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ORIGINAL PAPER

Genetic characterization and fine mapping of the blast resistance locus *Pigm(t)* tightly linked to *Pi2* and *Pi9* in a broad-spectrum resistant Chinese variety

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Abstract The identification and utilization of broad-spectrum resistance genes have been proven the most effective and economical approach to control rice blast disease. To understand the molecular mechanism of broad-spectrum resistance to rice blast, we conducted genetic and fine mapping analysis of the blast resistance gene in a Chinese rice variety: Gumei 4 (GM4) identified with broad-spectrum resistance and used in rice breeding for blast resistance for more than 20 years. Genetic and mapping analysis indicated that blast resistance to nine isolates of different Chinese races in GM4 was controlled by the same dominant locus designated as *Pigm(t)* that was finely mapped to an approximately 70-kb interval between markers C5483 and C0428 on chromosome 6, which contains five candidate NBS–LRR disease resistance genes. The allelism test showed that *Pigm(t)* was either tightly linked or allelic to *Pi2* and *Pi9*, two known blast resistance genes. Mapping information also indicated that another blast resistance gene *Pi26(t)* might also be located at the same region. Candidate genes were identified by sequence analysis of the Nipponbare and *Pi9* locus and the corresponding region in GM4. Sequence divergence of candidate genes was observed between GM4 and model varieties Nipponbare and 9311, and *Pi9*. Our current study provides essential information and new genetic resource for the cloning of functional resistance gene(s) and for marker-assisted selection in rice breeding for broad-spectrum blast resistance.

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Introduction

Rice blast, caused by the fungus *Magnaporthe grisea*, is one of the most devastating rice diseases worldwide (Valent and Chumley 1991), which is responsible for large yield loss in epidemically favorable areas and crop seasons. *M. grisea* is known for its genetic instability and pathogenic variability, and rapid breakdown of resistant varieties (Ou 1979; Bonman et al. 1986). Thus, many resistant varieties often remain effective only for a few years until the advent of new dominant pathogenic races (Mackill and Bonman 1992). Therefore, identifying and using broad-spectrum resistance genes in rice breeding have been considered as the most effective and economical strategy to control the disease. The genetic characteristic of blast resistance in rice has been extensively studied (Kiyosawa 1972a, b; Mackill and Bonman 1992). To date, more than 40 major resistance (*R*) genes or loci against blast have been identified, of which many have been mapped on rice chromosomes based on morphological and molecular markers (Mackill and Bonman 1992; McCouch et al. 1994; Pan et al. 1996). It seems that there are

more blast resistance genes on chromosomes 6, 11 and 12. For example, *Pi2/Piz*, *Pi8*, *Pi9* and *Pi13(t)* are mapped on chromosome 6 (Yu et al. 1991; Pan et al. 1996, 1998; Amante-Bordeos et al. 1992); *Pi1*, *Pi7(t)*, *Pi18(t)*, *Pi44(t)*, *PiCO39(t)*, *Pif and Pik* on chromosome 11 (Yu et al. 1991; Wang et al. 1994; Ahn et al. 1996; Chen et al.1999; Chauhan et al. 2002); *Pita*, *Pi6(t)*, *Pi12(t) and Pi19(t)* on chromosome 12 (Kiyosawa 1972b; Inukai et al. 1996; McCouch et al. 1994; Iwata 1997). Molecular mapping of these *R* genes not only provides tools for marker-assisted selection in rice breeding for blast resistance, but also facilitates isolation and characterization of these genes at the molecular level. So far, three of these *R* genes, *Pib*, *Pita* and *Pi9*, have been isolated by the map-based cloning approach; all are members of a large *R* gene family encoding receptor proteins containing nucleotide-binding site and leucine-rich repeats (NBS–LRR) (Wang et al. 1999; Bryan et al. 2000; Qu et al. 2006). Interestingly, a single amino acid difference in Pita protein correlates with the gene-for-gene-specific characteristic of the *Pita/AVR-Pita* system (Bryan et al. 2000).

