Identification and fine mapping of qPBR10-1, a novel locus controlling panicle blast resistance in Pigm-containing P/TGMS line

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Received: 7 September 2021 / Accepted: 15 November 2021 / Published online: 30 November 2021 © The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract  Rice blast is one of the most widespread and devastating diseases in rice production. Tremendous success has been achieved in the identification and characterization of genes and quantitative trait loci (QTLs) conferring seedling blast resistance, however, genetic studies on panicle blast resistance have lagged far behind. In this study, two advanced backcross inbred sister lines (MSJ13 and MSJ18) were obtained in the process of introducing Pigm into C134S and showed significant differences in the panicle blast resistance. One F2 population derived from the crossing MSJ13/MSJ18 was used to QTL mapping for panicle blast resistance using genotyping by sequencing (GBS) method. A total of seven QTLs were identified, including a major QTL qPBR10-1 on chromosome 10 that explains 24.21% of phenotypic variance with LOD scores of 6.62. Furthermore, qPBR10-1 was verified using the BC1F2 and BC1F3 population and narrowed to a 60.6-kb region with six candidate genes predicted, including two genes encoding exonuclease family protein, two genes encoding hypothetical protein, and two genes encoding transposon protein. The nucleotide variations and the expression patterns of the candidate genes were identified and analyzed between MSJ13 and MSJ18 through sequence comparison and RT-PCR approach, and results indicated that ORF1 and ORF2 encoding exonuclease family protein might be the causal candidate genes for panicle blast resistance in the qPBR10-1 locus.

Keywords  Panicle blast resistance · Genotyping by sequencing (GBS) · Quantitative trait loci (QTLs) · Fine mapping · Candidate gene prediction

Introduction

Rice blast is one of the most widespread and devastating diseases of rice and causes yield losses between

Supplementary Information  The online version contains supplementary material available at https://doi.org/10.1007/s11032-021-01268-3.

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10 and 30% of rice production worldwide in epidemic years (Skamnioti and Gurr 2009). Domestically, major blast epidemics occurred in China have damaged 5.333 million hectares of paddy rice areas, resulting in more than 1.5 billion kilograms of yield loss in the past decade (He et al. 2014). Development of resistant cultivars by using resistance (R) genes in rice improvement breeding programs is the most economical and environmentally friendly method to combat the disease. However, many resistant cultivars carrying single dominant R genes is generally short-lived due to the dynamic changes in race (pathotype) and composition of the blast pathogen (Dean et al. 2005). Therefore, exploring rice resistance gene resources continuously, identification and utilization of broad-spectrum resistance genes against multiple isolates of Magnaporthe oryzae have been considered to be one of the best options for crop protection and blast management.

To date, more than 100 blast resistance loci or genes in rice have been identified on all rice chromosomes except chromosome 3 (Ashkani et al. 2016; Sharma et al. 2012), of which 28 major R genes and five partial R genes have been cloned and functionally validated (Wu et al. 2021). Among the R genes identified and cloned, many broad-spectrum R genes have been documented and validated, viz., Pi1, Pi2, Pi5, Pi9, Pi33, Pi40, Pi7, Pi-zt, and Pigm (Jeung et al. 2007; Wu et al. 2007), and six of which (Pi2, Pigm, Pi40, Pi9, Pi7, and Pi-zt) were different R gene alleles of the Piz locus located on the short arm near the centromere of rice chromosome 6 (Deng et al. 2006; Qu et al. 2006; Zhou et al. 2006). By BAC sequencing and gene knockout technology, Pi9 was the first cloned gene of the Piz locus, and the lines carrying Pi9 were highly resistant to 43 strains collected from 13 different countries (Qu et al. 2006). Using the same cloning strategy, Zhou et al. (2006) identified Pi2 from the gene cluster composed of nine tandemly arranged nucleotide-binding site and leucine-rich repeat (NBS-LRR) gene members, and studies showed that Pi2 was resistant to most of 455 isolates collected from different regions of the Philippines and the 792 isolates from 13 major rice regions of China (Chen et al. 1996). Due to the high homology in sequence and structure between Piz-t and Pi2, Piz-t was directly cloned using the in silico cloning method, and comparison revealed only 8 amino acid differences within three LRR domains between the Piz-t and Pi2 gene (Qu et al. 2006; Zhou et al. 2006). Especially, the broad-spectrum resistance gene Pigm identified from landrace Gumei 4 (GM4) has been shown to be completely resistant to 50 isolates originating from diverse Chinese and worldwide collections and was used as an excellent resistance resource in blast resistance breeding for more than 30 years in different varieties cultivated on large surfaces (Deng et al. 2017; Cesari and Kroj 2017). Deng et al. (2017) revealed that epigenetic regulation of Pi-gmR and Pi-gmS balances the blast resistance and yield in rice, in which Pi-gmR confer broad-spectrum resistance of GM4 and Pi-gmS increase rice production to counteract the yield lost caused by Pi-gmR.

