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ORIGINAL RESEARCH ARTICLE

Identification of bacterial blight resistance genes in rice landraces from Yunnan Province, China

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Abstract

Bacterial blight (BB), a serious bacterial disease caused by pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) affects rice growth and yield. Yunnan Province is regarded as a center of rice diversity in China and indeed the world, and has abundant rice landrace resources, which may offer prospective candidate donors in rice improvement and breeding. In this study, a set of 200 rice landraces were evaluated to determine their resistance to 10 pathogenic *Xoo* strains resistance by the leaf-clipping method. The results indicated that the tested rice landraces had different resistance levels against different *Xoo* strains. Multiple comparisons showed that the *Xoo* strain PXO99 was virulent to the tested rice landraces. Sixty-six rice landraces conferred resistance against at least one *Xoo* strain. These resistant rice landraces screened were then performed the presence of 14 cloned BB resistance genes by closely linked molecular markers and designed specific primers. The results showed that none of these resistant accessions contained *xa13*, *Xa21*, *Xa27*, and *Xa45(t)* homologous fragments, while 9, 24, 4, 7, 9, 15, 1, 5, 4 and 27 accessions contained *Xa1*, *Xa2/Xa31(t)*, *Xa14*, *Xa3/Xa26*, *Xa4*, *xa5*, *Xa7*, *Xa10*, *Xa23* and *xa25* homologous fragments, respectively. Sequence analysis further revealed that nucleotide variations around functional nucleotide polymorphisms region were observed within these accessions containing the *Xa1*, *Xa2/Xa31(t)*, *Xa14*, *Xa3/Xa26*, *Xa4*, *xa5*, *Xa10*, *Xa23* and *xa25* homologous fragments. These results along with phenotypic resistance spectrum supported that these accessions carried nine resistance homologous genes. Only one accession (Qishanggu_Wenshan) carried the *Xa7* resistance gene. We also found that some resistant rice landraces, especially Xilandigu_Baoshan, and Laoyaling_Lincang without the above resistance genes, which mediated broad spectrum resistance to multiple *Xoo* strains, were identified as potential sources for breeding rice lines resistance to BB.

Keyword Yunnan rice landraces · Bacterial blight · Resistance genes

Introduction

Rice (*Oryza sativa* L) is one of the most important domesticated food crops in the worldwide. Bacterial blight (BB) caused by pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is considered to be one of the most destructive disease of rice crop (Niño-Liu et al. 2006; Savary et al. 2019). *Xoo* races often make the breakdown of rice resistance by highly pathogenic variability in a short period, which attacks rice by principally depending on diverse effectors secreted through a type III secretion system (T3SS) (Gu et al. 2005). Transcription activator-like effectors (TALEs) as major virulence effectors of *Xoo* strains, primarily activate host gene expression by combining to specific promoter region (effector binding elements, EBEs) of host susceptibility genes or resistance genes, thereby triggering susceptibility

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or resistance symptoms, respectively (Römer et al. 2009; Ji et al. 2016). Rice, meanwhile, also constantly employs its own resistance genes to defend the fierce attacks from variable *Xoo* races (Ji et al. 2018). Nowadays, plant host resistance, chemical measures and nitrogen management have been employed to control BB caused by *Xoo* races in rice breeding practice (Sombunjitt et al. 2017; Chukwu et al. 2019). However, breeding new resistant rice cultivars would be an effective, economic and environmental approach to better control BB disease (Deng et al. 2018). Consequently, more researchers are interested in discovering available resistance genes for controlling the disease.

At present, more than 40 resistance genes have already been found to mediate rice resistance against *Xoo* race-specific (Neelam et al. 2020). However, many BB resistance genes are not cloned and characterized types of proteins due to their unknown sequences. A recent study reported that 5 additional cloned *Xa1* allelic resistance genes, *Xa2*, *Xa31(t)*, *Xa14, CGS-Xo1*$_{11}$, and *Xa45(t)* from different rice varieties had highly conserved structure, and the variable number of 93 amino acid residues (279 bp) ranging from 4 in XA14 to 7 in XA45(t) at their C-terminal tandem repeats (LRR region) can distinguish the type of *Xa1* alleles (Ji et al. 2020). Meanwhile, sequence analysis confirmed that *Xa2* gene was the same as *Xa31(t)* gene (Ji et al. 2020; Zhang et al. 2020); hence, we refer to this gene as *Xa2/Xa31(t)* in this paper. The *CGS-Xo1*$_{11}$ gene is considered to be *Xo1*, which confers resistance to *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*), but it is unclear whether they are the same gene (Ji et al. 2020). The other *Xa1* allelic genes conferred specific BB resistance against *Xoo* strains. Additionally, 12 cloned BB resistance genes, including *Xa1* (Yoshimura et al. 1998), *Xa3/Xa26* (Sun et al. 2004), *Xa4* (Hu et al. 2017), *xa5* (Iyer and McCouch. 2004), *Xa7* (Chen et al. 2021), *Xa10* (Tian et al. 2014), *xa13* (Chu et al. 2006), *Xa21* (Song et al. 1995), *Xa23* (Wang et al. 2015), *xa25* (Liu et al. 2011), *Xa27* (Gu et al. 2005) and *xa41(t)* (Hutin et al. 2015) have been reported for encoding different types of proteins. Out of these genes, *Xa1* and its allelic genes (*Xa2/Xa31(t)*, *Xa14, Xa45(t)*), *xa5*, *Xa7*, *Xa10*, *xa13*, *Xa23*, *xa25, Xa27* and *xa41(t)* conferring race-specific resistance to certain *Xoo* are tightly interrelated with TALEs from the pathogen, but the three other resistance genes *Xa3/Xa26*, *Xa4*, and *Xa21*, encoding kinase proteins, are not induced by TALEs (Jiang et al. 2020). These resistance genes are important resources for rice breeding, such as pyramiding rice cultivars with different resistance genes have been applied to increase the resistance against *Xoo* (Sombunjitt et al. 2017; Sutrisno et al. 2018). Thus, it is essential to identify the present of the resistance genes in rice germplasm. However, the selection of rice varieties comprising resistant genes is difficult and time-consuming through the traditional approach alone. Molecular markers within closely linked to the target genes can be used to identify germplasm with one or more resistance genes (Perumalsamy et al. 2010). The application of gene-linked/specific molecular markers should be complemented by gene homologous cloning method and sequences analysis to effectively analyze the type of resistance genes (Jiang et al. 2019).

