# 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 some *Fusarium* species and leads to cases of severe yield reduction and seed contamination. Identifying resistance genes/QTLs from wheat germplasm may help to improve FHB resistance in wheat production. **Methods.** Our study evaluated 205 elite winter wheat cultivars for FHB resistance. A high-density 90K SNP array was used for genotyping the panel. A genome-wide association study (GWAS) from cultivars from three different environments was performed using a mixed linear model (MLM). **Results.** Sixty-six significant marker-trait associations (MTAs) were identified (P<0.001) on fifteen chromosomes that explained the 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. Six MTAs at 92 cM on chromosome 7B were found in cultivars from two different 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 in this study. 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, or chitinase activity in wheat, but further validation in needed. In addition, a total of five favorable alleles associated with wheat FHB 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

20  **Background.** *Fusarium* head blight (FHB) is a disease affecting wheat spikes caused by some
21  *Fusarium* species and leads to cases of severe yield reduction and seed contamination.
22  Identifying resistance genes/QTLs from wheat germplasm may help to improve FHB resistance
23  in wheat production.
24  **Methods.** Our study evaluated 205 elite winter wheat cultivars for FHB resistance. A high-
25  density 90K SNP array was used for genotyping the panel. A genome-wide association study
26  (GWAS) from cultivars from three different environments was performed using a mixed linear
27  model (MLM).
28  **Results.** Sixty-six significant marker-trait associations (MTAs) were identified ($P<0.001$) on
29  fifteen chromosomes that explained the phenotypic variation ranging from 5.4 to 11.2%. Some
30  important new MTAs in genomic regions involving FHB resistance were found on chromosomes
31  2A, 3B, 5B, 6A, and 7B. Six MTAs at 92 cM on chromosome 7B were found in cultivars from
32  two different environments. Moreover, there were 11 MTAs consistently associated with
33  diseased spikelet rate and diseased rachis rate as pleiotropic effect loci and *D_contig74317_533*
34  on chromosome 5D was novel for FHB resistance. Eight new candidate genes of FHB resistance
35  were predicated in wheat in this study. Three candidate genes, *TraesCS5D02G006700*,
36  *TraesCS6A02G013600*, and *TraesCS7B02G370700* on chromosome 5DS, 6AS, and 7BL,
37  respectively, were perhaps important in defending against FHB by regulating intramolecular
38  transferase activity, GTP binding, or chitinase activity in wheat, but further validation in needed.
39  In addition, a total of five favorable alleles associated with wheat FHB resistance were

40    discovered. These results provide important genes/loci for enhancing FHB resistance in wheat
41    breeding by marker-assisted selection.
42    **Keywords**
43    Wheat; Fusarium head blight; GWAS; SNP; MTA.
44

## Introduction

46         Wheat (*Triticum aestivum* L.), one of the three major food crops, is grown worldwide as an
47    essential food source and fodder. Therefore, maintaining consistent wheat production has
48    become a frequent focus of agricultural experts worldwide. Wheat is susceptible to both biotic
49    (diseases, insect pests, etc.) and abiotic (drought, freezing damage, etc.) stresses because of its
50    long growing phase. *Fusarium* head blight (FHB), also known as scab, is an infection of wheat
51    spikes mainly caused by Fusarium species. FHB is a quantitative trait controlled by
52    multiple genes that are affected by both the environment and genetics (Buerstmayr et al. 2015;
53    Liu et al. 2016b; Bai et al. 1994). This disease has become an important disease in the Yellow
54    and Huai River Valleys of China (Zhu et al. 2018), and seriously threatens wheat production and
55    processing. This disease not only causes severe wheat yield reduction but also contaminates
56    wheat seeds with deoxynivalenol (DON) toxins (Bai et al. 1994).  DON, also known as
57    vomitoxin, is regarded as a teratogen, neurotoxin and immunosuppressant, that can cause adverse
58    health effects (Ennouari et al., 2013). To control human exposure to DON, the Food and Drug
59    Administration (FDA, 2010) has set an advisory limit of 1 mg/kg for finished wheat products. In
60    addition to negatively affecting wheat production in the middle and lower Yangtze River Valley
61    region of China, FHB has become more common during the past 20 years in the Yellow and
62    Huai River Valley regions as a result of climatic and tillage system changes. Moreover, it has
63    become the most destructive spike disease in the world because no completely resistant varieties
64    have been found thus far, which seriously threatens food production and food security. Breeding
65    tolerant or fully resistant varieties and discovering resistance genes are the most effective ways
66    to solve the problem of FHB.
67         The interactions between genotype and environment have a substantial impact on FHB, a
68    complex trait with a quantitative nature. Previous research has demonstrated that FHB resistance
69    is influenced by plant height, heading date, blooming period, extrusion, etc. The weather (sunny
70    or wet) during blossoming is crucial for the development of this illness. Genetic linkage analysis
71    has been used to study FHB resistance extensively in wheat, and numerous QTLs (quantitative
72    trait loci)  (more than 400 scattered on 21 chromosomes) related to FHB resistance have been
73    reported (Ma et al. 2020). Five categories of FHB resistance currently exist: type I resistance to
74    initial spike infection, type II resistance to spread spike infection, type III resistance to
75    accumulation of mycotoxins, type IV resistance to kernel infection, and type V resistance to
76    yield reduction (Mesterházy 1995). Type I resistance and type II resistance were distinguished in
77    the seminal study by Schroeder and Christensen (1963). Almost all reports on FHB resistance
78    have been type II. Recently, numerous small-effect Type II FHB QTLs were reviewed by
79    Buerstmayr et al. (2019). Seven genes (*Fhb1* to *Fhb7*) for FHB resistance have been found, and

