 2775-2796. https://doi.org/10.1007/s00122-020-03631-y
534 Venske E, dos Santos RS, Farias DdR, Rother V, da Maia LC, Pegoraro C, Costa de
535 Oliveira (2019) A Meta-analysis of the QTLome of Fusarium head blight resistance in bread
536 wheat: refning the current puzzle. Front Plant Sci 10:727.
537 https://doi.org/10.3389/fpls.2019.00727
538 Verges, V. L., Brown-Guedira, G. L., & Van Sanford, D. A. (2021). Genome-wide
539 association studies combined with genomic selection as a tool to increase Fusarium head blight
540 resistance in wheat. Crop Breeding, Genetics and Genomics, 3(4).
541 Wang H, Sun S, Ge W, Zhao L, Hou B, Wang K, Lyu Z, Chen L, Xu S, Guo J, Li M, Su P,
542 Li X,Wang G, Bo C, Fang X, Zhuang W, Cheng X, Wu J, Dong L, Chen W, Li W, Xiao G, Zhao
543 J, Hao Y, Xu Y, Gao Y, Liu W, Liu Y, Yin H, Li J, Li X, Zhao Y, Wang X, Ni F, Ma X, Li A,
544 Xu S, Bai G, Nevo E, Gao C, Ohm H, Kong L (2020) Horizontal gene transfer of Fhb7 from
545 fungus underlies Fusarium head blight resistance in wheat. Science 368(6493): eaba5435.
546 https://doi.org/10.1126/science.aba5435
547 Wu, L., Zhang, Y., He, Y., Jiang, P., Zhang, X., & Ma, H. (2019). Genome-wide
548 association mapping of resistance to Fusarium head blight spread and deoxynivalenol
549 accumulation in Chinese elite wheat germplasm. Phytopathology, 109(7), 1208-1216.

Xue SL, Li GQ, Jia HY, Xu F, Lin F, Tang MZ, Wang Y, An X, Xu HB, Zhang LX, Kong ZX, Ma ZQ. (2010) Fine mapping Fhb4, a major QTL conditioning resistance to Fusarium infection in bread wheat (Triticum aestivum L.). Theor. Appl. Genet. 121, 147–156. https://doi.org/10.1007/S00122-010-1298-5

Xue SL, Xu F, Tang MZ, Zhou Y, Li GQ, An X, Lin F, Xu HB, Jia HY, Zhang LX, Kong ZX, Ma ZQ. (2011) Precise mapping Fhb5, a major QTL conditioning resistance to Fusarium infection in bread wheat (Triticum aestivum L.). Theor. Appl. Genet. 123(6):1055-1063. https://doi.org/1055-1063. 10.1007/s00122-011-1647-z

Yu JB (2007) Identification of new sources and mapping of QTL for FHB resistance in Asian wheat germplasm. Dissertation, Kansas State University

Zhang AM, Yang WL, Li X, Sun JZ (2018) Current status and perspective on research against Fusarium head blight in wheat. Hereditas 040(010):858-873 (in Chinese). https://doi.org/10.16288/j.yczz.18-252

Zhang SQ, Sun Y, Guo Y, Sun DY (2006) Cell wall linked receptor like kinase(WAK): plant cell wall and important cytoplasmic proteins. Chinese Sci Bull (01):1 (in Chinese). https://doi.org/10.1360/csb2006-51-1-1

Zhao Y, Wang D, Ji M, Tian J, Ding H, Deng Z (2023) Transcriptome Dynamic Analysis Reveals New Candidate Genes Associated with Resistance to Fusarium Head Blight in Two Chinese Contrasting Wheat Genotypes. International Journal of Molecular Sciences. 24(4):4222. https://doi.org/10.3390/ijms24044222

Zhu Z, Chen L, Zhang W, Yang L, Zhu W, Li J, Liu Y, Tong H, Fu L, Liu J, Rasheed A, Xia X, He Z, Hao Y, Gao C (2020) Genome-Wide Association Analysis of Fusarium Head Blight Resistance in Chinese Elite Wheat Lines. Front Plant Sci 27(11):206. https://doi.org/10.3389/fpls.2020.00206

Zhu ZW, Xu DA, Cheng SH, Gao CB, Xia XC, Hao YF, He ZH (2018) Characterization of Fusarium head blight resistance gene Fhb1 and its putative ancestor in Chinese wheat germplasm. Acta Agron Sin 44(4): 473-482 (in Chinese). https://doi.org/10.3724/SP.J.1006.2018.00473

Figure 1

Whole genome association analysis QQ map (left) and Manhattan plot of disease spikelet rate (right).

