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ORIGINAL ARTICLE

Genome-wide association mapping for resistance to bacterial blight and bacterial leaf streak in rice

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Abstract

Main conclusion **Using genome-wide SNP association mapping, a total of 77 and 7 loci were identified for rice bacterial blight and bacterial leaf streak resistance, respectively, which may facilitate rice resistance improvement.**

Abstract Bacterial blight (BB) and bacterial leaf streak (BLS) caused by Gram-negative bacteria *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *X. oryzae* pv. *oryzicola* (*Xoc*), respectively, are two economically important diseases negatively affecting rice production. To mine new sources of resistance, a set of rice germplasm collection consisting of 895 re-sequenced accessions from the 3000 Rice Genomes Project (3 K RGP) were screened for BB and BLS resistance under field conditions. Higher levels of BB resistance were observed in *aus*/*boro* subgroup, whereas the *japonica*, *temperate japonica* and *tropical japonica* subgroups possessed comparatively high levels of resistance to BLS. A genome-wide association study (GWAS) mined 77 genomic loci significantly associated with BB and 7 with BLS resistance. The phenotypic variance (R^2) explained by these loci ranged from 0.4 to 30.2%. Among the loci, 7 for BB resistance were co-localized with known BB resistance genes and one for BLS resistance overlapped with a previously reported BLS resistance QTL. A search for the candidates in other novel loci revealed several defense-related genes that may be involved in resistance to BB and BLS. High levels of phenotypic resistance to BB or BLS could be attributed to the accumulation of the resistance (*R*) alleles at the associated loci, indicating their potential value in rice resistance breeding via gene pyramiding. The GWAS analysis validated the known genes underlying BB and BLS resistance and identified novel loci that could enrich the current resistance gene pool. The resources with strong resistance and significant SNPs identified in this study are potentially useful in breeding for BB and BLS resistance.

Keywords Rice · Bacterial blight · Bacterial leaf streak · Disease resistance · GWAS · Candidate genes

Introduction

The ancient domesticated crop, rice (*Oryza sativa* L.) is one of the most widely cultivated grain crops all over the world and contributes significantly to global food security (Khush 2005). Diseases caused by bacterial, fungal, and

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viral pathogens pose continuous threats to crop production and lead to significant yield losses worldwide (Ou 1985). Bacterial blight (BB) and bacterial leaf streak (BLS) caused by Gram-negative bacteria *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *X. oryzae* pv. *oryzicola* (*Xoc*), respectively, are two important diseases with frequent outbreak in tropical and temperate regions resulting in considerable damage to rice production annually (Niño-Liu et al. 2006). BB and BLS can cause severe yield loss of up to 50% and 32%, respectively, depending on the rice variety, growth stage, the geographic location and environmental conditions (Niño-Liu et al. 2006). Chemical control is the most common management practices to mitigate BB and BLS, but increases the cost and leads to environmental risks. Deployment of host resistance is widely accepted as the most effective strategy to relive threats due to these diseases. Identification and characterization of loci or genes for disease resistance are indispensable for both deeply understanding the genetic architecture of phenotypic variation and efficiently developing resistant rice varieties.

Host resistance to BB has been extensively studied over last several decades. To date, at least 46 BB resistance (*R*) genes resistant to various strains of *Xoo* have been identified in rice (Neelam et al. 2020; Jiang et al. 2020; Chen et al. 2020). Sixteen of them including *Xa1*, *Xa2/Xa31*, *Xa3/Xa26*, *Xa4*, *xa5*, *Xa10*, *xa13*, *Xa14*, *Xa21*, *Xa23*, *xa25*, *Xa27*, *xa41*, *Xa45*, *CGS-Xo1*$_{11}$ and *Xa7* were cloned (Song et al. 1995; Yoshimura et al. 1998; Iyer and McCouch 2004; Sun et al. 2004; Gu et al. 2005; Chu et al. 2006; Xiang et al. 2006; Yang et al. 2006; Liu et al. 2011; Tian et al. 2014; Hutin et al. 2015; Wang et al. 2015a; Hu et al. 2017; Ji et al. 2020; Zhang et al. 2020a, b; Chen et al. 2021; Luo et al. 2021). Some of these genes have been widely applied in rice breeding programs, such as *Xa3/Xa26* and *Xa4*, which play an important role in rice breeding and production in China (Gao et al. 2010; Hu et al. 2017). However, the rapid genetic evolution of the bacteria often leads to frequent breakdown of *R* genes after several years of large-scale commercial use (Vera-Cruz et al. 2000). The breakdown of resistance contributed by *Xa3/Xa26* and *Xa4* has been observed in many rice-growing regions (Wang et al. 2005). To effectively control the disease, continuous efforts of enriching the pool of *R* genes and pyramiding multiple *R* genes into elite varieties are crucial.

Compared to BB, knowledge on genetic mapping of BLS resistance loci is very limited due to lack of highly resistant rice varieties. A few of quantitative trait loci (QTL) were reported (Tang et al. 2000; Zheng et al. 2005; Chen et al. 2006; Bossa-Castro et al. 2018). A QTL, *qBlsr5a*, with relatively larger effect on the short arm of chromosome 5 was finely mapped to a 30-kb interval (Xie et al. 2014). Coincidentally, the BB resistance gene *xa5*, encoding a small (γ) subunit of the conserved general transcription factor TFIIA, was located in the same region and proved to be responsible for the *qBlsr5a* effect (Xie et al. 2014, 2019; Yuan et al. 2016). Additionally, a recessive locus *bls1* and a dominant locus *Xo1* conferring high-level race-specific resistance to BLS were identified from Guangxi common wild rice and American heirloom rice variety Carolina Gold Select, respectively (He et al. 2012; Triplett et al. 2016). It is interesting that a non-host *R* gene, *Rxo1*, encoding a nucleotide-binding and leucine-rich repeat domain (NLR) protein, was isolated from maize and confers qualitative resistance to BLS in rice (Zhao et al. 2005). However, strong rice resistance against *Xoc* is rarely reported and the genetic basis of resistance remains unintelligible.

Quantitative trait locus mapping in bi-parental population is a conventional linkage-based approach extensively used to identify loci for traits of interest. This approach has some limitations, such as low allelic diversity and low recombination events in many cases. Construction of a mapping population is also labor-intensive and time-consuming. In contrast, the genome-wide association studies (GWAS) are based on linkage disequilibrium (LD) to dissect the genetic basis of complex traits in a large collection of germplasm accessions with broad diversity, accumulating more recombination events than bi-parental mapping populations and resulting in higher resolution (Nordborg and Tavaré 2002; Yu and Buckler 2006). The availability of explosive genome sequences and high-density SNP information for rice in combination with recent advances in study on the molecular interactions between plant and pathogens enable GWAS a very powerful tool for identification of loci involved in rice disease resistance. Genetic architecture of rice resistance to diverse diseases, including blast, bacterial blight, *bakanae* and false smut, has been investigated through GWAS in several studies (Wang et al. 2014; Kang et al. 2016; Mgonja et al. 2016; Raboin et al. 2016; Zhu et al. 2016; Dilla-Ermita et al. 2017; Zhang et al. 2017; Li et al. 2018, 2019; Long et al. 2020). Many candidate genes were identified through GWAS and some of them were cloned and characterized (Li et al. 2019; Liu et al. 2020).

