Pyramiding Three QTL Improved *Fusarium* Head Blight Resistance in Wheat (*Triticum Aestivum* L.)

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Research Article

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Abstract

Fusarium head blight (FHB) is a destructive fungal disease and a major threat to wheat production and food safety worldwide. Mapping of FHB-resistant quantitative trait loci (QTL) has facilitated marker-assisted breeding (MAS). To make better use of the genes/loci from different resistant sources, it is desirable to understand their combined effects on FHB resistance and agronomic traits in different genetic backgrounds. In this study, several FHB resistant donors were first characterized, including Yangmai 18 (YM18) with *Fhb1*, Zhenmai 9 (ZM9) and Yangmai 16 (YM16) both with *QFhb.yaas-2DL* and *QFhb.yaas-3BL*, Ningmai 13 (NM13) with *Fhb1* and *QFhb.yaas-3BL*. TaHRC-KASP, KASP-QFhb-2DL and KASP-QFhb-3BL were used for *Fhb1*, *QFhb.yaas-2DL* and *QFhb.yaas-3BL*, respectively, and they were screened in 77 cultivars/lines. Using the three KASP markers, we developed three RIL populations using MAS from crosses YM18/ZM9, YM18/YM16 and YM18/NM13. F₅ populations were investigated for their FHB severity, along with major agronomic traits. Of these three QTL, *Fhb1* was the most effective in reducing FHB damage, followed by *QFhb.yaas-2DL* and *QFhb.yaas-3BL*. The lines with all three QTL showed significantly more strong resistance than those with only two or one QTL. *Fhb1* might have negative effect on kernel number per spike (NKS), but not have significant effect on thousand kernel weight (TKW) and yield. On the other hand, *QFhb.yaas-3BL* might have positive effect on TKW and yield, and *QFhb.yaas-2DL* might have positive effect on spike number per plant (NSP) and yield. The addition of these two or three QTL shows positive effects on yield under different genetic backgrounds. Three elite lines, YM18/ZM9-12, YM18/YM16-18 and YM18/YM16-22, with similar FHB resistance to Sumai 3 (SM3) and superior yield to Yangmai 20 (YM20) across multiple environments, have the potential to be released as commercial varieties.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the major staple cereal crops that support food security of the world (Li et al. 2019b). *Fusarium* head blight (FHB), mainly caused by *Fusarium graminearum* (Fg), is a devastating disease for wheat, corn, and other grain crops (Kage et al. 2017). Infected grains contain toxins, which are harmful to humans and livestock (Foroud et al. 2008). Even the most efficient fungicides could not keep the toxin levels below the critical threshold particularly on susceptible or moderate susceptible cultivars under epidemic conditions (Mesterhazy et al. 2011). Enhancing plant resistance through genetic breeding provides a promising and environmentally friendly approach to reduce losses caused by FHB. To date, numerous quantitative trait loci (QTL) for FHB have been documented. *QTL-Fhb1* and *QTL-Fhb2* derived from Sumai 3 (SM3) and Wangshuibai are two major QTL for type II FHB resistance. *His* and *PFT* have been identified as the functional genes of *Fhb1* (Rawat et al. 2016; Li et al. 2019a; Su et al. 2019). *Fhb2* has been fine mapped but not yet been cloned (Zhang et al. 2021b). Another FHB resistance gene *Fhb7* was cloned based on assembled genome of *Thinopyrum elongatum*, and was confirmed to encode a glutathione S-transferase (GST) that confers broad resistance to *Fusarium* species by detoxifying trichothecenes via de-epoxidation (Wang et al. 2020). However, FHB resistance is controlled by hierarchies of genes, and introgression of a single gene is often insufficient

(Zhang et al. 2021b). Several genes such as *Rht-B1* (on chromosome 4B) and *Rht-D1* (4D), *Ppd-D1* (2DS), *Vrn-A1* (5AL) and *Vrn-B1* (5BL), which are associated with plant height and heading time, can also influence FHB resistance indirectly (Buerstmayr et al. 2019), which makes the simultaneous improvement of FHB resistance and yield difficult due to their potential negative relationship (Yoshida et al. 2005; Yi et al. 2018).