E1: the experimental field of Shandong Agricultural University in 2017; E2: the greenhouse of Shandong Agricultural University in 2017; E3: the experimental field of Shandong Agricultural University in 2016. 1-21： 1A, 1B, 1D, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 4B, 4D, 5A, 5B, 5D, 6A, 6B, 6D, 7A, 7B, 7D.

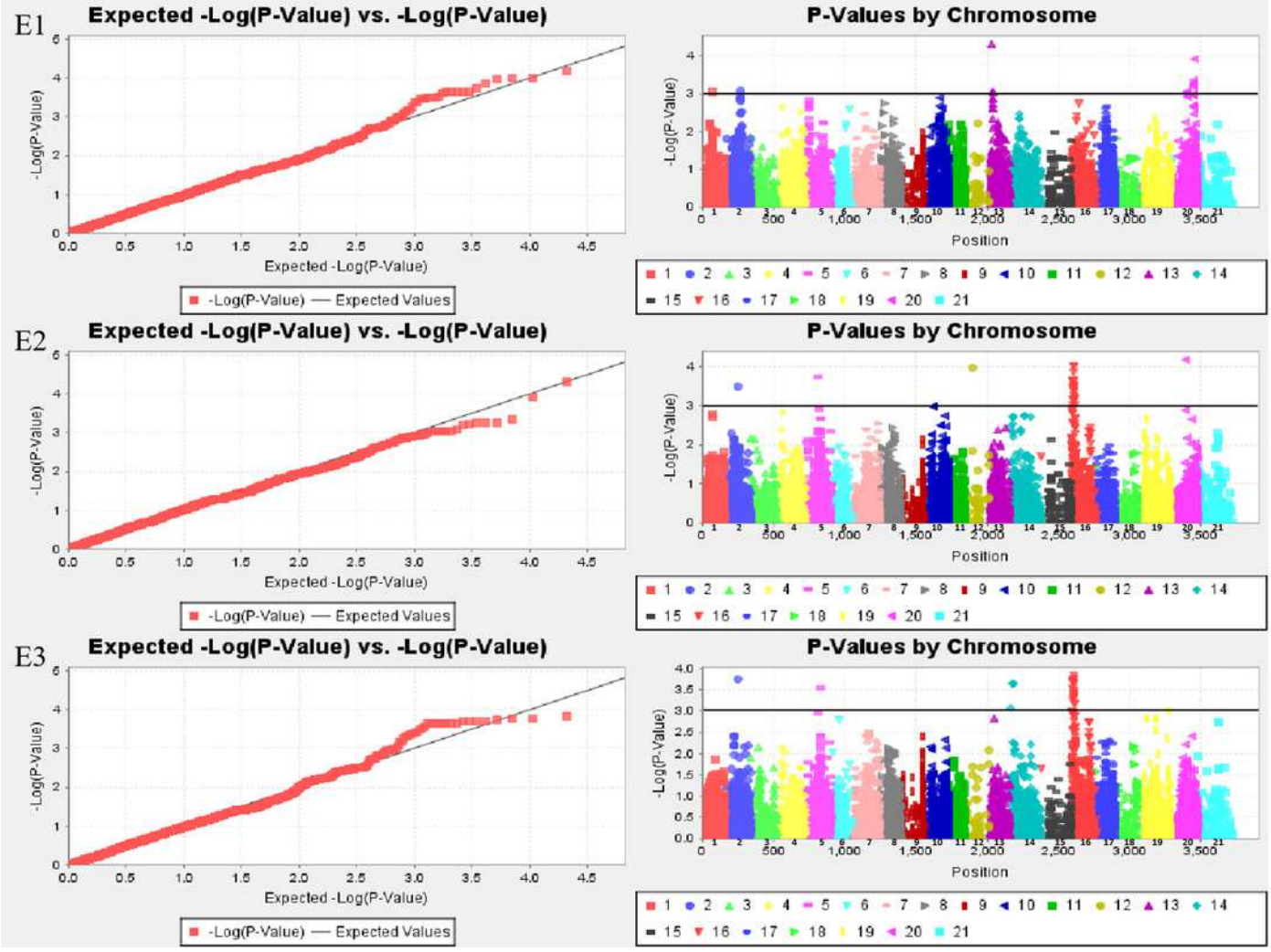

Table 1(on next page)