Due to race specificity, most of the mapped *R* genes confer resistance only to a certain number of isolates or races. It is well recognized that these genes will certainly provide broad-spectrum resistance when pyramided into the same rice variety. Nevertheless, the identification of *R* genes or loci with broad-spectrum resistance will provide important genetic resources for rice breeding. It has been shown that five *R* genes, *Pi1* (Yu et al. 1991), *Pi2* (Chen et al. 1996), *Pi3*/*Pi5* (Jeon et al. 2003), *Pi9* (Liu et al. 2002) and *Pi33* (Berruyer et al. 2003), probably confer broad-spectrum resistance. For example, *Pi2* was shown to confer resistance to 455 isolates collected from different regions of Philippines and most of the 792 isolates from 13 major rice regions of China (Chen et al. 1996, 1999). In a test with a total of 43 isolates from 13 countries, the *Pi9*-bearing lines were resistant to all isolates, and the lines carrying *Pi2* were resistant to 36 isolates (Liu et al. 2002). *Pi9* is located on the region containing a cluster of multiple NBS–LRR genes, *NBS2-Pi9*, a solo member of the *Pi9* gene cluster, confers broad-spectrum resistance to rice blast. However, the molecular basis of *NBS2-Pi9* in broad-spectrum blast resistance remains to be elucidated (Qu et al. 2006).

To understand the molecular mechanism of broad-spectrum resistance to rice blast, we widely screened Chinese varieties for broad-spectrum blast resistance. Gumei 4 (GM4), an *indica* variety from Sichuan, China, has been grown in different blast nurseries as a broad-spectrum and durable resistant control for more than 20 years. The resistant spectrum of GM4 is broader than those of *Pi1, Pi2, Pi3*, making it a good genetic resource in the resistance-breeding program (Peng et al. 1996; Shen et al. 2004). In an international cooperative project for rice blast resistance, this variety was observed to be highly resistant or immune to all 29 isolates tested, making it the top of the resistant source list screened from 156 varieties (Shen et al. 2004). In this study, we systematically analyzed the genetic behavior of resistance of GM4 to different blast isolates, and fine-mapped its resistance gene/locus, tentatively designated as *Pigm(t)* on chromosome 6 within 70-kb containing a gene cluster of 5 NBS–LRR candidate *R* genes based on Nipponbare sequence. Genetic analysis indicated that *Pigm(t)* was tightly linked or allelic to *Pi2* and *Pi9*.

Materials and methods

Plant materials and mapping population

The F_1, F_2, BC_1F_1 populations were derived from the crosses between GM4 (*indica*) and three susceptible (S) varieties Cpslo17 (*javanica*), Maratelli (*japonica*) and Suyunuo (*indica*). The BC_1F_2 populations were developed from the BC_1F_1 resistant individuals. These populations were used for genetic analysis. A total of 306 recessive (susceptible) individuals from the F_2 population of the cross between GM4 and Cpslo17 were used for preliminary mapping of the resistance gene, additional 1,250 recessive individuals from the F_2 and BC_1F_2 populations derived from the cross between GM4 and Maratelli were used for fine mapping of the resistance gene. In addition, the F_2 populations from the crosses between GM4 and 75-1-127 containing *Pi9*, and C101A51 containing *Pi2* were constructed for allelism test.

Blast inoculation and disease evaluation

Nine blast isolates of different races avirulent to GM4 were used in this study (Table 1), seven of these isolates, CH109, CH199, 01-19, CH174, CH63, CH102 and CH131 were collected and identified from different rice-cultivated regions of China, isolates 101/1/1 and 101/4/8 were two stable progenies from the cross of Chinese isolate CH72 × Thailand TH12. All these isolates have genetic differentiation and belong to different blast lineages (Shen et al. 1998, 2004). Two-week-old seedlings were spray-inoculated with spore suspensions (1×10^5 spores/ml) in a dew growth chamber for 24 h in darkness at 26°C, and were subsequently kept at 12/12 h (day/night), 26°C and 90% relative