80  *Fhb1*, *Fhb2*, *Fhb4*, and *Fhb5* are on chromosomes 3BS, 6BS, 4BL, and 5A, respectively in
81  common wheat; however, the remaining genes, *Fhb3*, *Fhb6*, and *Fhb7*, were derived from
82  related wheat species (Cainong et al., 2015; Jia et al., 2018; Li et al., 2019; Qi et al., 2008; Su et
83  al., 2019; Wang et al., 2020; Xue et al., 2010; Xue et al., 2011). The *Fhb1* gene has been widely
84  dissected and sequenced to discover a pore-forming toxin-like (*PFT*) gene that is responsible for
85  FHB resistance (Rawat et al. 2016). Later, another new gene was discovered for *Fhb1*, encoding
86  a putative histidine-rich calcium-binding protein (*His* or *TaHRC*) that was adjacent to *PFT* (Li et
87  al. 2019; Su et al. 2019). This research has led to the development of *Fhb1* function markers that
88  are being employed in molecular breeding to improve scab resistance. However, it appears that
89  the mechanisms by which *His* and *TaHRC* impart resistance are distinct. Therefore, more
90  research on this gene is still required to understand its molecular mechanisms (Li et al. 2019; Su
91  et al. 2019). Recently the candidate gene for *Fhb7* was determined and cloned, which revealed
92  that it encoded a glutathione (GST), Which can detoxify trichothecene toxins (Wang et al. 2020).
93  Its resistance depends on a reduction in pathogen growth in spikes, which is different from the
94  resistance of *Fhb1*. The remaining five FHB genes, however, have not yet been cloned.
95      Some significant loci for resistance have been discovered in addition to these seven FHB
96  genes. For instance, *QFhb.mgb-2A* was identified as a *WAK2* gene (Giancaspro et al. 2016), and
97  the function of *WAK2* in FHB resistance was validated (Gadaleta et al. 2019). Another important
98  locus on chromosome 2DL was the transcription factor *TaWRKY70*, which regulates the
99  expression of metabolite biosynthetic genes including *TaACT*, *TaDGK*, and *TaGLI* to influence
100 FHB resistance (Kage et al. 2017a, b). Using two recombinant inbred line (RIL) populations with
101 one common parent, named AC Barrie, from Canadian spring wheat, *QFhb.mcb-3B*, *QFhb.mcb-*
102 *6B*, and *QFhb.mcb-5A.1* were mapped to the expected locations of *Fhb1*, *Fhb2*, and *Fhb5*,
103 respectively (Thambugala et al. 2020). On chromosome 5B, the prominent resistance gene,
104 *QFhb.mbr-5B* was found to explain up to 36% of the phenotypic variation (Thambugala et al.
105 2020).
106     With the development of genomics, growing research has been conducted on the use of
107 genome-wide association studies (GWASs) to analyze wheat FHB resistance. One study (Zhu et
108 al. 2020) used a mixed linear model (MLM) to consistently identify five quantitative trait loci
109 (QTL) related to FHB on chromosome arms 1AS, 2DL, 5AS, 5AL, and 7DS. These QTLs
110 accounted for 5.6, 10.3, 5.7, 5.4, and 5.6% of the variation in phenotype, respectively. Tessmann
111 et al. (2019) used GWAS (based on 2-yr entry means) to identify 16 significant ($p < 0.001$)
112 single nucleotide polymorphisms (SNPs) associated with disease traits on multiple
113 chromosomes. Single nucleotide polymorphism association ranged from −2.14 to 4.01% of the
114 mean of a given trait. Another GWAS identified 26 loci (88 marker–trait associations), which
115 explained 6.65–14.18% of the phenotypic variances. The associated loci were distributed across
116 all chromosomes except 2D, 6A, 6D and 7D (Hu et al. 2020). In addition, numerous recent
117 studies have used GWAS in FHB resistance (Verges et al. 2021; Gaire et al. 2021; Ghimire et al.
118 2022; Wu et al. 2019). It appears that using GWAS to investigate wheat FHB is a very promising
119 endeavor.

