Identification of blast resistance QTLs based on two advanced backcross populations in rice

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Abstract

Background: Rice blast is an economically important and mutable disease of rice. Using host resistance gene to breed resistant varieties has been proven to be the most effective and economical method to control rice blast and new resistance genes or quantitative trait loci (QTLs) are then needed.

Results: In this study, we constructed two advanced backcross population to mapping blast resistance QTLs. CR071 and QingGuAi were as the donor parent to establish two BC 3 F 1 and derived BC 3 F 2 backcross populations in the Jin23B background. By challenging the two populations with natural infection in 2011 and 2012, 16 and 13 blast resistance QTLs were identified in Jin23B/CR071 and Jin23B/QingGuAi population, respectively. Among Jin23B/CR071 population, 3 major and 13 minor QTLs have explained the phenotypic variation from 3.50% to 34.08% during six observation times. And, among Jin23B/QingGuAi population, 2 major and 11 minor QTLs have explained the phenotypic variation from 2.42% to 28.95% during six observation times.

Conclusions: Sixteen and thirteen blast resistance QTLs were identified in Jin23B/CR071 and Jin23B/QingGuAi population, respectively. QTL effect analyses suggested that major and minor QTLs interaction is the genetic basis for durable blast resistance in rice variety CR071 and QingGuAi.

Introduction

Rice (*Oryza sativa*) is a staple food crop for more than 50% of the world's population. Rice blast, caused by the fungal pathogen *Magnaporthe oryzae*, is one of the most serious diseases of rice in tropical and temperate areas of the world. Improving disease resistance in crops is crucial for stable food production. Nevertheless, using host resistance gene (R gene) to breed resistant varieties has been proven to be the most effective and economical method to control rice blast, and gene pyramiding is a promising method for providing broad-spectrum and durable resistance (Fukuoka et al. 2009; Liu et al. 2016; Tabien et al. 2002).

So far, over 100 blast resistant genes or quantitative trait loci (QTL) have been identified (Su et al. 2015; Vasudevan et al. 2016; Xiao et al. 2017; Zheng et al. 2016). Among them, 37 genes have been cloned (Wang et al. 2017; Wang et al. 2019; Zhao et al. 2018), and most of them belong to the nucleotide-binding site (NBS) leucine-rich repeat (LRR) gene family. Many of these R genes are clustered in the rice genome, especially on chromosomes 6, 11, and 12. Notably, at least 11 R genes have been identified, including Pi2, Pi9, Pi22, Pi25, Pi26, Pi40, Pi42, Pigm, Piz, Pizt, and Pi50, which are concentrated as gene clusters in the short-arm region near the centromere of chromosome 6. Of these, Pi2, Pi9, Pi50, Pigm, and Pizt have been cloned and have shown broad-spectrum resistance (Deng et al. 2017; Qu et al. 2006; Su et al. 2015; Zhou et al. 2006). It had been reported that at least 7 R genes were located in the long-arm of rice chromosome 11, including Pik, Pi-kg(t), Pikm, Pik-h, Pik-p, Pi54 and Pi1 (Ashikawa et al. 2008; Hua et al. 2012; Pan et al. 1998; Sharma et al. 2010; Yuan et al. 2011; Zhai et al. 2011; Zhai et al. 2014). More than 20 R gene were located on rice chromosome 12, most of them were located near the centromere of chromosome 12, including Pi-ta, Pi-ta², Pi-taⁿ, Pi19, Pi20, Pi30, Pi31 and Ptr (Bryan et al. 2000; Hayashi et al. 1998; Imbe et al. 1997; Sallaud et al. 2003; Zhao et al. 2018).

Although the use of race-specific resistance genes is a major strategy for disease control, these genes are vulnerable to counter evolution of pathogens. Consequently, most varieties lose their resistance after a few years because of new *M. oryzae* races. Many studies indicated that the genetic control of blast resistance is complex and involves both major and minor resistance genes with complementary or additive effects, as well as environmental interactions (Bonman 1992; Li et al. 2007; Li et al. 2011; Wang et al. 1994; Wu et al. 2005). New resistance genes are then needed, thus continuing a cycle referred to as an evolutionary "arms race" between crops and pathogens (Jones and Dangl. 2006). Quantitative trait loci, which usually have smaller individual effects than R genes but confer broad-spectrum or non-race-specific resistance, can contribute to durable disease resistance (Kou and Wang. 2010). Thus, the discovery and use of novel QTLs and development of broad-spectrum resistant varieties are urgent goals in breeding for blast resistance in rice.