Genome sequences of rice are becoming increasingly available. The 3000 Rice Genomes Project (3 K RGP) was launched and successfully accomplished by the scientists from Chinese Academy of Agricultural Sciences (CAAS), the Beijing Genomics Institute (BGI)-Shenzhen and the International Rice Research Institute (IRRI). A total of 3010 accessions originating from 89 countries were re-sequenced using the Illumina NGS technology, with an average depth of 14.3×, which provides a valuable resource for rice genomics research and breeding (The 3,000 rice genomes project 2014; Wang et al. 2018). The objectives of this study were to identify novel resistance sources and loci for BB and BLS resistance. Thus, we evaluated the

BB and BLS resistance of 895 re-sequenced accessions, a subset of the germplasm selected from the 3 K RGP, to multiple strains under natural conditions. Then, GWAS was performed to detect associations between the disease reactions and high-density SNP markers. The results may facilitate genetic improvement of rice resistance against BB and BLS.

Materials and methods

Plant materials

A total of 895 geographically diverse *O. sativa* germplasm accessions included in the 3 K RGP (The 3,000 rice genomes project 2014) were provided by the International Rice Research Institute (IRRI). These accessions, including *indica* (371), *japonica* (60), *temperate japonica* (143), *tropical japonica* (181), *intermediate* (38), *aus/boro* (79), and *basmati/sadri* (23), displayed obvious variation in many agronomic traits. Based on the feasibility or practicability of the field experiments, we randomly selected 407, 271 and 528 of the 3 K RGP accessions for evaluation of BB resistance at the seedling, BB resistance at the adult, and BLS resistance at the adult stage, respectively (Table S1).

Evaluation of BB and BLS resistance under field conditions

All of the tested accessions were planted in field at the experimental station of the Huazhi Bio-tech Company Ltd., Changsha, China (28° 18′ N and 113° 18′ E). BB and BLS resistance evaluations were performed in the cropping season of 2017 and 2018, respectively. The experimental field was bordered with plastic film with 2 m high to prevent the spread of inoculum. The investigation of BB resistance was conducted in 2017 using the randomized complete block design with two replications, while the observation of BLS resistance was carried out in 2018 using the randomized complete block design with one replication. To evaluate the seedling-stage BB resistance of 407 accessions, 50 seeds per accession were sown in a seedling nursery. The seedlings were inoculated with the Philippine strain PXO99 (race P6) and Chinese strain FuJ (race C8) at the three to four-leaf stage, using the method described in Kauffman et al. (1973). Briefly, the bacterial strains were cultured on peptone sucrose agar medium at 30 °C for 3 days, and each inoculum was prepared by suspending the bacterial mass in sterile water at a concentration of $OD_{600} = 1.0$. Twenty healthy plants of each accession were selected and 1–2 uppermost fully expanded leaves of each plant were inoculated using scissors dipped in bacterial suspensions to clip 1–2 cm of the leaf tip. Plant reactions to disease infection were evaluated three weeks after inoculation by measuring lesion length (LL, cm). For each replication, the average LL per accession was calculated based on five longest lesions from at least three individual plants. The average LL of two replications for each accession was used for normalization through rankTransPheno function in the FRGEpistasis R package and subsequent association analysis. To evaluate the adult-stage BB resistance of 271 accessions, 8 healthy plants per accession at the tillering or booting stage (about 60 days after sowing) were inoculated with the Philippine strains PXO99 (race P6), PXO61 (race P1) and Chinese strains FuJ (race C8), YuN24 (race C9), and disease reactions were investigated with the same method used at the seedling stage. Seven more *Xoo* stains from China and Philippines were used for analysis of resistance spectra. To evaluate BLS resistance of 528 accessions, eight healthy plants per accession were inoculated with Chinese strains HAB8-47 and HNB1-19 through the penetration method using a needleless syringe at the tillering or booting stage (about 60 days after sowing). One inoculation per leaf was performed in two uppermost fully expanded leaves of the main stem. LL was measured three weeks after inoculation. The mean value of six longest lesions from at least three individual plants for each accession was used for normalization and subsequent association analysis.

The resistance level of accessions was scored based on the LL. For BB, accessions with $LL < 5$ cm, $5\text{ cm} \le LL < 10$ cm, $10\text{ cm} \le LL < 15$ cm, and $LL \ge 15$ cm were rated as resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S), respectively (Dilla-Ermita et al. 2017). For BLS, we used the criterion described by Wonni et al. (2015) with some modifications. Accessions with $LL < 0.5$ cm, $0.5\text{ cm} \le LL < 1.0$ cm, $1.0\text{ cm} \le LL < 1.5$ cm, and $LL \ge 1.5$ cm were rated as resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S), respectively.

SNP genotyping data and population structure

The SNP genotyping data of the tested accessions were retrieved from Rice SNP-Seek Database (https://snp-seek.irri.org) (Mansueto et al. 2017). SNP markers with a minor allele frequency (MAF) < 5% and call rate < 20% were removed from the dataset. The most widely applied method for analysis of population structure is principal components analysis (PCA). PCA-based methods use markers (SNPs in most cases) to infer orthogonal axes of continuous variation, called principal components that reduce the data to few variables explaining most of the variation of the genetic information. Here, PCA was performed using the program

SMARTPCA (Patterson et al. 2006). First five principal components (PCs) explained the most variations were used as covariates to correct population structure in association studies and first two principal components (PCs) were visualized using the package matplotlib of Python (Hunter 2007).

Genome-wide association analysis

GEMMA software and the mixed linear model (MLM) with the control of structure (*Q* matrix consisting of the first five PCs) and kinship (*K* matrix) were used to determine the association between SNP markers and the observed phenotypic traits (Zhou and Stephens 2012). To reduce the false-positive rate, SNP-trait associations were adjusted by Bonferroni correction. After Bonferroni correction, the cutoff for statistical significance was determined to be $P < 4.57 \times 10^{-6}$, 4.17×10^{-6}, 4.37×10^{-6} for BLS, BB at the adult stage and BB at the seedling stage, respectively (Lander and Kruglyak 1995). Manhattan plots and QQ plots were generated using the package matplotlib of Python (Hunter 2007). Phenotypic variation (R^2) explained by multiple SNPs in each significant locus was estimated by stepwise regression using R software (Zhou et al. 2017).

Analysis and annotation of significant association signals

We used R package Big-LD with threshold 0.6 of r^2 for LD block partitioning (Kim et al. 2018). LD blocks containing significantly associated SNPs were defined as the candidate genomic loci. The SNP with most significant association in a block was determined as the lead SNP. Genes in the blocks with significant SNPs were considered as the candidates for BB and BLS resistance. Gene-based SNP annotation was performed using the IRGSP-1.0 (Ensembl release 41) of the rice genome in the program SnpEff (Cingolani et al. 2012). Information of the reported BB resistance genes near the hits was retrieved from the literature, the Q-TARO (http://qtaro.abr.affrc.go.jp/) and funRiceGenes database (http://funricegenes.ncpgr.cn/) (Yamamoto et al. 2012; Yao et al. 2018). The Rice Annotation Project (RAP) identification numbers for the candidate genes involved in BB and BLS resistance are summarized in Table S6.