120    Therefore, our study evaluated 205 elite winter wheat accessions for FHB resistance. A
121    high-density 90K SNP array was used to genotype the panel. A genome-wide association study
122    (GWAS) in three environments was performed using a mixed linear model (MLM). The
123    objective of this study was to identify some novel genomic regions associated with the type II
124    resistance of wheat in different environments, and to predict candidate genes for loci associated
125    with these traits, which could improve wheat FHB resistance by molecular breeding in the future.
126

## Materials & Methods

### Plant materials

129    The association mapping panel of 205 wheat accessions for GWAS comprised 77 released
130    cultivars, 55 founder parents including 2 lines from Mexico and France, and 73 breeding lines
131    from 10 provinces that represent the major winter wheat production regions in China (Chen et al.
132    2016).
133    Sumai 3 was selected as an FHB highly resistant check, Yangmai158 as a moderately
134    resistant check, and Ningmaizi 22 as a moderately susceptible check.

### Growth conditions

136    The materials were grown in the field and greenhouse of Shandong Agricultural University
137    (117°16′ E, 36°17′ N) during the 2015–2016 and 2016–2017 cropping seasons, hereafter referred
138    to as 2016 and 2017, respectively. The terms E1, E2, and E3 represent the experimental field of
139    Shandong Agricultural University in 2017, the greenhouse of Shandong Agricultural University
140    in 2017, and the experimental field of Shandong Agricultural University in 2016, respectively. In
141    the field, a randomized block design was used, with two replications. All lines were planted in 2
142    m plots with three rows uniformly spaced at 25 cm intervals. Each row contained 70 seeds
143    evenly distributed seeds. The local recommended field crop management practices were
144    followed and no pests or diseases were found in the field. However in the greenhouse, the seeds
145    of materials were germinated and vernalized for an additional 4 weeks (4 °C, 12 h light/dark
146    regime) before being transferred to the greenhouse. Plants were potted in a mixture of compost,
147    sand chalk and common soil. Each plant was planted in an individual pot (the diameter was 30
148    cm, and the depth was 25 cm, with four seedlings) and in three replications (pots). The
149    temperatures of the greenhouse were gradually increased from 15°C/13°C during day/night to
150    20°C/18°C, with a 16 h/day photoperiod at the time of anthesis. The growing conditions in the
151    greenhouse have been described in our previous article (Zhao et al. 2023).

### Inoculum preparation and inoculation

153    In this study, the conidiospore suspension of 7136, F301, F609, and F15 virulent strains of
154    *F. graminearum*, was obtained with the courtesy of Nanjing Agricultural University. The
155    pathogen was propagated in mung bean medium (Buerstmayr et al. 2000) and incubated on a
156    shaker at 150 rpm under 25 °C for 4–5 days. After culturing and filtering, the mass of conidia
157    was examined under a microscope, and then, the four pathogen strains were mixed equally and
158    stored at 4 °C for later use. The preparation of *F. graminearum* was also the same as in our
159    previous study (Zhao et al. 2023).

160    Wheat was inoculated with 10 μl of the *F. graminearum* conidia suspension (50,000
161    spores/mL) applied to a pair of florets in the middle of the spikeduring flowering (Guo et al.
162    2015). Ten spikes were inoculated per line from each replicate. The whole wheat spike was then
163    covered with a self-sealing bag to retain moisture, and had the self-sealing bag was removed
164    after 3 days. The disease symptoms were investigated on Day 21 after inoculation, and the
165    diseased spikelet rate (DSR), diseased spike rachis rate, and disease index (DI) were calculated.
166    Both the field nursery and greenhouse experiments followed this method. All spikes were
167    classified into five classes of disease severity according to the diseased spikelet rate (DSR): 0%
168    (class 0), 1–25% (class 1), 26–50% (class 2), 51–75% (class 3), and 76–100% (class 4) (Lu et al.
169    2001). The disease index (DI) was calculated based on the rules for monitoring and forecasting
170    wheat head blight (Chinese Standard: GB/T 15796-2011).