Most blast resistance genes confer complete and race-specific resistances that the highly variable fungus can overcome the R gene effects within 2 or 3 years after planting (Wang et al. 2017). The resistance conferred by R genes often do not support sustainable crop production. However, resistance controlled by partially effective resistance genes is often considered to be non race-specific and therefore durable. Durable resistance is the main goal of rice breeding, repeated observations generally suggest that cultivars carrying partial resistance maintain resistance for a long time, possibly because of decreased selection pressure upon the pathogen. CR071 and QingGuAi are indica rice (*Oryza sativa* L.) cultivar that has provided a high level of durable resistance to blast over past decades,

and has been used as a donor of blast resistance in breeding in China. To understand the genetic mechanism of blast resistance in CR071 and QingGuAi, two advanced backcross population BC₃F₁ and derived BC₃F₂ population from Jin23B/CR071 and Jin23B/QingGuAi were studied for blast response under conditions of natural infection. The objective was to find blast resistance loci in the donor parents and to explain the underlying mechanism of resistance. Such results should be useful for improving blast resistance in rice breeding.

Materials And Methods

Experimental population

The blast resistant indica cultivar CR071 and QingGuAi which provided by the Enshi Academy of Agricultural Science, Hubei, China, were used as the donor parent. The blast susceptible indica cultivar Jin23B, the maintainer line for several elite hybrids in China, was used as the recurrent parent. Two backcross populations derived from the cross between Jin23B and CR071 and between Jin23B and QingGuAi were generated according to the plan outlined in Fig. 1. After the first cross, the F₁ generations were backcross to Jin23B, then the BC₁F₁ seeds were sown in a blast nursery in Xianfeng County, Hubei province, in 2010. Resistance plants identified by their leaf blast reactions were further backcrossed with the recurrent parent Jin23B, and BC₂F₁ plants were obtained. The BC₂F₁ seeds were sown in Hainan province in 2011 and were randomly crossed with Jin23B (Fig. 1), two backcross population contained 239 and 237 plants, respectively were obtained. The BC₃F₁ plants and parents were planted during the normal rice growing seasons (from mid-May to early October) at the experimental field of Xianfeng for phenotypic measurement in 2011 and selfed to produce BC₃F₂ families. In 2012, the BC₃F₂ families, each containing 12 plants, were grown in Xianfeng County for blast phenotyping. Varieties BL6 and CO39 were used as resistant and susceptible control.

Trait evaluations

All plants of the BC₃F₁ population were scored for leaf blast response at tillering and heading stages and were recorded for neck blast at maturation stages in 2011; the three traits were named 11RT (leaf blast response of plants at tillering, 2011), 11RH (leaf blast response of the plants at heading, 2011) and 11RN (neck blast of the plants at maturation, 2011). The BC₃F₂ populations were scored on an individual plant basis for leaf blast at tillering heading and for neck blast at the maturation stage, 2012, and results were named 12RT (leaf blast response of plants at tillering, 2012), 12RH (leaf blast response of plants at heading, 2012) and 12RN (neck blast of the plants at maturation, 2012). For the BC₃F₂ families, 24 plants of each family were planted in two row, and the middle 20 plants were scored for blast response. The mean of each family was used as raw data for QTL analysis. Lines developing asynchronously at the normal tillering or heading stages were excluded so that all data would reflect the same developmental stages of the plants. The most seriously diseased leaf of the top two or three new leaves was scored for each plant at each stage using the rating scale of Bonman et al. (1986), where 0 = no evidence of infection; 1 = brown specks smaller than 0.5 mm in diameter, no sporulation; 2 = brown specks about 0.5–1.0 mm in diameter, no sporulation; 3 = roundish to elliptical lesions, 1–3 mm in diameter, grey centre surrounded by brown margins, lesions capable of sporulation; 4 = typical spindle-shaped blast lesions capable of sporulation, 3 mm or longer; 5 = lesions as in 4 but about half of one to two leaf blades killed by coalescence of lesions. Reaction types 0, 1, 2 and 3 were considered resistant, and 4 and 5 as susceptible (Das et al. 2012). Neck blast severity was recorded as a percentage of infection on the neck of rice panicle at physiological maturity stage. The number of panicles showing symptoms of neck blast was expressed as percent infection. To induce infection by the pathogen, diseased straw collected during the previous year was evenly dispersed in each plot and the highly susceptible variety; CO39, was planted on both sides of each row and around the experimental population. Field management essentially followed normal agricultural practices with the exception of no use of bactericides.