Yangmai 158 (YM158) has moderate resistance to FHB and is a leading cultivar in the Middle and Lower Yangtze River Valleys from the late 1980s to 1990s (Zhu et al. 2019), and many cultivars/lines were derived from YM158, such as Yangmai 11 (YM11), Yangmai 12 (YM12) and Yangmai 16 (YM16). YM16 has become the most predominant cultivar in the Middle and Lower Yangtze River Valleys since 2010, due to its moderate FHB resistance, good quality, fine agronomic characters and high-yield potential. Five QTL for FHB resistance, *Qfhi.nau-2D*, *Qfhs.nau-2A*, *QFhb-5A*, *Qfhb.3AL*, *Qfhb.2DL* and *Qfhb.2DS* were identified from YM158 in three populations (Jiang et al. 2019; Yan et al. 2021; Zhang et al. 2021a). Besides, *QFhb.caas-4DS* at the *Rht-D1* locus, *QFhb.yaas-2DL*, *QFhb.yaas-3BL*, *QFhb.caas-3BL*, *QFhb.caas-6AL*, and *QFhb.caas-6BS* were also identified from YM16 (Hu et al. 2020a; Zhu et al. 2021). Five types of FHB resistance were deemed, i.e., types I, II, III, IV and V for resistance against initial infection, resistance to spread along the rachilla, low deoxynivalenol accumulation, lower kernel infection rate and host tolerance, respectively (Schroeder and Christensen 1963; Miller 1989; Mesterhazy 1995). *QFhb.yaas-2DL*, *QFhb.yaas-3BL* and *QFhb.yaas-4DS* were all identified both for type I and type II resistance (Srinivasachary et al. 2008, 2009; Hu et al. 2020a; Yan et al. 2021; Zhang et al. 2021a; Zhu et al. 2021). *QFhb.yaas-2DL*, *Qfhi.nau-2D* and *Qfhb.2DL* were mapped to the location near the major FHB resistant QTL derived from Wuhan-1 (Somers et al. 2003), CJ9306 (Jiang et al. 2007a, b) and YM158 (Zhang et al. 2020; Yan et al. 2021). *QFhb.yaas-3BL* and *QFhb.caas-3BL* were located at an overlapped physical region and may represent a same QTL (Hu et al. 2020a; Zhu et al. 2021). The resistant allele of *QFhb.yaas-4DS* and *QFhb.yaas-5BL* were confirmed to be in close linkage with *Rht-D1a* and *Vrn-B1a* in most cultivars/lines in the Middle and Lower Yangtze River Valleys (Rasheed et al. 2016; Hu et al. 2020a; Zhu et al. 2021). However, the effects of these resistant loci on FHB resistance and yield were not clear in breeding. The development of high-throughput molecular markers allows for efficient integration of genotypic information in cultivar development (Nan et al. 2020; Hagely et al. 2021). Development of genotypes by pyramiding desirable traits from different sources into elite genetic backgrounds have great potential to accelerate breeding for FHB resistance and high-yield. In this study, we investigated effects of different combinations of *Fhb1*, *QFhb.yaas-2DL* and *QFhb.yaas-3BL* on type II FHB resistance and yield related agronomic traits in three populations. Three lines with high FHB resistance similar to SM3, excellent agronomic traits and yield superior to YM20 were selected for potentially commercial release.

2. Materials And Methods

2.1. Plant materials and field trials

Yangmai 18 (YM18) was developed by Lixiahe Institute of Agricultural Sciences, Yangzhou, China. It combines high yield potential with high number of kernels per spike (NKS) and good resistance to

multiple diseases such as powdery mildew and FHB. Zhenmai 9 (ZM9), developed by Qiuling Institute of Agricultural Sciences, Zhenjiang, China, was an elite cultivar with high thousand kernel weight (TKW) and moderate resistance to FHB. YM16 with high yield potential and moderate FHB resistance was the most popular cultivar with a peak planting area of 0.39 Mha in 2013 (<http://202.127.42.47:6006/home/bigdataindex>). Ningmai13 (NM 13) was developed by Jiangsu Academy of Agricultural Sciences, Nanjing, China and widely grown in the Middle and Lower Yangtze River Valleys because of its high tillers number and yield stability. After detection of the KASP markers for *QFhb.yaas-4DS*, *QFhb.yaas-5BL* and *QFhb.yaas-6AS*, YM18, YM16, ZM9 and NM13 all carry resistance alleles of these three QTL (unpublished data).

YM16, ZM9 and NM13 were respectively crossed with YM18 to develop F_1 in 2014. After self-crossing, seeds of YM18/ZM9, YM18/YM16 and YM18/NM13 populations were sown in 2015 respectively. 885, 901 and 752 plants that had similar height, heading, and number of spikelets per spike to Yangmai 20 (YM20) were selected for further self-crossing in 2017. 372, 437 and 208 plants that had similar height, heading, and number of spikelets per spike to Yangmai 20 (YM20) were selected for further self-crossing in 2018 (Fig.1, Table 1). The seeds of the selected individual F_3 plants were sown in different plots, each having three 2.5-m rows with 0.2-m row spacing. About 30 seeds were planted per row. The plants outperformed similar to YM20 in agronomic traits were selected in 2019. In the 2019–2020 cropping season, two trials were set up for F_4 -derived F_5 populations. The trial for type II resistance evaluation had two blocks, each having two rows of 2.0-m in length with 0.2-m row spacing. About 20 seeds were planted per row. The trial for agronomic trait and yield evaluation had three blocks of five 3-m-row plots with a row spacing of 0.2-m, each row with 40 seedlings. A randomized complete block design with two replications was used. In the 2020–2021 cycle, the F_6 populations were sown in Yangzhou (YZ) and Gaoyou (GY) experimental bases of Lixiahe Institute of Agricultural Science, respectively with the same way as the trial for type II resistance evaluation and the trial for agronomic trait and yield evaluation in the 2019–2020 cycle. The field trials were managed in accordance to local practices. A total of 77 wheat varieties from Jiangsu and Hubei provinces were used for testing the effects of *Fhb1*, *QFhb.yaas-2DL* and *QFhb.yaas-3BL* in the type II experiments described above.

2.2. Phenotyping of FHB resistance

SM3 and Annong 8455 (AN8455) were used as the resistant and susceptible controls (CK), respectively, for FHB evaluation. Type II resistance was evaluated for selected lines using the single spikelet infusion method (Hu et al. 2020b). About 10 μ l mixed conidial suspension of *F. graminearum* containing 1,000 spores was injected into a flowering floret at a middle spikelet of a spike. Twenty spikes were inoculated in each plot. Humidity was kept steady by regular mist-irrigation. The number of infected spikelets per spike and the total number of spikelets of every tagged spike were recorded four weeks 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, i.e., highly resistant

($0 < \text{PSS} \leq 25\%$), moderately resistant ($25\% < \text{PSS} \leq 50\%$), moderately susceptible ($50\% < \text{PSS} \leq 75\%$), and highly susceptible ($75\% < \text{PSS} \leq 100\%$) (Li et al. 2016).