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

171

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

172

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

173
174    where i is the class of disease severity, hi is the number of wheat spikes in each class, and H
175    is the number of all investigated wheat spikes.
176    The standard of wheat FHB resistance was as follows: Immune (DI=0), High resistant (DI<
177    Sumai 3), Moderately resistant (Sumai 3< DI< Yangmai 158), Moderately susceptible (Yangmai
178    158< DI< Ningmaizi 22), High susceptible (DI> Ningmaizi 22).
179    **Genome-wide association analysis**
180    The genome-wide positions of SNPs were used in this study based on a consensus genetic
181    map of wheat (Wang et al. 2014). SNP markers, genotyping, and the population structure of the
182    samples have been previously reported (Chen et al. 2015; Chen et al. 2016; Chen et al. 2017). A
183    total of 24,355 mapped SNPs were used for MTA analysis. According to Chen et al (2017), the
184    association population was divided into four categories using STRUCTURE's maximum
185    membership probability (comprised of 43, 32, 105 and 25 varieties). Chen et al. (2016) also
186    reported LD values for different chromosomes.
187    Based on this information, significant marker–trait associations (MTAs) were identified
188    using a mixed linear model (MLM) in TASSEL3.0. The P value was used to determine whether a
189    QTL was associated with a marker, while the phenotypic variation explained (PVE) was used to
190    evaluate the magnitude of the MTA effects. SNPs with $P \leq 10^{-3}$ were considered to be
191    significantly associated with phenotypic traits, and SNPs with $P \leq 10^{-4}$ were considered to be
192    extremely significantly associated with phenotypic traits. Furthermore, when the marker was
193    detected in two or more environments at the same time, it was considered a stable MTA.
194    **Statistical analysis**
195    Analysis of variance (ANOVA) and correlations among phenotypic traits were carried out
196    using the statistical software SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

197 **Forecasting candidate genes for FHB resistance**
198     A BLAST (Basic Local Alignment Search Tool) search was performed on the International
199 Wheat Genome Sequencing Consortium database (RefSeq v1.0; https://urgi.versailles.inra.fr/blast/)
200 using the sequence of the significant SNP markers identified by GWAS. When an SNP marker
201 sequence from the IWGSC was 100% identical to any wheat contig, the sequence was extended
202 by 2 Mb for each marker using the IWGSC BLAST results. Afterward, the extended sequence
203 was used to run a BLAST search on the National Center for Biotechnology Information (NCBI)
204 database (http: //www.ncbi.nlm.nih.gov) and on Ensembl Plants
205 (http://plants.ensembl.org/Triticum_aestivum/Tools/Blast) to confirm possible candidate genes
206 and functions.
207 **Analysis of marker haplotype**
208     The effect of resistance genes was calculated by using the average diseased spikelet rate of
209 various gene combinations. Effect of resistance genes=(Average diseased spikelet rate of
210 materials carrying resistance gene - Average diseased spikelet rate of materials without
211 resistance gene)/Average diseased spikelet rate of materials without resistance gene.
212

213 # Results
214 **Phenotypic variation analysis of wheat FHB resistance**
215     The variation coefficient of the diseased wheat spikelet rate (DSR) was the highest in E2
216 (52.96%), followed by that in E3 (44.30%), and E1 (36.55%) (Table 1), indicating that DSR
217 genetic variation was abundant. The variance analysis of FHB resistance of the spikelet and
218 spike rachis indicated that significant differences were present between cultivars and
219 environments, and their interactions (Table 2). This illustrated that FHB resistance was a
220 quantitative trait affected not only by genotype but also by the environment. Furthermore, there
221 were significant positive correlation coefficients between the spikelet and spike rachis in the
222 three environments, indicating that the resistance trend of FHB was consistent between the
223 spikelet and spike rachis (Table 3).
224 **Marker–trait associations (MTAs) of FHB resistance**
225     Sixty-six MTAs associated with FHB resistance were distributed on chromosomes 1A, 1B,
226 2A, 2B, 2D, 3B, 3D, 4A, 5A, 5B, 5D, 6A, 6B, 7A, and 7B (Table S1; Table S2; Figure 1). The
227 phenotypic variation explained (PVE) of MTA loci to FHB resistance ranged from 5.45% to
228 11.20%, of which, 11 MTA loci were detected in both the spikelet and spike rachis. On
229 chromosome 7B, a novel genomic region from genetic position 92 to 103, significantly
230 associated with FHB resistance, was detected in all three environments. Moreover, there was one
231 major locus at genetic position 92 of chromosome 7B accounting for 11.20% of the phenotypic
232 variation in the spikelets, namely locus *BS00025286_51*, which was also detected for the spike
233 rachis, explaining 7.07% of its phenotypic variation. In E3, four loci on chromosome 7B were
234 found to be associated with both diseased spikelet rate and diseased spike rachis rate, but these
235 MTAs are located in the same region and may represent one QTL (Table 4; Table S2). In
236 addition, there were some genomic regions associated with FHB resistance on chromosomes 5B,