Table 1 Genetic and allelism analysis in populations to different isolates

Parents and generations	Isolates (races)	Resistant and susceptible individuals		Segregation ratio	X^2
		R	S		
GM4	CH109 (ZC13)	27	0		
Cplso17		0	30		
F_1		26	0		
BC_1F_1		42	34	1:1	0.84
BC_1F_2		773	232	3:1	1.89
F_2	CH109 (ZC13)[a]	282	85	3:1	0.66
	CH174 (ZB25)[a]	282	85	3:1	0.66
	CH131 (ZA1)[a]	282	85	3:1	0.66
GM4	101/1/1(ZA1)	30	0		
Cplso17		0	34		
F_2		69	22	3:1	0.05
GM4	101/4/8 (ZA9)	30	0		
Cplso17		0	28		
F_2		74	23	3:1	0.05
GM4	CH63 (ZG1)	27	0		
Cplso17		0	9		
F_2		86	23	3:1	0.78
GM4	CH199 (ZB1)	25	0		
Cplso17		0	31		
BC_1F_2		822	259	3:1	0.71
GM4	CH199 (ZB1)	32	0		
Maratelli		0	41		
F_1		30	0		
F_2		196	61	3:1	0.18
BC_1F_1		105	97	1:1	0.31
GM4	01–19 (ZB15)	24	0		
Maratelli		0	33		
F_2		176	54	3:1	0.28
BC_1F_1		122	108	1:1	0.85
GM4	CH102 (ZB13)	20	0		
Suyunuo		0	14		
F_1		25	0		
F_2		147	41	3:1	1.01
BC_1F_1		55	42	1:1	1.74
GM4	01–19(ZB15)	16	0		
75-1-127 (*Pi9*)		12	0		
C101A51 (*Pi2*)		19	0		
F_2 (GM4/75-1-127)		717	0		
F_2 (GM4/C101A51)		545	0		

[a] The same individuals were injection-inoculated with isolates on different tillers

humidity for 6 days. To analyze resistance response of the same individuals to different isolates, the inject-inoculation method was employed at the tillering stage as described (He and Shen 1989), each tiller of the same individuals was inoculated with one isolate by injecting 0.1 ml spore suspensions (2.5×10^4 spores/ml). After 7 days, lesion types on rice leaves were observed and scored from 0 (resistant) to 5 (susceptible) according to the standard scale described by Bonman et al. (1986).

Genetic and allelism analysis

The F_2, BC_1F_1, BC_1F_2 populations derived from the crosses of GM4 and susceptible parents were inoculated with different blast isolates, to determine inheritance of blast resistance in GM4. For allelism test, the F_2 populations from the crosses between GM4 and 75-1-127 containing *Pi9*, and C101A51 containing *Pi2* were inoculated with isolate 01-19 to observe resistance segregation.

BSA and linkage analysis

R and S DNA pools, respectively, representing 20 resistant and susceptible F_2 homozygous individuals confirmed in the F_3 generation were made for bulk segregant analysis (BSA) (Michelmore et al. 1991). A total of six pairs of R and S DNA pools were constructed from the F_2 populations of the cross of GM4 and

Cpslo17 inoculated with six different isolates. Total DNA was extracted and PCR was performed as previously described (Xu et al. 2004). A total of 120 SSR (simple sequence repeats) markers distributing evenly on 12 chromosomes released by the International Rice Microsatellite Initiative (IRMI) (http://www.gramene.org/microsat/) were used to screen for polymorphism between the R and S bulks. Polymorphic SSR markers associated with resistance in the bulks were confirmed by the analysis of recessive (susceptible) individuals. Mapmaker/Exp 3.0 was used to analyze the linkage between the molecular markers and the target gene using all mapping recessive individuals at a LOD threshold of 3.0 (Lincoln et al. 1992).

Fine mapping of *Pigm(t)*

Based on the SNPs (single nucleotide polymorphisms) and InDels (insertion/deletion polymorphisms) between the *japonica* (Nipponbare) (http://www.rgp. dna.affrc .go.jp/) and the *indica* (9311) (http://www. genomics .org.cn/) genomes, we designed new CAPS (cleaved amplified polymorphic sequence) and InDels markers for GM4 and Maratelli. In case that maker did not exhibit polymorphism between GM4 and Maratelli, we sequenced PCR products to search for SNPs between GM4 and Maratelli to develop new CAPS markers. The primer sequences, size of PCR products and enzymes used are listed in Table 2. Subsequently, a physical map spanning the *R* gene locus was constructed, based on the contig map (International Rice Genome Sequencing Project 2005).

Sequence analysis

The GM4 candidate *R* genes within the mapped region (see Results) were amplified by PCR using primers (Table 2), and sequenced by a 3,700 DNA sequencer