2.3. Agronomic trait and yield evaluation

YM20 was used as the control for agronomic trait and yield evaluation. Heading time (HT) and flowering time (FT) were recorded in the field, with HT being the number of days from sowing to heading of more than half of the plants in the plot, and FT being the number of days from sowing to flowering of more than half of the plants in the plot. Plant height (PH), spikelet number per spike (SNS), kernel number per spike (NKS), thousand kernel weight (TKW) and spike number per plant (NSP) of five plants randomly chosen from the middle row of each plot were measured at physiological maturity and the plot means were used in the analysis. PH was the total length of the aboveground part excluding the awn. SNS and NKS were counted from the main spikes. TKW and NSP were measured after oven-drying. TKW was determined by adding values of two samples of 500 kernels. The plants or lines with agronomic traits similar or superior to YM20 were progressed to next field trials. Each plot of F_5 and F_6 sown for yield measurement was manually harvested and naturally dried, and the moisture content and yield were measured, which was converted to yield of 13% moisture content.

2.4. Genotyping

Total genomic DNA was extracted from young leaves of each F_5 plot according to previously reported CTAB procedure (Ma et al. 1994). Polymorphism survey of YM18, ZM9, YM16, NM13 and YM20 were carried out with PCR markers that associated with two major type II FHB resistance QTL in common wheat, including WGRB619 (*Fhb1*), WGRB969 and WMC398 (*Fhb2*) (Zhang et al, 2021b). WSB and SM3 were used as positive control, AN 8455 was used as negative control. PCR was performed in Applied Biosystems™ ProFlex™ 96-Well PCR System (Thermo Fisher Scientific, MA, USA) following the procedure of Ma et al. (1996).

KASP assays were performed in 384-well PCR plates in a 5.0- μL volume with 2.5 μL of 2 $\times$ KASP Reaction Mix, 0.07 μL of assay mix, and 2.43 μL of genomic DNA (30 ng/ μL) (Jiang et al 2019). The PCR conditions were as follows: 94°C for 15 min; 10 touchdown cycles of 94°C for 20 s, 65–57°C for 60 s (decreasing by 0.8°C per cycle); and 32 cycles of 94°C for 20 s, and 55°C for 60 s. Fluorescence detection of the PCR reactions was performed with PHERAstar (BMG LABTECH, Germany). KlusterCaller software (LGC Genomics, Beverly, USA) was used (Jiang et al 2019). The KASP marker was validated in 77 cultivars/lines from Jiangsu and Hubei province.

2.5. KASP marker development and validation

To verify the effects of *QFhb.yaas-2DL* and *QFhb.yaas-3BL* identified in YM16 (Hu et al. 2020a; Zhang et al. 2020), we developed several KASP markers based on the neighboring SNP in the *QFhb.yaas-2DL* and *QFhb.yaas-3BL* intervals. Among them, two KASP markers KASP-QFhb-2DL and KASP-QFhb-3BL had SNP calls identical to their corresponding SNPs AX-110877743 and AX-109001202, which closely linked to the mapped QTL, and segregated best in the YM16/ZM895 RIL population (Hu et al. 2020a; Zhang et al. 2020). The AlleleFAM and AlleleHEX primers of KASP-QFhb-2DL were 5'-GAAGGTGACCAAGTTCATGCTCCAGCTAGGCTCTTCACACT-3' and 5'-GAAGGTCTGGAGTCAACGGATTCCAGCTAGGCTCTTCACACC-3', respectively, and the common primer was 5'-CGAGCCTCCCATATCCGTCT-3'. The AlleleFAM and AlleleHEX primers of KASP-QFhb-3BL were 5'-GAAGGTGACCAAGTTCATGCTCGGTCTCACAATGACATCAACG-3' and 5'-GAAGGTCTGGAGTCAACGGATTCTCGGTCTCACAATGACATCAACA-3', respectively, and the common primer was 5'-CTTGCAAACGATGAAGACGGC-3' (Hu et al. 2020a; Zhu et al. 2021). TaHRC-KASP for *Fhb1* reported by Su et al. (2018) was used in the present study

2.6. Statistical analysis

SPSS software (ver. 22.0) was used to perform analyses of variance (ANOVA), and the least square differences (LSD) method was used for multiple comparisons. The level of significance was $P < 0.05$ for all data analyses.

3. Results

3.1. Detection of the known type II FHB resistance QTL/gene in YM18, ZM9, YM16, and NM13

To find out whether four parents and YM 20 possess the known major type II FHB resistance QTL/genes, they were surveyed with the gene-specific marker of *Fhb1*, and flanking markers of *Fhb2*, using WSB, SM3 and AN 8455 as the references. Target band polymorphism of the *Fhb1* and *Fhb2* markers was revealed between WSB, SM3, YM18, ZM9, YM16, NM13, YM20 and AN 8455, indicating that YM18, NM13 possess *Fhb1*, while ZM9, YM16 and AN8455 do not possess *Fhb1*. Four parents and YM 20 all do not possess *Fhb2* (Fig. 2).