DNA markers

Among 1032 simple sequence repeat (SSR) markers, 182 were polymorphic between Jin23B and CR071, and 161 were polymorphic between Jin23B and QingGuAi, the level of polymorphism were 17.63% and 15.60%, respectively. A total of 145 and 113 polymorphic markers covering the whole rice genome were used to develop the genetic linkage map of Jin23B/CR071 and Jin23B/QingGuAi population. The RM marker series were searched in the available rice genomic database (<http://www.gramene.org>). Insertion/deletion markers were designed based on the references maps of Nipponbare and 9311. Genomic DNA was isolated from leaf tissues using the CTAB method. The SSR assay was performed with 4% urea polyacrylamide gels migration and silver staining as reported by Panaud et al (1996).

Genetic map construction and QTL analysis

A genetic linkage map was constructed using the Kosambi mapping function of MapMaker/Exp3.0 program (Lincoln et al. 1992). QTL analysis was performed by composite interval mapping (CIM) method using Windows QTL Cartographer 2.5 software (Wang et al. 2007) with a logarithm of odds (LOD) threshold of 2.5. The resistance score and genotype of each plant in the BC₃F₁ population were used for QTL analysis. For QTL detection of the BC₃F₂ population, the mean of each BC₃F₂ family was used as the row value and the genotypes of the BC₃F₁ plants were used as the genotypes of the BC₃F₂ families. Data analyses were performed using Microsoft Excel 2003 or SPSS 17.0. Correlations between the six traits in 2011 and in 2012 were examined by the Pearson correlation coefficient test. Genotypes of the BC₃F₁ population were determined using SSR markers.

Results

Measurements and relationship of the traits

The receptor parent Jin23B is an indica variety with susceptible performance to rice blast, and the donor parents CR071 and QingGuAi with resistance performance to rice blast. The leaf blast resistance score of Jin23B were 4.17 and 4.11 at tillering stage, and 4.22 and 4.19 at heading stage in 2011 and 2012 respectively (Table 1). The neck blast resistance of Jin23B were 91.7% and 93.7% in 2011 and 2012 respectively (Table 1). The leaf blast resistance score of CR071 and QingGuAi were 0.83 and 1.06 at tillering stage in 2011, and 0.97 and 1.11 at tillering stage in 2012 (Table 1). The leaf blast resistance score of CR071 and QingGuAi were 1.00 and 1.14 at heading stage in 2011, and 1.00 and 1.19 at heading stage in 2012 (Table 1). For neck blast resistance, CR071 and QingGuAi were 3.33% and 6.67% in 2011, and 4.67% and 8.33% in 2012 (Table 1). The resistance score between CR071 and Jin23B, QingGuAi and Jin23B were significant different at the corresponding measurement stages (Table 1). The resistance control BL6 and susceptible control CO39 had leaf blast scores of 1.20 and 4.60 at tillering stage and 1.30 and 5.00 at heading stage in 2011. And those for neck blast of BL6 and CO39 at maturation stage were 10.11% and 96.53% in 2011, and 12.10% and 100% in 2012.

Table 1
Statistical description of the parents and the backcross population in two years

Trait	Year	Parent			Jin23B/CR071 backcross population		Jin23B/QingGuAi backcross population	
		Jin23B	CR071	QingGuAi	Mean ± SD	Range	Mean ± SD	Range
RT	2011	4.17 ± 0.38	0.83 ± 0.38	1.06 ± 0.47	2.59 ± 1.12	0–5	2.30 ± 1.16	0–5
	2012	4.11 ± 0.32	0.97 ± 0.16	1.11 ± 0.40	2.58 ± 1.14	0.17–4.5	2.05 ± 1.05	0–4.75
RH	2011	4.22 ± 0.42	1.00 ± 0.23	1.14 ± 0.35	2.48 ± 1.25	0–5	2.37 ± 1.30	0–5
	2012	4.19 ± 0.40	1.00 ± 0.33	1.19 ± 0.40	2.59 ± 1.16	0–4.5	2.42 ± 1.08	0–4.58
RN	2011	91.7% ± 2.08%	3.33% ± 1.15	6.67% ± 0.58%	62.7% ± 40.7%	0–100%	71.3% ± 36.91%	0–100%
	2012	93.7% ± 2.31%	4.67% ± 1.52	8.33% ± 0.58%	54.8% ± 33.6%	0–100%	78.58 ± 32.23%	0–100%
Note: RT, leaf blast resistance at tillering stage. RH, leaf blast resistance at heading stage. RN, neck blast resistance at maturation stage.								

