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Genetic and physical mapping of *Pi37*(t), a new gene conferring resistance to rice blast in the famous cultivar St. No. 1

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Abstract The famous rice cultivar (cv.), St. No. 1, confers complete resistance to many isolates collected from the South China region. To effectively utilize the resistance, a linkage assay using microsatellite markers (SSR) was performed in the three F_2 populations derived from crosses between the donor cv. St. No. 1 and each of the three susceptible cvs. C101PKT, CO39 and AS20-1, which segregated into 3R:1S (resistant/susceptible) ratio, respectively. A total of 180 SSR markers selected from each chromosome equally were screened. The result showed that the two markers RM128 and RM486 located on chromosome 1 were linked to the resistance gene in the respective populations above. This result is not consistent with those previously reported, in which a well-known resistance gene *Pif* in the St. No. 1 is located on chromosome 11. To confirm this result, additional four SSR markers, which located in the region lanked by RM128 and RM486, were tested. The results showed that markers RM543 and RM319 were closer to, and RM302 and RM212 completely co-segregated with the resistance locus detected in the present study. These results indicated that another resistance gene involved in the St. No. 1, which is located on chromosome 1, and therefore tentatively designated as *Pi37*(t). To narrow down genomic region of the *Pi37*(t) locus, eight markers were newly developed in the target region through bioinformatics analysis (BIA) using the publicly available sequences. The linkage analysis with these markers showed that the *Pi37*(t) locus was mapped to a ≈ 0.8 centimorgans (cM) interval flanked by RM543 and FPSM1, where a total of seven markers co-segregated with it. To physically map the locus, the *Pi37*(t)-linked markers were landed on the reference sequence of cv. Nipponbare through BIA. A contig map corresponding to the locus was constructed based on the reference sequence aligned by the *Pi37*(t)-linked markers. Consequently, the *Pi37*(t) locus was defined to 374 kb interval flanking markers RM543 and FPSM1, where only four candidate genes with the resistance gene conserved structure (NBS-LRR) were further identified to a DNA fragment of 60 kb in length by BIA.

Keywords *Oryza sativa* L. Magnaporthe grisea · New resistance gene · Genetic and physical mapping · Bioinformatics analysis (BIA)

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Introduction

Rice blast, caused by the filamentous ascomycete *Magnaporthe grisea* (Hebert) Barr, is one of the most devastating diseases of rice and often reduces rice yields greatly in rice-growing countries under disease-conducive conditions (Ou 1985). Genetic resistance has been and undoubtedly will continue to be the major method of disease control for blast. The rice-*Magnaporthe grisea* pathosystem is also a model system to study plant-pathogen interactions (Valent 1990). In this pathosystem, race specific resistance follows the gene-for-gene relationship (Silue et al. 1992; Jia et al. 2000).

The genetics of blast resistance in rice has been extensively studied. To date, more than 40 major blast resistance genes have been identified (Chauhan et al. 2002; Berruyer et al. 2003; Sallaud et al. 2003; Liu et al. 2004; Liu et al. 2005; and see Pan et al. 1999 and Zhu et al. 2004 for reviews). Among them, only two resistance genes, *Pib* and *Pita*, have been isolated (Wang et al. 1999; Bryan et al. 2000). However, a number of blast resistance genes below have recently been finely

mapped, which should be regarded as an essential starting point to isolate these important genes through map-based cloning approach. Using identified random amplified polymorphic DNA (RAPD) and bacterial artificial chromosome (BAC) end markers, Liu et al. (2002) constructed a high-density map of the *Pi9*(t) locus, and demonstrated that the *Pi2*(t) and *Pi9*(t) are physically linked in ≈100-kb interval on rice chromosome 6. Meanwhile, Jiang and Wang (2002) identified a 118-kb DNA fragment covering the *Pi2*(t) locus through chromosome walking using BAC clones anchored by molecular markers tightly linked to the locus. Chauhan et al. (2002) genetically mapped a new rice blast resistance locus *Pi-CO39*(t) to a region of 1.2 cM in length on the short arm of rice chromosome 11 using simple sequence repeat or microsatellite (SSR), restriction fragment length polymorphism (SSR) and resistance gene analog (RGA) markers, and assembled three contigs of 180, 110 and 145-kb in the region by screening a large-insert genomic library of the donor cultivar (cv.) CO39 with the *Pi-CO39*(t) linked markers. Using rice genomic information deposited in internet and four mapping populations, Jeon et al. (2003) efficiently constructed a genetic and physical map of the *Pi5*(t) locus, in which the locus is located in a 170-kb binary bacterial artificial chromosome (BIBAC) contig on chromosome 9. In addition, they demonstrated that the *Pi5*(t) locus is identical to the *Pi3*(t) locus.