Statistical analysis

All the phenotypic screening experiments were performed with at least three replications. The mean values and standard errors were calculated using Microsoft Excel 2007. Statistical significance was tested by the one-way ANOVA followed by the Duncan's multiple range test using SPSS software. Significance was declared when $P < 0.05$ and indicated by different letters.

Results

Phenotypic evaluation of bacterial blight and bacterial leaf streak resistance

To evaluate the BB and BLS resistance of the selected germplasm accessions from 3 K RGP, four representative *X. oryzae* pv. *oryzae (Xoo)* strains (FuJ, YuN24, PXO99 and PXO61) and two *X. oryzae* pv. *oryzicola* (*Xoc*) strains (HAB8-47 and HNB1-19) were used for artificial inoculation under natural conditions in 2017 and 2018, respectively. Phenotypic reactions to BB infection at the seedling stage (BB-ss), BB and BLS at the adult stage (BB-as and BLS-as) were summarized in the supplementary tables (Tables S2, S3, S4). Distributions of disease reactions to all the BB strains at both seedling and adult stages were skewed toward susceptibility. A considerable proportion of tested accessions was susceptible/moderately susceptible to BB strains and higher proportions were observed for strains PXO99-ss and PXO99-as (85.4% and 95.5%), indicating that PXO99 was more virulent to the tested rice accessions in this study at both growth stages (Fig. 1a). However, some accessions showed high-level resistance. Three accessions, AUS 295 (IRGC 127184, *aus/boro*, Bangladesh), IR 70027-8-2-2-3-2 (IRGC 126028, *indica*, Philippines), SADA AUS (IRGC 127776, *aus/boro*, India), were resistant to the two *Xoo* strains at the seedling stage (Table S2). And three accessions, AUS 295 (IRGC 127184, *aus/boro*, Bangladesh), SAITA (IRGC 127778, *aus/boro*, Bangladesh), UCP 122 (IRGC 127871, *aus/boro*, Bangladesh), showed resistance to all four *Xoo* strains at the adult stage (Table S3). It is noteworthy that both SAITA and UCP 122 displayed resistance to FuJ and moderate resistance to PXO99 at the seedling stage, and AUS 295 was resistant to all the *Xoo* strains at both growth stages. We further investigated the resistance spectra and influence of plant development on BB resistance of the three accessions AUS 295, UCP 122 and SAITA. The results indicated that these three accessions conferred all-growth-stage resistance to FuJ and broad-spectrum resistance to 11 tested *Xoo* strains at the adult stage (Figs. S1, S2). For BLS, over half the tested accessions were resistant/moderately resistant to *Xoc* strains HAB8-47-as (55.9%) and HNB1-19-as (57.1%) (Fig. 1a). Twenty tested accessions including seven from *tropical japonica* subgroup, seven from *indica* subgroup, four from *japonica* subgroup, one from *intermediate*, and one from *aus/boro* subgroup, showed resistance to both of the two BLS strains (Table S4).

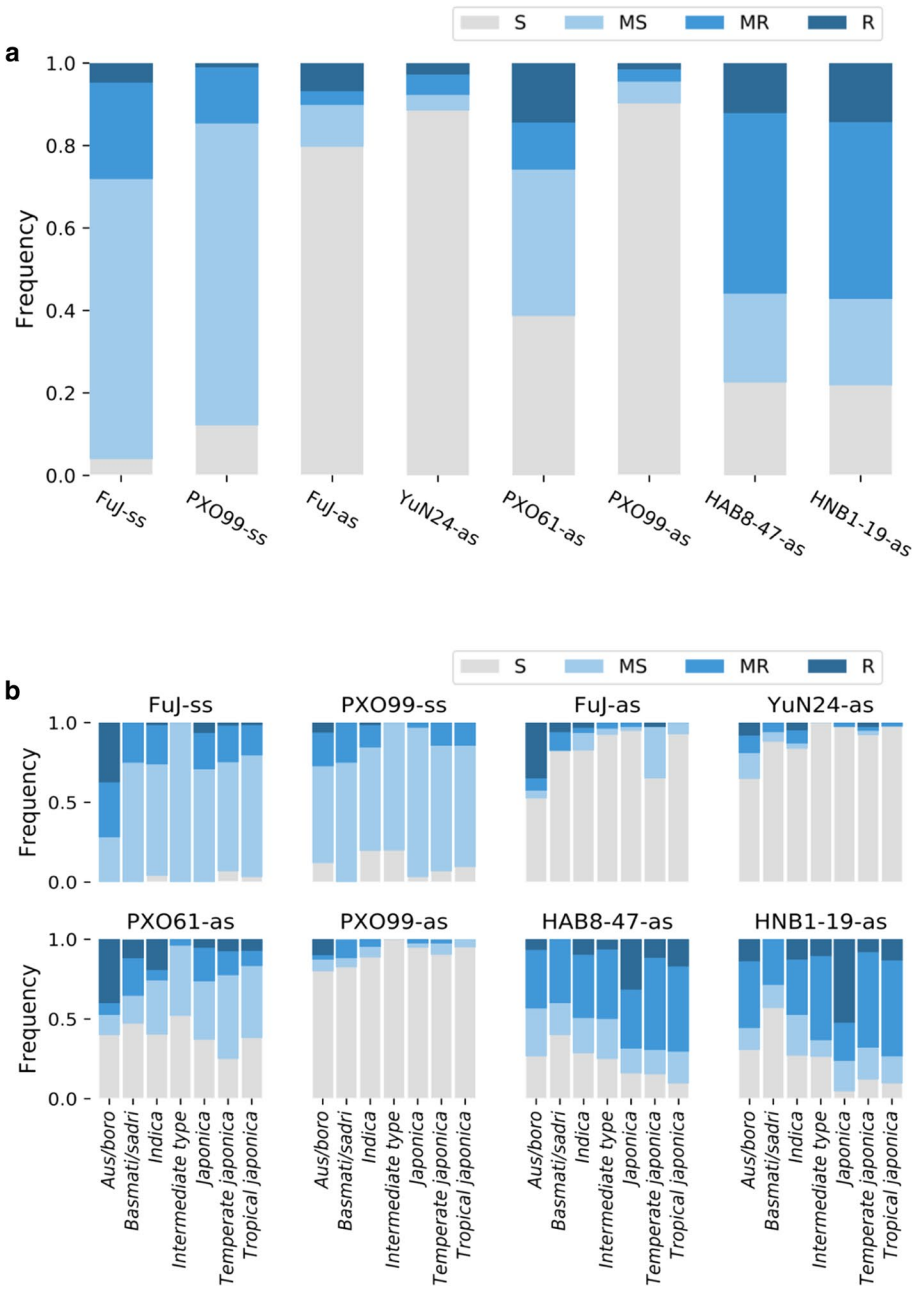

Fig. 1 Frequency distribution of responses to eight *Xoo* and *Xoc* strains in the tested rice accessions (**a**) and seven subgroups (**b**)

We then compared the disease severity among the seven subgroups of the observed germplasm accessions. The analysis revealed that 71.88%, 27.27%, 42.50%, 47.50%, 18.92% and 12.50% of the tested accessions were resistant or moderately resistant to FuJ-ss, PXO99-ss, FuJ-as, PXO61-as, YuN24-as and PXO99-as, respectively, in the *aus/boro* subgroup which was more resistant than other six subgroups against *Xoo* strains. But 100.00%, 100.00%, 96.30%, 96.00%, 100.00% and 100.00% of the tested accessions were susceptible or moderately susceptible to FuJ-ss, PXO99-ss, FuJ-as, PXO61-as, YuN24-as and PXO99-as, respectively, in the *intermediate* which was the most susceptible subgroup (Fig. 1b).