The broad-spectrum resistance of different multiple alleles of Piz locus and the diversity of their sequence and resistance spectrum suggest that they have great application potential. However, significant differences exist in resistance performance and resistance spectrum of these R genes under different genetic background (Wu et al. 2015), indicating that resistance performance of broad-spectrum resistance genes may require other regulatory factors (Zhou et al. 2019). OsRac1, a small GTPase, associates with and is activated by Pit at the plasma membrane. Once activated, OsRac1 induces reactive oxygen species (ROS) production and hypersensitivity reaction (HR), which contribute to Pit-mediated blast resistance (Kawano et al. 2010). In contrast to OsRac1, the transcription factor OsWRKY45 directly interacts with the coiled-coil (CC) domain of R protein Pb1 at the nucleus to induce quantitative blast resistance (Inoue et al. 2013). Similarly, the homeodomain-containing protein OsBIHD1 physically interacts with Pik-H4 by its CC domain and is required for Pik-H4–mediated resistance through ethylene-brassinosteroid pathway (Liu et al. 2017). Furthermore, Zhai et al. (2019) discover an RNA recognition motif (RRM) class of transcription factor PIBP1s, which directly interacts with the CC domain of PigmR, which could also activate the expression of defense genes OsWAK14 and OspAL1 directly.

Among various disease symptoms caused by M. oryzae, seedling blast and panicle blast are the most common, but panicle blast directly causes yield loss because it infects the top internodes or panicle of rice and results in barren panicles, chalky kernels, and sterile grain (Titone et al. 2015; Wu et al. 2017). However, the time-consuming and cumbersome
nature of inoculating rice panicles with *M. oryzae* has limited the focus of most studies to seedling blast resistance. Presently, only a few of R genes and QTLs (*qPbm11, Pb-bd1, Pi-jnw1, Pb1, Pi64*, and *Pi68*) were confirmed with resistance to panicle blast (Hayashi et al. 2010; Ishihara et al. 2014; Ma et al. 2015; Fang et al. 2019; Wang et al. 2016; Devi et al. 2020). However, the resistance to seedling and panicle blast is often inconsistent, and many varieties with high resistance to seedling blast show susceptible to panicle blast (Xiao et al. 2020). Multi-omics analysis also showed that distinct defense-related gene expression is induced by seedling blast and panicle blast, indicating that the genetic mechanisms of seedling blast and panicle blast resistance might differ and are independently controlled by different R genes (Liu et al. 2016; Yan et al. 2021). However, the current research on the molecular mechanism of rice blast resistance is all related to seedling blast (Li et al. 2017). Therefore, it is of great theoretical and practical value to identify the panicle blast resistance genes and analyze the molecular mechanism of panicle blast resistance regulation.

In our previous research, a set of NILs with six resistance alleles of the Piz locus (*Piz-t, Pi2, Pigm, Pi40, Pi9*, and *Piz*) were constructed with Yangdao 6 and 07GY31 as the recurrent parent, respectively. We also confirmed that *Pigm* had important application potential in breeding practice for conferring broad-spectrum resistance to seedling blast and panicle blast in *Xian* and *Geng* genetic background (Wu et al. 2016, 2017). However, in the process of introducing *Pigm* into C134S, an elite photoperiod and thermo-sensitive male sterile (P/TGMS) line widely used in two-line hybrid rice, two advanced backcross inbred sister lines (MSJ13 and MSJ18, BC2F7) were obtained, and resistance identification showed significant differences in the panicle blast resistance between the sister lines. Thus, we conclude that some of the genetic factors might be involved in the panicle blast resistance difference between the sister lines. In this study, QTL analysis was conducted with *F2* population deriving from the cross between MSJ13 and MSJ18 using GBS method, and a major QTL *qPBR10-1* on chromosome 10 was specifically identified. Additionally, *qPBR10-1* was verified among the BC1F2 and BC1F3 population and fine mapped within a 60.6-kb region between ID338 and K1401 markers, and putative candidate genes predicted underlying mapped QTLs that may be involved in genetic regulation of panicle blast resistance traits in *Pigm*-containing line.

