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

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Abstract

Background

Rice blast is one of the most widespread and devastating diseases in rice production. Tremendous success has been achieved in 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.

Results

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 F_2 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 7 QTLs were identified, including a major QTL *qPBR10-1* on chromosome 10 that explaining 24.21% of phenotypic variance with LOD scores of 6.62. Furthermore, *qPBR10-1* was verified via the BC_1F_2 and BC_1F_3 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.

Conclusions

A total of 7 QTLs conferring panicle blast resistance was identified from one F_2 population derived from the crossing between two advanced backcross inbred sister lines MSJ13 and MSJ18, which harbored the broad-spectrum resistance gene *Pigm*. A major QTL *qPBR10-1* was fine mapped in a 60.6-kb region with six candidate genes predicted, and *ORF1* and *ORF2* encoding exonuclease family protein might be the causal candidate genes for panicle blast resistance in the *qPBR10-1* locus through sequence comparison and RT-PCR approach.

Background

Rice blast is one of the most widespread and devastating diseases of rice and causes yield losses between 10 and 30% of rice production worldwide in epidemic years (Skamnioti and Gurr 2009). Domestically, major blast epidemics occurred in China has 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 of resistance (R) genes in rice improvement breeding

programms are 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 *M. 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 5 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*, *Piz*, *Piz-t*, *Pigm*, and others (Jeung et al. 2007; Wu et al. 2007), and six of which (*Pi2*, *Pigm*, *Pi40*, *Pi9*, *Piz* and *Piz-t*) 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 9 tandemly arranged NBS-LRR gene members, and studies showed that that *Pi2* was resistant to most of 455 isolates collected from different regions of 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 that 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* balance 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 suggesting 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), indicated 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 plasmalemma. Once activated, OsRac1 induces ROS production and HR, which contribute to Pit-mediated blast resistance (Kawano et al. 2010). In contrast to OsRac1, the transcription factor OsWRKY45 directly interacts with the CC domain of NLR 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 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 due to infect the top internodes or panicle of rice and result 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 leaf blast at the seedling stage show susceptibility to panicle blast at the heading stage (Xiao et al. 2020). Multi-omics analysis also showed that distinct defense-related gene expression is induced by seedling blast and panicle blast, indicated 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. 2020). 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, BC_2F_7) were obtained, and resistance identification showed significant differences in the panicle blast resistance between the sister lines, thus we conclude that some of genetic factors might be involved in the panicle blast resistance difference between the sister lines. In this study, QTL analysis were conducted with F_2 population deriving from a across between MSJ13 and MSJ18 using Genotyping by Sequencing (GBS) method, and a major QTL *qPBR10-1* on chromosome 10 was specifically identified. Additionally, *qPBR10-1* was verified among the BC_1F_2 and BC_1F_3 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.

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. One F_2 population consisting of 316 individual plants, derived from the cross of MSJ13 and MSJ18, was used for QTL mapping of panicle blast resistance. One F_2 individual plant with high panicle blast resistance was selected to obtain BC_1F_1 seeds by backcrossing with MSJ18, and then a BC_1F_1 individual plant with high panicle blast resistance was self-crossed to generate the BC_1F_2 and BC_1F_3 population for fine mapping of *qPBR10-1*. The donor GM4 and receptor C134S was 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, Jiangsu, Hunan, Hubei, Anhui and Sichuan provinces of China in 2010–2014 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 rice seedlings of GM4, MSJ13, MSJ18 and C134S was inoculated with a set of 80 *M. oryzae* isolates spore suspension (5×10^4 conidia/ml) in inoculation chambers, respectively, as the method described by Wu et al. (2016). After inoculation, the plants 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 photocycle at 90% relative humidity by intermittent spraying with water. After seven 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 to be 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, 316 F_2 individual plants, the homozygous recombinant lines advanced from recombinant individual plants, were inoculated with the isolate 85 − 14 by the injecting method as described as Wu et al. (2017). Three panicles for each F_2 individual plants were injected by 1 ml blast isolate 85 − 14 conidial suspension (5×10^4 conidia/ml) for QTL mapping, and 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 F_2 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 F_2 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 the further QTL analysis (Qin et al. 2018).

