Journal of Genetics and Genomics 50 (2023) 276–279

Contents lists available at ScienceDirect

Journal of Genetics and Genomics

Journal homepage: www.journals.elsevier.com/journal-of-genetics-and-genomics/

Letter to the editor

Identification and fine-mapping of quantitative trait loci (QTL) conferring rice false smut resistance in rice

Rice false smut (RFS) is a rice fungal disease caused by the biotrophic fungus *Ustilaginoidea virens* (Cooke) Takahashi. The pathogenic fungus specifically infects rice spikelets at the booting stage and coverts the infected spikelets into false smut balls consisting of hyphae and chlamydospores (Ashizawa et al., 2012; Sun et al., 2020). RFS not only causes yield loss of rice, but also produces various kinds of toxins like ustiloxins and ustilaginoidins, which are highly poisonous to human and livestock, posing a threat to food security and human health (Hu et al., 2020; Sun et al., 2022). In recent years, RFS is emerging as a major disease of rice worldwide, mainly due to the cultivation of high-yield hybrid rice (super rice) cultivars, increasing use of nitrogen fertilizers, and climate change (Brooks et al., 2009; Mohiddin et al., 2012; Sun et al., 2020). Currently, most rice cultivars grown in China are susceptible to false smut. Management of the disease mainly depends on chemical fungicides (Liang et al., 2014). However, heavy use of fungicides can cause environmental pollution and facilitate the emergence of fungicide resistance in the pathogenic fungus. Therefore, breeding rice cultivars with a high level of RFS resistance is an effective and environmentally-friendly way to control the disease, which requires identification and isolation of resistance genes or quantitative trait loci (QTL) for RFS in rice accession collection (Ge et al., 2022; Zhao et al., 2022).

Several QTLs conferring resistance to false smut have been identified in recent years. Using a recombinant inbred line (RIL) population derived from a cross between the resistance variety IR28 and the susceptible landrace Daguandao, two major resistance QTLs *qFSR11* and *qFSR12* along with five minor QTLs were identified (Li et al., 2011). Another research used a F_2 population of IR28 and HXZ and identified two QTLs on chromosome 5 (Andargie et al., 2018). In the introgression lines (ILs) derived from a cross between Teqing and Lemont, four major QTLs (*qFSR-6-7*, *qFSR-10-5*, *qFSR-10-2*, and *qFSR-11-2*) and six minor QTLs were detected (Zhou et al., 2014). In a F_2 population derived from the cross of Nanjing11 and CG3, a single QTL was mapped on chromosome 1 (Qiu et al., 2020). Using RILs derived from a cross of a resistant rice landrace MR183-2 with a highly susceptible line 08R2394, five resistance QTLs were detected on chromosomes 2, 4, 8, and 11 (Han et al., 2020). Several QTLs conferring false smut resistance have also been identified through genome-wide association study (GWAS), and 3 resistance loci were identified using a set of 315 rice accessions from the 3K rice database (Long et al., 2020). Another study used 125 lines from the global rice diversity panel to perform GWAS, which identified QTLs on chromosomes 2, 3, 4, 8, 9, and 11 (Hiremath et al., 2021). Most recently, a total of seven QTLs were mapped on rice chromosomes 2, 4, 5, 7, and 9 using a RIL population derived from a cross between a resistant line, RYT2668, and a highly susceptible variety, PR116 (Neelam et al., 2022). Therefore, it appears that QTLs are highly diverse among resistant varieties, which reflects the complexity of RFS resistance.

However, few RFS resistance QTLs have been fine mapped so far and no such single RFS resistance gene has been genetically identified. Fine mapping of RFS resistance genes remains difficult, mainly because the occurrence and severity of RFS are strongly affected by weather conditions during rice heading period (Jia et al., 2015). Therefore, individual plants with different heading dates can easily evade the disease outbreak, showing little or no disease phenotype even if they are highly susceptible. This can lead to false positive results or inconsistency of results in different seasons. Indeed, several previous studies reported that heading date has a significant correlation with disease severity, and the genes and QTLs affecting heading date could be phenotypically identified as RFS resistance QTLs (Zhou et al., 2014; Han et al., 2020). Therefore, the influence of heading date and environmental conditions should be eliminated to achieve reliable results, ensuring accurate mapping of RFS resistance QTLs. In this study, we addressed these difficulties with new disease evaluation and mapping strategies.