On the other hand, blast resistance in rice cvs is generally classified into two types, qualitative (complete) and quantitative (partial) (Yunoki et al. 1970; Bonman and Mackill 1988; Bonman 1992; Wang et al. 1994; Fukuoka and Okuno 2001; Zenbayashi et al. 2002). Most of the partial resistances are non-race specific, quantitative and polygenic (Fukuoka and Okuno 2001; Zenbayashi et al. 2002). However, there are some exception such as *Pif* in cv. St. No. 1 (Yunoki et al. 1970; Shinoda et al. 1971), *Pb1* in cv. Asanohikari (Fujii et al. 2000) and *pi21* in cv. Owarihatamochi (Fukuoka and Okuno 2001), which are single genes conditioning partial resistance to rice blast. Extensive pathotype tests showed that the St. No. 1 confers partial resistance to Japanese isolates (Ezuka et al. 1969a, b; Yunoki et al. 1970), but complete resistance to many Chinese isolates (Q. H. Pan, unpublished data). Previous study, however, did not identify the resistance gene(s) including the *Pif* gene through linkage analysis using St No. 1 as a resistance donor parent (Shinoda et al. 1971). To elucidate whether the *Pif* gene or other resistance gene(s) involved in the St. No. 1, which conveys complete resistance to many Chinese isolates, it is necessary to identify the resistance gene(s) in the various mapping populations using different isolates. Therefore, the objectives of the present study were to construct various mapping populations derived from crosses between the St. No. 1 and different highly susceptible cvs using different isolates, and to identify resistance gene(s) in the respective populations through segregation analysis using different isolates, and to construct a high-resolution map of the new resistance gene (if any) using PCR-based markers for subsequent marker-assisted gene pyramiding and cloning.

Material and methods

Plant materials, conidial inoculation and disease evaluation

St. No. 1, a *japonica* rice cv., was used as the donor parent, and crossed with three *indica* susceptible cvs, CO39, C101PKT and AS20-1, respectively. *M. grisea*

Table 1 Segregation and linkage analysis of the resistance gene *Pi37*(t) of *Magnaporthe grisea* in the three F_2 populations derived from crosses between the donor resistance cultivar St. No.1 and each of three susceptible cultivars, C101PKT, CO39 and AS20-1

Cross	Inoculation isolate[a]	Segregation model for resistance[b]			χ^2 for segregation ratio (3R : 1S)	Segregation model for marker genotype in the mapping population[c]			$r \pm$ SE[e]	Map distance (cM)
		R	S	Total		rrmm	rrmM	Total		
St. No. 1/C101PKT	CHL1159	1,172	389	1,561	0.003	211[d]	13	224	2.9 ± 1.1	2.9 ± 1.1
						194	30	”	6.7 ± 1.7	6.7 ± 1.7
St. No. 1/C101PKT	CHL1340	1,214	402	1,616	0.007	230	45	275	8.2 ± 1.7	8.3 ± 1.7
						234	41	”	7.5 ± 1.6	7.6 ± 1.6
St. No. 1/CO39	CHL1405	948	339	1,287	1.16	119	36	155	11.6 ± 2.6	11.8 ± 2.7
						130	25	”	8.1 ± 2.2	8.2 ± 2.3
St. No. 1/AS20-1	CHL1159	564	191	755	0.02	38	9	47	9.6 ± 4.3	9.7 ± 4.5
						46	1	”	1.1 ± 1.5	1.1 ± 1.5