Yunnan Province, known as one of the centers of rice genetic diversity, is alternately distributed with different mountains, plains and basins, and the diverse geographic and climatic conditions have contributed to the remarkably rich diversity of its rice landraces (Zeng et al. 2007). In comparison to modern rice cultivars, Yunnan rice landraces have many desirable agronomic traits, higher genetic diversity, various stress tolerance, as well as excellent resistance characteristics (Cui et al. 2016, 2017). However, only limited studies have been carried out to identify BB resistant varieties among the Yunnan rice landraces, such as Zhachanglong, a rice landrace from Yunnan Province, harbors *Xa3/Xa26*, *Xa22(t)*, and *Xa31(t)* resistance genes, which confers resistance to multiple Chinese *Xoo* strains (Sun et al. 2004; Wang et al. 2003, 2009). However, there is still insufficient information on the responses of Yunnan rice landraces against *Xoo* strains, and lack of information on the identification of BB cloned genes. In this work, we selected 200 representative rice landraces by previously collected from different regions in Yunnan. Evaluation of these rice landraces resistance to multiple *Xoo* strains was performed by using a scissors-based inoculation method under the same planting conditions. We then screened resistant rice landraces to identify the presence and type of previously cloned resistance genes, including *Xa1* and its allelic genes (*Xa2/Xa31(t)*, *Xa14*, *Xa45(t)*), *Xa3/Xa26*, *Xa4*, *xa5*, *Xa7*, *Xa10*, *xa13*, *Xa21*, *Xa23*, *xa25*, and *Xa27*, except for *xa41(t)* due to its nucleotide sequence unpublished. Our studies could provide a reference and valuable information for further utilizing Yunnan rice landraces in resistance breeding.

Materials and methods

Rice materials and Field planting

A total of 200 representative rice landraces were provided by Biotechnology & Genetic Germplasm Institute, Yunnan Academy of Agricultural Sciences, Yunnan, China (Table S1). The reference lines/varieties, IRBB1 (*Xa1*), IRBB4 (*Xa4*), IRBB3 (*Xa3*), IRBB5 (*xa5*), IRBB7 (*Xa7*), IRBB10 (*Xa10*), IRBB13 (*xa13*), IRBB21 (*Xa21*), Minghui63/Nipponbare (*xa25*) and *Oryza rufipogon* (*Xa23*), IRBB27 (*Xa27*) and a susceptible variety IR24 (none of these resistant genes), respectively, were used as control materials. Each accession with appropriate number of seeds was sown and then the seedlings were cultivated into

experimental field at Yunnan Academy of Agricultural Sciences, Yunnan, China, in the summer of 2017.

Xoo strains inoculation and phenotypic data analysis

Ten *Xoo* strains including one highly pathogenic strain from Philippines (PXO99), one representative race from Japan (T7147), two representative strains from China (C5 and C9), six pathogenic strains from Yunnan Province (Y8, YM1, YM187, HZHJ19, YJWS2 and YJDP2) were used to evaluate the resistance of rice landraces in Yunnan. All strains were cultured on Nutrient Agar medium by 28℃ for 48 h, then resuspended in double distilled water, and diluted to $OD_{600\ nm} = 0.8$ for inoculation by using the NanoDrop2000. Each rice accession at booting stage (panicle development) was respectively inoculated with *Xoo* strains by the leaf-clipping method at 15:00 ~ 17:00 (Jiang et al. 2019). Three replicates (three individual plants) for each accession were designed for each *Xoo* strain with five leaves inoculated per plant. Mock treatments with double distilled water and untreated plants served as the controls. Lesion lengths (cm) and leaf lengths (cm) were respectively measured across three leaves from each accession about 3 weeks after inoculation adopting the Standard Evaluation System (SES) for rice (IRRI, 2002). The leaf with lesion area 0–5% was scored 0, 6–10% was scored 1, 11–25% was scored 3, 26–50% was scored 5, 51–75% was scored 7, and 76–100% was scored 9. The lines with the score of 0, 1, 3, 5, 7 and 9 were considered as high resistance (HR), resistance (R), middle resistance (MR), middle susceptible (MS), susceptible (S), and high susceptible (HS), respectively. Analysis of variance (ANOVA) of lesion length rate (LLR) data was conducted by R programming language software (https://www.r-project.org). The R software package multcomp was used to conduct Tukey's multiple comparisons of the LLR between *Xoo* strains and varieties. The R software package ggplot2 was also performed to show the differences in the LLR values and disease levels among *Xoo* strains and varieties.