### Materials and methods

#### Plant material and pathogens

MSJ13, an advanced backcross inbred line derived from the process of introducing *Pigm* into C134S, showed high resistance to panicle blast. MSJ18, the sister line of MSJ13, was susceptible to the panicle blast (Additional file 1: Fig. S1). One *F2* population derived from the cross of MSJ13 and MSJ18, was constructed for QTL mapping of panicle blast resistance. One *F2* individual plant harbored the target region of *qPBR10-1* from MSJ13 and without other detected QTLs according the GBS results and was selected to obtain BC1F1 seeds by backcrossing with MSJ18, and then a BC1F1 individual plant with high panicle blast resistance was self-crossed to generate the BC1F2 and BC1F3 population for fine mapping of *qPBR10-1*. The donor GM4 and receptor C134S were used as the resistant and susceptible control, respectively.

A total of 80 isolates of *M. oryzae*, including the highly pathogenic differential isolate 85-14, were collected from Hainan, Guangdong, Guangxi, Zhejiang, Jiangxi, Hunan, Hubei, Anhui, and Sichuan provinces of China in 2010–2014 and were employed in this study (Additional file 1: Table S1). Single spore isolation, strain cultivation, and inoculum preparation were conducted as reported by Puri et al. (2009).

#### Inoculation and disease evaluation

Four-leaf stage of rice seedlings of GM4, MSJ13, and MSJ18, and C134S was inoculated with a set of 80 *M. oryzae* isolates spore suspension (5×10⁴ conidia/ml) in inoculation chambers, respectively, as the method described by Wu et al. (2016). After inoculation, the plants were incubated for 24 h in the dark in growth chambers maintained at 26 °C with relative humidity 95% and then transferred to a greenhouse under a 12-h light/12-h dark photocyte at 90%
relative humidity by intermittent spraying with water. After 7 days of inoculation, lesion scores of 0 to 5 were recorded according to the standard procedures (Mackill and Bonman 1992), where lines with scores of 0 to 2 were considered resistant (R) and 3 to 5 were considered as susceptible (S). Resistance was represented by resistance frequency (RF) as defined by Wu et al. (2016).

To evaluate the panicle blast resistance in the field, the GM4, MSJ13, MSJ18, and C134S, F2 individual plants, the homozygous recombinant lines advanced from recombinant individual plants, were inoculated with the isolate 85-14 by injecting method as described by Wu et al. (2017). For F2 population, 1000 F2 individual plants were transplanted into a paddy field as 50 rows and 20 plants per row with row spacing 13.3 cm × 25 cm. A total of 316 individual plants in the same developmental stage were selected for pathogenicity assay, and three panicles for each F2 individual plants were injected by 1 ml blast isolate 85-14 conidial suspension (5×10⁴ conidia/ml) for QTL mapping. For homozygous recombinant lines, plants were transplanted into a paddy field and each plot contained 6 rows and 10 plants per row with row spacing 13.3 cm × 25 cm. Fifteen booting panicles of each homozygous recombinant lines and the parents were injected with the isolate 85-14 for fine mapping. Diseased grain rates were evaluated based on visual assessment of disease severity 3 weeks after inoculation as described by Wang et al. (2016). The scores were ranged from 0 (without diseased grain) to 100% (100% diseased grains), where lines with scores of 0–40% were considered resistant (R) and 40.1–100% were considered to be susceptible (S).

GBS library construction and SNP identification

Genomic DNA of parents and 109 F2 individual plants (55 resistant plants and 54 susceptible plants) were extracted from 100 mg of leaf tissue using DNAsecure Plant Kit reagents (Qiagen). The quality of extracted genomic DNA was measured using BioPhotometer plus (Eppendorf). The GBS library was constructed as previously described by Poland et al. (2012). In brief, DNA samples were digested with restriction enzymes BamHI and MspI, and sequencing libraries were constructed using an Illumina HiSeq 2000 Sequencer (Illumina). The raw Illumina DNA sequence data (FASTQ file) were processed through the GBS analysis pipeline as implemented in TASSEL v3.0 software (Bradbury et al. 2007). The raw reads of 109 F2 individuals and their parents were sorted according to indices, and the high-quality SNPs between parents were called by alignment with Nipponbare reference genome MSU release 7 (Kawahara et al. 2013) using BWA package (Li and Durbin 2009). SNPs with read depth less than five and missing data>50 % were filtered out. Only the SNPs that were homozygous in either parent and polymorphic between the parents were prepared for further QTL analysis (Qin et al. 2018).

Genetic map construction and QTL analysis

A high-density genetic linkage map for the F2 population was constructed using inclusive composite interval mapping (ICIM) implemented in software IciMapping v4.0 (http://www.isbreeding.net/) (Meng et al. 2015). Recombination fractions were converted into centiMorgans (cM) using the Kosambi function, and the linkage map and SNP marker order were determined with a greedy algorithm. Location of QTL was described according to logarithm of odds (LOD) value, and the software was set LOD > 2.5 as a threshold which must be operated 1000 times at the p< 0.05 level. The contribution rate (PVE) was estimated as the percentage of variance explained by each QTL in proportion to the total phenotypic variance. Additive effect was estimated to find the positive or negative effect on the target trait (Qin et al. 2018).