Table 2 PCR-based markers linked to *Pigm(t)* locus

Marker	Forward primer (5′–3′)	Reverse primer (5′–3′)	PCR product size (bp)[e]	Enzyme
SRF52[a]	TTAGGATACGGCTTCTAGGC	CGTAATTGTTGCATATGGTG	251	
SRF5[a]	ATGTGGGAATTTCTAGCCCC	CCCTAGTTTTCCAAATGGCC	139	
SRF55[a]	ACGTCAGAATATCAACCATT	GCCCGTTCACTACTAGATAG	139	
SRF523[a]	AAAACCGACACGATACGGAG	CGTGGTGCCTCCTTTTAAAG	142	
C51[b]	AACAGGCAAGTCAAGGACCA	GAGGAGACACGACCCACCC	997	*Stu I*
C16315[b]	CCCTGGGAAGGCTTTATCT	GTCGTCCTCACAGACTTGGTT	872	*Pvu II*
C26348[b]	GGGGAGTATCTGCCTATCTG	CGTCACCACCTTATCGTTC	939	*Xba I*
C21602[b]	CGAGTCCATCAGAAACAGGC	TCAGGACAACCGACGCCAC	750	*Kpn I*
S53395[c]	GAGCGGGCAGTGAGGAGGGA	GCGCAAGCGAAACGGGAAA	563/620	
S95477[c]	AATTCTTCCACGCCCTAAT	AGATGGCATCACTTTCCTG	141/119	
C5483[b]	TTAGGCTGCTTGTCTTGGG	GGGAGGAGGAATGGTAGGAA	468	*EcoR I*
C29755[b]	GCTTTGCTTTCTCGGTTGA	ACTGCCCTGTTTGCGTCTA	1,114	*Xba I*
S150296[c]	AAAAGGCAAAGCCCATTAG	TTTCGCAATCAAGCGTAAA	321/272	
S41856[c]	GCATTAGAGCCAACACCTT	CGACCCAATTACTACAAGC	445/700	
C4217[c]	TCACGGCCAGATTTCGTTT	GTTCCCGCTCGTCATAGTT	576/533	
S652[c]	CTGAGCCTAATGGCATGTAC	AGAGGTTGACTGTGGGACTG	214/270	
S29742[c]	CAGTGAAACGAACGCTATG	AATAGGAAGGGTTGATGTTG	555/461	
S47656[c]	CGGGCTTCTTCTCCTCCTT	TCCGCAATCTATCTGTTATCCTC	427/581	
RM7311[a]	AGTGGTCGTTGAACTCGGAG	TCGTGGCGCCTTTAATCTC	147	
C0428[b]	AAGGTTCTCGTGGTTTCA	TCCCCATTGTTTATAGCAG	682	*Eco72 I*
PC22705[b]	CTAGCCTTCCGTCCTGTG	GAACTGCCCTTTCCCTCT	994	*Eco72 I*
C24901[b]	GGTTTGTACCCTTCTGTTC	ACCTCTGTTGTTAATCTTCG	986	*Cla I*
P236[d]	GAAAGGGCAGTTCAATAC	TGACTAGAGCCTGGAGGT	1,156	
P257[d]	TCACTTGGTTGAAGGGCTAA	TCGGAGGCTACGGAGATT	1,165	
P248[d]	ACCTCCAGGCTCTAGTCA	CCTCTGTTGTTAATCTTCG	1,063	
P28[d]	ATTTATCTGTCTTTCGCCTTGT	CCATGCTCAGCACCGTCT	1,176	
P34[d]	ACTCTTGTTATTTGCTGCTAT	TTGCCTAAACCACCCATC	1,027	
P35[d]	TGGTTTAGGCAAGACAGC	TCCAAGACCCGTAACATC	1,164	
P36[d]	TGGGAGATGGCTCTGATTT	TCTTTTCCAACAGGGGTGA	1,158	

[a] SSR marker

[b] CAPS marker

[c] InDels marker

[d] Sequencing primers of the *Pigm(t)* candidate genes

[e] PCR began with a denaturing step of 4 min at 94°C, followed by 35 cycles of 94°C for 45 s, and 56°C for 45 s and 72°C for 45 s, with a final extension step at 72°C for 10 min

(Applied Biosystems). Sequences of Nipponbare and 9311 in the mapped loci were obtained from the databases (http://www.rgp.dna.affrc.go.jp/) and (http://www.genomics.org.cn/). Sequences were analyzed and compared by the BLASTN search program (http://www.ncbi.nlm.nih.gov/BLAST/).