Genetic map construction and QTL analysis

A high-density genetic linkage map for the F_2 population was constructed using inclusive composite interval mapping (ICIM) implemented in software IciMapping v4.0 (http://www.isbreeding.net/). Recombination fractions were converted into centiMorgans (cM) using the Kosambi function, and the linkage map and SNP marker order was determined with a greedy algorithm. Location of QTL was described according to LOD (logarithm of odds) value, and the software was set LOD > 2.5 as a threshold which must be operate 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

30 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*, InDel and KASP markers within delimited interval were designed (Additional file 2: Table S2). A linkage map of 1682 individuals from the BC_1F_2 population was analyzed with four SSR markers and two InDel markers on chromosome 10 to ensure the presence of the major QTL *qPBR10-1*. A total of 3568 BC_1F_3 individuals were used to screen recombinants between the ID1846 and ID4789. In total, 5 types of recombinants were identified. Twenty progenies of each recombinant were planted and screened for homozygous plants from each group. These homozygous recombinant lines (BC_1F_3, BC_1F_4) 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), FGENSH (http://linux1.softberry.com/), and RiceGAAS (http://rgp.dna.affrc.go.jp) software. The DNA and full-length cDNA of six predicted genes from two parents were amplified by PCR methods and sequenced (General Biol, Anhui).

The immature panicles of MSJ13 and MSJ18 inoculated by blast isolate 85 − 14, were cuted-off and collected at 0 h, 24 h, 48 h, 72 h and 96 h after inoculation for the expression analysis of candidate genes. 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. Relative quantification of transcript levels of four candidate genes was obtained based on the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). All reactions were performed in three replicates. The primers for four candidate genes and *OsActin1* in RT-PCR assay are shown in Additional file 2: Table S2.

Data analysis

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 markers detection showed that *Pigm* was harbored in MSJ13 and MSJ18 (Fig. 1a). We also 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 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), indicated 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 inoculated 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 resistant to AX3-2 and GD1-6, but susceptible to 85 − 14, R5-1 and 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 genetic factors might be involved in the panicle blast resistance difference between MSJ13 and MSJ18.

GBSSNP identification and map construction

In order to identify the genetic factors, one F_2 population was constructed from an intra-specific cross of the sister lines (MSJ13×MSJ18), and 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 F_2 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 F_2 population were filtered out. Finally, a total of 2947 high-quality SNPs that are homozygous for each parent and show polymorphism between the parents were developed for further QTL-seq analysis.

With the advent of GBSSNP 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 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.

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 F_2 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 preliminary 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 BC_1F_2 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 BC_1F_3 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 genes 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 transposon protein. Compared the genomic sequences of these six candidate genes between two parents MSJ13 and MSJ18, *ORF1*, *ORF2* and *ORF4* showed differences, and *ORF3*, *ORF5* and *ORF6* presented no difference (Table 3).

The expression patterns of four functional candidate genes in MSJ13 and MSJ18 were detected in immature panicles inoculated by the blast with real-time PCR approach. *ORF1* was highly expressed, and slightly down-regulated and reached the bottom at 72 h in MSJ13, while *ORF1* displayed constitutive low-level expression in MSJ18 and dramatically down-regulated and reached the bottom at 72 h in MSJ18 (Fig. 5a). The relative expression level of *ORF2* also showed significant difference between MSJ13 and MSJ18. The expression of *ORF2* was slightly induced and reached the peak at 48 h, then decreased to the normal level in MSJ13, while *ORF2* was slightly down-regulated and reached the bottom at 48 h in MSJ18 (Fig. 5b). The expression 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.