The distributions of lesion scores as measures of blast response at the six measurement stages for the BC₃F₁ population in 2011 and BC₃F₂ population in 2012 are shown in Fig. 2. There was transgressive segregation in both directions for all traits. The Pearson correlation coefficients showed significant correlation ($p < 0.01$) between all six traits in both years (Table S1). Blast resistance of the BC₃F₁ population at the tillering and heading stages in 2011 had a remarkable positive relationship with blast resistance of the BC₃F₂ population at the heading and maturation stages in 2012 ($p < 0.01$). Resistance during different stages also exhibited significant relationships ($p < 0.01$). Leaf blast at the tillering and heading stages and neck blast at the maturation stage were significantly

correlated with each other, so we were able to predict the neck blast response level according to the leaf blast response at tillering under natural condition.

QTL mapping for blast resistance in Jin23B/CR071 population

A total of 16 QTLs for blast resistance were identified on chromosomes 1, 2, 3, 4, 6, 7, 8, 11 and 12 in the Jin23B/CR071 population in two years (Table 2; Fig. 3). The phenotypic variance explained by each QTL ranged from 3.50–34.08%.

Table 2
QTL mapping results from Jin23B/CR071 background population in two years

Trait	Chr	QTL	Position	LOD	Add	R ² (%)	Trait	Chr	QTL	Position	LOD	Add	R ² (%)
11RT							12RT	1	qBR1 ^a	RM237-RM486	7.39	-0.73 ^a	6.09
	2	qBR2-1	RM236-RM452	5.32	1.42	6.64		2	qBR2-1	RM236-RM452	6.17	1.63	8.17
								2	qBR2-2	RM324-RM341	4.73	1.20	5.77
								2	qBR2-3	RM530-RM213	4.13	0.92	9.15
	3	qBR3-3	SR49-RM426	20.15	1.58	25.42		3	qBR3-3	SR49-RM426	30.08	1.81	34.08
	6	qBR6	RM539-R19951	4.05	0.92	8.89		6	qBR6	RM539-R19951	10.02	1.92	20.40
								7	qBR7-1	RM501-RM542	3.47	1.51	10.09
								8	qBR8	RM72-RM404	4.04	1.31	6.29
	12	qBR12	RM179-YP6213	2.80	0.57	4.25		12	qBR12	RM179-YP6213	3.57	0.58	4.48
11RH	1	qBR1 ^a	RM237-RM486	9.34	-0.93	7.99	12RH	1	qBR1 ^a	RM237-RM486	8.35	-0.80	7.25
	2	qBR2-1	RM236-RM452	3.90	1.41	4.51		2	qBR2-1	RM236-RM452	5.75	1.41	6.38
	2	qBR2-2	RM324-RM341	4.03	1.12	4.89		2	qBR2-2	RM324-RM341	3.91	1.13	5.21
	2	qBR2-3	RM530-RM213	4.51	0.96	8.68		2	qBR2-3	RM530-RM213	3.23	0.84	7.47
	3	qBR3-3	SR49-RM426	23.67	1.76	25.83		3	qBR3-3	SR49-RM426	28.23	1.78	31.33
	6	qBR6	RM539-R19951	8.94	0.85	7.79		6	qBR6	RM539-R19951	7.80	0.95	10.40
	8	qBR8	RM72-RM404	6.78	1.71	7.00		8	qBR8	RM72-RM404	4.20	1.30	4.83
	12	qBR12	RM179-YP6213	3.28	0.59	3.90		12	qBR12	RM179-YP6213	2.93	0.53	3.50
11RN	1	qBR1 ^a	RM237-RM486	10.70	-0.36	8.12	12RN	1	qBR1 ^a	RM237-RM486	10.55	-0.35	9.48
	2	qBR2-3	RM530-RM213	3.61	0.28	7.10							
	3	qBR3-1	RM282-RM411	4.74	0.31	7.19		3	qBR3-1	RM282-RM411	7.50	0.23	7.02
	3	qBR3-2	RM411-RM487	4.12	0.23	3.91							

Note: ^a, the resistance effect of QTL come from Jin23B. 11RT and 12RT, leaf blast resistance at tillering stage in 2011 and 2012. 11RH and 12RH, leaf blast resistance at heading stage in 2011 and 2012. 11RN and 12RN, neck blast resistance at maturation stage in 2011 and 2012.