237   6A, 2A, and 3B, but they were found only in a single environment. The other six loci, including
238   *D_contig74317_533* on chromosome 5D, *Kukri_c14239_1995* on chromosome 6A,
239   *Kukri_c7087_896* on chromosome 3B, *RAC875_c35801_905* on chromosome 3D,
240   *BS00099729_51* on chromosome 5B, and *RAC875_c68525_284* on chromosome 6B, were also
241   determined to be associated with both the diseased spikelet rate and the diseased spike rachis
242   rate. The remaining MTA loci were detected only for a single trait in a single environment.
243   **Allelic variation analysis of MTA loci**
244       According to the PVE of MTA loci (Table S2), we selected 10 of markers and analyzed
245   their allelic variation (Table 4).. Alleles T and C of the marker, *Kukri_c14239_1995* on
246   chromosome 6A were associated with the largest phenotypic difference (0.2297). Specifically,
247   the phenotypic value of the diseased spikelet rate associated with *Kukri_c14239_1995-T* was
248   significantly higher than that associated with *Kukri_c14239_1995-C*, indicating that
249   *Kukri_c14239_1995-C* was better than *Kukri_c14239_1995-T* for FHB resistance (Table 4).
250   Furthermore, because allele C of *D_contig74317_533* showed a significantly higher diseased
251   spikelet rate than *D_contig74317_533-T*, allele T was deemed to be better for improving FHB
252   resistance. On chromosome 7B, allele C of *BS00025286_51* had a higher diseased spikelet rate
253   than allele T; thus, allele T for this locus was favorable for FHB resistance. Nevertheless, for the
254   other four loci on this chromosome, significant differences between the two alleles for the
255   diseased spikelet rate seemed to be at 5%. The smallest difference in diseased spikelet resistance
256   was observed between *Kukri_c7087_896-G* and *Kukri_c7087_896-A*, which indicated that this
257   locus affected FHB resistance to a smaller degree. Moreover, on chromosome 3D,
258   *RAC875_c35801_905-G* yielded better results than *RAC875_c35801_905-A* for FHB resistance.
259   **Prediction of candidate genes for some important loci**
260       Eight important candidate genes were screened for important loci significantly associated
261   with the diseased spikelet rate and diseased spike rachis rate in wheat (Table S3). One candidate
262   gene, *TraesCS3D02G326700* located on chromosome 3D is associated with actin-binding in
263   wheat. The candidate gene, *TraesCS5D02G006700* of the marker, *D_contig74317_533* on 5DS
264   was predicted in wheat, whose function was intramolecular transferase activity. Two candidate
265   genes *TraesCS6A02G013700* and *TraesCS6A02G013800*, predicted by *IAAV9150*, participate in
266   the ubiquitin-dependent ER-associated degradation (ERAD) pathway in wheat. The candidate
267   gene, *TraesCS6A02G013600* of *Excalibur_c20597_509* functions in GTP binding in wheat. On
268   chromosome 7BL, *TraesCS7B02G370700* of *BS00025286_51* is involved in the biological
269   process of defense response to fungi. There is one candidate gene, *TraesCS7B02G340200*, for
270   the three loci, *RAC875_c18043_369*, *RAC875_c18043_411*, and *Kukri_c4143_1055*, on
271   chromosome 7BL that was identified because of being in the same physical location. The
272   candidate gene, *TraesCS7B02G340100* of *RAC875_c5646_774* is associated with the
273   carbohydrate metabolic process in *Triticum aestivum*. By analyzing the homologous genes of
274   these candidate genes, we found that the functions or biological processes of most homologous
275   genes in other crops, such as *Japonica rice*, *Hordeum vulgare*, *Oryza sativa Indica*, *Oryza sativa*
276   *Japonica*, and *Arabidopsis thalian*, are involved in the defense response of fungi, which indicates

277    that these candidate genes may be related to FHB resistance, but this needs further verification in
278    wheat.