Linked molecular markers and designed specific primer

Total genomic DNA from the resistant rice landraces in Yunnan was isolated by using cetyltrimethylammonium bromide (CTAB) method, and was analyzed to determine the presence of 14 cloned BB genes *Xa1* and its allelic genes (*Xa2/Xa31(t)*, *Xa14* and *Xa45(t)*), *Xa3/Xa26*, *Xa4*, *xa5*, *Xa7*, *Xa10*, *xa13*, *Xa21*, *Xa23*, *xa25*, and *Xa27* by using gene-linked markers or gene-specific primers reported, respectively (Table S2). The tested rice landraces were initially considered to contain disease-resistant allele markers of these genes, and further used to identify the homologous fragments of these genes by designing specific primers based on their nucleotide sequences in ORF region and polymorphic locus region related to resistance function (Table S2; Fig. S2).

PCR amplification and detection

For each PCR, 25 μL reaction mixture consisted of 12.5 μL 2 × Phanta® Max Master Mix (Vazyme, China), 1 μL of each forward and reverse primer (10 μmol L^{-1}), 1 μL DNA (25 ng μL^{-1}), 9.5 μL ddH_2O. The PCR amplification program was as follows: 95℃ for 3 min; followed by 35 cycles at 95℃ for 15 s, 50–60℃ for 15 s, 72℃ for 30–60 s/kb, and finally extension at 72℃ for 5–10 min. The PCR amplified products were separated on 1%-3% (variable depending on product sizes) agarose gel electrophoresis. The presence of resistance genes in rice landraces were judged by comparing the fragments in corresponding control lines. If no or weak fragments on agarose gel, the above steps were repeated twice to ensure the accuracy of the results.

DNA sequencing analysis of selected rice landraces

The selected rice landraces carrying homologous fragments of resistance gene(s) around functional nucleotide polymorphisms region were then sequenced. The sequenced data were spliced into complete sequences by DNAMAN. The resistant and susceptible sequences of cloned genes were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/), except for *Xa10* recessive allele, for which no published sequence was available. The spliced sequences were compared with the cloned gene sequences by using the DNAMAN for multiple alignments, and their similarity/identity analysis were performed by using BLASTN programs (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Phenotypic resistance of rice landraces to *Xoo* strains

The phenotypic resistance levels and LLR of 200 rice landraces from different cities/states in Yunnan to 10 *Xoo* strains are shown in Table S1. The ANOVA results showed that there were significant difference between *Xoo* strains and rice varieties ($P < 0.01$) (Table 1), but no obvious difference related to geographic origins ($P > 0.1$). The ten *Xoo* strains had specific virulence to these tested rice landraces (Table S1). Multiple comparisons indicated that the average LLR of rice landraces inoculated with the PXO99 strain was 0.5, which was significantly higher than other strains (Fig. 1A). Among 200 rice landraces, 31 accessions

Table 1 ANOVA of lesion length rate caused by *Xoo* strains in Yunnan rice landraces

Variance source	*df*	*SS*	Mean Square	*F* value	*P* value
Strain	9	9.33	1.04	131.88	< 0.01
Variety	199	4.78	0.02	2.14	< 0.01
City/State	15	0.23	0.02	0.25	0.245
Error	1791	10.86	0.01		

were resistant to YM187 strain (15.5% of the tested accessions). For the other *Xoo* strains HZHJ19, YM1, T7147, Y8, C5, C9, YJWS2, YJDP2 and PXO99, the number of disease resistant accessions were 20, 18, 9, 9, 7, 5, 4, 2 and 2, accounting for 10.0%, 9.0%, 4.5%, 4.5%, 3.5%, 2.5%, 2.0%, 1.0% and 1.0% of the tested accessions, respectively (Fig. 1B).

In this study, 66 accessions were more than moderate resistance (MR) to at least one *Xoo* strain, but only Xilandigu_Baoshan, Huangxiangnuo_Honghe and Laoyaling_Lincang had broad spectrum resistance to multiple *Xoo* strains based on phenotypic data (Table S1). When we characterized the phenotypic resistance profiles of these disease-resistant rice landraces to *Xoo* strains, most accessions showed different resistance spectrum, only a few accessions had phenotypic resistance patterns similar to those of the resistant lines/varieties, IRBB1 (*Xa1*), IRBB3 (*Xa3/Xa26*), IRBB4 (*Xa4*), IRBB5 (*xa5*), IRBB10 (*Xa10*), IRBB13 (*xa13*), IRBB21 (*Xa21*), *Oryza rufipogon* Griff (*Xa23*), Minghui63/Nipponbare (*xa25*) and IRBB27 (*Xa27*), respectively (Table S1). Considering the differences or similarities of resistance responses with those of reference lines, we conducted analyses to detect whether these resistant accessions harbored any of the cloned BB resistance genes.