Trait	Chr	QTL	Position	LOD	Add	R ² (%)	Trait	Chr	QTL	Position	LOD	Add	R ² (%)
	4	qBR4	RM252- RM470	3.04	0.22	4.16							
	7	qBR7-1	RM501- RM542	28.26	0.54	28.48		7	qBR7-1	RM501- RM542	20.27	0.36	18.94
	7	qBR7-2	RM214- M5543	25.26	0.48	22.00		7	qBR7-2	RM214- M5543	17.44	0.37	18.94
	8	qBR8	RM72- RM404	3.33	0.37	4.06							
	11	qBR11- 1	RM181- RM120	27.25	0.58	28.16		11	qBR11- 1	RM181- RM120	21.57	0.47	26.19
	11	qBR11- 2	RM120- RM536	24.09	0.44	20.26		11	qBR11- 2	RM120- RM536	18.69	0.34	17.93
	11	qBR11- 3	RM21- RM590	8.02	0.53	17.48		11	qBR11- 3	RM21- RM590	5.07	0.41	15.94

Note: ^a, the resistance effect of QTL come from Jin23B. 11RT and 12RT, leaf blast resistance at tillering stage in 2011 and 2012. 11RH and 12RH, leaf blast resistance at heading stage in 2011 and 2012. 11RN and 12RN, neck blast resistance at maturation stage in 2011 and 2012.

For leaf blast resistance at tillering stage, nine QTLs were detected on chromosome 1, 2, 3, 6, 7, 8 and 12 (Table 2; Fig. 3). Among them, four QTLs, qBR2-1, qBR3-3, qBR6 and qBR12 were detected in both year, five QTLs, qBR1, qBR2-2, qBR2-3, qBR7-1 and qBR8 were detected only in 2012 (Table 2; Fig. 3). The QTLs flanked by SR49 and RM426 on chromosome 3, qBR3-3, was detected in both year and explained 25.42% of the phenotypic variation in 2011 and 34.08% of the phenotypic variation in 2012. A QTLs, qBR6, located between RM539 and R19951 on chromosome 6, was also detected in two years and explained 8.89% and 20.40% of the phenotypic variation, respectively. Two QTLs, qBR2-1 and qBR12, were located flanked by RM236-RM451 on chromosome 2, and RM179-YP6213 on chromosome 12, respectively. qBR2-1 was detected in both years and accounted for 6.64% and 8.17% of the phenotypic variation, respectively. Whereas qBR12 was detected in both year and accounted for 4.25% and 4.48% of the phenotypic variation.

For leaf blast resistance at heading stage, eight QTLs were detected on chromosome 1, 2, 3, 6, 8 and 12 (Table 2; Fig. 3), and all the QTLs were detected in both year. These QTLs explained the phenotypic variance ranged from 3.90–25.83% in 2011, and from 3.50–31.33% in 2012. Among them, the QTL, qBR3-3, flanked by SR49 and RM426 on chromosome 3, have the largest effect which explained 25.83% of the phenotypic variation in 2011 and 31.33% of the phenotypic variation in 2012.

For neck blast resistance at maturation stage, eleven QTLs were detected on chromosome 1, 2, 3, 4, 7, 8 and 11 (Table 2; Fig. 3). Among them, seven QTLs, qBR1, qBR3-1, qBR7-1, qBR7-2, qBR11-1, qBR11-2 and qBR11-3 were detected in both year, four QTLs, qBR2-3, qBR3-2, qBR4 and qBR8 were detected only in 2011 (Table 2; Fig. 3). These QTLs explained the phenotypic variance ranged from 3.91–28.48% in 2011, and from 7.02–26.19% in 2012. A QTL, qBR7-1, located between RM501 and RM542 on chromosome 7, explained 28.48% of the phenotypic variation, which have the largest effect in 2011. Whereas the QTL qBR11-1, located between RM181 and RM120 on chromosome 11, accounted for 26.19% of the phenotypic variation, which have the largest effect in 2012.

Among the sixteen QTLs in the Jin23B/CR071 population, the gene effect of fifteen QTLs come from donor parent CR071, in contrast, the gene effect of qBR1 come from Jin23B. The QTLs, qBR1, flanked by RM237 and RM486 on chromosome 1, was detected in both year at five measuring stage and explained phenotypic variations of 6.09% in 12RT, 7.99% in 11RH, 7.25% in 12RH, 8.12% in 11RN and 9.48% in 12RN, respectively (Table 2). Six QTLs, qBR2-1, qBR2-3, qBR3-3, qBR6, qBR8 and qBR12 were detected in both year at four measuring stage; two QTLs qBR2-2 and qBR7-1 were detected in both year at three measuring stage; five QTLs qBR3-1, qBR7-2, qBR11-1, qBR11-2 and qBR11-3 were detected in both year at two measuring stage and these QTLs only have the effect to neck blast in maturation stage; two QTLs, qBR3-2 and qBR4 were only detected in 11RN.