Through long-term screening of rice germplasm, we identified 2 resistant varieties of rice, Xiushui47 and Xiushui664. Because of the difficulty of artificial inoculation, we first established a procedure for accurate RFS resistance evaluation, which includes a two-year test in three natural nurseries (Fujian, Linan, and Shaoxing), accompanying with artificial spray and injection inoculation (Fig. S1). Xiushui47 and Xiushui664 showed high resistance to false smut for two consecutive years in which no false smut balls were detected on panicles, while FS159 and Teqing showed high susceptibility to false smut (Fig. S2). We also adjusted Xiushui47's sowing date such so it had a similar heading date as other lines. Artificial injection inoculation showed the same results, no false smut ball grew on the inoculated panicles of Xiushui47, few false smut balls grew on the inoculated panicles of Xiushui664, while FS159 and Teqing showed severe disease phenotype (Fig. S2). To quantitatively evaluate the resistance level of the four parent lines, we calculated the diseased spikelet rate of the lines (Fig. S2).

To find the most effective phenotypic trait to evaluate RFS resistance, we recorded the number of diseased panicles (DP) and the number of diseased spikelets on each panicle of the susceptible parent FS159. We then calculated total disease spikelets (TDS), highest disease spikelets (HDS), and diseased spikelets per diseased panicles (DSPDP). We found that although all the phenotypic traits varied significantly in different years/seasons, DSPDP was relatively more consistent compared with the other phenotypic traits, suggesting that DSPDP is affected less by growth environments, and had a