[a]Isolates, which are avirulent to St. No. 1 and virulent to C101PKT, CO39 and AS20-1, were selected for inoculations
[b]*R* Resistant; *S* susceptible
[c]The mapping populations, which consisted of the viable susceptible individuals, only, were subjected to subsequent linkage analysis; *rr* Phenotype for susceptible cultivar; *mm* homozygous genotype for susceptible parental cultivar; *mM* heterozygous genotype for both resistant and susceptible parental cultivars
[d]*Upper line* data from the farthest marker RM128 on centromere side; *lower line* data from the farthest marker RM486 on telomere side
[e]*r* Recombination frequency (percent)

isolates CHL1405, CHL1340 and CHL1159, which were collected from Guangdong (GD) province, China, and showed differential reactions on the parents, were selected to evaluate the resistance segregation of F_2 populations derived from the three crosses, respectively. Seedling growth, inoculum preparation and disease evaluation were performed as previously described (Pan et al. 2003).

Marker development and identification

Plant DNA was prepared from frozen leaves of rice plants using the CTAB method (Murray and Thompson 1980). Only PCR-based markers such as SSR, PSM (position-specific microsatellite, prefix FPSM) and STS (sequence-tagged site, prefix FSTS) were used in the current study. For SSR markers, the primer sets were adopted from the International Rice Microsatellite Initiative (IRMI, http://www.gramene.org; McCouch et al. 2002), and the detection procedures as described by Zhu et al. (2004). For further linkage analysis, FPSM and FSTS markers were developed in the target region defined by the SSR markers through bioinformatics analysis (BIA) using the publicly available reference sequences of the entire rice genome of two subspecies, i.e., *japonica* (cv. Nipponbare; http://rgp.dna.affrc.go.jp) and *indica* (cv. 93–11; http://www.genomics.org.cn). Particularly, primer sets for FPSM markers were designed based on the reference sequence of cv. Nipponbare using software tools, SSRIT (http://www.gramene.org/microsat/ssrtool) and Primer Premier 5.0 (PREMIER Biosoft International, http://www.premierbiosoft.com/). On the other hand, primer sets for FSTS markers were designed according to the sequence comparison between the two subspecies in the target region using a software tool, Pairwise BLAST (http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html). That is, a sequence, where exists a large deletion between the two subspecies, is considered as a candidate sequence for a putative FSTS marker. The detection procedures and conditions of FPSM and FSTS markers were shown in Table 2.

Genetic map construction

The F_2 populations being segregated into 3R:1S (resistant/susceptible) were subjected to construct genetic map of the resistance gene(s) of St. No. 1 with SSR markers (Table 1). The bulked-segregant analysis (BSA; Michelmore et al. 1991) combined with recessive-class analysis (RCA; Pan et al. 2003) was used to identify molecular markers linked to the resistance gene (Pan et al. 2003; Zhu et al. 2004). When linkage analysis showed similar results in the respective populations, these populations were pooled as a mapping population for the target gene. After determining the chromosomal location of the target gene, chromosome walking to the resistance gene was initiated from both sides with SSR markers, and followed by FPSM as well as FSTS markers.

Physical map construction

Since the genetic map of the target gene has been constructed by sequence-tagged markers such as SSR and STS, the physical map of the target gene can be subsequently constructed by BIA using BAC and PAC (P1-derived artificial chromosome) clones of cv. Nipponbare sequenced by the International Rice Genome Sequencing Project (IRGSP). That is, these clones were anchored with the target gene-linked markers and then aligned with the software tool, Pairwise BLAST.