QTL mapping for blast resistance in Jin23B/QingGuAi population

A total of 13 QTLs for blast resistance were identified on chromosomes 1, 2, 3, 4, 6, 7, 8, 11 and 12 in the Jin23B/QingGuAi population in two years (Table 3; Fig. 4). The phenotypic variance explained by each QTL ranged from 2.42–28.95%.

Table 3
QTL mapping results from Jin23B/QingGuAi background population in two years

Trait	Chr	QTL	Position	LOD	Add	R ² (%)	Trait	Chr	QTL	Position	LOD	Add	R ² (%)
11RT	6	qBR6	L6ID3F-ZH6111	19.39	1.40	24.93	12RT	1	qBR1-1	RM297-RM486	2.98	0.54	3.95
	7	qBR7-1	RM214-RM5543	7.83	0.89	8.87		6	qBR6	L6ID3F-ZH6111	13.73	1.14	19.91
	11	qBR11-1	RM229-RM457	5.17	1.08	6.57		7	qBR7-1	RM214-RM5543	3.18	0.55	4.12
								11	qBR11-2	RM457-YH43	6.94	34.7	5.49
11RH							12RH	4	qBR4-2	RM241-RM317	3.44	0.49	2.51
	6	qBR6	ZH6111-RM20069	28.39	1.90	36.48		6	qBR6	L6ID3F-ZH6111	28.35	1.31	25.27
	7	qBR7-1	RM214-RM5543	14.98	1.36	16.47		7	qBR7-1	RM214-RM5543	29.33	1.43	26.31
								11	qBR11-1	RM229-RM457	3.2	0.61	2.42
11RN							12RN	1	qBR1-2	RM5-RM488	10.9	24.76	8.55
								2	qBR2	ZH282-RM71	2.59	44.12	8.92
								3	qBR3	RM3441-RM232	4.46	50.77	12.72
	4	qBR4-1	RM471-RM241	3.69	42.68	12.31		4	qBR4-1	RM471-RM241	7.64	40.01	18.47
	4	qBR4-2	RM241-RM317	2.53	45.94	8.82		4	qBR4-2	RM241-RM317	6.76	46.65	16.92
	6	qBR6	L6ID3F-ZH6111	33.94	50.47	31.91		6	qBR6	L6ID3F-ZH6111	29.23	42.24	28.95
	7	qBR7-1	RM214-RM5543	30.17	50.02	27.94		7	qBR7-1	RM214-RM5543	20.75	35.94	18.71
								7	qBR7-2	RM432-RM21691	4.61	17.06	3.43
	8	qBR8	RM404-RM210	7.80	38.08	18.89							
								12	qBR12	RM5927-RM6296	8.02	-33.18 _a	9.58
Note: ^a , the resistance effect of QTL come from Jin23B. 11RT and 12RT, leaf blast resistance at tillering stage in 2011 and 2012. 11RH and 12RH, leaf blast resistance at heading stage in 2011 and 2012. 11RN and 12RN, neck blast resistance at maturation stage in 2011 and 2012.													

For leaf blast resistance at tillering stage, four QTLs were detected on chromosome 1, 6, 7 and 11 (Table 3; Fig. 4). Among them, three QTLs, qBR1-1, qBR6 and qBR7-1 were detected in both year, one QTLs, qBR11-2 was detected only in 2012 (Table 3; Fig. 4). A QTL, qBR1-1, located between RM297 and RM486 on chromosome 1, was detected in two years and explained 24.93% and 3.95% of the

phenotypic variation, respectively. The QTL flanked by L6ID3F and ZH6111 on chromosome 6, qBR6, was also detected in both year and explained 8.87% of the phenotypic variation in 2011 and 19.91% of the phenotypic variation in 2012. The QTL, qBR7-1, flanked by RM214 and RM5543 on chromosome 7 was detected in both years and accounted for 6.57% and 4.12% of the phenotypic variation, respectively. Whereas qBR11-2 was detected only in 2012 and accounted for 5.49% of the phenotypic variation.

For leaf blast resistance at heading stage, four QTLs were detected on chromosome 4, 6, 7 and 11 (Table 3; Fig. 4). Two QTLs, qBR6 and qBR7-1, were detected in both year and the QTLs explained the phenotypic variance were 36.48% and 16.47% in 2011, respectively, and 25.27% and 26.31 in 2012, respectively. Two QTL, qBR4-2 and qBR11-1, were located flanked by RM241-RM317 on chromosome 4, and RM229-RM547 on chromosome 11, respectively. The QTLs qBR4-2 and qBR11-1 were detected only in 2012 and accounted for 2.51% and 2.42% of the phenotypic variation, respectively.