Validation and fine mapping of qPBR10-1

Thirty simple sequence repeat (SSR) markers located in 1-Mb region including the marker interval of qPBR10-1 were selected and identified for their genetic polymorphisms between two parents, MSJ13 and MSJ18. Four markers (RM25375, RM25384, RM25387, and RM5806) showed polymorphisms between two parents. For fine mapping qPBR10-1, insertion-deletion (InDel) and competitive allele specific PCR (KASP) markers within delimited interval were designed (Additional file 2: Table S2). A linkage map of 1682 individuals from the BC1F2 population analyzed with four SSR markers and two InDel markers on chromosome 10 was constructed to ensure the presence of the major QTL qPBR10-1. A total of
3568 BC1F3 individuals were used to screen recombinants between ID1846 and ID4789. In total, five types of recombinants were identified. Twenty progenies of each recombinant were planted and screened for homozygous plants from each group. These homozygous recombinant lines (BC1F3, BC1F4) were tested for panicle blast resistance in 2019 and 2020. The average diseased grain rates value was used for fine mapping.

Prediction and expression analysis of candidate genes

Open reading frames in the target region of markers ID338 and K1401 on chromosome 10 were predicted by GENSCAN (http://genes.mit.edu), FGENESH (http://linux1.softberry.com/), and RiceGAAS (http://rgp.dna.affrc.go.jp) software. The DNA and full-length cDNA of four predicted genes from two parents were amplified by PCR methods and sequenced (General Biol, Anhui). The primers for gene amplification and sequence are shown in Additional file 2: Table S2.

The immature panicles of MSJ13 and MSJ18 inoculated by blast isolate 85-14 were cut off and collected at 0 h, 24 h, 48 h, 72 h, and 96 h after inoculation for the expression analysis of candidate genes as described by Fang et al. (2019). The expressions of four functional candidate genes were detected by real-time PCR methods as described by Huang et al. (2008). The rice housekeeping gene OsActin (LOC_Os03g50885) was used as an internal control.

The seedling blast resistance phenotypic and expression data were analyzed using Statistical Analysis System (SAS) software (Cary, NC, USA) and compared with Student’s t-test at the 5% and 1% levels of probability. Multiple comparisons were used to reveal differences among the tested lines for panicle blast disease severity, where p < 0.001 was used to measure statistical significance.

Results

The broad-spectrum resistance gene Pigm was successfully introduced into MSJ13 and MSJ18

MSJ13 and MSJ18 were obtained by successive backcrossing and inbreeding with introducing the broad-spectrum resistance gene Pigm into C134S, an elite P/TGMS line widely used in two-line hybrid rice, through molecular marker–assisted selection in 2013. Molecular marker detection showed that Pigm was harbored in MSJ13 and MSJ18 (Fig. 1a). We also

![Figure 1](image_url)

**Figure 1** The broad-spectrum resistance gene *Pigm* was successfully introduced in MSJ13 and MSJ18. a Molecular marker detection verified *Pigm* was harbored in MSJ13 and MSJ18. b Expression of *Pigm* detected in different tissues between MSJ13 and MSJ18 by RT-PCR. c Resistance frequency of test lines for seedling blast resistance. d Seedling blast resistance performance of test lines against the differential isolate 85-14. ** indicates significant differences between test lines at 1% levels, n.s. indicates no significant difference.
found that *Pigm* displayed constitutive expression in all tissues and showed equivalent expression patterns between MSJ13 and MSJ18 (Fig. 1b). A total of 80 isolates were collected from different ecological regions in southern China to evaluate seedling blast resistance of MSJ13 and MSJ18. The results showed that MSJ13 and MSJ18 exhibited an equivalent resistance RF with the donor parent GM4, with RFs of more than 97.50%, and significantly increased seedling blast resistance compared with the recurrent parent C134S (Fig. 1c, d), indicating that the broad-spectrum resistance gene *Pigm* was successfully introduced and could be normally expressed in MSJ13 and MSJ18.