https://doi.org/10.1016/j.jgg.2022.11.010

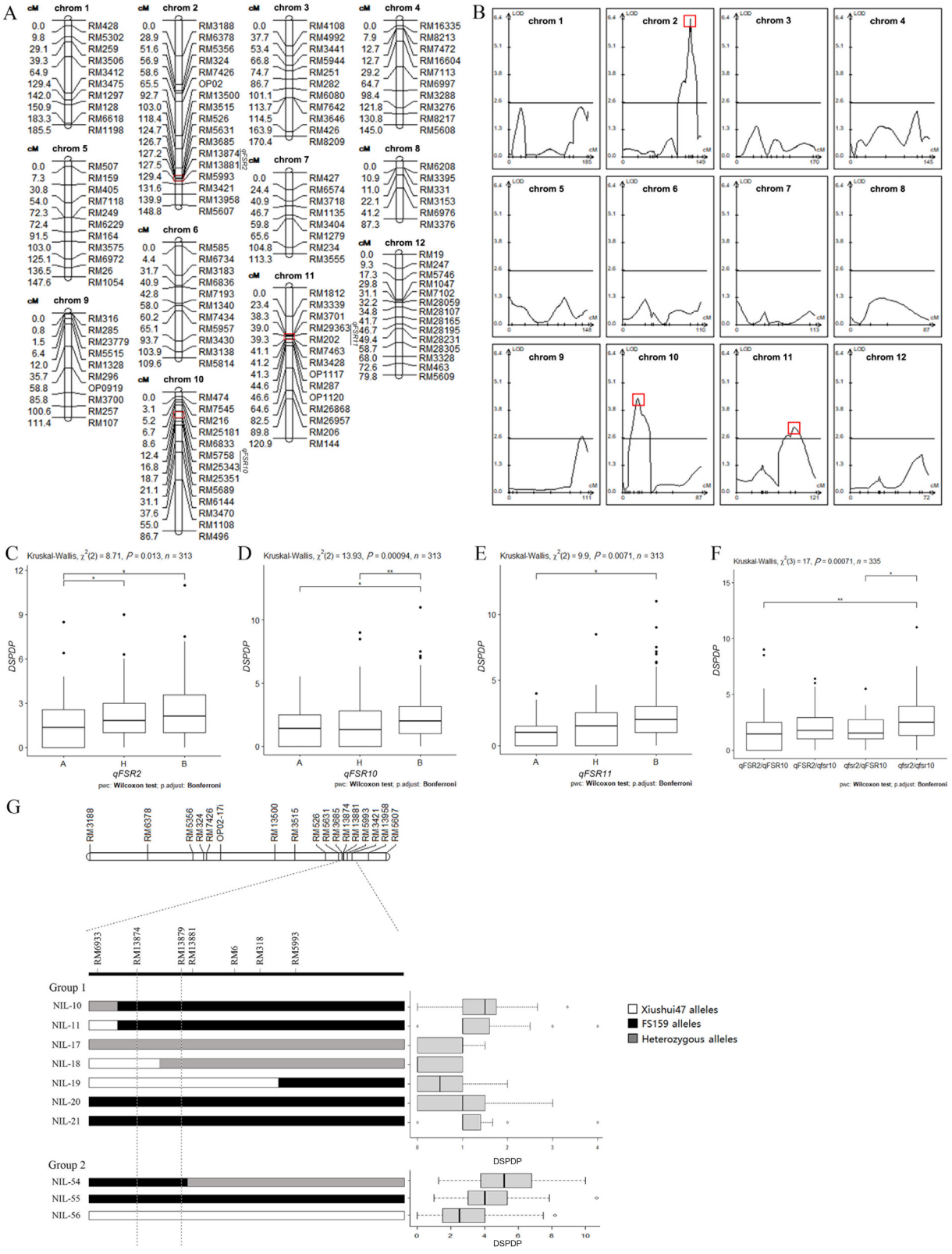

Fig. 1. Four QTLs for false smut resistance were identified on chromosomes 2, 10, and 11 using the BC_1F_2 and F_2 population derived from a hybrid of Xiushui47 and FS159, and *qFSR2* was finely mapped in the 88 kb region between markers RM13874 and RM13879 on chromosome 2. **A**: Linkage map was constructed by 134 SSR markers evenly distributed on 12 chromosomes. The locations of *qFSR2*, *qFSR10*, and *qFSR11* are indicated by red rectangles, corresponding markers are indicated by lines and labels. **B**: Compositive interval mapping of all chromosomes using the phenotypic trait DSPDP. The *qFSR2*, *qFSR10*, and *qFSR11* are indicated by red rectangles. **C**–**F**: Phenotype variance of *qFSR2* (**C**), *qFSR10* (**D**), and *qFSR11* (**E**) were analyzed in a BC_1F_2 population. A represents the allele of resistant parent Xiushui47; B represents the allele of susceptible parent FS159; H represents the hybrid type. **F**: *qFSR2* and *qFSR10* pyramided. **G**: Fine mapping of *qFSR2* by analysis of RFS resistance phenotype in the different NIL populations. White bars represent homozygous chromosomal segments derived from Xiushui47. Black bars represent homozygous chromosomal segments derived from FS159. Grey bars represent heterozygous chromosomal segments. The mapping interval of *qFSR2* is indicated by dashed lines. DSPDP, diseased spikelets per diseased panicles.

smaller deviation than the other three phenotypic traits (Fig. S3). Based on these findings, we used DSPDP as the main phenotypic trait to evaluate rice false smut resistance accurately.

We found that the occurrence and severity of RFS varied greatly under different temperature and weather conditions. To evaluate the environmental effect precisely, we recorded the heading date of each individual in a BC_1F_2 population and the weather, maximum temperature, minimum temperature, and relative humidity in the late booting stage (four days prior to the heading date) (Fig. S4). We found a significant negative correlation ($P < 0.001$) of disease severity with maximum temperature and a significant positive correlation ($P < 0.001$) of disease severity with relative humidity, while no significant correlation between disease severity and minimum temperature was detected. We observed the same results with different phenotypic traits (TDS, HDS, and DP) (Table S1).

To minimize the environmental effect, we planted seedlings at three dates in each season, with intervals of two weeks. The group that the susceptible parent FS159 and Teqing showing the most severe disease phenotype was selected for QTL mapping. Due to the segregation of heading date in mapping populations, we also selected the individuals with similar heading date for QTL mapping to minimize the environment influence. A total of 309 individuals of the F_2 population of Xiushui47/FS159 which had a similar heading date (around September 27, 2020) were genotyped with 134 polymorphic SSR markers to construct the linkage map (Fig. 1A). Four QTLs (designated as *qFSR2*, *qFSR9*, *qFSR10*, and *qFSR11*) were detected on chromosomes 2, 9, 10, and 11 using the phenotypic trait DSPDP (Fig. 1B). The four QTLs, *qFSR2*, *qFSR9*, *qFSR10*, and *qFSR11*, explained 8.8%, 4.5%, 5.3%, and 3.6% of the phenotype variation, respectively. Xiushui47 allele of *qFSR2*, *qFSR10*, and *qFSR11* conferred resistance to RFS, while Xiushui47 allele of *qFSR9* conferred susceptibility to RFS. We further compared the dominance effect and additive effect of those QTLs. *qFSR2* and *qFSR10* had a D/A of 0.76 and 0.29, indicating that they are semi-dominance loci. *qFSR11* had a D/A of 0.1, suggesting an additive locus.

