Genome-wide association mapping revealed syntenic loci QFhb-4AL and QFhb-5DL for Fusarium head blight resistance in common wheat (Triticum aestivum L.)

Wenjing Hu1,2,3,4, Derong Gao1,2,3, Hongya Wu1,2,3, Jian Liu1,2, Chunmei Zhang1,2,3, Junchan Wang1,2, Zhengning Jiang1,2, Yeyu Liu1,2, Dongsheng Li1,2, Yong Zhang1,2* and Chengbin Lu1,2,3*

Abstract

Background: Fusarium head blight (FHB), primarily caused by Fusarium graminearum, is a major threat to wheat production and food security worldwide. Breeding stably and durably resistant cultivars is the most effective approach for managing and controlling the disease. The success of FHB resistance breeding relies on identification of an effective resistant germplasm. We conducted a genome-wide association study (GWAS) using the high-density wheat 90 K single nucleotide polymorphism (SNP) assays to better understand the genetic basis of FHB resistance in natural population and identify associated molecular markers.

Results: The resistance to FHB fungal spread along the rachis (Type II resistance) was evaluated on 171 wheat cultivars in the 2016–2017 (abbr. as 2017) and 2017–2018 (abbr. as 2018) growing seasons. Using Illumina Infinium iSelect 90 K SNP genotyping data, a genome-wide association study (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, with those on chromosomes 1B, 4A, 5D and 7A being detected in both years. New loci for Type II resistance were found on syntenic genomic regions of chromosome 4AL (QFhb-4AL, 621.85–622.24 Mb) and chromosome 5DL (QFhb-5DL, 546.09–547.27 Mb) which showed high collinearity in gene content and order. SNP markers wsnp_ID_c4438_5568170 and wsnp_CAP11_c209_198467 of 5D, reported previously linked to a soil-borne wheat mosaic virus (SBWMV) resistance gene, were also associated with FHB resistance in this study.

Conclusion: The syntenic FHB resistant loci and associated SNP markers identified in this study are valuable for FHB resistance breeding via marker-assisted selection.

Keywords: Triticum aestivum L, Fusarium head blight (FHB); mixed linear model (MLM), Genome-wide association study (GWAS), Single nucleotide polymorphism (SNP)
Background

Common wheat (Triticum aestivum L.) is one of the most important cereals in the world and is the raw material for breads, biscuits, noodles and cakes [1]. Fusarium head blight (FHB), caused by Fusarium graminearum, is one of the most destructive fungal diseases in wheat, which spreads considerably due to farming practices and climate changes [2]. FHB does not only reduce grain yield and quality but also leads to infected kernels with excessive deoxynivalenol (DON), resulting in severe harm to human and animal health [3]. China has the largest wheat production and consumption suffering from severe FHB damages, especially in the Middle and Lower Reaches of the Yangtze River with its warm, humid environment. In recent years, FHB has become more serious and expanded in the major wheat production area of the Yellow and Huai River Valleys [4].

The most effective way for wheat producers to manage and control FHB is by breeding resistant cultivars. Great efforts have been made to find FHB resistance genes and understand the genetic mechanism of the resistance [5–9]. The genetic mechanisms for FHB resistance are complex, and the genotype by environment interaction has very strong effects on trait expression [10, 11]. Resistance to F. graminearum in wheat has been classified into five categories: (1) type I for resistance to initial infection by the pathogen, (2) type II for resistance to fungal spread along the rachis, (3) type III for resistance to kernel infection, (4) type IV for resistance to toxin accumulation, and (5) type V for tolerance [12, 13]. Many quantitative trait loci (QTL) have been identified for multiple types of FHB resistance in wheat with different magnitudes of effects [14–17]. Major and stable QTL often have large effects in multiple environments and are more valuable for practical breeding than minor QTL. However, major and stable QTL are rare for FHB resistance. Fhb1, identified from Chinese wheat Wangshuibai and Sumai 3 and located on chromosome 3BS, is the best characterized FHB resistance locus with major effect and stable resistance. Fhb1 was reported as a pore-forming toxin-like gene (PFT) QTL [18]. However, recent studies revealed an histidine-rich calcium-binding protein (His) was responsible for the Fhb1 resistance [19, 20]. A comprehensive discussion on the two studies has been revealed that the cultivars could be separated into two sub-populations (K = 2) (Fig. 3a, b). Subgroup 1 consists of 99 cultivars, mainly comprising varieties from Anhui, Jiangsu, Henan, Shaanxi and Hunan; subgroup 2 consists with moderate resistance to FHB have been approved to be released and become main cultivars [25]. Most of Yangmai-series cultivars don’t carry the Fhb1 locus [26], indicating that other FHB resistance genes may be present in these cultivars and can be more easily applied to breeding. Therefore, discovering more FHB-resistant germplasms and new FHB-resistant loci is essential for breeding wheat varieties with better FHB resistance.