Results

Blast resistance is conferred by a single locus *Pigm(t)* in GM4

Inoculation results of the F_1, BC_1F_1, F_2 and BC_1F_2 populations from the crosses of GM4 and the three susceptible parents with nine blast isolates were summed in Table 1. GM4 was resistant to all isolates, displayed no lesions at all in leaves. The three parents, Suyunuo, Cpslo17 and Maratelli, were highly susceptible to all isolates. The F_1 plants were resistant to all blast isolates tested, indicating that the resistance of GM4 is dominant (Table 1). Phenotypes of resistance and susceptibility in these F_2 and BC_1F_2 populations fitted the segregation ratio of 3:1, and these in the BC_1F_1 populations followed a 1:1 pattern. Therefore, single dominant genes, respectively, conferred resistance to nine different isolates in GM4, which belonged to the A, B, C and G Chinese race groups (Table 1). To confirm whether these single genes conferring resistance to different races is the same gene (locus), we performed injection inoculation in different tillers of the same F_2 individuals with three selected isolates/races: CH131 (ZA1), CH174 (ZB25) and CH109 (ZC13). The result showed that these plants exhibited the same resistant or susceptible pattern to the three races, documenting that a single dominant locus, tentatively named *Pigm(t)*, most likely confers broad-spectrum resistance to different races at least to ZA1, ZB25 and ZC13 in GM4 (also see below).

Pigm(t) locus was preliminarily mapped on chromosome 6

A total of 120 SSR markers distributing with 10–15 cM intervals on 12 chromosomes were employed to identify markers linked to *Pigm(t)* by BSA as described above. A polymorphic marker SRF5 on chromosome 6 associated with *Pigm(t)* was identified. To confirm the reliability of linkage between SRF5 and *Pigm(t)*, we analyzed the polymorphism of SRF5 in 30 resistant and susceptible homozygous individuals confirmed by F_3 progenies, and found that the SRF5 polymorphism was consistent with the phenotype. Based on this, we further screened 306 susceptible/recessive individuals using SRF5, which were identified from the F_2 population generated from the cross between GM4 and Cpslo17 and were confirmed in the F_3 progenies by inoculating with isolate CH109. A total of 53 recombinant events were observed. Additional 15 SSR markers were selected in a 40 cM region around SRF5, and were assayed for linkage with *Pigm(t)*. As a result, *Pigm(t)* was delimited to a 14.7 cM region between two SSR markers, SRF5 and SRF52. To further localize *Pigm(t)* to a smaller region on chromosome 6, we developed ten CAPS and InDels markers between SRF5 and SRF52 according to sequence information of the *japonica* Nipponbare and the *indica* 9311 genomes, and found four markers displaying polymorphism between GM4 and Cpslo17. Hence *Pigm(t)* locus was further delimited within a 4.3 cM region between the two makers, C26348 and S47656 (Fig. 1), where *Pi9*/*Pi2*, *Pi26(t)* also located, and *Pi25(t)* was mapped on a neighbor region (Liu et al. 2002; Wu et al. 2005).

Pigm(t) is tightly linked or allelic to *Pi2* and *Pi9*

Since two known broad-spectrum resistance genes, *Pi2* and *Pi9*, were also mapped to the same region of chromosome 6 (Liu et al. 2002), it is essential to study the linkage of the three genes. We constructed the F_2 populations of GM4 (*Pigm(t)*) × C101A51(*Pi2*) and GM4 (*Pigm(t)*) × 75-1-127 (*Pi9*) with 545 and 717 individu-

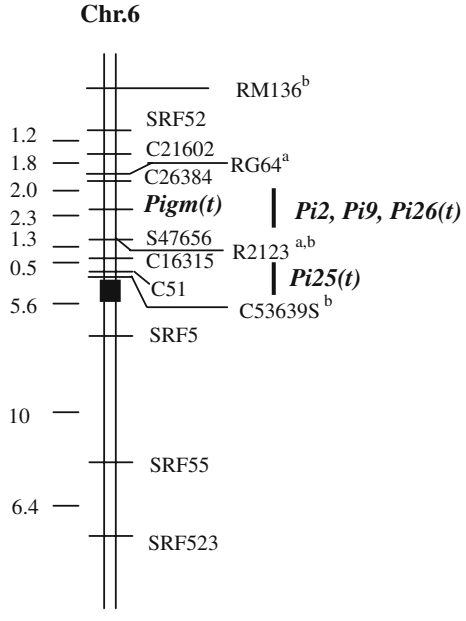

Fig. 1 Preliminary genetic map of *Pigm(t)* on chromosome 6. The positions of other resistance genes *Pi2*, *Pi9*, *Pi25(t)* and *Pi26(t)* and markers were integrated on the basis of reported data from the following sources: *a* Liu et al. (2002); *b* Wu et al. (2005)

als, respectively. Allelism test showed that all F_2 individuals were resistant, no susceptible plant segregated (Table 1). Therefore, we speculated that *Pigm(t)*, *Pi2* and *Pi9* are either tightly linked or allelic. Similar to our observation, *Pi2* and *Pi9* were also considered tightly linked (Liu et al. 2002).