3.2. Validation of KASP markers

The screening results of the effects of *Fhb1*, *QFhb.yaas-2DL* and *QFhb.yaas-3BL* were presented in Table S1. The results showed that the FHB resistance of cultivars/lines carrying *QFhb.yaas-2DL* or *QFhb.yaas-3BL* were significantly ($P < 0.05$) higher than those carrying none of the genes; but the effects of *QFhb.yaas-2DL* or *QFhb.yaas-3BL* were significantly ($P < 0.05$) lower than those of *Fhb1* (Table S1, Fig. 3). The FHB severity of accessions with *QFhb.yaas-3BL* alone was the highest among all QTL/gene

combination, and those harboring *Fhb1*, *QFhb.yaas-2DL* and *QFhb.yaas-3BL* had the lowest average FHB severity (Table S1, Fig. 3).

The validation of KASP markers in the 77 cultivars/lines (6 from Hubei, and 71 from Jiangsu) showed that YM18 carried *Fhb1* alone, NM13 carried *Fhb1* plus *QFhb.yaas-3BL*, ZM9 and YM20 carried *QFhb.yaas-2DL* and *QFhb.yaas-3BL*, SM3 carried *Fhb1* plus *QFhb.yaas-2DL* and *QFhb.yaas-3BL*, whereas AN 8455 did not carry any of the above resistance QTL (Table S1). Nineteen accessions from Jiangsu carried *Fhb1*, while 3 accessions from Hubei and 49 accessions from Jiangsu carried *QFhb.yaas-2DL*, 3 accessions from Hubei and 37 cultivars/lines from Jiangsu carried *QFhb.yaas-3BL*. All accessions from Hubei did not carry *Fhb1*. The genotype result indicated that the KASP markers for *Fhb1*, *QFhb.yaas-2DL* and *QFhb.yaas-3BL* were consistent with previous report (Su et al. 2018; Zhu et al 2018; Zhu et al 2020; Zhu et al 2021)

3.3. Development of lines by agronomic trait evaluation and FHB marker-assisted selection

Based on the results of self-crossing and performance of several traits such as PH, HT, FT, NSP, and seed infection similar or superior to YM20 had been selected and grown continuously since F₂ generation (Fig. 1). 32, 43 and 13 plots of three F₅ populations were conducted for genotyping and investigating agronomic traits and yield, respectively. The genotypic results, FHB resistance status, agronomic characters and yield of three F₅ populations were depicted in Fig. 4 and Table S2–S3.

3.4. FHB resistance with different QTL combinations in three RIL populations

The FHB resistance condition and QTL constitution of the selected F₅ lines were presented in Table S3. For YM18/ZM9 and YM18/YM16 populations, as expected, lines harboring all three QTL had the highest FHB resistance with an average PSS of 6.43% and 7.59%, respectively, which were similar to SM 3 (Table 2, Table S1, S3). The average PSS of the lines carrying *Fhb1* plus *QFhb.yaas-2DL* and *Fhb1* plus *QFhb.yaas-3BL* were both significantly lower ($P < 0.05$) than those carrying *QFhb.yaas-2DL* plus *QFhb.yaas-3BL* in these two populations, respectively. Lines carrying *QFhb.yaas-2DL* alone had the higher FHB severity ($P < 0.05$) than those carrying *QFhb.yaas-3BL* alone in these two populations, respectively. Lines harboring none of the resistance QTL had the highest FHB severity ($P < 0.05$) in these two populations, respectively. For YM18/ZM9 population, lines carrying *Fhb1* alone had significantly higher FHB resistance ($P < 0.05$) than those carrying *QFhb.yaas-2DL* alone or *QFhb.yaas-3BL* alone. For YM18/YM 16 population, lines carrying *Fhb1* alone had similar FHB resistance to those carrying *QFhb.yaas-2DL* alone plus *QFhb.yaas-3BL*, and slightly more resistance than lines carrying *QFhb.yaas-2DL* alone. In YM18/NM13 population, the average PSS of the lines carrying *Fhb1* alone was 29.36%, and lines carrying

Fhb1 plus *QFhb.yaas-3BL* had significantly lower FHB severity with an average PSS of 20.89% ($P < 0.05$). None of the combinations had similar FHB resistance to SM3 in this population ($P < 0.05$) (Table 2).

3.5. The effects of FHB QTL on yield-related traits and yield

The performances of agronomic traits and yield of the selected lines were assessed and exhibited in Table S2–S3. For YM18/ZM9 and YM18/YM16 populations, lines carrying *Fhb1* alone had the lowest NKS ($P < 0.05$). Lines carrying *QFhb.yaas-2DL* alone, *QFhb.yaas-3BL* alone, and *QFhb.yaas-3BL* plus *QFhb.yaas-2DL* had similar NKS, while higher than those carrying none of the resistance QTL, *Fhb1* plus *QFhb.yaas-3BL*, *Fhb1* plus *QFhb.yaas-2DL* and all three resistance QTL. For YM18/ZM9 populations, no significant difference in NSP were observed among the lines carrying different QTL combinations except those carrying none of the resistance QTL and *QFhb.yaas-2DL* alone. Lines carrying *QFhb.yaas-2DL* alone had the highest NSP ($P < 0.05$), while those carrying none of the resistance QTL had the lowest NSP ($P < 0.05$). For YM18/YM16 population, lines carrying *QFhb.yaas-2DL* alone, *QFhb.yaas-3BL* alone and *Fhb1* plus *QFhb.yaas-2DL* had the highest NSP ($P < 0.05$). Lines carrying *Fhb1* plus *QFhb.yaas-3BL*, *QFhb.yaas-3BL* plus *QFhb.yaas-2DL* and all three resistance QTL had similar NSP, while higher than those carrying *Fhb1* alone. Lines carrying none of the resistance QTL had the lowest NSP ($P < 0.05$) compared with others. For YM18/ZM9 population, lines carrying *QFhb.yaas-3BL* alone had the highest TKW ($P < 0.05$) compared to others. Lines carrying *Fhb1* plus *QFhb.yaas-3BL*, *QFhb.yaas-3BL* plus *QFhb.yaas-2DL* and all three resistance QTL had slightly higher TKW than those carrying none of the resistance QTL, *Fhb1* alone, *QFhb.yaas-2DL* alone and *Fhb1* plus *QFhb.yaas-2DL*. For YM18/YM16 population, lines carrying *QFhb.yaas-2DL* alone, *QFhb.yaas-3BL* alone, *Fhb1* plus *QFhb.yaas-3BL*, *QFhb.yaas-3BL* plus *QFhb.yaas-2DL* and all three resistance QTL had similar TKW, and all with the highest TKW ($P < 0.05$) compared to others. Lines carrying *Fhb1* plus *QFhb.yaas-2DL* had slightly higher TKW than those carrying none of the resistance QTL and *Fhb1* alone. For YM18/ZM9 and YM18/YM16 populations, lines carrying none of the resistance QTL and *Fhb1* alone had the lowest yield ($P < 0.05$) compared with others. No significant difference in yield were observed among other lines with different QTL combinations (Table 3–4). For YM18/NM13 population, no significant difference in yield and its related traits was observed among the lines carrying different QTL combinations (Table 5).