As to the resistance to BLS strains, out of the tested accessions in *japonica*, 68.42% and 76.19% were resistant or moderately resistant to HAB8-47 and HNB1-19, respectively. Among the tested accessions in *temperate japonica*, 69.23% and 68.00% were resistant or moderately resistant to HAB8-47 and HNB1-19, respectively. In the *tropical japonica* subgroup, 70.48% and 73.33% of the tested accessions were resistant or moderately resistant to HAB8-47 and HNB1-19, respectively. Thus, the *japonica*, *temperate japonica* and *tropical japonica* subgroups were relatively

resistant to BLS strains. But the 60.00% and 71.43% of the tested accessions in *basmati/sadri* subgroup were susceptible or moderately susceptible to HAB8-47 and HNB1-19, respectively, and thus was the most susceptible subgroup (Fig. 1b).

Genome-wide association analysis

To exclude the possibility of population stratification causing false-positive association, we conducted principal component analysis (PCA) of the 407, 271 and 528 accessions for phenotypic scores of BB at the seedling and adult stages, and BLS at the adult stage, respectively (Fig. 2). PCA showed that the randomly selected sample accessions were evenly distributed among all the 3 K RGP germplasm accessions, suggesting that these three sets of sample accessions could represent the genetic variance of this unprecedented genomics resource in rice. To identify genomic loci associated with BB and BLS resistance, we performed GWAS using the SNP datasets and the phenotypic data. Totally, 823 SNPs clustering in 84 genomic loci were found to be associated with the observed phenotypic traits (Fig. 3; Table 1; Table S4). Among these loci, 77 across 12 chromosomes were significantly associated to BB resistance explaining 0.4–30.2% of the phenotypic variation, including 24 (10 and 14 associated with resistance to FuJ-ss and PXO99-ss, respectively) with resistance at the seedling stage, and 53 (13, 30, 6 and 4 associated with resistance to FuJ-as, PXO61-as, YuN24-as and PXO99-as, respectively) with resistance at the adult stage. These loci explained together 30.51% (FuJ-ss), 31.99% (PXO99-ss), 67.41% (FuJ-as), 74.48% (PXO61-as), 11.76% (YuN24-as), and 33.21% (PXO99-as) of the phenotypic variation for resistance reaction, respectively. Several hotspots of significantly associated SNPs were identified. A total of 343 SNPs at 4 loci (BBRAL8, BBRAL30, BBRAL7, BBRAL68) significantly associated with BB resistance were clustered in an approximately 245-kb region (259,484–504,974 bp) on chromosome 5, accounting for 41.70% of all the associated SNPs. A cluster of 5 resistance-associated loci, BBRL39, BBRL40, BBRL41, BBRL42 and BBRL43, spanned a region of approximately 462-kb interval (27,364,589–27,827,470 bp) on chromosome 11, and contained 202 SNPs. Moreover, an interval encompassing 84-kb (2,733,731–2,818,547 bp) on chromosome 12 harbored the locus BBRAL13 containing 61 SNPs.

In contrast to BB, only 17 significantly associated SNPs in 7 loci including 1 and 6 for resistance to HAB8-47 and HNB1-19, respectively, were detected. These loci could explain 0.65% and 25.11% of the phenotypic variance for reaction to infection of BLS strains HAB8-47 and HNB1-19, respectively. The locus BLSRAL2 demonstrated the highest level of associations with 6 SNPs.

Validation of significant SNP-resistance associations for BB and BLS

To understand effects of the allelic variation on BB or BLS resistance, we selected 5 loci associated with the highest number of SNPs on Chromosome 5, 11, and 12. The most significantly associated SNP on each locus was considered as the candidate resistance allele. It is shown that there are five SNPs significantly associated with the resistance in the natural population (Fig. 4a). Among the 7 subgroups of rice accessions, *R* alleles in BBRAL8, BBRAL13 and BBRAL30 are more abundant in *aus*/*boro* accessions. We found 35.90%, 30.00% and 33.33% of accessions from *aus*/*boro* subgroup carry the *R* alleles in BBRAL8, BBRAL13 and BBRAL30, respectively. In BBRAL39 and BBRAL41, *indica* accessions harbors more *R* alleles than other subgroups (Fig. 4b). Additionally, we investigated the average number of *R* alleles of the five loci in the subgroups of accessions. The analysis indicated that *aus*/*boro* had the highest

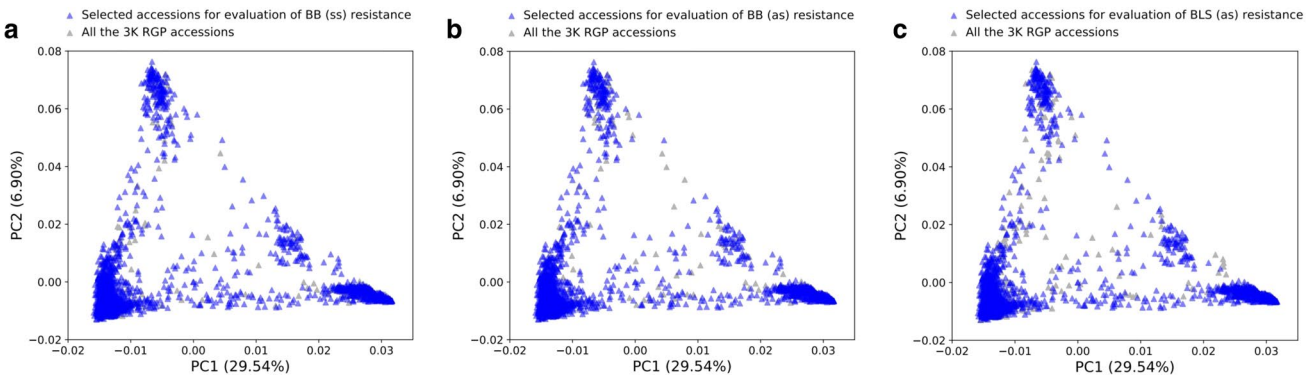

Fig. 2 Principal component analysis plots for the first two components of rice 3 K RGP accessions. PCA analysis of 407 rice accessions for evaluation of BB resistance at the seedling satge (**a**), 271 rice accessions for evaluation of BB resistance at the adult satge (**b**) and 528 rice accessions for evaluation of BLS resistance at the adult satge (**c**)