Genotypic detection of cloned BB resistance genes in disease-resistant rice landraces

The availability of tightly linked molecular markers or reported gene-specific primers were exploited to detect the type of resistance genes of interest. To estimate the frequency of BB cloned resistance genes in the 66 resistant rice landraces, we first screened these resistant rice landraces by using the reported gene-linked markers or gene-specific primers of 14 cloned BB resistance genes, *Xa1* and its allelic genes (*Xa2/Xa31(t)*, *Xa14* and *Xa45(t)*), *Xa3/Xa26*, *Xa4*, *xa5*, *Xa7*, *Xa10*, *xa13*, *Xa21*, *Xa23*, *xa25*, and *Xa27*, respectively. The results indicated that *Xa1* and its allelic genes (*Xa2/Xa31(t)*, *Xa14*, and *Xa45(t)*), *Xa3/Xa26*, *Xa4*, *xa5*, *Xa7*, *Xa10*, *Xa23*, *xa25*, and *Xa27* genes could be detected their corresponding resistant allele markers in 56% (37), 10.6% (7), 47% (31), 22.7% (15), 12.1% (8), 7.6% (5), 6.1% (4), 40.9% (27), and 53% (35) accessions, respectively, while *xa13* and *Xa21* genes were absent in these rice landraces (Fig. S1, Table S3). Interestingly, we found that 63 accessions were comprised two or more disease-resistant allele markers (Table S3), but most of them showed narrower spectrum resistance to the tested *Xoo* strains in this study, especially those carrying the *xa5*, *Xa23*, and *Xa27* resistant allele markers (Table S1). On the contrary, 3 accessions (Lengshuigu_Yuxi, Xilandigu_Baoshan and Laoyaling_Lincang) were found not to contain the above disease resistance allelic markers, but 2 accessions (Xilandigu_Baoshan and Laoyaling_Lincang) of them had broad-spectrum resistance (Table S1; Table S3). In general, a rice cultivar with multiple resistances genes provides more resistance than a single resistance gene (Singh et al. 2001; Rajpuroit et al. 2010). On the basis of this result, it was possible that some of the resistant allele markers detected in our study were false positive fragments.

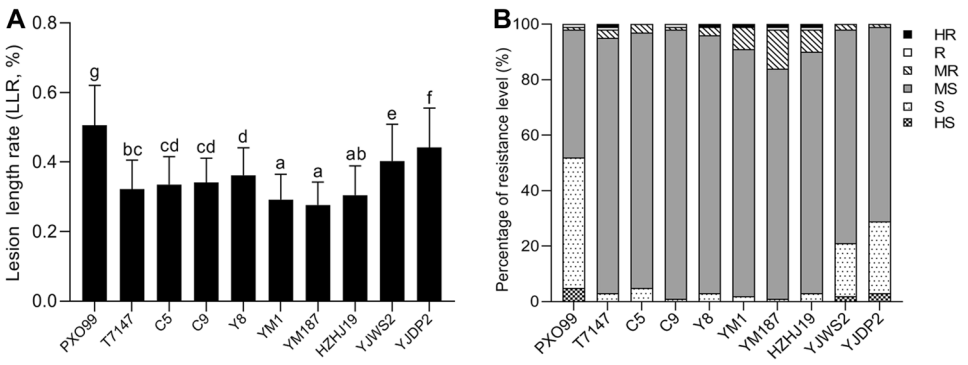

Fig. 1 Comparison of lesion length rate (LLR) and percentage of resistance reaction to 10 *Xoo* strains in rice landraces. (**A**) The average LLR were analyzed in rice landraces. The different letters above the bars indicated significant difference (P < 0.01). (**B**) Different resistance level of rice landraces to different strains. (HR) high resistance; (R) resistance; (MR) medium resistance; (MS) medium susceptible; (S) susceptible; (HS) high susceptible

To avoid whether our results included false positive allele markers with the same sizes as resistance genes, we chose the rice landraces with the above resistant allele markers and 2 accessions (Xilandigu_Baoshan and Laoyaling_Lincang) without the above resistance allele markers to further evaluate the homologous fragments of resistance genes using the designed specific primers (Fig. S2; Table S1). *Xa1* and its allelic genes can be distinguished mainly by the variable number of LRR region at their C-terminal tandem repeats (Ji et al. 2020; Zhang et al. 2020). We designed two pairs of primers to amplify the ORF and LRR regions of *Xa1* allelic genes in 37 accessions, respectively. The results indicated that 9 accessions were detected to contain *Xa1* expected homologous fragments, 24 accessions were detected to contain *Xa2/Xa31(t)* expected homologous fragments, 4 accessions were detected to contain *Xa14* expected homologous fragments, no accessions were detected to contained *Xa45(t)* expected homologous fragments (Fig. 2A; Table S3). Similarly, we also designed specific primers for *Xa3/Xa26*, *Xa4*, *xa5*, *Xa10*, *Xa23*, *xa25*, and *Xa27* to amplify their homologous fragments depending on their nucleotide sequences in ORF region and polymorphic loci region related to resistance function. The results showed that all 7 accessions with *Xa3/Xa26* resistant allele marker (Fig. 2B), 15 accessions with *xa5* resistant allele marker (Fig. 2C), 5 accessions with *Xa10* resistant allele marker (Fig. 2D), 4 accessions with *Xa23* resistant allele marker (Fig. 2E), 27 accessions with *xa25* resistant allele marker were amplified to have expected homologous fragments of their corresponding resistance genes (Fig. 2F), respectively. For *Xa4*, 9 of 31 accessions with resistant allele marker had the homologous fragments of *Xa4* resistance gene, whereas 22 accessions were absent the same fragments as IRBB4 (Fig. 2G; Table S3). For *Xa7*, 1 of 8 accessions with resistant allele marker had the homologous fragments of *Xa7* resistance gene (Fig. 2H; Table S3). For *Xa27*, all 35 accessions with resistant allele marker could not amplify the expected fragments as their resistant/susceptible lines (Table S3). We also found that two broad-spectrum resistant accessions, Xilandigu_Baoshan and Laoyaling_Lincang without resistant allele markers and the susceptible control IR24 were detected to carry *Xa3/Xa26*, *xa5*, *xa25* homologous fragments (Fig. 2). Although some false positive resistant allelic accessions were excluded by homologous gene cloning method, the phenotypic resistance of some accessions with resistant homologous fragments was different from that of the resistance control lines/varieties in this study (Table S1). We suspected that the phenotypic resistance difference in rice might be affected by nucleotide sequence variations, especially functional nucleotide polymorphisms region related to resistant/susceptible gene (Fujino et al. 2011; Wu et al. 2013), besides its own genetic background (Cao et al. 2007; Zhou et al. 2009).