3.6. Development of lines with similar FHB resistance to SM3

In F_5 populations, six lines, YM18/ZM9-5, YM18/ZM9-12, YM18/YM16-15, YM18/YM16-18, YM18/YM16-22 and YM18/YM16-24 carrying three resistance QTL, had similar FHB resistance to SM3 (Fig. 5–6). YM18/ZM9-12, YM18/YM16-18 and YM18/YM16-22 showed consistently superior yield (2586.22g/plot–2639.58g/plot) to YM20 (2397.07g/plot–2450.49g/plot) while growing in two environments for evaluating the type II FHB resistance and yield in 2021. The result showed that the three lines had superior yield to YM20 and similar FHB resistance to SM3 in both YZ and GY (Table 6).

4. Discussion

4.1. Utilization of *QFhb.yaas-2DL* and *QFhb.yaas-3BL* in FHB resistance breeding

Fhb1 is a major gene with the highest and most stable effect for FHB resistance in wheat (Su et al. 2019). *Fhb1* has been used in FHB resistance breeding in the USA, Canada, Europe and other countries (Buerstmayr et al. 2019). Some leading cultivars such as Alsen (led in 2002–2006), Glenn (2007–2011), Barlow (2012–2015) and SY Soren (2016) were SM3-derived varieties (Hao et al. 2019). However, SM3 and its derivatives have not been successfully used in China so far because of the difficulty in simultaneous improvement of the FHB resistance and agronomic traits. One single resistant source or gene/locus used in genetic improvement may have reduced genetic diversity and prevented further enhance of yield (Zhang et al. 2021b). YM18 and NM13 had *Fhb1* from NM9, which was derived from a Japanese landrace wheat Xifeng that served as the main source of *Fhb1* in China and is different from SM3. *QFhb.yaas-3BL* from YM16 was identified as a new major FHB resistant QTL for both type II and type I (Hu et al. 2020a; Zhu et al. 2021). *QFhb.yaas-2DL* from YM16 and YM158 was also regarded as a stable FHB resistant QTL for both type II and type I (Hu et al. 2020a; Yan et al. 2021).

Here, a set of 77 wheat cultivars/lines from Jiangsu and Hubei, two provinces that heavily affected by FHB on wheat production, were tested with the KASP markers to assess the occurrence of *QFhb.yaas-2DL* and *QFhb.yaas-3BL*. We also screened *Fhb1* in the panel to rule out the influence of this QTL. *Fhb1* plus *QFhb.yaas-2DL* and *Fhb1* plus *QFhb.yaas-3BL* showed similar effects on FHB resistance, and higher than *Fhb1* alone and *QFhb.yaas-2DL* plus *QFhb.yaas-3BL*. Among the cultivars/lines which carried all three QTL, SM3 showed highest resistant to FHB compared to others. Therefore, we reasoned that SM3 might carry other resistant gene/loci like *Fhb2* for improving its resistance (Zhang et al. 2018; Anderson et al. 2012; 2019).

The genotypes with the combination of the three FHB QTL had significantly higher resistance than those with only *QFhb.yaas-2DL* or *QFhb.yaas-3BL*. The average FHB severity of four materials carrying *Fhb1* alone was slightly lower than the average of genotypes carrying both *QFhb.yaas-2DL* and *QFhb.yaas-3BL*. *QFhb.yaas-2DL* and *QFhb.yaas-3BL* were widespread in Jiangsu and Hubei provinces, accounting for 67.53% and 51.95% of the materials, respectively. *Fhb1* only accounted for 24.68%, and all were derived from NM9. The materials carrying *QFhb.yaas-2DL* plus *QFhb.yaas-3BL* accounted for 36.36%, significantly more than other two combinations (*Fhb1* plus *QFhb.yaas-2DL*, and *Fhb1* plus *QFhb.yaas-3BL*). This indicated that *QFhb.yaas-2DL* and *QFhb.yaas-3BL* from YM16 were frequently selected in local breeding programs, being introduced to many cultivars/lines through phenotypic selection of previous conventional breeding. The KASP markers used in this study can be effectively utilized in MAS for FHB resistance.