Fine mapping of blast resistance gene and candidate gene analysis

Based on the preliminary mapping result (Fig. 1), we further conducted fine mapping of *Pigm(t)* with a new mapping population derived from the cross of GM4 and Maratelli that showed rich polymorphism within the mapping region. Through systemic search of the sequence information of Nipponbare and 9311 available in the databases, we developed 14 new CAPS and InDels markers to narrow down the region encompassing *Pigm(t)* locus between the two flanking markers C26348 and S47656, and subsequently screened a total of 1,556 susceptible individuals using the new polymorphic markers. Finally, two recombinant events were detected with marker C5483, and eight were found with marker C0428 (Figs. 2a, b; 3b). Three markers, S29742, PC22705, C24901 (Table 2), were identified to co-segregate with *Pigm(t)* (Fig. 3b). Our further mapping with other five pairs of S and R DNA pools to other five different isolates confirmed that the *Pigm(t)* locus confers resistance to different isolates (races) tested in the variety because allelic form of the markers associated with resistance was amplified in all R bulks but not in S bulks (Fig. 2c). These results indicate that the *Pigm(t)* locus confers broad-spectrum resistance against all isolates tested in GM4.

The markers tightly linked to the *Pigm(t)* locus were mapped on BAC/PAC clones of Nipponbare, a contig map spanning the *Pigm(t)* locus was constructed (Fig. 3a). As a result, the *Pigm(t)* locus was localized in a 70-kb interval of the Nipponbare sequence flanked by the markers C5483 and C0428 on PAC AP005659 located in 58.7 cM of chromosome 6 (Fig. 3b), which contains a gene cluster with six predicated NBS–LRR resistance genes according to the sequences of Nipponbare (Fig. 3c), where *Pi9*/*Pi2* also located according to the mapping and functioning information (Liu et al. 2002; Qu et al. 2006). The sixth candidate resistance gene is a pseudogene without obvious open reading frame, and its corresponding sequence was unavailable in the 9311 genome. Sequence alignment of the five putative resistance genes (accession numbers Os06g17880, Os06g17900, Os06g17920, Os06g17930 and Os06g17950) showed identities of 95–99% between the Nipponbare and 9311 copies, respectively

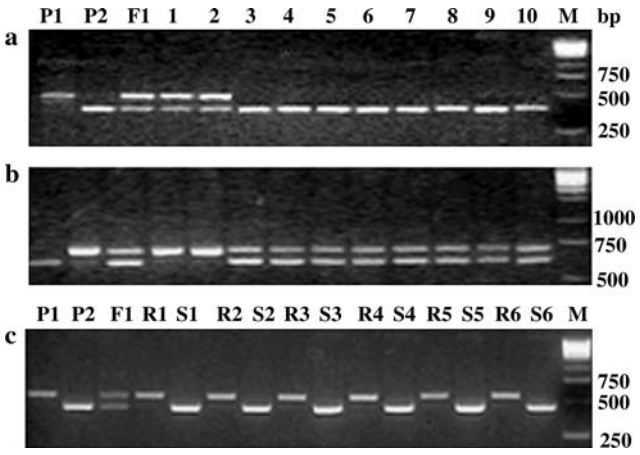

Fig. 2 **a, b** Polymorphism detected by flanking CAPS markers C5483 (**a**) and C0428 (**b**) in ten recombinant individuals from the mapping population. The PCR products of C5483 and C0428 were cleaved by *EcoR* I and *Eco72* I, respectively. Lanes: *P1* GM4; *P2* Maratelli; *F1* cross progeny of P1and P2; *1–10* recombinant individuals. **c** The pattern of the co-segregating InDels marker S29742 amplified in different resistant and susceptible pools. The pairs of R1–S1 to R6–S6 represent homozygous resistant and susceptible pools from the F_2 population inoculated with isolate CH109, CH63, 101/1/1, 101/4/8, 01–19 and CH199, respectively

(Fig. 3c). Our preliminary BAC screening and sequencing of the GM4 genome indicated that the *Pigm(t)* locus of GM4 has a similar *R* gene cluster within the same region, which shows a similar arrangement of the NBS–LRR members, and share high identities (> 90%) to the *Pi9* and Nipponbare clusters in the same loci (data not shown). Most of the candidate *R* genes were expressed in GM4 including a transcript similar to the *NBS2-Pi9* transcript that is an ortholog to the Nipponbare transcript AK067966 (Qu et al. 2006).