Functional nucleotide sequences analysis of resistance genes in selected rice landraces

Xa1 and its allelic resistance genes can be distinguished on the basis of the substructure of variable 93-aa (279 bp) tandem repeats at the LRR domain, which are to be likely involving the strength of rice-*Xoo* resistance interactions (Ji et al. 2020; Zhang et al. 2020). We selected all 9 accessions with *Xa1* (2010 bp), 24 accessions with *Xa2/Xa31(t)* (1731 bp) and 4 accessions with *Xa14* (1572 bp) to compare the sequence identity of LRR region, respectively. These accessions sequenced were 99.8% identical to *Xa1*, 99.8% identical to *Xa2*, and 99.3% identical to *Xa14* (Fig. S3). The results, along with phenotypic data, indicated that these accessions carried *Xa1* homologous allelic resistance genes. A total of 9 accessions comprising *Xa3/Xa26* homologous fragments including 2 accessions, Xilandigu_Baoshan and Laoyaling_Lincang without resistant allele marker around the resistant and susceptible function region (Xiang et al. 2006; Hur et al. 2013) were sequence alignment. Seven accessions had the same TGCA (+452–456 bp) in exon 1 region as that in the *Xa3/Xa26* resistant allele, but 2 accessions with susceptible allele marker had AATC characteristic of the *xa3/xa26* susceptible allele (Fig. S4). *Xa4* and its recessive allele *xa4* exists one base substitution (+456 C → G) in the exon 1 coding region, resulting in the replacement of aspartic acid (D) residue with glutamic acid (E). Residue D45 is speculated to be associated with resistance phenotype (Hu et al. 2017). Comparison sequences analysis revealed nine accessions were the same as *xa4* (C), with a few base deletions and substitutions in the promoter region (Fig. S5).

xa5 is a natural allele of dominant susceptible gene *Xa5*. Sequence analysis of 15 accessions with resistant allele marker and two accessions, Xilandigu_Baoshan and Laoyaling_Lincang with susceptible allele marker revealed that two nucleotide (TC) substitutions were identical with *Xa5* in polymorphic loci related to resistance function, and single base mutation was appeared in partial accessions (Fig. S6). These results confirmed that these accessions contained *Xa5* homologous gene, but not *xa5* resistance gene. *Xa7*, *Xa10* and *Xa23* are three executor resistance genes. *Xa7* is a recently cloned executor resistance gene, which has two types of EBE sequences in the promoter region, namely Type I (IRBB7, DV85, and AUS 308) and Type II (Lao Zao Gu, MOTIA, and DANGAR). And they have the same CDS sequences (Chen et al. 2021). One accession (Qishanggu_Wenshan) amplified sequence was identical to that of Type I (IRBB7) (Fig. S7). Unlike other cloned BB resistance genes, the susceptible allele sequence of *Xa10* has not been published. Sequence analysis indicated that five accessions were equal to *Xa10* at coding region, but had a few base mutations and substitutions in the specific TALE AvrXa10 binding