Genome-wide association studies (GWAS), based on linkage disequilibrium (LD) has been widely used to discover various quantitative traits associated nucleotide polymorphisms in plants. For example, using a panel of 192 bread wheat cultivars from southwest China, 57, 27, 30, and 34 single nucleotide polymorphism (SNP) were identified for associations with plant height (PH), grain protein content (GPC), thousand kernel weight (TKW) and sodium dodecyl sulfate (SDS) content, respectively [27]. One hundred-twenty consistent loci were detected using SNP-GWAS and Haplotype-GWAS, and 78 were potentially new [28]. The recently released reference genome sequence of Chinese Spring [29] provides an elite platform for detecting genes significantly associated with linked markers with known physical positions in the genome and promoting the molecular breeding process [30]. In this study, we report a GWAS analysis of FHB resistance using a set of 171 common wheat cultivars with 90 K SNP genotyping and 2 year’s phenotyping data. The aims of this study were to identify stable loci for FHB resistance using GWAS and better understand the genetic basis of FHB resistance in natural population.

Results

Phenotypic variation

Continuous variation for percentage of symptomatic spikelets (PSS) was observed at the GWAS panel in both 2017 and 2018 growing seasons, from highly resistant (PSS < 25%) to highly susceptible (PSS > 75%)(Fig. 1). The disease symptom was more severe in 2018 growing season (Fig. 2a). Wheat cultivars from different provinces of China exhibited different levels of resistance to FHB(Fig. 2b). Cultivars from Hunan and Jiangsu provinces exhibited consistently highly resistant to FHB in two seasons, whereas cultivars from Shandong province showed the highest susceptibility.

Population structure analysis

To estimate the sub-populations of the 171 wheat cultivars, population structure analysis was performed using 1676 polymorphic SNP markers distributing on 21 wheat chromosomes with $r^2$ values > 0.2. The results indicated that the cultivars could be separated into two sub-populations (K = 2) (Fig. 3a, b). Subgroup 1 consists of 99 cultivars, mainly comprising varieties from Anhui, Jiangsu, Henan, Shaanxi and Hunan; subgroup 2 consists
72 cultivars (Additional file 1: Table S1), most of which were from Henan, Jiangsu, Shandong, Shanxi. Wheat cultivars from Anhui and Hunan were all clustered into subgroup 1.

**Linkage disequilibrium (LD) analysis**

The filtered markers from the 90 K SNP genotyping arrays were used to calculated LD decay for the A, B, and D sub-genomes separately as well as the whole genome. 38.9% of all pairs of loci had significant LD ($P < 0.001$) with an average $r^2$ of 0.281 from 23,556 polymorphic SNPs which distributed at the genome-wide level. The B sub-genome contained the largest number of significant markers (50.0%), followed by A (39.7%) and D (24.0%) sub-genomes. The highest LD decay distance was present in the D sub-genome and the lowest was found in the B sub-genome. The average LD decay distance was ~10.5 Mb for the whole genome and 10, 9.5, and 12 Mb for A, B, and D sub-genomes, respectively (Fig. 3c).

**Marker-trait associations**

Association analysis was conducted using PSS data across 2 years and 23,556 filtered markers. Altogether, 26 loci (88 MTAs, $P < 10^{-3}$) with phenotypic variances explained ($R^2$) ranging from 6.64–14.18% were identified across all of the chromosomes except for 2D, 6A, 6D and 7D (Fig. 4a, b). Among these, 41, 32 and 15 significant markers were located on the A, D and B sub-
More FHB MTAs were found on chromosomes 1A, 1B, 1D, 2A, 3B, 4A and 5D. Twenty-eight MTAs located on chromosomes 1B (1), 4A (7), 5D (19) and 7A (1) were consistently identified in both seasons and could be considered as stable QTL (Table 1). SNP GENE-0293_154 located on 1B explained 6.91–7.18% of phenotypic variances ($R^2$). Seven and 19 SNPs located on 4AL and 5DL chromosomes explained phenotypic variances ($R^2$) ranging from 9.36–11.63% and 8.11–14.18%, respectively. The SNP BobWhite_c22875_239 located on 7A could explain 8.12–8.53% of phenotypic variances ($R^2$).

Due to the high level of LD in wheat, the SNP clusters identified on chromosomes 4AL ($QFhb-4AL$) from 621.85 Mb to 622.24 Mb and 5DL ($QFhb-5DL$) from 546.09 Mb to 547.27 Mb most likely represented chromosome regions containing significant FHB associated loci, respectively. Haplotype analyses of the associated markers revealed three haplotype groups (Fig. 5a). Haplotype 1 consisted of 149 cultivars with an average PSS of 48.92% over 2 years, in which 24 were resistant, 55 were moderately resistant, and 70 were susceptible. Haplotype 2 consisted of 19 cultivars with an average PSS of 19.94% over 2 years, and 12 of them were resistant and 7 were moderately resistant. Haplotype 3 comprised three resistant cultivars with an average PSS of 11.52%. The results indicated that other resistant genes also existed in the cultivars of Haplotype 1 (Table 2, Additional file 1: Table S3). Interestingly, each haplotype contains wheat cultivars with same associated SNPs on both $QFhb-4AL$ and $QFhb-5DL$ simultaneously (Fig. 5b).