Discussion

The availability and utilization of the sequence information for the rice whole-genome of the two subspecies, *japonica* cv. Nipponbare and *indica* cv. 9311, greatly accelerate the fine-scale genetic mapping of important rice genes. Newly developed CAPS and InDels markers are powerful tools for the map-based cloning strategy. In this paper, we have reported the identification and mapping of a broad-spectrum blast resistance locus *Pigm(t)* in a Chinese elite genetic resource, by analyzing R and S DNA pools to different isolates. In the modern sustainable agriculture, broad-spectrum disease resistance is essential for crop breeding. However, no much information is available for rice molecular breeding for broad-spectrum resistance to blast, the most destructive fungal disease in China. Our high-resolution genetic map at the *Pi-gm(t)* locus and a

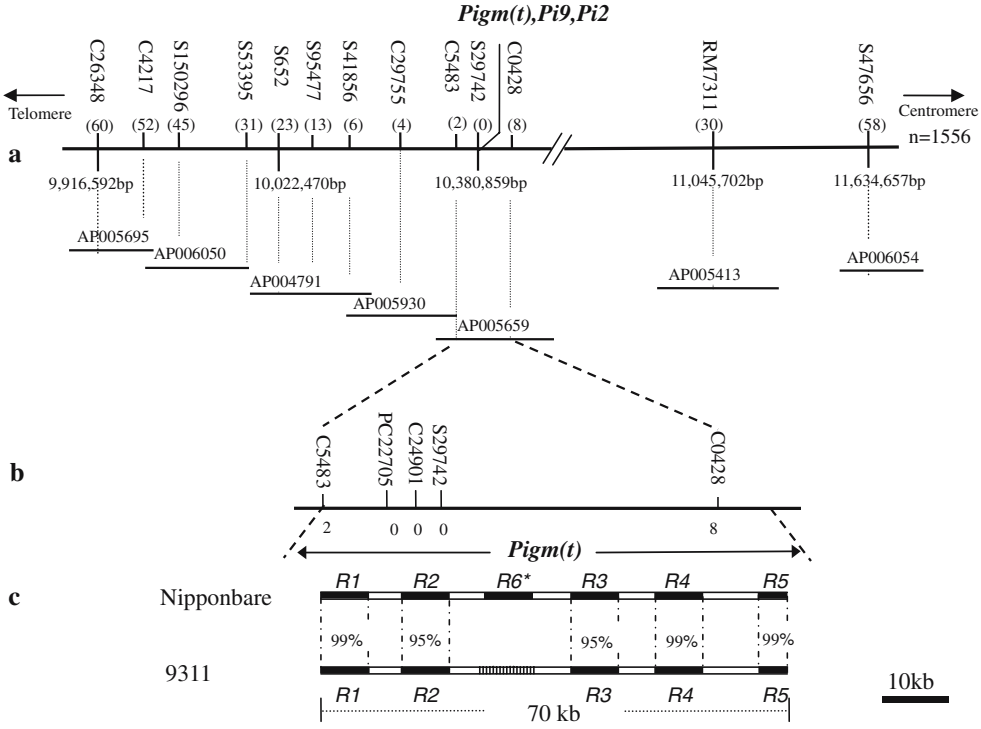

Fig. 3 Genetic and physical maps of the region covering the *Pigm(t)* locus. **a** A contig map spanning the *Pigm(t)* locus was constructed with 1,556 susceptible individuals, with the chromosome orientation indicated. The *numbers in parentheses* above the map are the numbers of recombinants detected between the corresponding markers and the *Pigm(t)* locus. The *numbers below the map* indicated positions of the corresponding markers on chromosome 6 based on sequenced map of IRGSP (International Rice Genome Sequencing Project 2005). The BAC and PAC clones of Nipponbare anchored by the corresponding markers are shown. The *dashed lines* designate the relative positions of the corresponding markers in BAC or PAC clones. **b** The fine physical map of the *Pigm(t)* locus between the markers C5843 and C0428. **c** Putative R genes and sequence similarity of the corresponding *Pigm(t)* loci in the genomes of *japonica* Nipponbare and *indica* 9311. The GenBank accession numbers for *R1–R6* are Os06g17880, Os06g17900, Os06g17920, Os06g17930 Os06g17950 and Os06g17910, respectively. *R6* is a pseudogene in Nipponbare and was not found in 9311 because of sequencing gap (indicated by *lines*)

number of CAPS and InDels markers tightly linked to *Pigm(t)* will also facilitate marker-assisted selection of broad-spectrum resistance gene in rice breeding. Actually, new elite restorer lines for hybrid rice have been developed with *Pigm(t)* and other desirable agronomic traits of GM4 (Zhu et al., unpublished data). In addition, the physical localization of this locus to an approximate 70-kb DNA region represents a significant step toward the final cloning and functional characteristics of this *R* locus.