Phenotypic variation of wheat diseased spikelets rate

1 **Table 1** Phenotypic variation of wheat diseased spikelets rate

Environment	Change	Mean	Standard deviation	Coefficient of variation
E1	0.0611~1	0.7721	0.28	36.55%
E2	0.0477~1	0.6388	0.34	52.96%
E3	0.0111~1	0.7494	0.25	44.30%

2 [a] **E1:** the experimental field of Shandong Agricultural University in 2017; **E2:** the greenhouse of Shandong Agricultural

3 University in 2017; **E3:** the experimental field of Shandong Agricultural University in 2016.

4

Table 2(on next page)

ANOVA of wheat diseased spikelet and spike rachis rate in different environments

* indicated significant at the 0.05 level (2-tailed).

1 **Table 2** ANOVA of wheat diseased spikelet and spike rachis rate in different environments

Source	Dependent variable	Type III Sum of Squares	Degree of freedom	Mean Square	*F*-value
Varieties	Spikelet	94.062	204	0.461	16.421*
	Spike rachis	88.842	204	0.435	19.698*
Environment	Spikelet	5.262	2	2.631	93.709*
	Spike rachis	5.505	2	2.752	124.493*
Varieties * Environment	Spikelet	85.307	406	0.21	7.483*
	Spike rachis	83.735	406	0.206	9.329*
Error	Spikelet	34.425	1226	0.028	
	Spike rachis	27.105	1226	0.022	
Total	Spikelet	1128.175	1839		
	Spike rachis	1182.519	1839		

2 * indicated significant at the 0.05 level (2-tailed).

3

Table 3(on next page)

The correlation coefficients of spikelet and spike rachis in three environments, respectively

[a] E1, E2 and E3 were the same as the Table 1. ** Correlation is significant at the 0.001 level(2-tailed); * Correlation is significant at the 0.05 level(2-tailed).

1 **Table 3** The correlation coefficients of spikelet and spike rachis in three environments, respectively

Environment[a]		E1		E2		E3	
	Variable	Spikelet	Spike rachis	Spikelet	Spike rachis	Spikelet	Spike rachis
E1	Spikelet	1					
	Spike rachis	0.881**	1				
E2	Spikelet	0.318**	0.203**	1			
	Spike rachis	0.355**	0.239**	0.902**	1		
E3	Spikelet	0.263**	0.205**	0.224**	0.142**	1	
	Spike rachis	0.233**	0.202*	0.205**	0.118*	0.986**	1

2 [a] E1, E2 and E3 were the same as the Table 1. ** Correlation is significant at the 0.001 level(2-tailed); * Correlation is

3 significant at the 0.05 level(2-tailed).

4

Table 4(on next page)

Phenotypic effect of alleles for the relatively stable loci of disease spikelet rate

Table 4 Phenotypic effect of alleles for the relatively stable loci of disease spikelet rate