MSJ13 and MSJ18 exhibited significant resistant difference in panicle blast resistance

However, in the process of breeding practice, we have noticed that MSJ13 and MSJ18 presented significant difference in panicle blast resistance inoculated with mixed isolates of *M. oryzae* in 2013–2015 (Additional file 3: Table S3). Then, the panicle blast resistance reaction of the experimental lines was evaluated through inoculating with five representative isolates of *M. oryzae* individually and mixed isolates. The results showed that the panicle blast resistance performance of MSJ13 was consistent with the donor parent GM4 and exhibited resistance reaction against the five isolates and mixed isolates. However, MSJ18 only showed to be resistant to AX3-2 and GD1-6, but susceptible to 85-14, R5-1, WS8-3, and the mixed isolates (Fig. 2A). Specifically, MSJ18 also presented a more serious panicle blast phenotype compared with GM4 and MSJ13 against the differential isolate 85-14 and other isolates of *M. oryzae* (Fig. 2A, B). Therefore, we conclude that some of the genetic factors might be involved in the panicle blast resistance difference between MSJ13 and MSJ18.

### GBS-SNP identification and map construction

In order to identify the genetic factors, one F2 population was constructed from an intra-specific cross of the sister lines (MSJ13×MSJ18), and 316 individual plants in the same developmental stage were inoculated with the isolate 85-14. After phenotypic identification, 55 highly resistant individual plants and 54 extremely susceptible individual plants were selected to construct the GBS library with MSJ13 and MSJ18. The GBS data of 109 F2 individuals and their parents were sorted according to indices, and the high-quality SNPs between parents were called by alignment with Nipponbare reference genome MSU release 7 using BWA package, and a total of 9692 SNP molecular markers were obtained. Of these, 6745 SNPs observed with the low coverage sequencing and missing data>50% in the F2 population were filtered out. Finally, 2947 high-quality SNPs that are homozygous for each parent and show polymorphism between the parents were developed for further QTL-seq analysis.

![Figure 2](image_url)

**Figure 2** Significant differences in the panicle blast resistance against blast isolates were observed between MSJ13 and MSJ18. A Diseased grain rate of test lines for panicle blast resistance. B Panicle blast resistance performance of MSJ13 and MSJ18 against the differential isolate 85-14. Entries with different letters were statistically significantly different at *p* < 0.001 level.
With the advent of GBS-SNP data, a high-density linkage map was constructed (Fig. 3a). The total length of the linkage map was 1985.49 cM with Chr.4 (338.62 cM) being the largest and Chr.2 (82.72 cM) being the smallest. The number of markers per linkage group varied from 72 (Chr.2) to 435 (Chr.5), with an average of 245.58 markers per linkage group. The average marker density was 1.48/cM with Chr.5 being the most dense (2.09/cM) and Chr.8 being the least (0.68/cM). A summary of the constructed genetic map is presented in Table 1.

![Figure 3](https://example.com/figure3.png)

**Figure 3** Mapping of QTLs controlling panicle blast resistance in F₂ population derived from MSJ13 X MSJ18 using GBS method and the LOD scores of qPBR10-1. a Genetic linkage map of rice constructed using the F₂ population derived from the parental lines MSJ13 X MSJ18. b LOD scores of qPBR10-1 controlling panicle blast resistance in F₂ population

**Table 1** Summary of SNP markers characteristics in the F₂ population

| Chromosome | No. of SNP markers used | Chromosome length coverage (bp) | Genetic length (cM) | No. of SNP markers/cM | Minimum interval (cM) | Maximum interval (cM) |
|------------|-------------------------|---------------------------------|--------------------|-----------------------|-----------------------|-----------------------|
| 1          | 287                     | 43,237,333                      | 149.24             | 1.92                  | 0.22                  | 9.98                  |
| 2          | 72                      | 35,875,736                      | 82.72              | 0.87                  | 0.27                  | 7.19                  |
| 3          | 276                     | 36,405,799                      | 187.36             | 1.47                  | 0.28                  | 8.01                  |
| 4          | 428                     | 35,501,387                      | 338.62             | 1.26                  | 0.27                  | 6.88                  |
| 5          | 435                     | 29,507,277                      | 208.47             | 2.09                  | 0.24                  | 4.35                  |
| 6          | 306                     | 30,869,147                      | 169.32             | 1.81                  | 0.27                  | 13.09                 |
| 7          | 189                     | 29,582,943                      | 152.12             | 1.24                  | 0.21                  | 8.7                   |
| 8          | 73                      | 28,399,689                      | 107.38             | 0.68                  | 0.27                  | 10.57                 |
| 9          | 217                     | 22,779,506                      | 121.54             | 1.79                  | 0.25                  | 6.49                  |
| 10         | 346                     | 23,117,196                      | 191.35             | 1.81                  | 0.28                  | 11.55                 |
| 11         | 145                     | 28,973,227                      | 158.64             | 0.91                  | 0.28                  | 5.33                  |
| 12         | 173                     | 27,488,377                      | 118.73             | 1.46                  | 0.27                  | 6.75                  |
| Total      | 2947                    | 371,737,617                     | 1985.49            | -                     | -                     | -                     |
| Average    | 245.58                  | 30,978,134.75                  | 165.46             | 1.48                  | 0.27                  | 8.24                  |
QTL mapping for panicle blast resistance trait