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 seedling blast susceptible varieties have shown the resistance to panicle blast, and the other of 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 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 resistant to AX3-2 and GD1-6, but susceptible to 85 − 14, R5-1 and 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 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 *Pb1* 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 both effective resistance to seedling blast and panicle blast (Ma et al. 2015). The other is the genetic mechanisms of seedling blast and panicle blast resistance might differ in distinct defense pathway. OsGF14b, a quantitatively blast resistance gene in rice, plays opposite roles in seedling blast and panicle blast resistance, 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. 2020). Transcriptome analysis also identified a large number of genes were up-regulated involved in detoxification, cell wall synthesis and modifications 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 is introduced into a genomic fragment, so the gene could present a large additive effect in breeding. However, great interaction effect with genome background cause 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 5 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 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 differentially expressed 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 participate in individually or combination with each other to regulate *Pigm*-mediated panicle blast resistance through CRISPR/Cas9 and transgenic complementary methods.

Declarations

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Authors' contributions

YW and AL conceived and designed the experiments;NX, YL, QG, YN, LY, YC, 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 authors read and approved the final manuscript.

Availability of data and materials

All relevant data are provided as Tables within the paper and in the Supporting information files

Ethics approval and consent to participate

There has no ethics problems involved in this article.

Consent for publication

These co-authors involved in the paper all consent to publish this article on Rice.

Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, table 1, 2, 3 is only available as a download in the Supplemental Files section.

Figures

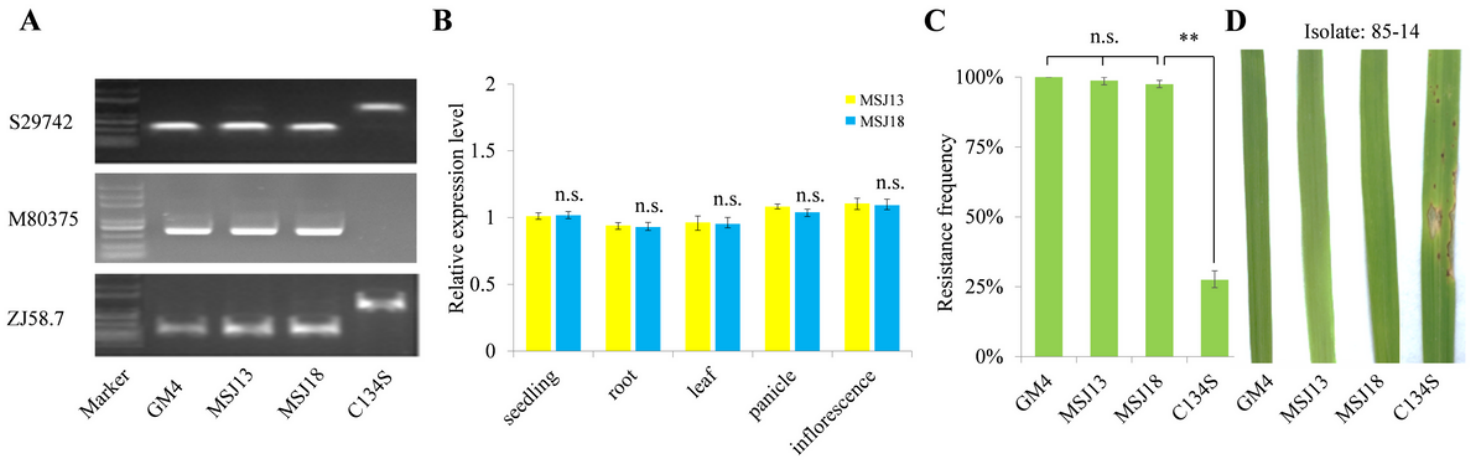

Figure 1

The broad-spectrum resistance gene Pigm was successfully introduced in MSJ13 and MSJ18. a Molecular markers detection verified Pigm was harbored in MSJ13 and MSJ18. b Expression of PigmR 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.