Locus	Chromosome	Allele	Variety number	Environment[a]			Average	Difference[b]
				E1	E2	E3		
D_contig74317_533	5D	CC	177	0.7829	0.7585	0.764	0.7685Aa	0.0989
		TT	28	0.6983	0.6519	0.6586	0.6696Bb	
Kukri_c14239_1995	6A	TT	192	0.795	0.754	0.762	0.7703Aa	0.2297
		CC	11	0.4593	0.619	0.5435	0.5406Bb	
BS00025286_51	7B	CC	125	0.8001	0.7584	0.7484	0.7689a	0.096
		TT	52	0.6529	0.6632	0.7025	0.6729b	
Kukri_c7087_896	3B	GG	14	0.8014	0.8043	0.7106	0.7721a	0.0185
		AA	191	0.7691	0.7393	0.7524	0.7536a	
RAC875_c35801_905	3D	AA	186	0.7841	0.7521	0.7547	0.7636Aa	0.094
		GG	19	0.6463	0.6635	0.699	0.6696Bb	
RAC875_c68525_284	6B	AA	22	0.7774	0.7489	0.7525	0.7596a	0.0437
		GG	183	0.7209	0.7018	0.725	0.7159b	
Kukri_c4143_1055	7B	AA	104	0.7907	0.7624	0.7661	0.7731a	0.0379
		CC	101	0.7505	0.7244	0.7306	0.7352b	
RAC875_c18043_369	7B	CC	104	0.7915	0.763	0.768	0.7741a	0.0402
		TT	100	0.7502	0.7229	0.7287	0.7339b	
RAC875_c18043_411	7B	AA	105	0.7914	0.764	0.7695	0.7749a	0.041
		GG	100	0.7502	0.723	0.7287	0.7339b	
RAC875_c5646_774	7B	GG	104	0.7915	0.763	0.768	0.7742a	0.0391
		AA	101	0.7505	0.7244	0.7306	0.7351b	

[a] E1, E2 and E3 were same as Table 1. [b] Difference between alleles. **A and B:** Different capital letters indicate significant difference between alleles at one locus at $P \leqslant 0.01$; **a & b:** Different lowercase letters indicate significant difference between alleles at one locus at $P \leqslant 0.05$

Table 5(on next page)

Disease resistance statistics of different resistant QTL combinations

“+” represents the allele for improving scab resistance; **“-”** represents the allele that reduces the resistance.

Table 5 Disease resistance statistics of different resistant QTL combinations

	D_contig74317_533	*Kukri_c14239_1995*	*BS00025286_51*	*RAC875_c35801_905*	*Kukri_c4143_1055*	Variety number	Mean DSR	Effect of resistance genes	Standard deviation	*F*-value	*P*-value
Genotype	-	-	-	-	-	38	0.848	.	0.148	2.551	0.001**
	+	-	-	-	-	9	0.866	0.022	0.081		
	-	+	-	-	-	3	0.815	-0.038	0.088		
	-	-	+	-	-	28	0.713	-0.159	0.237		
	-	-	-	+	-	2	0.922	0.087	0.047		
	-	-	-	-	+	62	0.777	-0.084	0.230		
	+	-	+	-	-	1	0.725	-0.145	.		
	+	-	-	-	+	1	0.330	-0.611	.		
	-	-	+	+	-	2	0.905	0.068	0.089		
	-	-	+	-	+	10	0.684	-0.193	0.343		
	-	-	-	+	+	2	0.892	0.052	0.051		
	+	+	-	+	-	2	0.186	-0.781	0.017		
	+	+	-	-	+	1	0.726	-0.144	.		
	+	-	+	-	+	2	0.498	-0.413	0.491		
	+	-	-	+	+	3	0.609	-0.281	0.304		
	-	+	+	+	-	2	0.617	-0.272	0.068		
	-	+	+	-	+	1	0.844	-0.005	.		
	-	-	+	+	+	3	0.593	-0.300	0.270		
	+	+	+	-	+	1	0.210	-0.752	.		
	+	-	+	+	+	1	0.40	-0.524	.		

"+" represents the allele for improving scab resistance; **"-"** represents the allele that reduces the resistance.

Table 6(on next page)

Haplotype of marker associated with FHB resistance and corresponding carrier materials

1 **Table 6** Haplotype of marker associated with FHB resistance and corresponding carrier materials

D_contig74317_533	*Kukri_c14239_1995*	*BS00025286_51*	*RAC875_c35801_905*	*Kukri_c4143_1055*	Number of resistance alleles	Variety	Disease spikelet rate	FHB resistance
T	C	C	G	A	3	B70	0.1979	HR
						B72	0.1739	HR
T	C	C	A	C	3	B97	0.7256	MS
T	T	T	A	C	3	B179	0.1501	HR
T	T	C	G	C	3	B131	0.3217	MR
						B200	0.5782	MS
C	C	T	G	A	3	B44	0.6654	MS
						B196	0.5691	MS
C	T	T	G	C	3	B16	0.3519	MR
						B68	0.5425	MS
T	C	T	A	C	4	B202	0.2100	HR
T	T	T	G	C	4	B34	0.4037	MS

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