**Discussion**

FHB resistance loci identified by GWAS

QTL for *Fusarium* head blight resistance have been extensively reported using different mapping populations and mapping platforms. From more than 250 documented QTL conferring FHB resistance, only $Fhb1$-$Fhb7$ have been proven to be major effects QTLs. $Qfhs.nau-6B$ ($Fhb2$), $Qfhi.nau-4B$ ($Fhb4$), and $Qfhi.nau-5A$ ($Fhb5$) were fine mapped in the 2.2 cM, 0.14 cM, and 0.09 cM interval [16]. $Fhb1$ has been cloned recently [18–20]. In current study, four loci (28 MTAs) were identified on chromosomes 1B, 4A, 5D and 7A in two seasons. In comparison to the SNP GENE-0293_154 on chromosome 1B identified for type II FHB resistance in this study, a minor QTL for type II resistance was found in a
Fig. 4 Manhattan plots from genome-wide association scan for FHB severities among 171 wheat accessions in (a) 2017 and (b) 2018. Dashed horizontal line is the significant threshold level. (c) Numbers of significant FHB associated markers on different chromosomes.
similar physical position from Chinese wheat landrace Huangfangzhu [31]. Two loci located on 4AL and 5DL chromosomes at physical intervals of 0.39 Mb and 1.18 Mb, respectively, were related to type II resistance with variation (\(R^2\)) of 9.36–11.63% and 8.11–14.18%, respectively. The SNP BobWhite_c4438_162 itself on 5DL could explain 8.89–14.18% variation.

QTL for FHB resistance on chromosome 4AL have been reported from European wheat cultivars. Holzapfel et al. [32] identified two FHB resistance QTL on chromosome 4AL from a French cultivar (Apache) linked with XP7452–646 and a German cultivar (Pirat) linked with XP7553–254.AR. Another QTL, QFHs.fal-4AL, has been mapped on 4AL at a physical position 357.2 Mb from a Swiss winter wheat cultivar (Arina) [33]. FHB QTL identified on 4AL at a physical position from 621.85 Mb to 622.24 Mb in the current study is different from the reported ones and should be new FHB resistance loci. A type II resistance QTL from FHB-resistant wheat cultivar Chokwang (Korea) was mapped on 5DL and linked to the SSR marker Xbarc239 [34, 35] with a physical position of 420.96 Mb. Jia et al. [36] reported a QTL on chromosome 5D linked with Xgwm358 with a physical position of 120.61 Mb. Since no QTL for Type II resistance has ever been reported on chromosome 5DL at the physical interval of 546.09 Mb to 547.27 Mb, QFHb-5DL is likely to be a new FHB resistance locus.

### Table 1: FHB resistance loci revealed by GWAS in 2 years

| Locus      | Marker                | Chr | Position (Mb) | P value       | \(R^2(\%)\) | Allele            | Resistant allele |
|------------|-----------------------|-----|---------------|---------------|--------------|-------------------|-----------------|
| QFHb-1B.2  | GENE-0293_154         | 1B  | 549.47        | 6.20E-04/7.50E-04 | 6.91–7.18  | C/T               | C               |
| QFHb-4A    | BS00011469_51         | 4A  | 621.85        | 1.00E-04/2.40E-04 | 8.37–9.36  | C/T               | C               |
|            | Excalibur_c22724_85   | 4A  | 622.2         | 2.00E-05/1.10E-04 | 9.33–11.63 | C/T               | T               |
|            | Kukri_c24695_273      | 4A  | 622.2         | 2.00E-05/1.10E-04 | 9.33–11.63 | A/G               | A               |
|            | Kukri_c1073_91        | 4A  | 622.24        | 2.00E-05/1.00E-04 | 9.65–11.53 | C/T               | T               |
|            | Excalibur_c667_961    | 4A  | 622.24        | 2.00E-05/1.10E-04 | 9.33–11.63 | A/G               | A               |
|            | Excalibur_c687_907    | 4A  | 622.24        | 2.00E-05/1.10E-04 | 9.33–11.63 | C/T               | T               |
|            | Excalibur_c687_886    | 4A  | 622.24        | 2.00E-05/1.10E-04 | 9.33–11.63 | A/G               | G               |
| QFHb-5D    | BobWhite_c13030_406   | SD  | 546.09        | 2.00E-05/1.30E-04 | 9.37–11.65 | A/G               | G               |
|            | BS00079676_51         | SD  | 546.65        | 2.00E-05/1.10E-04 | 9.33–11.63 | A/G               | A               |
|            | RAC875_c13169_459     | SD  | 546.65        | 2.00E-05/1.10E-04 | 9.33–11.63 | C/T               | C               |
|            | D_G488501A1E4G_122    | SD  | 546.65        | 2.00E-05/1.10E-04 | 9.33–11.63 | C/T               | C               |
|            | wsnp_JD_c4438_5568170 | SD  | 546.69        | 8.00E-05/1.60E-04 | 10.76–11.72 | A/G/R             | A               |
|            | wsnp_JD_c4438_5567972 | SD  | 546.69        | 2.00E-05/1.10E-04 | 9.33–11.63 | A/G               | A               |
|            | wsnp_JD_c4438_5567834 | SD  | 546.69        | 9.00E-05/4.10E-04 | 9.33–11.63 | C/T/Y             | C               |
|            | BobWhite_c4438_162    | SD  | 546.69        | 7.00E-06/2.50E-04 | 8.89–14.18 | A/G               | G               |
|            | IACX10520             | SD  | 546.69        | 2.00E-05/1.10E-04 | 9.33–11.63 | C/T               | T               |
|            | BS00088587_51         | SD  | 546.69        | 2.00E-05/1.10E-04 | 9.33–11.63 | G/T               | G               |
|            | D_G05L2Z01CBWNE_99    | SD  | 546.7         | 2.00E-05/1.10E-04 | 9.33–11.63 | C/T               | T               |
|            | Kukri_c5528_603       | SD  | 546.7         | 2.00E-05/1.10E-04 | 9.33–11.63 | C/T               | C               |
|            | Excalibur_c42190_383  | SD  | 546.91        | 4.10E-04/4.40E-04 | 9.55–9.57  | A/G/R             | A               |
|            | Excalibur_c28592_377  | SD  | 546.91        | 9.00E-05/3.00E-04 | 8.11–9.47  | C/T               | T               |
|            | Excalibur_c28592_173  | SD  | 546.91        | 4.10E-04/4.40E-04 | 9.55–9.57  | C/T/Y             | T               |
|            | Excalibur_c14043_548  | SD  | 546.91        | 9.00E-05/3.00E-04 | 8.11–9.47  | C/T               | C               |
|            | CAP8_c145S_89         | SD  | 547.27        | 2.00E-05/1.10E-04 | 9.33–11.63 | C/T               | T               |
|            | wsnp_CAP11_c209_198467| SD  | 547.27        | 2.00E-05/1.30E-04 | 9.37–11.65 | A/G               | A               |
|            | BS00011794_51         | SD  | 547.27        | 2.00E-05/1.30E-04 | 9.37–11.65 | C/T               | T               |
| QFHb-7A    | BobWhite_c22875_239   | 7A  | 661.3         | 2.20E-04/3.00E-04 | 8.12–8.53  | C/T               | C               |