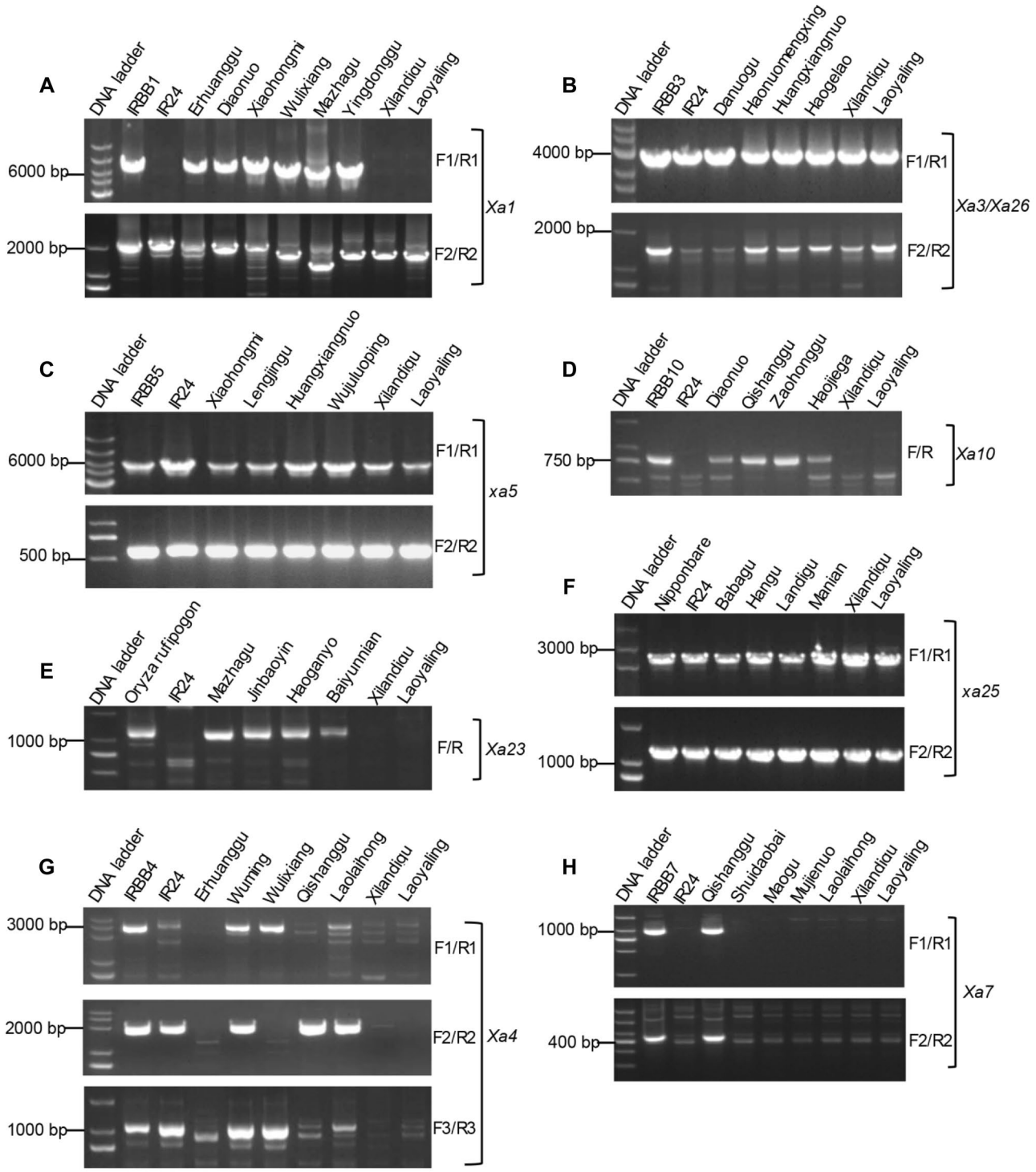

Fig. 2 Detection of homologous fragments of BB cloned resistance genes in rice landraces (Partial accessions were shown here). (**A**) the ORF (F1/R1) and LRR (F2/R2) region amplification of *Xa1* and its allelic genes (*Xa2*, *Xa14* and *Xa45(t)*); (**B**) the ORF (F1/R1) and functional polymorphic region (F2/R2) amplification of *Xa3/Xa26* gene; (**C**) the ORF (F1/R1) and functional polymorphic region (F2/R2) region amplification of *xa5* gene; (**D**) the promoter region and coding region (F/R) amplification of *Xa10* gene; (**E**) the promoter region and coding region (F/R) amplification of *Xa23* gene; (**F**) the ORF (F1/R1) and functional polymorphic region (F2/R2) region amplification of *xa25* gene; (**G**) the ORF (F1/R1 and F2/R2) and functional polymorphic region (F3/R3) region amplification of *Xa4* gene; (**H**) the promoter region and coding region (F1/R1) and functional polymorphic region (F2/R2) amplification of *Xa7* gene

element ($EBE_{AvrXa10}$) position associated with *Xa10* resistance expression (Fig. S8). Similarly, *Xa23* and its susceptible allele *xa23* share identical coding sequence, but *xa23* lacks cognate TALE AvrXa23 binding element ($EBE_{AvrXa23}$) due to 7 bp polymorphism bases insertion in the promoter region. All four accessions were found to have the same sequence as *xa23* in the $EBE_{AvrXa23}$ region (Fig. S9). These evidences confirmed that 4 accessions carried *xa23* homologous allele gene, instead of *Xa23* resistance gene. *xa25* and its dominant susceptible allele *Xa25* have four types of *Xa25/xa25* alleles, including two recessive *xa25* resistance alleles (Minghui63 and $OsSWEET13_{Nip}$/$OsSWEET13_{Kit}$) and two dominant *Xa25* susceptible alleles ($OsSWEET13_{ZS97}$ and $OsSWEET13_{IR24}$), are identified as dependent on EBE variability at promoter region correlated with gene function (Zhou et al. 2015; Cheng et al. 2017; Xu et al. 2019). All 27 accessions with *xa25* resistance alleles shared cognate $xa25_{Nip}$ ($OsSWEET13_{Nip}$) EBE sites but also base changes in promoter region, and were considered to have $xa25_{Nip}$ ($OsSWEET13_{Nip}$) type homologous gene (Fig. S10), whereas Xilandigu_Baoshan and Laoyaling_Lincang with *Xa25* susceptible allele marker had $Xa25_{IR24}$ and $Xa25_{ZS97}$ EBE sites in corresponding region, respectively.