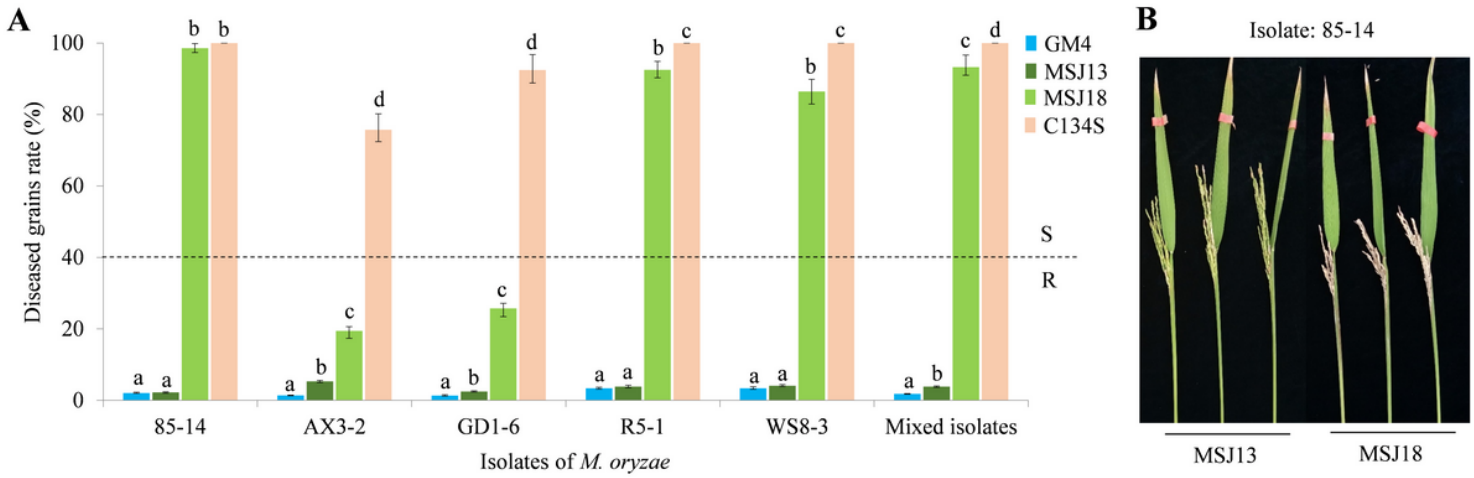

Figure 2

Significant differences in the panicle blast resistance against blast isolates were observed between MSJ13 and MSJ18. a Diseased grains 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.