For neck blast resistance at maturation stage, ten QTLs were detected on chromosome 1, 2, 3, 4, 6, 7 and 12 (Table 3; Fig. 4). Among them, four QTLs, qBR4-1, qBR4-2, qBR6 and qBR7-1 were detected in both year, and explained the phenotypic variance were 12.31%, 8.82%, 31.91% and 27.94%, respectively, in 2011, and were 18.47%, 16.92%, 28.95% and 18.71%, respectively, in 2012. Six QTLs, qBR1-2, qBR2, qBR3, qBR7-2, qBR8 and qBR12 were detected only in one year (Table 3; Fig. 4), and explained the phenotypic variance were 8.55%, 8.92%, 12.72%, 3.43%, 18.89% and 9.58%, respectively.

Among the thirteen QTLs in the Jin23B/QingGuAi population, the gene effect of twelve QTLs come from donor parent QingGuAi, in contrast, the gene effect of qBR12 come from Jin23B. The QTLs, qBR12, flanked by RM5927 and RM6296 on chromosome 12, was detected only in 2012 at maturation stage and explained phenotypic variations of 9.58% (Table 2). Two QTLs, qBR6 and qBR7 were detected in both year at six measuring stage; one QTL, qBR4-2, was detected in both year at three measuring stage; two QTLs, qBR4-1 and qBR11-2 were detected in both year at two measuring stage; eight QTLs, qBR1-1, qBR1-2, qBR2, qBR3, qBR7-2, qBR8, qBR11-1 and qBR12 were only detected in one measuring stage.

Discussion

QTL mapping using advanced backcross population was proposed as an effective molecular breeding technique for incorporating valuable genes from exotic sources into an adapted background (Eizenga et al. 2013; Tanksley and Nelson 1996). With this method, QTL from the donor parents are detected by backcrossing with the adapted parent to eliminate most of the unwanted genes from the donor parent. In addition, using advanced backcross population can accelerate the crop improvement process because near-isogenic lines containing the desired QTL (genes) from the donor in the background of the recurrent parent can be selected from the advanced backcross population. Several studies have been performed to identify QTLs in advanced backcrossing. For example, Thomson et al. (2003) mapped quantitative trait loci for yield, yield components and morphological traits in rice using a BC₂F₁ and BC₂F₂ population by composite interval mapping and Windows QTL Cartographer 1.21. And Eizenga et al. (2013) mapped sheath blight and blast quantitative trait loci in two different advanced backcross populations. In our study, we developed two advanced backcross population with the objective of introgression of useful resistance genes from CR071 and QingGuAi into Jin23B. Using this strategy, we mapped 16 blast resistance QTLs from the Jin23B/CR071 population and 13 blast resistance QTLs from the Jin23B/QingGuAi population. We also obtained maintainer lines resistant to blast in the background of Jin23B, which can be used for rice blast resistance breeding.

To date, over 100 blast resistant genes or QTLs have been identified (Su et al. 2015; Vasudevan et al. 2016; Xiao et al. 2017; Zheng et al. 2016). Among them, 37 genes have been cloned (Wang et al. 2017; Wang et al. 2019; Zhao et al. 2018). Many of these resistance genes are clustered on rice chromosomes 6, 11 and 12. Notably, at least 11 resistance genes—including Pi2, Pi9, Piz, Pizt, Pigm, Pi22, Pi25, Pi26, Pi40, Pi42 and Pi50—are concentrated in the short-arm region near the centromere of chromosome 6. In this study, the resistance QTL qBR6 located between RM539 and R19951 in the Jin23B/CR071 population, and the QTL qBR6 located between L6ID3F and ZH6111 in the Jin23B/QingGuAi population were overlapped the gene cluster on chromosome 6. QTLs qBR1 was located between RM237 and RM486 on chromosome 1 in the Jin23B/CR071 population, and the QTL qBR1-2 was located between RM297 and RM486 in the Jin23B/QingGuAi population, in this region, blast resistance gene Pi-t, Pi37, Pish and Pi35(t) had previously been reported (Hayashi and Yoshida 2009; Lin et al. 2007; Nguyen et al. 2006; Takahashi et al. 2010). QTLs qBR2-3 located between RM530 and RM213 on chromosome 2 in the Jin23B/CR071 population, was close to the cloned gene Pib (Wang et al. 1999). The neck blast resistance QTLs, qBR11-1, was located between RM21 and RM590 on chromosome 11 in the Jin23B/CR071 population, in this region, blast resistance gene Pikm, Pik-h and Pik-p had previously been reported (Ashikawa et al. 2008; Yuan et al. 2011; Zhai et al. 2014).