Discussions

Some previous studies have reported phenotypic resistance to BB in rice by the leaf-clipping method (Fred et al. 2016; Jiang et al. 2019). Molecular markers can greatly facilitate the development of new disease resistant materials, and effectively detect the presence of BB resistance genes in rice (Ullah et al. 2012; Hur et al. 2013). Several studies have been reported to detect the genotype of resistance genes in rice by using gene-linked molecular markers (Singh et al. 2001; Hajira et al. 2016). For example, gene-linked markers were used to detect *Xa4*, *xa5*, and *Xa21* resistance genes in Pakistan rice (Sabar et al. 2016), and *xa5*, *xa13*, *Xa21* and *Xa27* resistance genes in wild rice species (Xia et al. 2010). However, little information is known about the resistance phenotypes and specific resistance genes of Yunnan rice landraces to BB. In the present study, when we characterized the phenotypic resistance response of 200 rice landraces to ten different *Xoo* strains, 33% (66) showed specific resistance reactions against at least one strain. The PXO99 strain showed stronger pathogenicity than the other strains towards these tested rice landraces. Jiang et al. (2019) reported that PXO99 also exhibited high virulence in wild rice species. Out of the 66 resistant rice landraces identified in this study, 63 accessions contained the combination of two or more BB resistance allele genes by using gene specific molecular markers/primers reported. Rice cultivar comprising multiple resistant genes tend to show a broader spectrum compared with cultivars with a single resistance gene (Huang et al. 1997; Rajpurohit et al. 2010; Luo et al. 2012). In this study, however, most accessions carrying multiple resistance alleles, such as Erhuanggu_Kunming, Wuming_Dali, Lizihong_Yuxi, and Wulixiang_Wenshan only showed moderately resistance to a single *Xoo* strain. Two rice landraces, Xilandigu_Baoshan and Laoyaling_Lincang, showed a broad spectrum resistance, despite the lack of resistant alleles. When considering phenotypic differences, the influence of rice genetic background should be taken into account, such as *Xa3/Xa26*, *Xa4* had different levels of in different rice materials (Cao et al. 2007; Zhou et al. 2009). Sequence variations in resistant alleles can also influence the phenotypic differences (Konishi et al. 2008; Fujino et al. 2011; Wu et al. 2013).

Our results confirmed that many of rice landraces possessed one or more *Xa1*, *Xa2/Xa31(t)*, *Xa3/Xa26*, *Xa4*, *xa5*, *Xa10*, *Xa14*, *Xa23*, and *xa25* homologous genes, but did not necessarily show strong resistance because of nucleotide changes in their coding sequences and/ or promoter region. These results have provided the important information for the identification of BB resistance genes in Yunnan rice landraces. *Xa1* allelic genes belong to nucleotide-binding and leucine-rich repeat (NLR) class of typical resistance genes, and confer similar resistance to BB (Ji et al. 2020). Some recent studies revealed that *Xa1* could recognize multiple intact TALEs to mediate broad-spectrum resistance against *Xoo* (Ji et al. 2016; Tang et al. 2019). However, most Asian *Xoo* races harbor a set of truncated TALEs (also termed as interfering TALEs, iTALEs), which interfere with the recognition of total TALEs by XA1, and suppresses resistance mediated by *Xa1* (Ji et al. 2016). The resistant reference line IRBB1 (*Xa1*) showed narrow-spectrum resistance in this study. Although all 37 rice landraces with *Xa1* allelic genes shared higher sequence identity at the *Xa1* allele, there were some base mutations in LRR region that may contribute to phenotypic differences in resistance. The three dominant resistance genes *Xa3/Xa26*, *Xa4*, and *Xa21*, encoding kinase proteins, are not induced by TALEs, of which *Xa3/Xa26* and *Xa21* involve receptor-like kinases (RLK) proteins that confer race-specific resistance to multiple *Xoo* strains, while *Xa4*, encoding wall-associated kinase (WAK) protein, is an unusual resistance gene of rice. In this study, *Xa3/Xa26* and *Xa4* homologs were found in 7, and 9 accessions, while *Xa21* was absent in all resistant rice landraces. Other studies also reported that *Xa21* resistance gene was not widely distributed in rice (Xia et al. 2010; Jiang et al. 2019). *Xa3* and *Xa26* have been confirmed as actually the same gene and renamed *Xa3/Xa26* (Sun et al. 2004; Xiang et al. 2006). *Xa3/Xa26*-mediated resistance to *Xoo* strain is affected by rice genetic background, and IRBB3 show higher resistance against *Xoo* strains than Minghui 63 (Cao et al. 2007; Zhou et al. 2009). Consistent with this, we observed that a weaker

resistance spectrum in Minghui63 than IRBB3 in the study (Table S1). Moreover, compared with the control line IRBB3 and Minghui63, rice landraces with *Xa3/Xa26* homologs showed different resistance spectrum. We speculated that they might be related to their own genetic backgrounds. *Xa4* locates closely to the *Xa3/Xa26* gene locus and confers a race-specific resistance against *Xoo* in rice (Ma et al. 1999; Sun et al. 2003; Zhang et al. 2009), which has been widely utilized in rice breeding project since 1970s. However, some rice resistant varieties with only *Xa4* have become susceptible to *Xoo* in Asia (Krattinger et al. 2017). In the present study, the reference line IRBB4 (*Xa4*) was susceptible to the PXO99, T7147, C5, C9, HZHJ19, YJWS2 and YJDP2 strains, and only showed moderate resistant to YM1 and YM187 strains in this study.

xa5 encoding transcription factor IIA gamma subunit (TFIIAγ) protein, is distinct from its susceptible gene *Xa5* by two nucleotide (TC) substitutions, resulting in an amino acid variation from valine acid (V) to glutamic acid (E) at 39th position (V39E) related to gene expression in rice (Chen et al. 1997; Iyer and McCouch 2004). The *xa5* resistance gene confers BB broad resistance, which depends on TALEs-mediated resistance against *Xoo* race, but differs from the *SWEET* gene in rice (Jiang et al. 2006; Huang et al. 2016). In our studies, the tested rice landraces with the *xa5* resistant allele had a narrower resistance spectrum than the control line IRBB5 (*xa5*), and existed two nucleotide substitutions (TC) with the same as *Xa5*. Previous studies suggested that Pakistani rice varieties possessed *xa5* gene (Naveed et al. 2010; Sabar et al. 2016), but neither of those studies sequenced the allele. Two recessive resistance genes *xa13* and *xa25,* belonging to nodulin MtN3/SWEET family, are promoter variant alleles of the dominant susceptible genes *Xa13* (also known as *Os8N3/SWEET11*) and *Xa25* (also known as *Os12N3/SWEET13*) in the EBE regions, respectively (Yang et al. 2006; Yuan et al. 2013; Zhou et al. 2015; Zaka et al. 2018). *xa13* was absent in any of the resistant rice landraces tested in this study. Other studies reported that other rice varieties were rarely found the existence of *xa13* (Hajira et al. 2016; Jiang et al. 2019). *xa25* was first identified in indica variety Minghui63 (Chen et al. 2002; Liu et al. 2011). Previous studies have reported that two types of recessive *xa25* alleles are Minghui63 and $OsSWEET13_{Nip}$/$OsSWEET13_{Kit}$ from Nipponbare, Kitaake as resistance genes, while two dominant *Xa25* alleles are $OsSWEET13_{ZS97}$ from Zhenshan97 and $OsSWEET13_{IR24}$ from IR24 as susceptibility genes (Zhou et al. 2015; Cheng et al. 2017). In this study, the rice landraces with *xa25* resistance alleles contained $xa25_{Nip}$ ($OsSWEET13_{Nip}$) type homologous gene.