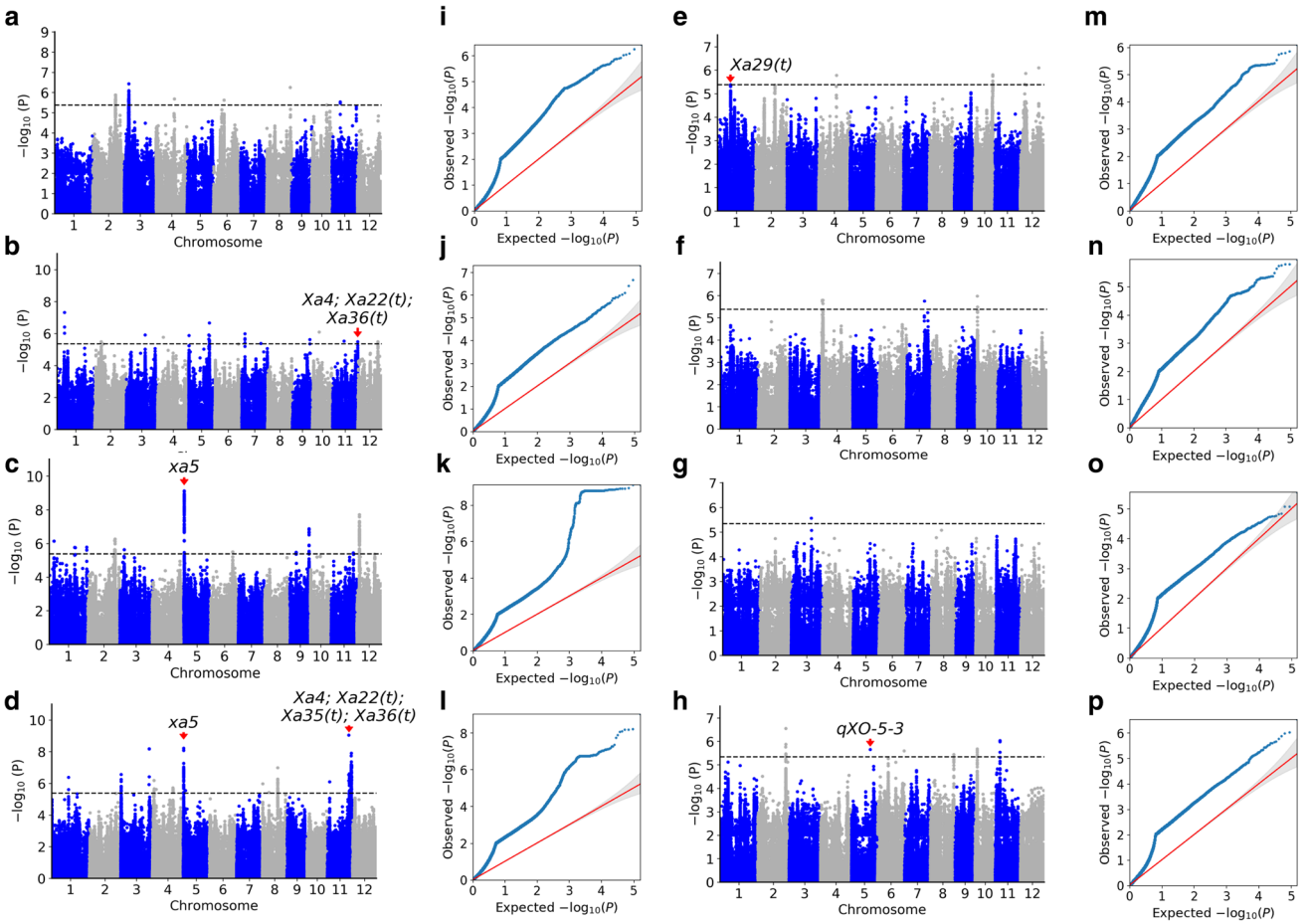

Fig. 3 Genome-wide association scan of rice BB and BLS resistance. Manhattan plots for eight *Xoo* and *Xoc* strains: **a** FuJ-ss; **b** PXO99-ss; **c** FuJ-as; **d** PXO61-as; **e** PXO99-as; **f** YuN24-as; **g** HAB8-47-as; **h** HNB1-19-as. Negative $\log_{10}$-transformed *P* values from a genome-wide scan are plotted against position on each of 12 chromosomes. Black horizontal dashed line indicates the genome-wide significance threshold. The red arrows indicate that the identified loci are co-localized with previously mapped or cloned resistance *Xa* genes or QTL. Quantile–quantile plot for the eight strains: **i** FuJ-ss; **j** PXO99-ss; **k** FuJ-as; **l** PXO61-as; **m** PXO99-as; **n** YuN24-as; **o** HAB8-47-as; **p** HNB1-19-as

frequency of *R* alleles (1.05), whereas 0.35, 0.51, 0.04, 0.16, 0.15, 0.05 were observed in *basmati/sadri*, *indica*, *intermediate*, *japonica*, *temperate japonica* and *tropical japonica*, respectively. Furthermore, we analyzed the SNP haplotypes of the 84 associated loci in the rice accessions that displayed broader spectrum and high levels of resistance to BB or BLS strains. The results showed that the average frequency of the *R* alleles for 3 BB-resistant accessions is 77.1% and 20 BLS-resistant accessions is 67.2% (Fig. 5). These results demonstrate that the *R* alleles are highly enriched in these resistant accessions which are valuable resistant resources for rice BB and BLS resistance breeding.

Candidate genes involved in the BB and BLS resistance

Out of the 7 loci associated with resistance to BLS, one was co-localized with a previously reported QTL, *qXO-5-3* (Table 1) (Bossa-Castro et al. 2018). Only one significant SNP was detected in this locus (BLSRAL3). A total of 20 genes were identified in BLSRAL3 based on haplotype block structure analysis and gene annotation information (Table S6). Interestingly, one of them, *Os05g0439400*, encoding ubiquitin E3 ligase, was proved to positively regulate rice resistance to BB (Ishikawa et al. 2014). Another locus, BLSRAL2, which contains 6 SNPs shows highly significant and the strongest association with BLS resistance. None of BLS-resistant QTLs has been previously located in this region, suggesting that BLSRAL2 represents a new genomic region associated with BLS resistance. Among the

Table 1 Loci significantly associated with the bacterial blight or bacterial leaf streak resistance