*a Markers were detected at the threshold -log10 (\(P\)) = 3.0
*b Chr, Chromosome
*c Physical positions of SNP markers based on wheat genome sequences from the International Wheat Genome Sequencing Consortium (IWGSC, http://www.wheatgenome.org/)
*d Percentage of phenotypic variance explained by the MTA
![Image of a bar graph showing the distribution of cultivar numbers across different resistance levels: resistant (24), moderately resistant (55), moderately susceptible (46), and susceptible (24).](image)

![Image of haplotype sequences: Haplotype 1 (TCGCCA), Haplotype 2 (CTATTG), Haplotype 3 (TTATTG), Haplotype 1 (AGTTGGTACTCTGCCTCGC), Haplotype 2 (GACCAACGTGCTATTCTAT), Haplotype 3 (GACCAACGTGCTGCTTTAT).](image)

Fig. 5 (See legend on next page.)
5DL were reported to be closely linked to a soil-borne wheat mosaic virus (SBWMV) resistance gene *Sbwm1* [37]. The SNPs have been developed into breeder-friendly Kompetitive Allele-Specific Polymerase chain reaction (KASP) markers for effectively distinguish resistant and susceptible alleles of *Sbwm1* in a diverse wheat panel in breeding programs. It would be interesting to verify whether these KASP markers can be used in marker-assisted selection of FHB resistance in wheat breeding. Furthermore, *BobWhite_c22875_239* was found associated with type II resistance on chromosome 7AL at 661.3 Mb that is about the same proximal region of a reported QTL *QFhb-nau-7A* from Wangshuibai [38, 39] (Additional file 1: Table S4).

**QFhb-4AL and QFhb-5DL are located on syntenic genomic regions**

We detected two loci significantly associated with FHB resistance on 4AL and 5DL at a physical intervals of 0.39 Mb and 1.18 Mb, respectively. LD of markers and FHB severity analysis indicated that each haplotype contains wheat cultivars with associated SNP on both *QFhb-4AL* and *QFhb-5DL* simultaneously. Gene annotations of the genomic intervals revealed homologous gene pairs between 4AL and 5DL. Highly collinearity in gene order and content were observed for the two FHB resistant QTL regions, even through large fragment insertions/deletions were also presented (Additional file 1: Table S5; Fig. 6).

Wheat has experienced structural evolution involving chromosome translocation of 4A, 5A, and 7B. The 4AL/5AL translocation taken place at the diploid level and existed both in *T. monococcum* and *T. aestivum*, followed by a 4AL/7BS translocation, a pericentric inversion (4AS;4AL) and a paracentric inversion (4AL;4AL) that occurred in the tetraploid progenitor of hexaploid wheat [40]. Recently, Dvorak et al. [41] reassessed the evolution of wheat chromosomes 4A, 5A and 7B after sequence comparison of wild emmer wheat and *Aegilops tauschii*. They found that the 596.20–631.84 Mb genomic region of 4A pseudomolecule was derived from ancestral 5AL with nested inversion and is corresponding to the end of the * Ae. tauschii* arm 5DL. The two FHB associated loci on 4AL (621.81–622.49 Mb) and 5DL (546.45–546.92 Mb) are located on the syntenic block with sequence inversion (Fig. 6), providing further information of this structure rearrangement containing important genes for agronomic trait.