With the help of ICIM method implemented in software IciMapping v4.0, seven QTLs significantly related to panicle blast resistance were detected in the F2 segregation population, which were located on chromosomes 1, 2, 3, 9, and 10, respectively, and explained 7.88–24.21% of phenotypic variation. Especially, a major QTL was detected between marker interval Chr10P13890948-Chr10P14272420 on chromosome 10, designated as qPBR10-1. It could explain 24.21% of phenotypic variance with LOD scores of 6.62 (Fig. 3b; Table 2) and was subsequently verified and identified.

Fine mapping of qPBR10-1

According to the QTL mapping results, qPBR10-1 was preliminarily mapped in the region near intermediate location of the long arm of rice chromosome 10 (Fig. 4a). To validate the major QTL qPBR10-1, the target mapping interval was further amplified to 1Mb including the marker interval of Chr10P13890948-Chr10P14272420, and polymorphic markers were designed and selected in this target region (Additional file 2: Table S2). In 1682 BC1F2 segregated population, 3, 2, 0, 4, and 5 recombinants were identified by the markers RM25375, RM25384, RM25387, ID1846, ID4789, and RM5806, respectively. The results showed that qPBR10-1 could be mapped in the region of markers ID1846 and ID4789 (Fig. 4b).

A large BC1F3 population consisting of 3568 individuals was developed to narrow the region of qPBR10-1. Twenty-one recombinants were identified between ID1846 and ID4789 markers, including 3, 0, 3, 4, 0, and 11 recombinants identified by markers ID1846, K394, ID338, K1401, K1407, ID1286, and ID4789, respectively (Fig. 4c). Based on the genotypes, these 21 recombinants were classified into five groups. For each group, we selected the homozygous recombinant lines advanced from recombinant individual plants. These five groups of homozygous recombinant lines were inoculated with blast isolate 85-14 to evaluate their panicle blast resistance phenotypes in 2019 and 2020. The qPBR10-1 was finally narrowed in the 60.6-kb region between markers ID338 and K1401.

Candidate gene prediction and their expression

According to the MSU Rice Genome Annotation Project Database (http://rice.plantbiology.msu.edu), six open reading frames (ORFs) were predicted within the 60.6-kb region located in the qPBR10-1 locus (Fig. 4d). Six ORFs with functional annotation showed that ORF1 and ORF2 (LOC_Os10g26720 and LOC_Os10g26730) encode exonuclease family protein, ORF3 and ORF4 (LOC_Os10g26740 and LOC_Os10g26750) encode hypothetical protein, and ORF5 and ORF6 (LOC_Os10g26760 and LOC_Os10g26770) encode En/Spm sub-class transposon protein. Except for ORF5 and ORF6, the genomic sequences of four candidate genes were compared between two parents MSJ13 and MSJ18; ORF1, ORF2, and ORF4 showed differences; and ORF3 presented no difference (Table 3).

The expression patterns of four functional candidate genes in MSJ13 and MSJ18 were detected in immature panicles inoculated with the isolate 85-14 using real-time PCR approach. ORF1 was highly expressed and slightly downregulated and reached the bottom at 72 h in MSJ13, while ORF1 displayed constitutive low-level expression in MSJ18 and dramatically downregulated and reached the bottom at 72 h in MSJ18 (Fig. 5a). The relative expression level of ORF2 also showed significant difference between

| Table 2 | Identification of QTLs for traits related to panicle blast resistance |
|---|---|---|---|---|---|
| QTLs | Chr. | Pos. (cM) | Marker interval | LOD | PVE (%) | Add |
| qPBR1-1 | 1 | 18.34 | Chr1P2580906-Chr1P2986432 | 2.92 | 9.38 | −0.12 |
| qPBR1-2 | 1 | 69.78 | Chr1P14582463-Chr1P16286739 | 3.23 | 11.24 | −3.64 |
| qPBR2-1 | 2 | 81.67 | Chr2P35028269-Chr2P35163637 | 3.42 | 12.35 | −0.35 |
| qPBR3-1 | 3 | 15.92 | Chr3P3670546-Chr3P4786854 | 2.71 | 7.88 | 0.78 |
| qPBR3-2 | 3 | 87.38 | Chr3P18244638-Chr3P20284683 | 3.87 | 10.26 | −1.89 |
| qPBR9-1 | 9 | 37.43 | Chr3P8671284-Chr3P11642988 | 4.16 | 11.36 | −2.37 |
| qPBR10-1 | 10 | 150.65 | Chr10P13890948-Chr10P14272420 | 6.62 | 24.21 | −3.21 |
MSJ13 and MSJ18. The expression of ORF2 was slightly induced and reached the peak at 48 h and then decreased to the normal level in MSJ13, while ORF2 was slightly downregulated and reached the bottom at 48 h in MSJ18 (Fig. 5b). The expressions of ORF3 and ORF4 were not prominently induced and were smooth over time in MSJ13 and MSJ18 (Fig. 5c, d). Taken together with genomic sequence comparison and expression patterns, it indicated that ORF1 and ORF2 encoding exonuclease family protein might be the causal candidate genes for panicle blast resistance in the qPBR10-1 locus.