Locus	Strain	Stage	Chromosome	Number of significant SNP in LD block	Lead SNP position (bp)	LD block (bp)	$-\log_{10}(P)$ of lead SNP	Known *R* gene or locus
BLSRAL1	HAB8-47	Adult stage	3	1	23,367,279	22,866,078–23,572,102	5.57	
BLSRAL2	HNB1-19	Adult stage	2	6	32,176,706	31,447,481–32,248,698	6.55	
BLSRAL3	HNB1-19	Adult stage	5	1	21,624,608	21,505,165–21,644,915	5.65	*qXO-5-3*
BLSRAL4	HNB1-19	Adult stage	6	1	30,508,855	30,493,380–30,551,197	5.59	
BLSRAL5	HNB1-19	Adult stage	8	1	26,860,989	26,860,857–26,861,993	5.44	
BLSRAL6	HNB1-19	Adult stage	10	4	2,477,571	2,374,365–2,521,758	5.68	
BLSRAL7	HNB1-19	Adult stage	11	3	5,415,033	5,407,599–5,468,482	6.04	
BBRAL1	FuJ	Adult stage	1	1	5,359,513	5,343,291–5,421,945	6.15	
BBRAL2	FuJ	Adult stage	1	1	28,429,652	28,427,790–28,430,589	5.76	
BBRAL3	FuJ	Adult stage	1	1	29,214,777	29,043,296–29,246,939	5.75	
BBRAL4	FuJ	Adult stage	1	2	42,048,686	42,040,353–42,105,516	5.78	
BBRAL5	FuJ	Adult stage	2	14	30,584,224	29,741,841–30,782,710	6.27	
BBRAL6	FuJ	Adult stage	3	1	4,879,521	4,314,446–6,162,098	5.63	
BBRAL7	FuJ	Adult stage	5	4	355,801	351,780–356,185	7.38	
BBRAL8	FuJ	Adult stage	5	186	368,109	356,754–475,460	9.13	*xa5*
BBRAL9	FuJ	Adult stage	6	1	24,741,856	24,332,006–24,865,759	5.50	
BBRAL10	FuJ	Adult stage	9	2	6,953,507	6,941,116–7,013,523	5.48	
BBRAL11	FuJ	Adult stage	9	11	21,271,979	21,132,666–21,353,698	6.87	
BBRAL12	FuJ	Adult stage	11	1	24,971,424	24,963,580–25,122,509	5.44	
BBRAL13	FuJ	Adult stage	12	61	2,803,033	2,733,731–2,818,547	7.71	
BBRAL14	PXO61	Adult stage	1	1	19,452,606	19,439,001–19,504,281	6.37	
BBRAL15	PXO61	Adult stage	1	1	19,428,125	19,428,125–19,428,125	5.62	
BBRAL16	PXO61	Adult stage	3	2	525,566	525,191–533,517	5.58	
BBRAL17	PXO61	Adult stage	3	4	544,958	533,700–545,871	5.82	
BBRAL18	PXO61	Adult stage	3	6	546,721	545,883–610,933	6.56	
BBRAL19	PXO61	Adult stage	3	1	32,520,753	32,497,656–32,547,448	5.92	
BBRAL20	PXO61	Adult stage	3	3	32,623,317	32,620,714–32,628,009	8.18	
BBRAL21	PXO61	Adult stage	4	7	1,880,139	1,811,942–2,015,778	6.18	
BBRAL22	PXO61	Adult stage	4	1	23,612,201	23,610,282–23,615,793	5.60	
BBRAL23	PXO61	Adult stage	4	1	23,644,331	23,640,191–23,691,793	5.72	
BBRAL24	PXO61	Adult stage	4	1	23,711,538	23,709,771–23,714,193	5.64	
BBRAL25	PXO61	Adult stage	4	1	23,744,927	23,721,946–23,819,615	5.44	
BBRAL26	PXO61	Adult stage	4	1	4,309,064	4,309,064–4,309,064	5.62	
BBRAL27	PXO61	Adult stage	4	1	23,624,508	23,624,508–23,624,508	5.60	
BBRAL28	PXO61	Adult stage	4	1	23,693,611	23,693,611–23,693,611	5.53	
BBRAL29	PXO61	Adult stage	4	1	23,695,925	23,695,925–23,695,925	5.55	

Table 1 (continued)

Locus	Strain	Stage	Chromosome	Number of significant SNP in LD block	Lead SNP position (bp)	LD block (bp)	$-\log_{10}(P)$ of lead SNP	Known *R* gene or locus
BBRAL30	PXO61	Adult stage	5	152	420,429	356,754–475,460	8.22	*xa5*
BBRAL31	PXO61	Adult stage	5	1	2,518,553	2,467,590–2,521,871	5.56	
BBRAL32	PXO61	Adult stage	8	1	529,945	493,976–532,544	5.97	
BBRAL33	PXO61	Adult stage	8	1	7,623,083	7,615,447–7,757,411	5.43	
BBRAL34	PXO61	Adult stage	8	20	17,562,286	17,551,274–17,674,478	6.98	
BBRAL35	PXO61	Adult stage	11	1	2,586,360	2,574,730–2,645,073	6.10	
BBRAL36	PXO61	Adult stage	11	3	24,705,712	24,705,450–24,706,636	9.04	
BBRAL37	PXO61	Adult stage	11	14	25,258,809	25,250,702–25,264,574	6.61	
BBRAL38	PXO61	Adult stage	11	2	26,303,687	26,273,879–26,335,216	5.60	
BBRAL39	PXO61	Adult stage	11	92	27,414,010	27,364,589–27,440,642	7.74	
BBRAL40	PXO61	Adult stage	11	23	27,482,608	27,478,616–27,498,150	6.33	
BBRAL41	PXO61	Adult stage	11	70	27,574,964	27,570,533–27,647,374	7.90	
BBRAL42	PXO61	Adult stage	11	1	27,661,823	27,648,392–27,679,631	6.34	*Xa4; Xa22(t); Xa35(t); Xa36(t)*
BBRAL43	PXO61	Adult stage	11	16	27,819,879	27,792,852–27,827,470	7.15	*Xa4; Xa22(t); Xa35(t); Xa36(t)*
BBRAL44	PXO99	Adult stage	1	1	14,777,476	14,769,121–14,858,187	5.38	*Xa29(t)*
BBRAL45	PXO99	Adult stage	4	1	20,067,971	19,932,981–20,093,107	5.78	
BBRAL46	PXO99	Adult stage	10	7	20,876,399	20,849,019–20,889,064	5.81	
BBRAL47	PXO99	Adult stage	12	1	5,116,604	4,780,734–5,174,459	5.40	
BBRAL48	PXO99	Adult stage	12	1	6,217,077	6,211,016–6,244,451	5.86	
BBRAL49	PXO99	Adult stage	12	1	21,121,974	21,116,177–21,125,050	6.10	
BBRAL50	YuN24	Adult stage	4	2	628,507	58,714–844,289	5.80	
BBRAL51	YuN24	Adult stage	4	2	1,412,961	1,333,398–1,444,265	5.80	
BBRAL52	YuN24	Adult stage	7	1	20,792,596	20,731,208–20,795,945	5.76	
BBRAL53	YuN24	Adult stage	10	2	123,305	45,299–159,456	5.98	
BBRAL54	FuJ	Seedling stage	2	10	25,951,997	25,872,158–26,003,350	5.88	
BBRAL55	FuJ	Seedling stage	2	2	26,068,343	26,039,501–26,078,877	5.87	
BBRAL56	FuJ	Seedling stage	2	1	26,081,089	26,080,899–26,094,348	5.63	
BBRAL57	FuJ	Seedling stage	2	1	26,171,439	26,116,980–26,198,071	5.68	
BBRAL58	FuJ	Seedling stage	3	26	5,214,259	4,177,116–6,162,098	6.43	
BBRAL59	FuJ	Seedling stage	4	1	20,907,587	20,891,640–20,938,493	5.68	
BBRAL60	FuJ	Seedling stage	6	1	12,064,814	11,999,586–13,060,781	5.62	
BBRAL61	FuJ	Seedling stage	8	1	26,904,920	26,881,796–26,907,896	6.25	
BBRAL62	FuJ	Seedling stage	10	1	17,278,310	17,262,693–17,460,155	5.39	

Table 1 (continued)