The hypothetical proteins were predicted for the 4AL and 5DL syntenic blocks (Table 3). Two kinase proteins, homologous to PTI1-like tyrosine-protein kinase 1 and Putative receptor protein kinase ZmPK1, proved to be associated with plant disease resistance were annotated in the corresponding genomic regions (Additional file 1: Tables S4 and S5). Protein kinases (PKs) are important for transmembrane signaling that regulates plant development and adaptation to diverse environmental conditions [42]. Several kinase proteins have been reported related to plant innate immunity. For example, the combination of a kinase and a putative START lipid-binding domain is necessary to confer wheat rust resistance of *Yr36* [43]. Wheat stripe rust resistance gene *Yr15* (WTK1) [44] and barley (*Hordeum vulgare L.*) stem rust (*P. graminis* f. sp. *tritici*) resistance gene *Rpgl* [45] contain a structure with tandem kinase domains. A maize wall-associated kinase protein (*ZnWAK*) was reported to confer quantitative resistance to maize head smut [46] and the PTI-like kinase (*ZmPtIa*) was known to play an important role in the signaling pathway that facilitates pollen performance and male fitness [47].

**Table 2** Descriptive statistics of the three haplotypes for FHB severities

| Haplotype* | No.b | Minimum (%) | Maximum (%) | Mean c (%) | Standard deviation | Variance |
|------------|------|-------------|-------------|------------|--------------------|----------|
| Haplotype1 | 149  | 7.71        | 94.73       | 48.92A     | 22.29              | 496.69   |
| Haplotype2 | 19   | 4.29        | 41.25       | 19.94B     | 11.36              | 129.06   |
| Haplotype3 | 3    | 7.3         | 16.58       | 11.52B     | 4.7                | 22.09    |

* Three haplotype groups revealed through haplotype analyses of the associated markers
b No. Number of cultivars
* indicates extremely significant differences at 0.01 significance level among parents and controls (*P* < 0.01)
Graminearum was required for much more developmental processes linked to sexual reproduction, plant infection, and cell wall integrity [48]. The glycogen synthase kinase gene orthologous to mammalian GSK3 was an significant virulence factor and Fgk3 glycogen synthase kinase was also important for growth, pathogenesis, conidiogenesis, DON production and stress responses in *F. graminearum* [49]. Taken the potential importance of kinase proteins in FHB resistance synthetic loci identified on 4AL and 5DL, the wheat homologs of PTI1-like tyrosine-protein kinase 1 and putative receptor protein kinase ZmPK1 might be considered as candidates of FHB resistance and need further characterization.

**Conclusions**

In the present study, we identified 26 FHB resistance loci using the wheat 90 K SNP assay, and four stable loci were detected in both seasons. Two new FHB resistance loci on 4AL and 5DL were found to be located on syntenic genomic regions, indicating that these regions contain important genes valuable for future research and breeding application. The SNP markers significantly associated with the FHB resistance could be used to develop diagnostic markers for marker associated selection of FHB resistance breeding.

**Methods**

**Plant materials**

An association panel comprising 171 wheat cultivars was used for SNP genotyping and 2 years FHB resistance phenotyping. Among them, three cultivars were derived from Italy, Mexico and Japan, and the other 168 cultivars were collected from 8 provinces at winter wheat region in Northern China and 9 provinces from Southern China (Additional file 1: Table S1). All wheat accessions are collected under permission from the National Genebank of China, Chinese Academy of Agricultural Sciences and Jiangsu Academy of Agricultural Sciences. The population was planted at Wanfu Experimental Station, Institute of Agricultural Sciences of the Lixiahe, Yangzhou, Jiangsu Province, China (altitude 8 m, latitude 32.24°N, annual rainfall about 1000 mm, growing season from early November to the next May) for 5 years, and flowering date were recorded every year. The 171 wheat cultivars displayed a difference of less than 4.0 days on average in flowering date between the earliest cultivar and the latest cultivar.

Field experiments were designed as randomized complete blocks with two replicates per year. The cultivars in each replication were sown in two rows of 133 × 25 cm with 40 seeds per row. The field trials were in accord with local practices management.