Majority of plant disease resistance genes appear to occur in the form of complex clusters that contain multiple copies of closely related sequences in rice and other plants (Michelmore and Meyers 1998; Monosi et al. 2004; Wisser et al. 2005). A striking example in rice is the bacterial blight resistance gene *Xa21* family that comprises eight members in a 230-kb region (Song et al. 1995). The *Arabidopsis RPP1* resistance locus contains four tightly linked genes, and three of which encode functional R proteins that recognize distinct *Peronospora parasitica* avirulence determinants (Botella et al. 1998). Similarly, the *Mla, Dm3, Mi* and *Cf-4/9* resistance genes are all members of clustered gene families (Parniske et al. 1997; Meyers et al. 1998; Milligan et al. 1998; Wei et al. 1999). Thus, it has been known that some *R* genes in a cluster may confer resistance to multiple pathogens as well as multiple variants of the single pathogen (Botella et al. 1998; van der Vossen et al. 2000).

In our current study, the orthologous locus of *Pigm(t)* contains five putative NBS–LRR disease resistance genes in the Nipponbare genome (Fig. 3c), although Nipponbare was susceptible to the all isolates tested in our experiment. Our preliminary sequence analysis of BAC clones and PCR products from GM4 indicated that the GM4 genome also harbors a similar gene cluster in the same region, but with quite a number of sequence changes of the GM4 *Pigm(t)* cluster in comparison with those of 9311 and Nipponbare, including InDels and SNPs (data not shown). We postulate that the Nipponbare locus might have lost resistance to blast during the *R* locus evolution as observed in other *R* loci (Hulbert et al. 2001). The identification of the functional *Pigm(t)*

gene through transgenic complementation will provide essential information whether the *Pigm(t)* gene confers broad-spectrum disease resistance in transgenic plants as its donor, or other *R* genes or loci also contribute to broad-spectrum disease resistance observed in GM4.

Similarly, *Pi9* has been identified as *NBS2-Pi9* in a 76-kb BAC contig containing an NBS–LRR like resistance gene cluster (Qu et al. 2006). Our study has documented that *Pigm(t)* is located on the same *R* gene cluster to *Pi2* and *Pi9*. However, sequencing analysis revealed that much sequence divergence exists between the corresponding *Pigm(t)* candidate gene and *NBS2-Pi9*, including a number of SNPs and InDels. In addition, another blast resistance gene, *Pi26(t)* from another *indica* durable resistant variety Gumei 2 (GM2), was roughly mapped around this region (Wu et al. 2005). GM2 and GM4 were selected from the same cross of Gulong 13 × Meike 138; both the parents are blast resistant. We presume that *Pi26(t)* might also be the same locus to *Pigm(t)/Pi2/Pi9*. However, our previous study showed that GM2 and GM4 exhibited different resistance spectra to blast isolates (Shen et al. 2004), suggesting that the two varieties may have different resistance genes at different loci. Furthermore, *Pi2* was derived from the Southern America resistant breeding line 5137 (Mackill and Bonman 1992); *Pi9* was introgressed into the cultivar IR31917 from the wild rice *O. minuta* (Amante-Bordeos et al. 1992). Therefore, it is suggested that these *R* loci might have been created through re-assorting of genetic variation (Michelmore and Meyers 1998; Hulbert et al. 2001). However, one cannot exclude the possibility that these varieties may have different genes (or alleles) at several loci that confer resistance to different blast races. Functionality and comparison of these orthologous genes, *Pigm(t)*, *Pi2*, *Pi9* and *Pi26(t)*, will provide molecular evidence whether all these genes are the same or allelic with certain sequence divergence that may contribute to their slight difference in resistance spectrum, and also the basis of broad-spectrum resistance to blast and evolutionary mechanism of the *R* gene cluster in rice genome. The identification and recognition of corresponding fungal *Avr* genes will provide a further clue on the mechanism of broad-spectrum disease resistance. Nevertheless, functional characterization of the *Pigm(t)* gene will certainly provide essential as well as rich information for engineering new varieties with broad-spectrum disease resistance in agriculture.

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