4.2. Effects of different QTL combinations on FHB severity

Except *Fhb1* and *Fhb2*, other type II resistance QTL could explain minor or unstable effects on FHB resistance (Zhou et al. 2003; Yang et al. 2005; Ma et al. 2006a; Ma et al. 2006b; Pumphrey et al. 2007). Previous studies had shown that *QFhs.nau-2DL* might have a higher effect on FHB resistance than *Fhb1* in several genetic backgrounds (Agostinelli et al. 2012), and the effect of *Fhb1* plus *QFhs.nau-2DL* was the most resistant compared with that of other combinations (Clark et al. 2016). In this study, the effects of pyramiding three QTL were consistent with the result of 77 cultivars/lines, YM18/ZM9 and YM18/YM16 populations. The results showed that introducing any of these three QTL could significantly reduce the FHB severity. The lines carrying *Fhb1* plus *QFhb.yaas-2DL* and *Fhb1* plus *QFhb.yaas-3BL* had the significantly lower FHB severity than those carrying *QFhb.yaas-2DL* plus *QFhb.yaas-3BL*, which was correspond with the result that the resistance effect of *Fhb1* was the highest among the three resistance QTL. In YM18/NM13 population, *QFhb.yaas-2DL* was absent in the parents and populations and no lines had the same FHB resistance as SM3, indicating that the absence of *QFhb.yaas-2DL* has a significant effect on improvement of FHB resistance in this population. For three RIL populations, among the lines carrying *Fhb1* alone, no lines had the same level of FHB resistance as YM18. We reasoned that YM18 could harbor other undetected resistance genes/QTL besides *Fhb1*.

4.3. Effects of different QTL combinations on yield-related traits and yield

For YM18/ZM9 and YM18/YM16 populations, lines carrying *Fhb1* alone had the lowest NKS, and introducing *Fhb1* could slightly reduce NKS in all QTL combinations, indicating that *Fhb1* had negative effects on NKS. Lines carrying none of the resistance QTL had the lowest NSP in two populations, and introducing any of the resistance QTL might increase NSP, indicating that all these three resistance QTL did not have significant effect on NSP. Lines carrying *QFhb.yaas-3BL* alone had the highest TKW, and lines carrying none of the resistance QTL and *Fhb1* alone both had the lowest TKW. We reasoned that *Fhb1* might do not have significant effect on TKW in two populations, while *QFhb.yaas-3BL* had positive effect on TKW in two populations. *QFhb.yaas-2DL* had positive effects on TKW in YM18/YM16 population, but not in YM18/ZM9 population, indicating that the effects of *QFhb.yaas-2DL* on TKW might depend on the genetic background. Lines carrying none of the resistance QTL and *Fhb1* alone both had the lowest yield compared with others in two populations, indicating that *Fhb1* did not have significant effect on yield, while *QFhb.yaas-3BL* or *QFhb.yaas-2DL* had positive effects on yield in two populations, respectively. Therefore, introducing *QFhb.yaas-3BL* or *QFhb.yaas-2DL* could increase yield. For YM18/NM13, as the selected lines were limited and absence of the lines those carrying none of the resistance QTL, no significant difference in yield and yield-related traits were observed between the lines carrying *Fhb1* alone and *Fhb1* plus *QFhb.yaas-3BL*. No lines had similar or superior yield to YM20 in YM18/NM13 population. The reason probably be that we chose the plants from F₂ to F₄ generations with the similar or superior agronomic traits to YM20 in this study, so as shown in other reports, negative effects of resistance genes/loci on other traits could be overcome by retaining enough genetic variation and directional selection (Balut et al. 2013; Clark et al. 2016; Li et al. 2019c). Furthermore, the results

suggest that it is difficult to obtain lines with target agronomic traits and yield from the breeding populations derived from the parents with close genetic backgrounds.

4.4. Strategy for FHB resistance breeding

The lines or cultivars carrying *Fhb1* or other FHB resistance QTL have been used as resistance donors, and common wheat lines or cultivars as recipients in previous FHB resistance breeding programs (Bernardo et al. 2014). It was reported that FHB resistance achieved in backcrossed lines heavily depended on the resistance level of the recipient cultivar/line (Salameh et al. 2011). In this study, all the parents from the three tested populations, YM18, ZM9, YM16 and NM13, were used as both resistant donors and recipient cultivars. After selection of agronomic traits in early generation, high-generation homozygous lines were evaluated for FHB resistance based on their phenotype and genotype. YM18 and NM13 both had the background of NM9, but ZM9 and YM16 had not. It was noted that the number of selected lines with good agronomic traits and resistance loci were different for the three populations. For example, YM18/YM16 population had the most elite lines, while YM18/NM13 population had the least. The latter had no lines with the same level of FHB resistance as SM3, with only a few elite lines proceed to the next trial. Therefore, it is feasible to improve FHB resistance without reducing yield potential when *Fhb1* plus other resistance locus/genes are introgressed into elite cultivars or lines. On other hand, it is more efficient to improve FHB resistance by single crosses using resistance QTL donors with good agronomic traits and high-yield potential as parents. The lines YM18/ZM9-12, YM18/YM16-18 and YM18/YM16-22 developed in this study that showed similar FHB resistance to SM3 with good agronomic traits could be crossed with each other to develop FHB resistant varieties in the future.