**Phenotyping**

All cultivars were inoculated in growing seasons 2016–2017 (abbr. as 2017) and 2017–2018 (abbr. as 2018) with four *F. graminearum* strains (F4, F15, F34, and F0609), kindly provided by Prof. Huaigu Chen from Jiangsu Academy of Agricultural Sciences, Nanjing, China. Ten
| Marker                  | Chr  | Position (cM) | Adjacent *T. aestivum* gene | Predicted function | Identity (%) | Orthologous gene |
|------------------------|------|---------------|-----------------------------|-------------------|--------------|------------------|
| GENE-0293_154          | 1B   | 549.47        | –                           | –                 | –            | –                |
| BS00011469_51          | 4A   | 621.85        | *TraesCS4A02G341700*        | PTI1-like tyrosine-protein kinase | 100          | LOC109767070    |
| Excalibur_c22724_85    | 4A   | 622.20        | *TraesCS4A02G507700LC*      | protein FAR1-RELATED SEQUENCE 6-like | 96.62        | LOC10976821     |
| Excalibur_c687_961     | 4A   | 622.24        | *TraesCS4A02G342500*        | putative receptor protein kinase ZmPK1 | 99.25        | TRRIUR3_00021   |
| BobWhite_c13080_406   | 5D   | 546.09        | *TraesCS5D02G329700*        | uncharacterized protein | 100          | LOC10976624     |
| BS00079676_51 RAC875_c13169_239 D_GABX540A1A4_GS_122 | 5D   | 546.65        | *TraesCS5D01G338000*        | putative RNA-binding protein Luc7-like 2 | 100          | LOC10976817     |
| wsnp_ID_c4438_5568170  | 5D   | 546.69        | *TraesCS5D02G306000*        | putative receptor protein kinase ZmPK1 | 100          | LOC10976820     |
| D_GDS7ZLN01CBWNE_99 Kuki_c5528_203 | 5D   | 546.70        | *TraesCS5D02G307000*        | probable ion channel CASTOR 1 | 100          | LOC10976818     |
| Excalibur_c42190_338   | 5D   | 546.91        | *TraesCS5D02G331200*        | PTI1-like tyrosine-protein kinase | 100          | LOC109767070    |
| CAR8_c145_89 wsnp_CAPI1_c209_198467 BS00011794_51 | 5D   | 547.30        | *TraesCS5D02G321000*        | uncharacterized protein | 99           | LOC109767042    |
| BobWhite_c22875_239    | 7A   | 661.30        | *TraeCS7A02G464800*         | probable polyamine transporter At3g13620 | 98.73        | LOC109739139    |

*a* Markers were detected at the threshold -log10 (*P*) = 3.0

*b* Chr, Chromosome

c Physical positions of SNP markers based on wheat genome sequences from the International Wheat Genome Sequencing Consortium (IWGSC, [http://www.wheatgenome.org/](http://www.wheatgenome.org/))

d *T. aestivum* gene transcripts and their domains were explored in Ensembl (using the transcript table link)

e The sequences of *T.aestivum* gene were blasted in the NCBI ([http://www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)), databases to identify putative gene functions.
spikes per row were inoculated at the late-heading stage by injecting 10 μL of macroconidial suspension (1.0 × 10^5 conidia/ml) into a single floret in the middle of each spike based on the flowering time.

The disease nursery was mist-irrigated for 5 min every 30 min from 7:00 am to 6:00 pm each day to ensure the inoculated spikes fully infected under high humid conditions [50]. The number of infected spikelets and the total number of spikelets of every tagged spike were recorded 25 days after inoculation. The average percentage of symptomatic spikelets (PSS) was calculated as the measure of FHB severity. All tested accessions were classified into four classes based on FHB severity, resistant (0 < PSS ≤ 25%), moderately resistant (25% < PSS ≤ 50%), moderately susceptible (50% < PSS ≤ 75%) and susceptible (75% < PSS ≤ 100%) [51].

Genotyping and SNP calling
Genomic DNA was extracted from fresh leaves of field grown non-infected plants at seedling stage using the CTAB method [52]. The association mapping population was genotyped from the wheat Illumina 90 K iSelect array with 81,587 SNPs (Wang et al. 2014) at the Biotechnology Center, Department of Plant Sciences, University of California, USA, using the Illumina SNP genotyping platform and BeadArray Microbead Chip [53]. To avoid spurious marker-trait associations (MTAs), SNP markers with minor allele frequencies (MAF) < 0.05 and missing data > 10% were excluded from subsequent analyses. The physical positions of SNP markers were obtained from Chinese Spring reference genome sequences at the International Wheat Genome Sequencing Consortium website (IWGSC, http://www.wheatgenome.org/).

Population structure analysis and linkage disequilibrium
Population structure was estimated using Structure 2.3.4 with 1676 polymorphic SNP markers distributed on all 21 wheat chromosomes with r^2 < 0.2, based on the Bayesian cluster analysis [54]. Six runs of Structure were performed with a K between 1 and 11, using the admixture model with 100,000 replicates each for burn-in and MCMC. The optimal K-value was determined using the ΔK method [55]. Linkage disequilibrium (LD) among markers was computed by the full matrix and sliding window options in Tassel v5.0 and the program Structure v2.3.4. The R^2 showing the variation explained by the SNP were recorded [58]. SNPs with an adjusted -log_{10}(P-value) ≥ 3.0 were regarded as significant associated with FHB resistance. Significant SNP markers within one LD on the same chromosome were considered to represent one locus. Haplotype analyses of the significant SNPs were performed with Haplovie v4.2 [59].

Identification of candidate genes
To identify the candidate genes linked to significant SNPs, the physical positions of the markers preceded by the chromosome name were taken to Ensembl (https://urgi.versailles.inra.fr/gb2/gbrowse/wheat_survey_sequence_annotation), and the genes in the same genetic positions were considered. The intervals were then explored for predicted genes and annotations. For genes that are unavailable from the IWGSC annotations, we evaluated orthologous genes (proteins) in related species with reported predicted functions using the comparative genomics tool in Ensembl. When the genes had less than 70% similar ortholog in the annotated genomes of related species in Ensembl, the sequence of the T. aestivum gene was taken to search highly similar sequences using NCBI and basic local alignment search tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blat.cgi).