The other four dominant resistance genes *Xa7*, *Xa10*, *Xa23* and *Xa27* are known as executor dominant resistance genes, which differ from typical resistance gene, encoding relatively small proteins in rice (Porter et al. 2003; Zhang et al. 2015). Our studies indicated that Executor resistance genes were not widely distributed in Yunnan rice landraces. Similarly, Yang et al. (2019) reported that *O. officinalis* populations in Yunnan Province with strong resistance to *Xoo* strains were absent the Executor resistance genes. Executor genes confer broad and durable resistance by cognate TALE-dependent binding to their respective EBE in the promoter elements, leading to hypersensitive response in rice plants (Cui et al. 2017). The resistant and susceptible alleles of executor genes encode the identical protein, but they differ from each other in the promoter region correlated with nucleotide sequence polymorphisms. *Xa10* gene expression depends on directly interaction between its promoter and AvrXa10, but its recessive allele has not been reported in rice (Gu et al. 2008; Wang et al. 2017). We found that rice landraces with *Xa10* homologous gene had sequence variations in their cognate $EBE_{AvrXa10}$ positions. In addition, they contributed narrower phenotypic resistance than that of the control line IRBB10. *Xa23* is from *O. rufipogon* with an extremely broad spectrum resistance to BB (Wang et al. 2005). *Xa23* and its recessive gene *xa23* have the same sequence in their coding regions, but *xa23* lacks the binding elements $EBE_{AvrXa23}$ in its promoter region, which is closely related to disease resistance in rice (Wang et al. 2015). Our findings indicated that the $EBE_{AvrXa23}$ were absent from the tested rice landraces, and their resistance phenotype was narrower than *O. rufipogon*. A previous study revealed that the $EBE_{AvrXa23}$ is present only in the promoter region of the dominant resistance gene *Xa23* (Cui et al. 2013). *Xa27* is first reported and cloned executor resistance gene, and its specific expression is related to sequences in its promoter region like other executor genes (Gu et al. 2004; Bimolata et al. 2013). In the present study, 35 accessions were detected to lack of *Xa27* homologs, although they possessed *Xa27* resistance allele markers. By comparison, Xia et al. (2010) reported that *O. rufipogon* was found to harbor *Xa27* resistance gene by using its molecular maker, whereas no further analyzed that *O. rufipogon* carried the type of *Xa27* gene. *Xa7* is a newly cloned executor resistance gene by three different research groups, respectively (Chen et al. 2021; Luo et al. 2021; Wang et al. 2021). Just like *Xa10*, *Xa23* and *Xa27*, *Xa7* BB resistance is lost by a variation consisting of an 11-bp insertion and a base substitution (G to T) in EBE_{AvrXa7} region. Most cultivars, landraces, and wild accessions are lack of the *Xa7* gene (Wang et al. 2021). Our results also indicated that the *Xa7* gene was absent in most of the tested Yunnan rice landraces, only Qishanggu_Wenshan contained this gene and had a similar resistance spectrum to IRBB7 (Table S1). A notable finding in our studies is that Xilandigu_Baoshan and Laoyaling_Lincang with none of the above cloned resistance genes still have stable broad-spectrum in the later resistance identification (data not shown).

Conclusions

We have used a combination of phenotypic and genotypic identification methods to analyze the BB resistance levels and the BB cloned resistance genes of Yunnan rice landraces. A total of 200 representative rice landraces from different origins had been described as the distinct phenotypic resistance reactions towards 10 *Xoo* strains, of which 66 accessions were screened to confer specific resistance against at least one strain. The 66 resistant accessions were performed to analyze the distribution of 14 cloned BB resistance genes by linked molecular markers and homologous gene sequence method. These resistant accessions lacked of *xa13, Xa21, Xa27 and Xa45(t)* resistance genes. There were 9, 24, 4, 7, 9, 15, 5, 4, 27 resistant accessions containing *Xa1*, *Xa2/Xa31(t)*, *Xa3/Xa26*, *xa4*, *Xa5*, *Xa10*, *Xa14*, *xa23* and $xa25_{Nip}$ homologous genes, respectively. Only Qishanggu_Wenshan had *Xa7* resistance gene. Remarkably, Xilandigu_Baoshan, and Laoyaling_Lincang conferring high and broad resistance to BB have potential applications as donor varieties for rice breeding. This work provides the important information about the BB resistance levels and the distribution of BB resistance homologous genes in rice landraces from Yunnan Province, China.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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