279    **Analysis of marker haplotype and resistance**

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

294

295    # Discussion

296          The majority of earlier researchers focused on finding the significant genes/loci of FHB via
297    QTL mapping or association mapping to improve the resistance to FHB in wheat cultivars
298    (Loeffler et al. 2009; Li et al. 2011; Venske et al. 2019). Previous findings suggested that
299    practically all wheat chromosomes were implicated (Yu et al. 2007; Ma et al. 2020), but
300    chromosomes 3B, 4B, 5A, and 6B seemed to be important because of the *Fhb1*, *Fhb2*, *Fhb4*, and
301    *Fhb5* genes (Loeffler et al. 2009; Zhang et al. 2018). Five QTLs were discovered by GWAS
302    analysis on chromosome arms 1AS, 2DL, 5AS, 5AL, and 7DS (Zhu et al. 2020). Of these, the
303    locus on chromosome 5B, for decreasing the amount of deoxynivalenol may be novel. However,
304    in our study, fifteen chromosomes were involved in the MTA loci, and some important genomic
305    regions involved in FHB resistance were found on chromosomes 2A, 3B, 5B, 6A, and 7B. The
306    significance of chromosome 3B for FHB resistance was further supported by this result. Six
307    MTAs at 92 cM were also discovered on chromosome 7B in two different contexts. Of these, the
308    BS00025286 51 locus explained 11.20% of the phenotypic variation and five MTAs were
309    consistently associated with the diseased spikelet rate and diseased rachis rate. It appeared that
310    FHB resistance was relevant in this region. More intriguingly, five SNP markers
311    (*Kukri_c14239_1995* on chromosome 6A, *Kukri_c7087_896* on chromosome 3B,
312    *RAC875_c35801_905* on chromosome 3D, *BS00099729_51* on chromosome 5B, and
313    *RAC875_c68525_284* on chromosome 6B) in this study have appeared in previous reports
314    (Venske et al. 2019), which indicated the reliability of our results. There was one novel locus
315    *D_contig74317_533* on chromosome 5D found for FHB resistance.

316    According to previous research, plant height had an impact on FHB resistance under field
317    conditions. QTL mapping showed that approximately 40% of the QTLs for plant height
318    overlapped with the QTLs for FHB resistance on 14 chromosomes (Buerstmayr et al. 2019). Five
319    QTLs for FHB resistance were discovered by Zhu et al. (2020), among which *QFhb.hbaas-5AS*
320    had a significant correlation with plant height. Thirty-eight MTA loci for plant height were
321    discovered on chromosomes 1B, 2A, 2B, 3A, 3B, 3D, 4A, 4B, 5A, and 6D by Chen et al. (2015)
322    using a panel of 205 wheat accessions for the GWAS analysis of plant height. Of these, 11 loci
323    were detected on chromosome 6D in two or more environments (Chen et al. 2015). However in
324    this study, there were five MTA loci for FHB resistance that showed no significant relationship
325    with plant height, that is, *D_contig74317_533* on chromosome 5D, *Kukri_c14239_1995* on 6A,
326    *RAC875_c35801_905* on 3D, and *Kukri_c4143_1055* and *BS00025286_51* on 7B, which
327    indicated that these loci can be flexibly used in breeding. Additionally, wheat materials with the
328    aforementioned loci were screened, and by using molecular marker-assisted selection, they can
329    be incorporated into major Chinese planted varieties without compromising plant height.
330    In fact, plant disease resistance is a complex molecular process controlled by genes (Ma et
331    al. 2020). Only a few significant genes were found, although researchers have discovered
332    hundreds of QTLs scattered across wheat, including 21 chromosomes from common wheat
333    varieties or related species (Buerstmayr et al. 2009; Liu et al. 2009). Nevertheless, it has become
334    simpler to find additional genes as a result of the advances in molecular technology and the
335    wheat genome sequence (both major and minor). Moreover, the isolation and functional
336    verification of FHB resistance genes are beneficial to understanding the pathogenesis and
337    resistance mechanism of wheat FHB at the molecular level (Liu et al. 2016b). Previous research
338    has demonstrated that the mechanism of the genes/loci identified in FHB resistance could
339    involve a complicated signal transduction pathway and be associated with the synergistic effect
340    of many protein factors (Zhang et al.  2018; Liu et al. 2016a; Dweba et al. 2017). For example,
341    the genes encoding a 12-oxophytodienoate reductase-like protein identified in the region of
342    *QFh.hbaas-1AS* may be related to the biosynthesis or metabolism of signaling molecules,
343    oxylipins, such as jasmonic acid (JA) (Ding et al. 2011; Qi et al. 2016). These genes were
344    discovered to encode several different proteins, including receptor-like kinase, UDP-
345    glycosyltransferase, pathogenesis-related protein 1, and glucan endo-1,3-beta-glucosidase (PR2).
346    (Anand et al. 2003; Pan et al. 2018; Ma et al. 2020).
347    In this study, the candidate genes on chromosome 3D encoded UDP-glycosyltransferase
348    activity and were related to the defense response to biotic stimulus (Li et al. 2017). This
349    indicated that this gene may enhance resistance to FHB because this protein could detoxify both
350    DON and NIV produced by *F. graminearum* (Poppenberger et al. 2003; Zhu et al. 2020). By
351    performing homologous gene detection on the *D_contig74317_533* locus of chromosome 5D, the
352    genes were found to have homologous nucleic acid binding and defense functions in barley,
353    *Arabidopsis*, indica rice, japonica rice, and wild rice. Of these, the *AT2G39510* gene is related to
354    the activity of glutamine transmembrane transporter protein. Previous studies have shown that
355    the glutamine-gated ion channel is related to the function of the *Fhb5* gene, which can control