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12870-019-2177-0.

Additional file 1: Table S1 171 wheat accessions used in the genome-wide association study (GWAS) for FHB severities and their origins, Table S2 Marker-trait associations (MTAs) for FHB resistance in 171 wheat accessions identified by the Tassel v5.0, Table S3 Cultivars belonging to different haplotype and their FHB severities, Table S4 Physical positions of reported FHB resistance QTL related to the current study, Table S5 The associated regions with FHB resistance with the same function exists in the corresponding sections of 4A and 5D.

Abbreviations
DON: Deoxynivalenol; FHB: Fusarium head blight; GPC: Grain protein content; GWAS: Genome-wide association study; His: Histidine-rich calcium-binding protein; LD: Linkage disequilibrium; MAF: Minor allele frequencies; MAS: Marker-assisted selection; MLM: Mixed linear model; MTA: Marker-trait association; PFT: Pore-forming toxin-like gene; PH: Plant height; PSS: The percentage of symptomatic spikelets; QTL: Quantitative trait loci; R^2: Phenotypic variance explained; SDS: Sodium dodecyl sulfate; SNP: Single nucleotide polymorphism; TKW: Thousand kernel weight

Acknowledgments
The authors are grateful Dr. Jindong Liu, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences for critical review of this manuscript.
Author's contribution
WJH carried out the experiments and wrote the paper. DRG and HYW participated in the field trials and assisted in revising the paper. JL, CMZ, JCW, ZN, YLY and DSL participated in the field trials. YZ and CBL designed the experiment and assisted in analyzing the data and writing the paper. All authors read the final version of this manuscript and approved it for publication.

Funding
This work was supported by the National Natural Science Foundation of China (31901544), National Key Research and Development Program of China (2017FY1000801, 2016YFD0101802), the Natural Science Foundation of Jiangsu Province (BK20171279), the China Agricultural Research System (CARS-03-03B). Each of the funding bodies granted the funds based on a research proposal. National Key Research and Development Program of China (2017YFD0100801, 2016YFD0101802) assisted in designing of the study and collection, analysis, and interpretation of data and in writing the manuscript. Other fundings had no influence over the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Availability of data and materials
The phenotypic data of the current study is available in the Additional file 1: Table S1. The data sets supporting the results of this research could be obtained within the article and its additional files. Any other datasets used and/or analyzed are available upon request.

Ethics approval and consent to participate
We declare that these experiments comply with the ethical standards and national guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Institute of Agricultural Sciences for Lixiahe Region in Jiangsu, Yangzhou 225007, China. 2Key Laboratory of Wheat Biology and Genetic Improvement–Rutkoski J, Benson J, Jia Y, Brown-Guedira G, Jannink J-L, Sorrells M. 2. Nopsa JFH, Baenziger PS, Eskridge KM, Peiris KHS, Dowell FE, Harris SD, Curtis T, Halford NG. Food security: the challenge of increasing wheat yield for Low & Middle Yangtze Valley, Ministry of Agriculture and Rural Affairs, Yangzhou, China. 3Jiangsu Key Laboratory of Crop Genomics and Molecular Breeding, Yangzhou, China. 4Collaborative Innovation Center of Henan Grain Crops, Henan Agricultural University, Zhengzhou 45002, Henan, China.

Received: 25 July 2019 Accepted: 29 November 2019
Published online: 20 January 2020