Rice cultivars with durable blast resistance have been recognized in several production systems. The durable resistance of these cultivars is associated with polygenic partial resistance that shows no evidence of race specificity. This partial resistance is expressed as fewer and smaller lesions on the leaf blade but latent period does not appear to be an important component. Many blast resistant varieties with single resistance genes lose resistance after a few years; that is, they have or had nondurable resistance (Babujee and Gnanamanickam 2000). Varieties with durable resistance may contain more than one resistance gene (Zhu et al. 2012). Many studies have been performed to pyramid resistance genes into rice varieties. Hittalmani et al. (2000) pyramided three blast resistance genes Pi1, Piz-5 and Pita into rice variety CO39 and Jiang et al. (2012) pyramided three blast resistance genes Pi1, Pi2 and D12 into rice variety Jin23B. Their results confirmed that pyramiding of blast resistance genes is an effective way to develop highly resistant varieties. In our study, the donor parent CR071 and QingGuAi have a high level and durable resistance to blast over past decades, and have been used as donor parent for blast resistance in breeding in China. In the study, two advanced backcross populations were constructed for analyses the genetic mechanism of blast resistance in CR071 and QingGuAi, and major and minor blast resistance QTLs were identified in the donor parents. QTL effect analyses suggested that major and minor QTLs interaction is the genetic basis for durable blast resistance for CR071 and QingGuAi in the past decade in Wuling mountain area in China.

Conclusions

Overall, the mapping results showed that sixteen blast resistance QTLs were identified in the Jin23B/CR071 backcross population, in which, one QTL, qBR1, was detected in both year at five measuring stage; six QTLs, qBR2-1, qBR2-3, qBR3-3, qBR6, qBR8 and qBR12 were detected in both year at four measuring stage; nine QTLs were detected at one to three measuring stage. Thirteen blast resistance QTLs were identified in Jin23B/QingGuAi population, in which, two major QTLs, qBR6 and qBR7 were detected in both year at six measuring stage and eleven QTLs were detected at one to three measuring stage. Major and minor effect QTLs were identified in the two populations, which conferring resistance to blast together at different measuring stage.

Abbreviations

11RT: Leaf blast resistance at tillering stage in 2011; 11RH: Leaf blast resistance at heading stage in 2011; 11RN: Neck blast resistance at maturation stage in 2011. 12RT: Leaf blast resistance at tillering stage in 2012; 12RH: Leaf blast resistance at heading stage in 2012; 12RN: Neck blast resistance at maturation stage in 2012. CIM method: Composite interval mapping method; NBS-LRR gene: Nucleotide-binding site leucine-rich repeat gene; QTLs: Quantitative trait loci; R gene: Resistance gene; SSR: Simple sequence repeat.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The data sets supporting the results of this article are included within the article and its supporting files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YH designed the experiments. HJ, YF, and LQ performed the experiments. GG and QZ helped with field management. HJ, YF and QL analyzed the data. HJ and YH wrote the manuscript. All authors approved the manuscript.

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Figures

Figure 1

Strategy to develop mapping populations for blast resistance QTLs

Figure 2

Frequency distribution of blast resistance of the Jin23B/CR071 (a, b, c) and Jin23B/QingGuAi (d, e f) population in 2011 and 2012. 11RT and 12RT, leaf blast resistance at tillering stage in 2011 and 2012. 11RH and 12RH, leaf blast resistance at heading stage in 2011 and 2012. 11RN and 12RN, neck blast resistance at maturation stage in 2011 and 2012. Blue pillar and green pillar indicate the frequency of BC3F1 and BC3F2 population, respectively.

Figure 3

Distribution of QTLs for blast resistance in the Jin23B/CR071 population on the genetic linkage map. 11RT and 12RT, leaf blast resistance at tillering stage in 2011 and 2012. 11RH and 12RH, leaf blast resistance at heading stage in 2011 and 2012. 11RN and 12RN, neck blast resistance at maturation stage in 2011 and 2012.

Figure 4

Distribution of QTLs for blast resistance in the Jin23B/QingGuAi population on the genetic linkage map. 11RT and 12RT, leaf blast resistance at tillering stage in 2011 and 2012. 11RH and 12RH, leaf blast resistance at heading stage in 2011 and 2012. 11RN and 12RN, neck blast resistance at maturation stage in 2011 and 2012.

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