**Table 3** Predicted candidate genes of qPBR10-1

| Number | Annotated genes | Position       | Protein length (AA) | Putative protein function                      | Sequence difference between two parents |
|--------|-----------------|----------------|---------------------|------------------------------------------------|----------------------------------------|
| ORF1   | LOC_Os10g26720  | 13,945,597–13,952,212 | 436            | Exonuclease family protein, putative, expressed | Yes                                    |
| ORF2   | LOC_Os10g26730  | 13,954,485–13,958,089 | 493            | Exonuclease family protein, putative, expressed | Yes                                    |
| ORF3   | LOC_Os10g26740  | 13,965,403–13,966,443 | 56             | Hypothetical protein, expressed protein         | No                                     |
| ORF4   | LOC_Os10g26750  | 13,978,470–13,979,462 | 117            | Hypothetical protein, expressed protein         | Yes                                    |

**Figure 4** Fine mapping of qPBR10-1. a qPBR10-1 was preliminary located in the region near intermediate location of the long arm of rice chromosome 10. b Fourteen recombinants were screened from 1682 BC$_1$F$_2$, and qPBR10-1 was located between markers ID1846 and ID4789, numbers below the ledge and are the recombination events in the mapping populations. c Twenty-one recombinants were screened from 3568 BC$_1$F$_3$ population, and qPBR10-1 was finally narrowed in the 60.6-kb region between markers ID338 and K1401; numbers below the ledge are the recombination events in the mapping populations. d Six candidate ORFs were predicted and the arrows represent the direction of ORFs.
Discussion

Blast disease caused by *M. oryzae* mainly occurs in two forms: seedling blast and panicle blast. Because it takes longer to study the panicle blast resistance and it is troubled in evaluation of panicle blast, most of the research on blast resistance in rice has focused on the seedling blast resistance. However, panicle blast is the major cause of yield loss, and the research on panicle blast resistance has been receiving increasing attention in recent years. Following with the deepening of seedling and panicle blast resistance, the researchers found that some of the seedling blast–susceptible varieties have shown the resistance to panicle blast, and the other seedling blast–resistant cultivars become susceptible to panicle blast (Manojkumar et al. 2020; Puri et al. 2009). In this study, we also observed that GM4 and MSJ13 showed outstanding performance in seedling blast resistance and also exhibited effective panicle blast resistance against five individual isolates and mixed isolates. However, MSJ18, with seedling blast RFs of more than 97.50%, only showed panicle blast resistance to AX3-2 and GD1-6, but susceptible to 85-14, R5-1, WS8-3, and the mixed isolates (Fig. 2A, B). Presently, the mismatch in results on seedling and panicle blast resistance could be explained in two aspects. The one is organ-specific R gene expression at different developmental stages that might affect the resistance difference between seedling blast and panicle blast. For example, *Pi9* is highly resistant to seedling blast, but is susceptible to panicle blast, and the RT-PCR results showed that the relative expression level of *Pi9* in panicles was only 47.3% as compared to that in seedling leaves (Liu et al. 2021). Similarly, the blast resistance gene *Pbl* exhibited excellent panicle blast but was susceptible to seedling blast due to lower gene expression in vegetative growth stage compared with reproductive growth stage (Hayashi et al. 2010). Meanwhile, the *Pi64* gene with similar high expression at seedling and heading stages showed effective resistance to both seedling blast and panicle blast (Ma et al. 2015). The other is the genetic mechanisms of seedling blast and panicle blast resistance that might differ in distinct defense pathway. *OsGF14b*, a quantitatively blast resistance gene in rice, plays opposite roles in seedling blast and panicle blast resistance and appears to positively regulate the expression of genes involved in the auxin and jasmonic acid (JA) signaling pathway, accompanied by the reprogramming of the phenylpropanoid and diterpenoid pathways, but negatively regulate the expression of genes involved in the salicylic acid (SA) signaling pathway (Liu et al. 2016; Yan et al. 2021). Transcriptome analysis also identified that a large number of genes were upregulated to be involved in detoxification, cell wall synthesis, and modification pathway in panicle blast resistance, which were rarely reported in seedling blast resistance (Kumar et al. 2021).