356  Ca$^{2+}$ influx (Dennison et al. 2000; Kugler et al. 2013). It was also found that Ca$^{2+}$ was involved
357  in early signaling defense against FHB (Ding et al. 2011). Recent research has revealed that
358  wall-associated kinase (WAK) is a receptor-like protein kinase, that is involved in signal
359  transduction and the defense response of plants (Zhang et al. 2006; Hu et al. 2010). In this study,
360  it was shown that the gene *Traescs6A02G013600* contains homologous genes in Arabidopsis and
361  Japonica rice, some of which encode members of the receptor-like cytoplasmic kinase (RLCK)
362  and wall-associated kinase (WAK) families. Therefore, it is possible that this gene contributes to
363  wheat FHB resistance, but more research is needed. Additionally, earlier research has
364  demonstrated that the pathogenesis-related protein (PR) chitinase participates in the plant's
365  fundamental defense mechanism and starts to accumulate during pathogen infection (Ma et al.
366  2020). Fortunately, the gene, *TraesCS7B02G370700* of *BS00025286_51* on the 7BL
367  chromosome was also found to be associated with chitinase activity and the defense response for
368  fungi in our study. Meanwhile, the eight candidate genes identified were associated with either
369  calcium ion binding or GTP binding, which has been shown to be involved in the early response
370  of wheat to *F. graminearum* infection by salicylic acid (SA) and Ca$^{2+}$ signals (Ding et al. 2011).
371  Given that Ca$^{2+}$ signal transduction was discovered to be crucial for the transcriptional
372  reprogramming of innate plant immunity (Boudsocq et al. 2010), it is possible that these
373  candidate genes associated with Ca$^{2+}$ signals will be crucial in protecting against FHB in wheat.

## Conclusions

375  In this study, sixty-six significant marker–trait associations (MTAs) were identified
376  (*P*<0.001) on fifteen chromosomes, explaining phenotypic variation ranging from 5.4% to
377  11.2%. Some genomic regions involved in FHB resistance were found on chromosomes 2A, 3B,
378  5B, 6A and 7B. There were eleven MTAs consistently associated with the disease spikelet rate
379  and disease rachis rate as pleiotropic effect loci. Eight new candidate genes of FHB resistance
380  were predicted in wheat. Of these, three genes *TraesCS5D02G006700*, *TraesCS6A02G013600*
381  *and TraesCS7B02G370700* on chromosomes 5DS, 6AS and 7BL, respectively, possibly
382  defending against FHB by regulating intramolecular transferase activity, GTP binding, and
383  chitinase activity in wheat. In addition, a total of five favorable alleles associated with wheat
384  FHB resistance were discovered in this study. In the materials with multiple favorable alleles, the
385  resistance was mostly moderately resistant or moderately susceptible.