References
1. Curtis T, Halford NG. Food security: the challenge of increasing wheat yield and the importance of not compromising food safety. Ann Appl Biol. 2014; 164(3):354–72.
2. Noppla JPH, Baenzerger PS, Eskinridge KM, Peiris KHS, Dowell FE, Harris SD, Wengjun SN. Differential accumulation of deoxynivalenol in two winter wheat cultivars varying in FHB phenotype response under field conditions. Can J Plant Pathol. 2012;34(3):380–9.
3. Rutkoski J, Benson J, Jia Y, Brown-Guedira G, Jannink J-L, Sorrells M. Evaluation of genomic prediction methods for Fusarium head blight resistance in wheat. Plant Genome. 2012;5(2):51–61.
4. Ren J, Wang Z, Du Z, Che M, Zhang Y, Quan W, Wang Y, Jiang X, Zhang Z. Detection and validation of a novel major QTL for resistance to Fusarium head blight from Triticum aestivum in the terminal region of chromosome 7DL. Theor Appl Genet. 2019;132(1):241–55.
5. Bai G, Shaner G. Scab of wheat: prospects for control. Plant Dis. 1994;78(8):760–6.
6. Bai G, Shaner G. Management and resistance in wheat and barley to Fusarium head blight. Annu Rev Phytopathol. 2004;42:135–61.
7. Buerstmayr H, Ban T, Anderson JA. QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. Plant Breed. 2009;128(1):1–26.
8. Gilbert J, Haber S. Overview of some recent research developments in Fusarium head blight of wheat. Can J Plant Pathol. 2013;35(2):149–74.
9. Chen Y, Wang J, Yang N, Wen Z, Sun X, Chai Y, Ma Z. Wheat microbe-immune bacteria can reduce virulence of a plant pathogenic fungus by altering histone acetylation. Nat Commun. 2018;9(1):3429.
10. Campbell KA, Leps PE. Allocation of resources: sources of variation in fusarium head blight screening nurseries. Phytopathology. 1998;88(10):1078–86.
11. Fuentes RG, Mickelson HR, Busch RH, Dill-Macky R, Evans CK, Thompson WG, Wiersma JV, Xie W, Dong Y, Anderson JA. Resource allocation and cultivar stability in breeding for Fusarium head blight resistance in spring wheat. Crop Sci. 2005;45(5):1965–72.
12. Mesterhazy A, Bartok T, Mirocha CG, Komoroczy R. Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. Plant Breed. 1999;118:97–110.
13. Mesterhazy A. Types and components of resistance to Fusarium head blight of wheat. Plant Breed. 1995;114(5):377–86.
14. Petersen S, Lyerly JH, Maloney PV, Brown-Guedira G, Cowger C, Costa JM, Dong Y, Murphy JP. Mapping of Fusarium head blight resistance quantitative trait loci i n winter wheat cultivar NC-Neuse. Crop Sci. 2016; 56(4):1473–93.
15. Amuda MP, Brown P, Brown-Guedira G, Krill AM, Thumber C, Merrill KR, Foresman BJ, Kolb FL. Genome-wide association mapping of Fusarium head blight resistance in wheat using genotyping-by-sequencing. Plant Genome. 2016;9(1). https://doi.org/10.3835/plantgenome2015.04.0028.
16. Jia H, Zhou J, Xue S, Li G, Yan H, Ran C, Zhang Y, Shi J, Jia L, Wang X, et al. A journey to understand wheat fusarium head blight resistance in the Chinese wheat landrace Wangshuibai. Crop J. 2016;8(1):418–59.
17. Giancaspro A, Gove SL, Zito D, Blanco A, Gadaleta A. Mapping QTLs for Fusarium head blight resistance in an interspecific wheat population. Front Plant Sci. 2016;7:1381.
18. Rawat P, Pumfrey MO, Liu S, Zhang X, Tiwari VK, Ando K, Trick HN, Bockus WW, Akhunov E, Anderson JA, et al. Wheat Fhb1 encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain conferring resistance to Fusarium head blight. Nat Genet. 2016;48(12):1576–80.
19. Su Z, Bernardo A, Tian B, Chen H, Wang S, Ma H, Cai S, Liu D, Zhang L, Di T, et al. A deletion mutation in TaHRC confers Fhb1 resistance to Fusarium head blight in wheat. Nat Genet. 2019;51(7):1099–105.
20. Li G, Zhou J, Jia H, Gao Z, Fan M, Luo Y, Zhao P, Xue S, Li N, Yuan Y, et al. Modification of a histidine-rich calcium-binding protein gene in wheat confers resistance to Fusarium head blight. Nat Genet. 2016;48(12):1576–80.
21. Lagudah ES, Krattinger SG. A new player contributing to durable Fusarium resistance. Nat Genet. 2019;51(7):1070–1.
22. McDonald BA, Linde C. Pathogen population genetics, evolutionary potential, and durable resistance. Annu Rev Phytopathol. 2002;40:349–79.
23. Miedaner T, Korzun V. Marker-assisted selection for disease resistance in wheat and barley breeding. Phytopathology. 2012;102(6):560–6.
24. Hao Y, Rasheed A, Zhu Z, Wu LF, He Z. Harnessing wheat Fhb1 for fusarium resistance. Trends Plant Sci. 2019. https://doi.org/10.1016/j.tplants.2019.10.006.
25. Zhu Z, Hao Y, Mengmu Q, Bai G, Humphreys G, Cloutier S, Xia X, He Z. Breeding for resistance to Fusarium head blight in the Global North: China, USA, and Canada. Crop J. 2019. https://doi.org/10.1002/cj.201906003.
26. Rasheed A, Wen W, Gao F, Zhai S, Jin H, Liu J, Guo Q, Zhang Y, Dreisigacker S, Xia X, et al. Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. Theor Appl Genet. 2016; 129(10):1843–60.
27. Liu J, Feng B, Xu Z, Fan X, Jiang F, Jin X, Cao J, Wang F, Liu Q, Yang L, et al. A genome-wide association study of wheat yield and quality-related traits in Southwest China. Mol Breed. 2017;38(1):11.
28. Li F, Wen W, Liu J, Zhang Y, Cao S, He Z, Rasheed A, Jin H, Zhang C, Yan J, et al. Genetic architecture of grain yield in bread wheat based on genome-wide association studies. BMC Plant Biol. 2019;19(1):168.
29. International Wheat Genome Sequencing C, Investigators IRp, Appels R, Eversole K, Feuillet C, Keller B, Rogers J, Stein N, Investigators iW-GAP, Pozniak C, et al. Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science. 2018;361(6403). https://doi.org/10.1126/science.aar7191.
30. Juliana P, Singh RP, Singh PK, Poland JA, Bergstrom GG, Huerta-Espino J, Bhavani S, Cress J, Sorrells ME. Genome-wide association mapping for resistance to leaf rust, stripe rust and tan spot in wheat reveals potential candidate genes. Theor Appl Genet. 2018;131(7):1405–22.