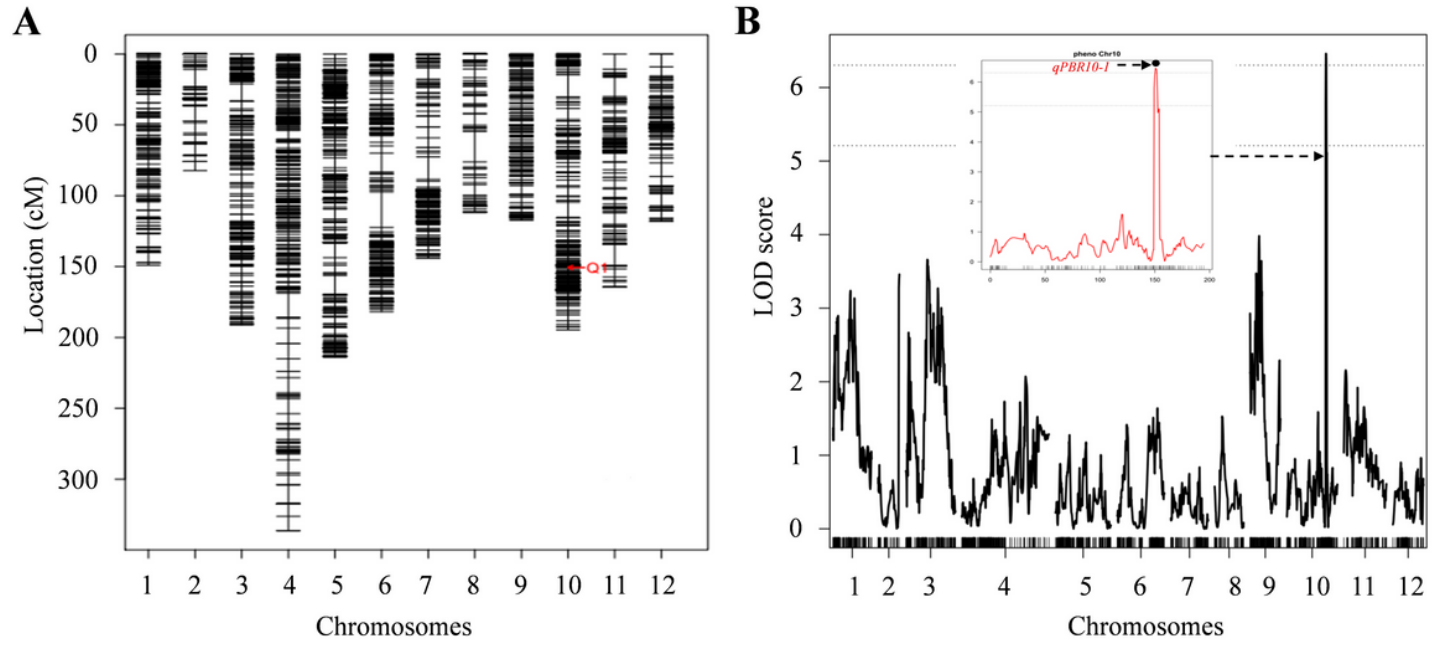

Figure 3

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.