We also used other phenotypic traits to perform QTL mapping. *qFSR2* and *qFSR10* were also detected using TDS and HDS, suggesting that those two QTLs might be major loci conferring consistent resistance to RFS (Table S2). Additionally, we performed QTL mapping using the F_2 population of Xiushui664/Teqing. Two QTLs were mapped on chromosome 2 and chromosome 11, respectively. The QTL on chromosome 2 was close to *qFSR2* and was likely the same locus. The QTL on chromosome 11 was a different locus from *qFSR11*, which we named *qFSR11-2* (Fig. S5). We thus chose *qFSR2* for further fine mapping because it showed the highest LOD and explained the highest percentage of phenotype variation.

We then verified the phenotype of *qFSR2*, *qFSR10*, and *qFSR11* in a BC_1F_2 population of Xiushui47/FS159 in 2021. Nonparametric tests (Kruskal-Wallis test and Wilcoxon test) were used because the phenotypic traits were not normally distributed. All three QTLs showed significant phenotypic difference between the resistance and the susceptible allele (Fig. 1C–1E), showing that these QTLs conferred consistent resistance in different years. We also found that the pyramided lines containing *qFSR2*/*qFSR10* showed higher resistance than that of individual QTL lines, indicating that *qFSR2*/*qFSR10* have additive effects on RFS resistance (Fig. 1F).

For fine mapping of *qFSR2*, 4584 F_5 individuals were screened with 9 SSR markers near *qFSR2*. Twenty recombinant lines with crossover between those markers were screened out of the F_5 individuals. Those recombinant lines were self-crossed twice to generate the F_7 NILs. To avoid the influence of the other two QTLs, lines with susceptible *qFSR10* and *qFSR11* alleles were selected. Ten recombinant lines showed significant disease phenotype. Due to the segregation of heading date, these lines were divided into two groups. Group 1 consisted of seven NILs with similar heading date, and group 2 consisted of three NILs with another heading date. The disease severity of lines in group 1 was moderate, while those in group 2 were severe. In group 1, NIL-10 and NIL-11 were derived from the same F_5 line. Both lines displayed a crossover between the markers RM6933 and RM13874. NIL-17 to NIL-21 were derived from the same F_5 line. NIL-18 displayed a crossover between the markers RM13874 and RM13879, and line NIL-19 displayed a crossover between the markers RM318 and RM5993. In group 2, NIL-54, NIL-55 and NIL-56 were derived from the same F_5 line. NIL-54 displayed a crossover between the markers RM13879 and RM13881 (Fig. 1G). We found that in group 1, NIL-10, NIL-11, NIL-20, and NIL-21 showed the same level of susceptibility, while NIL-17, NIL-18, and NIL-19 were more resistant, indicating that *qFSR2* is located in the downstream of RM13874. In group 2, NIL-54 and NIL-55 were susceptible while NIL-56 was more resistant, indicating that *qFSR2* is located in the upstream of RM13881. Together, these key recombinants suggested that *qFSR2* is likely located between RM13874 and RM13879, in the 88-kb region on chromosome 2 (Fig. 1G).

Genomic sequence analysis revealed several allelic variances in this mapping interval between the resistant and susceptible parents. Further functional identification of the causal gene for *qFSR2* will be conducted independently. We also compared our mapping results with previous reports and found that *qFSR2* and *qFSR10* are newly mapped, and have no overlap with previous results. *qFSR11* is close to the *qFSR11-1* mapped by Li et al. (2011), suggesting that the loci might be the same locus. *qFSR11-2* is close to the *qFSR11-1* mapped by Han et al. (2020), suggesting that they may be another locus.

In conclusion, four RFS resistance QTLs have been identified in this study. We fine-mapped *qFSR2* to an 88-kb interval and screened the candidate genes in this region. This result will essentially facilitate map-based cloning and molecular marker-assisted breeding of RFS resistance rice.

Conflict of interest

The authors declare that they have no conflicts of interests.

Acknowledgments

This work was supported by grants from the Strategic Priority Research Program of Chinese Academy of Sciences (XDA24 010304 and XDB27040201), the National Natural Science Foundation of China (32088102, 31720103913, U20A2021). We thank Professor Jianjun Wang and Professor Xiaoming Zhang from Zhejiang Academy of Agricultural Sciences for helping us in evaluation of disease resistance. We thank Professor Jianjun Wang from Zhejiang Academy of Agricultural sciences and Professor Changdeng Yang from China National Rice Research Institute for providing the rice germplasm. We thank Zhejiang Academy of Agricultural Sciences, Enshi Academy of Agricultural Sciences, and Fujian Academy of Agricultural Sciences for providing the natural nurseries.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgg.2022.11.010.

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7 October 2022
Available online 7 December 2022

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