Locus	Strain	Stage	Chromosome	Number of significant SNP in LD block	Lead SNP position (bp)	LD block (bp)	$-\log_{10}(P)$ of lead SNP	Known *R* gene or locus
BBRAL63	FuJ	Seedling stage	11	2	9,462,561	9,456,365–9,468,380	5.53	
BBRAL64	PXO99	Seedling stage	1	3	8,539,889	8,141,633–8,654,918	7.32	
BBRAL65	PXO99	Seedling stage	2	1	7,371,946	7,370,039–7,556,607	5.49	
BBRAL66	PXO99	Seedling stage	3	1	21,965,065	21,914,259–22,070,217	5.91	
BBRAL67	PXO99	Seedling stage	4	1	6,419,603	6,415,712–6,419,960	5.77	
BBRAL68	PXO99	Seedling stage	5	1	379,097	259,484–504,974	5.87	*xa5*
BBRAL69	PXO99	Seedling stage	5	2	23,113,744	22,705,619–23,132,338	5.91	
BBRAL70	PXO99	Seedling stage	5	5	23,776,550	23,408,261–24,071,020	6.67	
BBRAL71	PXO99	Seedling stage	7	4	3,538,562	3,359,706–3,554,443	5.99	
BBRAL72	PXO99	Seedling stage	7	1	21,953,542	21,834,219–21,962,514	5.39	
BBRAL73	PXO99	Seedling stage	9	1	19,953,253	19,819,533–20,171,306	5.62	
BBRAL74	PXO99	Seedling stage	10	1	7,826,720	7,752,672–7,829,896	6.10	
BBRAL75	PXO99	Seedling stage	11	1	13,186,228	13,069,083–13,639,575	5.53	
BBRAL76	PXO99	Seedling stage	11	1	28,805,397	28,804,757–28,808,377	5.49	*Xa4; Xa22(t); Xa36(t)*
BBRAL77	PXO99	Seedling stage	12	2	23,024,010	23,001,064–23,036,551	5.49	

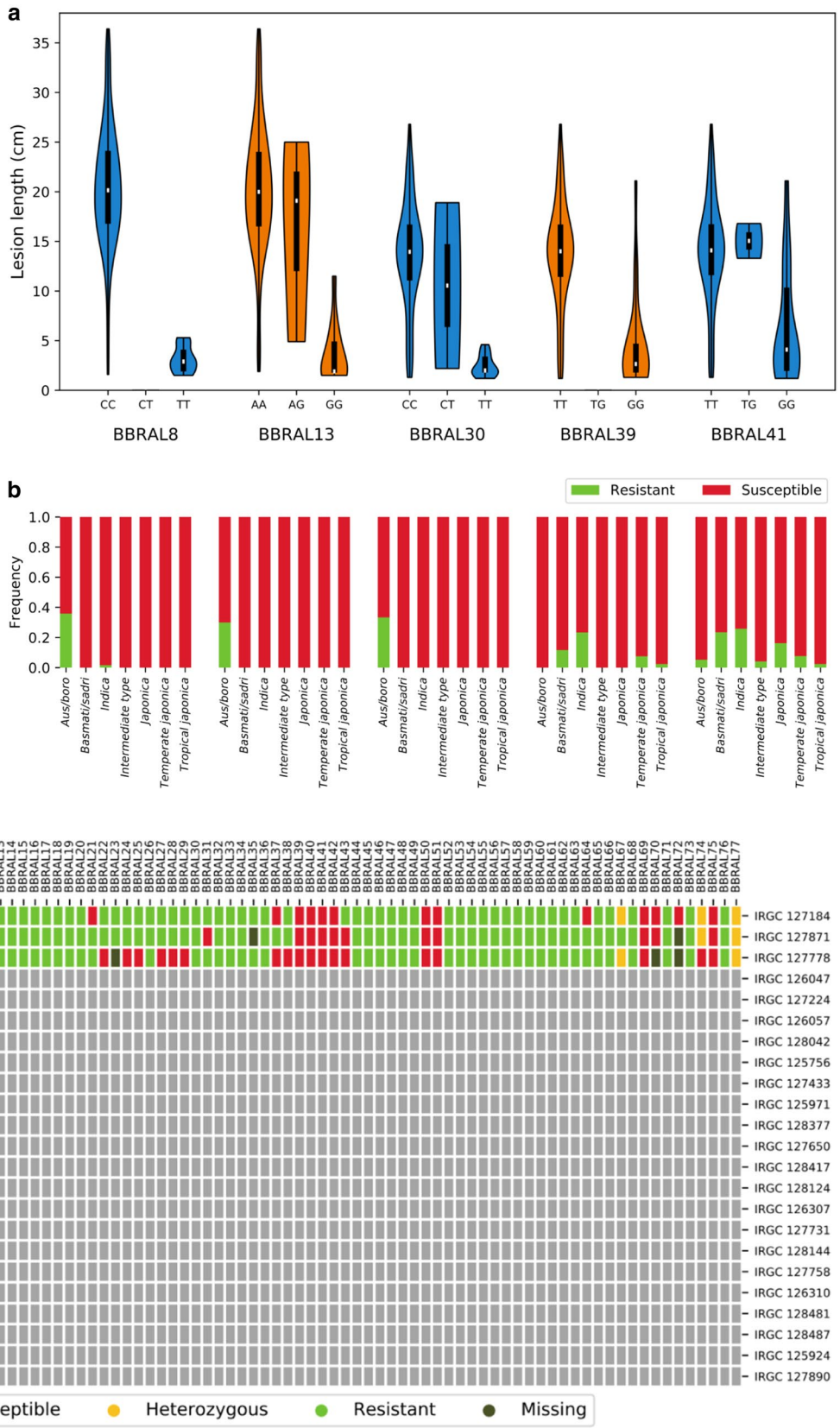

Fig. 4 Phenotypic distributions for the alternative alleles at the most significant SNPs of five associated loci in the hotspot regions (**a**) and distribution of resistance alleles among different subgroups (**b**)

Fig. 5 Heat map showing the SNP haplotypes of the 3 highly BB resistant accessions and 20 highly resistant cultivars in 77 BBRALs and 7 BLSRALs, respectively

121 genes identified in this locus, *Os02g0759400*, a RING-H2-type zinc finger protein gene, could be induced by pathogen infections (Meng et al. 2006), which may be involved in the rice disease resistance.