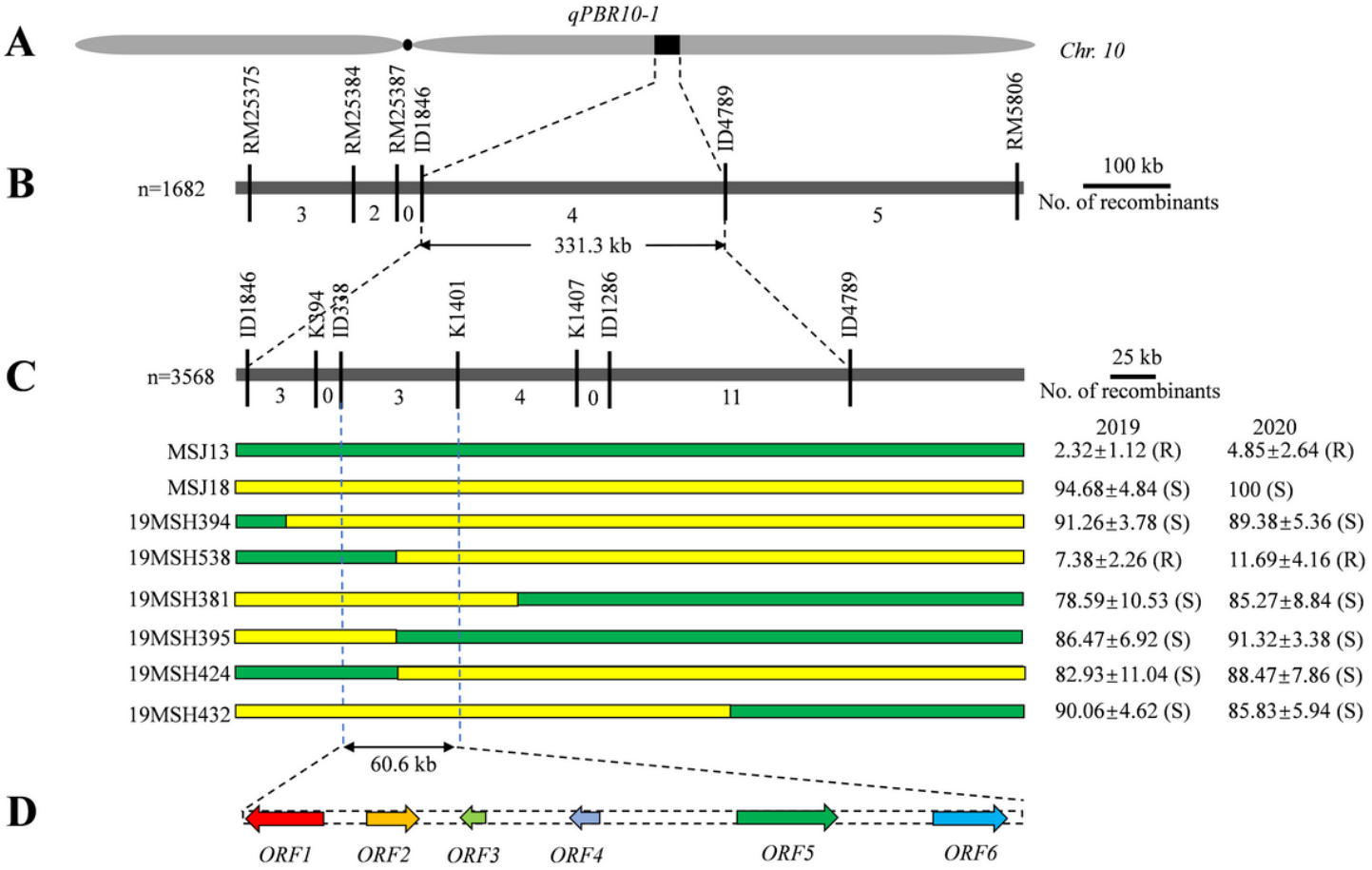

Figure 4

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 BC1F2, and qPBR10-1 was located between markers ID1846 and ID4789. c Twenty-one recombinants were screened from 3568 BC1F3 population, and qPBR10-1 was finally narrowed in the 60.6 kb region between markers ID338 and K1401. d Six candidate ORFs were predicted and the arrows represent the direction of ORFs.

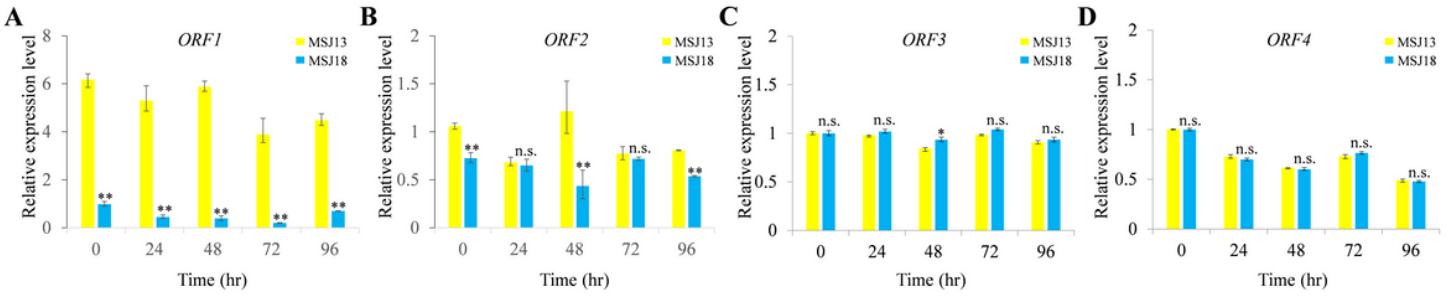

Figure 5

Expression patterns of candidate genes between MSJ13 and MSJ18 in immature panicles by RT-PCR methods. a Expression analysis of four functional ORF1 after blast inoculations for 0 h, 24 h, 48 h, 72 h and 96 h between MSJ13 and MSJ18. b Expression analysis of ORF2 after blast inoculations for 0 h, 24 h, 48 h, 72 h and 96 h between MSJ13 and MSJ18. c Expression analysis of ORF3 after blast inoculations for 0 h, 24 h, 48 h, 72 h and 96 h between MSJ13 and MSJ18. d Expression analysis of ORF4 after blast inoculations for 0 h, 24 h, 48 h, 72 h and 96 h between MSJ13 and MSJ18. ** indicates significant differences between test lines at 1% levels, * indicates significant differences between test lines at 5% levels, n.s. indicates no significant difference.

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