5. Conclusion

It would be very difficult to develop wheat variety with high resistance to FHB by using *Fhb1* genes alone. In addition, two QTL *QFhb.yaas-2DL* and *QFhb.yaas-3BL* identified from YM16 were frequently used in previous breeding programs. Concomitant analysis of QTL effects in three populations showed that *QFhb.yaas-2DL* and *QFhb.yaas-3BL* could play key roles like *Fhb1* in FHB resistance. *Fhb1* might have negative effect on NKS, but not have significant effect on TKW and yield. On the other hand, *QFhb.yaas-3BL* might have positive effect on TKW and yield, and *QFhb.yaas-2DL* might have positive effect on NSP and yield. Pyramiding these two or three QTL show positive effects on yield under different genetic backgrounds. Moreover, three lines YM18/ZM9-12, YM18/YM16-18 and YM18/YM16-22 with high FHB resistance were obtained along with good agronomic traits. This study indicates that having the genetic background of high yield potential and moderate FHB resistance, evaluation of agronomic traits at early generation and pyramiding resistance loci/genes of different sources could be an effective approach for developing FHB resistance varieties.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All datasets used and/or analyzed are available upon request.

Competing interests

The authors declare no competing interests.

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Contributions

W. J. Hu proposed the project concept, carried out the experiments and wrote the paper. L. P. Fu, D. R. Gao and Y. Zhang assisted in performing the experiments and participated in the field trials. L. P. Fu, D. R. Gao and C. M. Zhang participated in the field trials and data collection. All authors read and approved the final manuscript and its publication.

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Tables

Table 1 Population size and the number of plants/plots selected in each generation

	Combination	Generation	No. of plants
Population 1	YM18/ZM9	F ₂	885
		F ₃	372
			No. of plots
		F ₄	105
		F ₅	32
			No. of plants
Population 2	YM18/YM16	F ₂	901
		F ₃	437
			No. of plots
		F ₄	133
		F ₅	43
			No. of plants
Population 3	YM18/NM13	F ₂	752
		F ₃	208
			No. of plots
		F ₄	45
		F ₅	13

YM18 represents Yangmai 18; ZM9 represents Zhenmai 9; YM16 represents Yangmai 16; NM13 represents Ningmai 13.

Table 2 Effects of the QTL combination on FHB severity in the three F₅ RIL populations

	YM18/ZM9	YM18/YM16	YM18/NM13
QTL/QTL combinations ^a	FHB severity ^b (%)	FHB severity ^b (%)	FHB severity ^b (%)
None	66.31a	53.22a	
<i>Fhb1</i>	25.15d	25.23cd	29.36a
<i>QFhb-2DL</i>	31.36c	28.16c	–
<i>QFhb-3BL</i>	47.38b	34.48b	–
<i>Fhb1+QFhb-2DL</i>	17.21e	19.64d	–
<i>Fhb1+QFhb-3BL</i>	19.33e	20.68d	20.89b
<i>QFhb-2DL+QFhb-3BL</i>	28.23c	25.73c	–
<i>Fhb1+QFhb-2DL+QFhb-3BL</i>	6.43f	7.59e	–

^a *QFhb-2DL* indicates *QFhb.yaas-2DL*, *QFhb-3BL* indicates *QFhb.yaas-3BL*, respectively.

^b Different letters following mean values indicate significant differences among genotypes ($P < 0.05$).

YM18 represents Yangmai 18; ZM9 represents Zhenmai 9; YM16 represents Yangmai 16; NM13 represents Ningmai 13.

Table 3 Effects of the QTL combinations on yield-related agronomic traits in YM18/ZM9 population

QTL/QTL combinations ^a	Kernel number per spike ^b	Spike number per plant ^b	Thousand kernel weight ^b (g)	Observed 3m ² -Yield ^b (g/plot)
None	43.16ab	7.64b	40.53b	2052.23b
<i>Fhb1</i>	41.91b	8.20ab	39.65b	2038.10b
<i>QFhb-2DL</i>	45.20a	8.99a	41.50b	2464.07a
<i>QFhb-3BL</i>	44.99a	8.23ab	45.16a	2403.70a
<i>Fhb1+QFhb-2DL</i>	43.32ab	8.23ab	41.34b	2304.33a
<i>Fhb1+QFhb-3BL</i>	42.94ab	8.16ab	42.05ab	2270.63ab
<i>QFhb-2DL+QFhb-3BL</i>	44.76a	8.59ab	42.91ab	2452.61a
<i>Fhb1+QFhb-2DL+QFhb-3BL</i>	42.91ab	8.49ab	42.22ab	2498.22a

^a *QFhb-2DL* indicates *QFhb.yaas-2DL*, *QFhb-3BL* indicates *QFhb.yaas-3BL*, respectively.

^b Different letters following mean values indicate significant differences among genotypes ($P < 0.05$).

Table 4 Effects of the QTL combinations on yield-related agronomic traits in YM18/YM16 population

QTL/QTL combinations ^a	Kernel number per spike ^b	Spike number per plant ^b	Thousand kernel weight ^b (g)	3m ² -Yield ^b (g/plot)
None	42.52ab	6.66c	39.66b	1912.04b
<i>Fhb1</i>	41.93b	7.42bc	39.63b	2020.97b
<i>QFhb-2DL</i>	44.80a	8.90a	43.02a	2497.03a
<i>QFhb-3BL</i>	45.13a	8.59a	45.27a	2498.81a
<i>Fhb1+QFhb-2DL</i>	43.68ab	8.82a	42.33ab	2422.05a
<i>Fhb1+QFhb-3BL</i>	44.03ab	7.86ab	44.23a	2360.17a
<i>QFhb-2DL+QFhb-3BL</i>	44.76a	8.39ab	45.21a	2483.74a
<i>Fhb1+QFhb-2DL+QFhb-3BL</i>	41.95b	8.41ab	43.93a	2529.99a

^a *QFhb-2DL* indicates *QFhb.yaas-2DL*, *QFhb-3BL* indicates *QFhb.yaas-3BL*, respectively.