*Pigm* is known as a durable blast resistance gene and was used as an excellent resistance resource in blast resistance breeding for more than 30 years in different varieties cultivated on large surfaces (Cesari and Kroj 2017). Introduction of *Pigm* into elite rice varieties by marker selection breeding has been actively and successfully carried out (Dai et al. 2018; Tian et al. 2016). Traditionally, a functional gene region was introduced into a genomic fragment, so the gene could present a large additive effect in breeding. However, great interaction effect
with genome background causing differential phenotypic effects was observed (Wu et al. 2015; Cao et al. 2007). In this study, MSJ18, an advanced backcross inbred line derived from the process of introducing Pigm into C134S, despite possessing Pigm, do not exert panicle blast resistance as its sister line MSJ13. Then, we attempted to solve the conundrum of the absence of panicle blast resistance in the MSJ18 line. Genetic analysis of MSJ13 and MSJ18 revealed that seven QTLs were involved in panicle blast resistance on five rice chromosomes. Among them, one QTL (qPBR3-1) negatively affected the resistance, despite the presence of Pigm. Six QTLs (qPBR1-1, qPBR1-2, qPBR2-1, qPBR3-2, qPBR9-1, qPBR10-1), on the other hand, had a positive influence on Pigm-mediated panicle blast resistance (Table 2), so it is expected that these QTLs will specifically change Pigm-mediated resistance individually or in combination with others in the C134S genetic background.

Here, we focused on the major QTL qPBR10-1, which could explain 24.21% of phenotypic variance with LOD scores of 6.62. At last, qPBR10-1 was mapped in a region of 60.6 kb between markers ID338 and K1401, and there were six candidate genes predicted. Among these six candidate genes, ORF1 and ORF2 encode exonuclease family protein, ORF3 and ORF4 encode hypothetical protein, and ORF5 and ORF6 encode transposon protein. As reported previously, exonuclease family protein is a member of the Rad2 family of exonucleases and possesses 5→3 double-stranded DNA exonuclease and flap endonuclease activities (Tran et al. 2004). It is known to be involved in multiple pathways for DNA metabolism and repair, including mismatch repair, mitotic and meiotic recombination, Okazaki fragment maturation, response to UV damage, and telomere processing and maintenance (Goellner et al. 2015; Hu et al. 2016). Literature reports indicated that exonuclease family protein is involved in the innate immune response in virus and human, such as the exonuclease domain of the Lassa virus nucleoprotein which is critical to avoid RIG-I signaling and to inhibit the innate immune response (Reynard et al. 2014). TREX1, an exonuclease homolog in human, mutations in TREX1 gene cause a spectrum of autoimmune disorders, including Aicardi-Goutieres syndrome, familial chilblain lupus, and retinal vasculopathy with cerebral leukodystrophy, and are associated with systemic lupus erythematosus in human (Grieves et al. 2015; Gunther et al. 2015; Lee-Kirsch et al. 2007; Richards et al. 2007). However, the report of the exonuclease family protein involved in the innate immune response in plants was limited. In this study, genomic sequence comparison and expression profiles of the candidate genes showed that differences in genomic sequences and differential expression of ORF1 and ORF2 between MSJ13 and MSJ18 implied that ORF1 and ORF2 encoding exonuclease family protein might be the causal candidate genes for panicle blast resistance in the qPBR10-1 locus. In our future research, we will further validate which candidate gene participates individually or in combination with each other to regulate Pigm-mediated panicle blast resistance through CRISPR/Cas9 and transgenic complementary methods.

**Author contribution** YW and AL conceived and designed the experiments; NX, YL, QG, YN, LY, CP, XZ, NH, CZ, HJ, JL, WS, and ZC performed the experiments; YN and CL analyzed the data; YW and AL wrote the paper. All the authors read and approved the final manuscript.

**Funding** This work was supported by the National Natural Science Foundation of China (31801342, 31971868), the Natural Science Foundation of Jiangsu Province, China (BK20181216); the Key Studying and Developing Project of Jiangsu Province for Modern Agriculture (BE2020318, BE2019339-3); Rice Industry Technology System of Yangzhou Comprehensive Experimental Station, Yangzhou, Jiangsu Province, China (CARS-01-60); and the Key Studying and Developing Project of Yangzhou for Modern Agriculture (YZ2019035).

**Declarations**

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

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