Among the 77 loci associated with resistance to BB, 7 were co-localized with the known *Xa* genes (Table 1). BBRAL8, which contains the most significant SNP explaining up to 30.15% of the phenotypic variation, overlapped with the well characterized BB resistance gene *xa5* (*Os05g0107700*). This *xa5* encoding the transcription factor IIA gamma subunit 5 (TFIIAγ5) is a widely used recessive *R* gene in rice resistance breeding programs. Similarly, BBRAL30 and BBRAL68 were localized in the same genomic region. The lead SNP in BBRAL30 explained 29.39% of the phenotypic variation. In contrast, the only significant SNP in BBRAL68 explained 1.84% of the phenotypic variation and this locus may play minor role in the resistance to PXO99-ss. The results agreed with previous studies, that PXO99 was compatible on *xa5*, whereas PXO61 and FuJ were incompatible (Liu et al. 2007). In the hotspot region on chromosome 11, 5 loci (BBRL39, BBRL40, BBRL41, BBRL42 and BBRL43) involving 202 SNPs significantly associated with BB resistance spanned an approximately 462-kb genomic region, which was overlapped with 4 *Xa* genes, including *Xa4*, *Xa22(t)*, *Xa35(t)* and *Xa36(t)*. In addition to *Xa4*, which encodes a cell wall-associated kinase and improves the BB resistance by strengthening the cell wall (Hu et al. 2017), other three genes were not yet isolated (Wang et al. 2003; Guo et al. 2010; Miao et al. 2010). Twenty-five annotated genes were identified in the region including three NB-ARC genes (*Os11g0686900*, *Os11g0686500*, *Os11g0678400*) (Table S6). NB-ARC and NBS-LRR encoding genes are the most prevalent and ancient *R* gene families in plants. Moreover, 61 SNPs significantly associated with BB resistance were clustered in hotspot region (BBRAL13) on chromosome 12, which was mapped far from any currently known BB resistance gene and likely represent a novel resistance locus. Eight functionally annotated genes were found in this region. However, none of them were reported to be related to rice disease resistance (Table S6). These candidate genes need to be functionally verified and annotated.

Discussion

The main objectives of the current study were to identify novel germplasm with high-level and broad-spectrum resistance against *Xoo* and *Xoc*, and novel loci for BB and BLS resistance by GWAS approach. We randomly selected and constructed three sets of rice accessions, which were inoculated with 2 *Xoo* strains at the seedling stage, 4 *Xoo* strains and 2 *Xoc* strains at the adult stage, respectively. Most of the tested accessions showed moderate susceptibility or susceptibility against all the *Xoo* strains at both seedling and adult stages. Among the inoculated *Xoo* strains, PXO99 showed the highest virulence. We found 85.4% and 95.5% of the accessions were susceptible or moderately susceptible to PXO99 at the seedling stage and adult stage, respectively. However, over half of the tested accessions were resistant or moderately resistant to the two *Xoc* strains. Among the seven rice subgroups, *aus*/*boro* accessions showed relatively high frequency of BB resistance to all the strains at both seedling and adult stages. This result agreed with the previous reports that *aus*/*boro* accessions were eltite resistant resources for BB (Sidhu et al. 1978; Dilla-Ermita et al. 2017; Li et al. 2018). Dilla-Ermita et al. (2017) reported that 96% of the tested *aus* genotypes exhibited resistance to both strains of race 9, PXO339 and PXO349. Similarly, *aus* subgroup was proved to be more resistant to *Xoo* race C1 in another study (Li et al. 2018). Based on the phenotypic screening, we obtained three and 20 accessions exhibiting high-level resistance against BB and BLS, respectively. The three BB resistant accessions conferred not only all-growth-stage resistance, but also broad-spectrum resistance. (Tables S2, S3; Figs. S1, S2).

GWAS approach has recently been widely used to dissect the genetic architecture of rice resistance to diverse pathogens. However, most of the studies focused on rice blast disease, caused by the fungal pathogen *Magnaporthe oryzae* (Wang et al. 2014, 2015b; Shinada et al. 2015; Kang et al. 2016; Mgonja et al. 2016; Raboin et al. 2016; Zhu et al. 2016; Lin et al. 2018; Li et al. 2019; Lu et al. 2019; Liu et al. 2020; Zhang et al. 2020a, b). There are few reports on application of GWAS to identify loci associated with BB and BLS resistance (Dilla-Ermita et al. 2017; Zhang et al. 2017; Bossa-Castro et al. 2018; Li et al. 2018; Kim and Reinke 2019). Using an *indica*/*aus* panel and an *indica* subset, 15 loci associated with resistance to *Xoo* strains from the Philippines were detected (Dilla-Ermita et al. 2017). Zhang et al. (2017) identified twelve loci containing 121 significantly associated signals in an *indica* population consisting 172 accessions. Li et al. (2018) identified 15 loci associated with the resistance against *Xoo* race C1 using 267 rice accessions from Rice Diversity Panel 1 (RDP1). Additionally, researchers used multi-parent advanced generation intercross (MAGIC) populations to map the QTL or genes conferring resistance to BB and BLS through combining GWAS with QTL mapping (Kim et al. 2019). To our knowledge, the present work represents the first report on identification of resistant loci against BB at the seedling stage and BLS using natural population through a GWAS approach. In this study, a total of 84 loci were identified, including 7 for BLS resistance, 24 for BB resistance at the seedling stage, and 53 for BB resistance at the adult stage. Among them, one BLSAL and seven BBRALs were overlapped with

previously reported genes/QTLs. On chromosome 5, a hotspot region containing 343 SNPs significantly associated with BB resistance, was co-localized with an extensively studied and used gene *xa5* (Jiang et al. 2020). It is interesting that, the frequency distribution of resistant alleles in this region was higher in *aus/boro* subgroup (35.90%) than any other subgroups. Our resluts agreed wtih the earlier studies that *xa5* is prevalent in *aus*/*boro* (Garris et al. 2003). Therefore, *xa5* is the most probable candidate gene for this region. However, the single *xa5* gene would not confer resistance to the *Xoo* strains PXO99 and YuN24. Thus, other *R* genes may exist in the accessions with broad-spectrum resistance, such as Aus 295, UCP122 and SAITA. In another hotspot region on chromosome 11, 5 BBRALs involving 202 associated SNPs were overlapped with 4 *Xa* genes, including *Xa4*, *Xa22(t)*, *Xa35(t)* and *Xa36(t)*. To date, over 20 QTL conferring BLS resistance have been reported (Bossa-Castro et al. 2018; Wang et al. 2020). Two of them, *qBlsr5a* (*xa5*) and *Xo1* were successfully isolated (Xie et al. 2014; Ji et al. 2020; Read et al. 2020). Among seven associated loci identified, only BLSRAL3 was found to co-localize with a fomerly described QTL *qXO-5–3* on chromosome 3. Interestingly, *qXO-5–3* was identified to confer resistance to both *Xoo* and *Xoc* strains (Bossa-Castro et al. 2018).

Analysis of the associated loci in these resistant accessions indicated that the high-level resistance was due to the accumulation of *R* alleles. It suggested that pyramiding of the resistant alleles may achieve effective resistance to BB and BLS. In addition, the lead SNPs at the five loci in hotspot regions were analyzed across all the tested accessions. The *aus*/*boro* subgroup contains more *R* alleles, and thus demonstrates high frequency of BB resistance.

Artificial inoculation of *Xoo* and *Xoc* strains under field conditions allowed effective identification of resistant accessions to BB and BLS. Application of GWAS in this study revealed 7 and 77 SNP loci associated with BLS and BB resistance, respectively. This study provides new insights into the genetic architecture of BLS resistance and BB resistance at two growth stages in rice. The findings in our study may facilitate the rice breeding for BB and BLS resistance. Future studies will focus on fine mapping of these resistant loci, validating the effects and functional characterization of the candidate genes.

Author contribution statement JHP, XLL and YZY conceived and designed the study. NJ, YL, YLS, ZWL, YLX, ZZH, YTW and YL conducted the field experiments. JF, QZ and KW conducted the GWAS. NJ, JF, QZ, YL and JHP wrote the manuscript. All authors read and approved the final version of the manuscript.

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Declarations

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

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