^b Different letters following mean values indicate significant differences among genotypes ($P < 0.05$).

Table 5 Effects of the QTL combinations on yield-related agronomic traits of YM18/NM13 population

QTL/QTL combinations ^a	Kernel number per spike ^b	Spike number per plant ^b	Thousand kernel weight ^b (g)	3m ² -Yield ^b (g/plot)
<i>Fhb1</i>	42.25a	8.54a	39.97a	2112.14a
<i>Fhb1+QFhb-3BL</i>	43.09a	9.03a	39.88a	2202.21a

^a *QFhb-2DL* indicates *QFhb.yaas-2DL*, *QFhb-3BL* indicates *QFhb.yaas-3BL*, respectively.

^b Different letters following mean values indicate significant differences among genotypes ($P < 0.05$).

Table 6 Evaluation of FHB resistance and yield for the selected three lines

	Name	3m ² - Yield of YZ ^{ab} (g/plot)	3m ² - Yield of GY ^{ab} (g/plot)	Diseased spikelets per spikelet of YZ ^{ab}	FHB severity of YZ ^{ab} (%)	Diseased spikelets per spikelet of GY ^{ab}	FHB severity of GY ^{ab} (%)
CK1	SM3	1498.35c	1586.28c	1.28d	6.74d	1.55d	8.80d
CK2	AN8455	2195.43b	2220.65b	14.67a	68.16a	15.79a	75.26a
CK3	YM20	2450.49b	2397.07b	6.09b	29.99b	6.15b	32.11b
Parent 1	YM18	2396.45b	2444.62b	4.07c	20.16c	3.56c	22.12c
Parent 2	ZM9	2267.84b	2364.73b	6.31b	28.68b	7.11b	29.14b
Parent 3	YM16	2428.63b	2445.68b	4.98b	23.73b	5.97b	25.84b
Parent 4	NM13	2286.94b	2349.23b	5.56b	24.95b	5.41b	26.11b
Population 1	YM18/ZM9-12	2597.34a	2603.29a	1.45d	7.25d	2.04d	9.16d
Population 2	YM18/YM16-18	2608.50a	2639.58a	2.01d	9.12d	2.08d	10.08d
	YM18/YM16-22	2629.16a	2586.22a	1.59d	7.36d	1.95d	9.15d

^a Different letters following mean values indicate significant differences among lines ($P < 0.05$).

^bYZ and GY mean the populations were sown in Yangzhou (YZ) and Gaoyou (GY) experimental base of Lixiahe Institute of Agricultural Science, respectively.

SM3 represents Sumai 3; AN8455 represents Annong 8455; YM20 represents Yangmai 20; YM18 represents Yangmai 18; ZM9 represents Zhenmai 9; YM16 represents Yangmai 16; NM13 represents Ningmai 13.

Figures

Figure 1

Procedure for development of lines with improved FHB resistance. YM18 represents Yangmai 18; ZM9 represents Zhenmai 9; YM16 represents Yangmai 16; NM13 represents Ningmai 13; SM3 represents Sumai 3; YM20 represents Yangmai 20.

Figure 2

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Band patterns of markers linked to *Fhb1* (a) and *Fhb2* (b and c), in WSB, SM3, YM18, ZM9, YM16, NM13, YM20 and AN8455. The target bands are indicated by red arrows. Size marker: DL2000 (M). PCR products were separated in 1% agarose gel (WGRB619) or 8% non-denatured polyacrylamide gels with an acrylamide and bis-acrylamide ratio of 29:1 (WGRB969, WMC398).

YM18 represents Yangmai 18; ZM9 represents Zhenmai 9; YM16 represents Yangmai 16; NM13 represents Ningmai 13; SM3 represents Sumai 3; YM20 represents Yangmai 20; AN8455 represents Annong8455.

Figure 3

Effects of alleles for *Fhb1*, *QFhb-2DL* and *QFhb-3BL* on FHB severity (PSS) in 77 wheat cultivars. *QFhb-2DL* indicates *QFhb.yaas-2DL*, *QFhb-3BL* indicates *QFhb.yaas-3BL*, respectively.

“No.” means Number of cultivars/lines. Different letters above the bars indicate significant differences among genotypes ($P<0.05$).

Figure 4

Kompetitive Allele Specific PCR array in F_5 RIL populations. a: TaHRC-KASP; b: KASP-QFhb-2DL; c: KASP-QFhb-3BL. Dark blue dots represent the resistant homozygous alleles, dark red dots represent the susceptible homozygous alleles, green dots represent the heterozygous alleles, pink dots in (a), (b) and (c) are water controls.

Figure 5

Evaluation of FHB severity for the three selected lines, four parents and CK in two environments (Yangzhou and Gaoyou). Different letters above the bars indicate significant differences among

genotypes in the three population, respectively ($P < 0.05$). PSS represents the average percentage of symptomatic spikelets

Figure 6

The phenotypic response of the spikelets in the lines with similar FHB resistance to SM3. From left to right: AN 8455 (susceptible control), YM18/ZM9-12, YM18/YM16-18, YM18/YM16-22 and SM3 (resistant control). Photos were taken three weeks after single floret inoculation. (a), (b), (c), (d) and (e) show the condition of the inoculation site for AN 8455, YM18/ZM9-12, YM18/YM16-18, YM18/YM16-22 and SM3, respectively. The red arrows indicate the site of the inoculation.

Supplementary Files

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