386

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391

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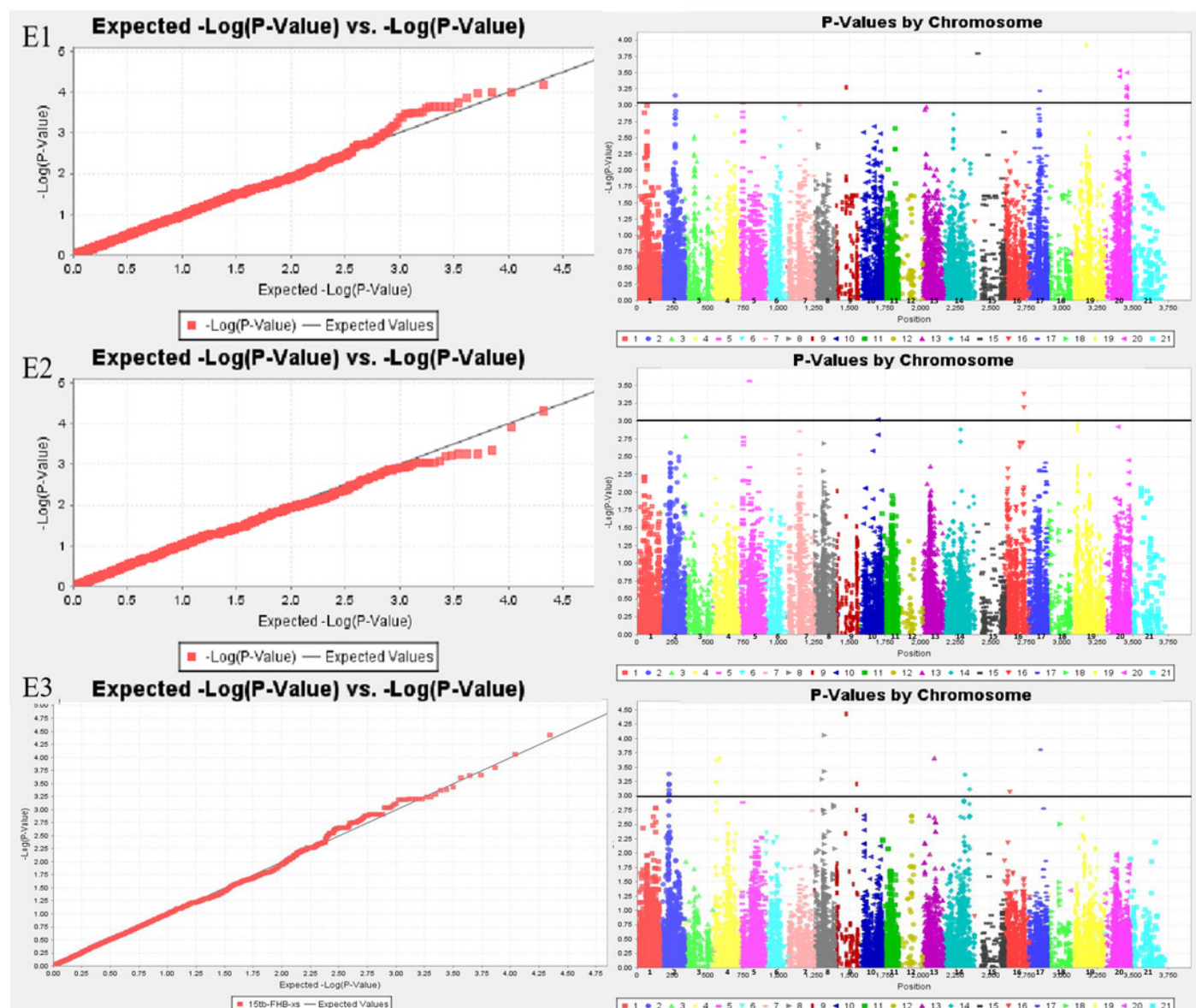
# Figure 1

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.

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.

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).

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).

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

1          **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 | |

2          [a] E1, E2 and E3 were same as Table 1. [b] Difference between alleles. **A and B:** Different capital letters indicate significant

3      difference between alleles at one locus at $P \leqslant 0.01$; **a & b:** Different lowercase letters indicate significant difference between

4      alleles at one locus at $P \leqslant 0.05$

Disease resistance statistics of different resistant QTL combinations

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

1     **Table 5** Disease resistance statistics of different resistant QTL combinations

| Genotype | D_contig7 4317_533 | Kukri_c14 239_1995 | BS000252 86_51 | RAC875_c 35801_905 | Kukri_c41 43_1055 | Variety number | Mean DSR | Effect of resistance genes | Standard deviation | F-value | P-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | - | - | - | - | - | 38 | 0.848 | . | 0.148 | | |
| | + | - | - | - | - | 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 | 2.551 | 0.001** |
| | - | - | - | + | + | 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 | . | | |

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

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 |