Results

Mapping population construction

Three F_2 populations derived from crosses between St. No. 1 and the three susceptible cultivars, C101PKT, CO39 and AS20-1, were inoculated with *M. grisea* isolates CHL1159, CHL1340 and CHL1405, respectively (but the first one was inoculated with CHL1159 and CHL1340). All the F_2 populations segregated into

Table 2 Primer sequence of position-specific microsatellite (FPSM) and sequence tagged site (FSTS) markers developed in the study

Marker name	Forward (5′–3′)	Reverse (5′–3′)	PCR	Gel	Products size (bp)
FPSM1	TTGAACATGATCCACCCCAC	ATTCCCGTAGCCGTAGAGTC	A	I	177
FPSM2	GAAGGTCCATCAAACGCTGC	CTCGCGGACAAGACGATACG	A	I	206
FPSM3[a]	CCACCACATGTCTTCCTAC	GAAGACACCACCTCACCTGC	A	I	156
FPSM4	CCTTCCAGTCCTCGTTATCG	CCACGCGACCCTGTTTGAGA	A	I	244
FSTS1	CGCTGCATGGCACTAACCCT	CAAGAGGCTGGAACAGACAC	B	II	164
FSTS2	GGAACTGCGGCGAAAGGAAT	TCAGGAAGCCGTACATTAGG	B	II	1,285
FSTS3	GCCGCTGTGGCCTCGTCAATC TACATCAAG	AAGGAAGAGGAGATCGCTA TCGGAGGGGCA	C	III	6,968
FSTS4	CAGGCTCAGGAACGACACG	GCTACGACGCGCTGTGGAAT	A	I	127

A After preheating 4 min at 94°C, 35 PCR cycles (30 s at 94°C, 45 s at 55°C, 1 min at 72°C), followed by 5 min at 72°C; *B* after preheating 4 min at 94°C, 30 PCR cycles (30 s at 94°C, 30 s at 66°C, 1.5 min at 72°C), followed by 7 min at 72°C; *C* after preheating 4 min at 94°C, 30 PCR cycles (30 s at 94°C, 7 min at 68°C), followed by 10 min at 72°C

I 8% acrylamide; *II* 3% acrylamide; *III* 0.8% agarose

[a]Monomorphic marker correspond to the parents

3R:1S, indicating that the resistance gene(s) detected in the respective populations is a single dominant resistance gene against different isolates selected from GD, China (Table 1). Since the resistance gene detected in the respective F_2 populations, which were derived from the same donor cv. St. No. 1, was linked to SSR markers RM128 and RM486, these populations were combined as a mapping population for the target gene (Table 1 and also see below). Consequently, a population consisting of 701 viable susceptible and 85 resistant F_2 plants randomly selected was constructed for genetic and physical mapping of the target gene.

Genetic map construction

To determine chromosomal position of the target gene, at first, a total of 180 known SSR markers selected from all the 12 rice chromosomes with an intervals of ≈10 cM were tested in the respective F_2 populations, through the BSA approach. The results showed that the two markers RM128 and RM486, located on chromosome 1, showed positive polymorphisms for the resistance gene in all the F_2 populations. To confirm the polymorphic markers, the plants consisting of the resistant and susceptible pools in the respective populations were tested individually. The results showed that both markers co-segregated with the resistance gene in the respective populations (data not shown). Then, the RCA approach was employed to efficiently determine the linkage relationships between the resistance gene and each of these two markers. A total of 701 extremely susceptible individuals derived from the three populations were subjected to linkage analysis with the two SSR markers. The results showed that 103 and 97 distinct recombinants were identified at RM128 and RM486 loci, respectively (Table 3). It revealed that the resistance gene identified in the respective populations is not the *Pif* gene on chromosome 11, but is another new resistance gene. It is flanked by RM128 on centromere side and RM486 on telomere side with genetic distances of 7.3 and 6.9 cM, respectively (Fig. 1a). As to our knowledge, no resistance gene to blast was described previously in this region. This new gene is, therefore, tentatively called *Pi37*(t), because *Pi36*(t) was recently identified on chromosome 8 in cv. Q61 (Liu et al. 2005). Chromosome walking to the *Pi37*(t) locus was, therefore, started from the two loci with a total of 200 recombinants occurred in the target region (Table 3).

To find additional markers flanking the *Pi37*(t) locus, four SSR markers, RM543, RM302, RM212 and RM319, and one STS marker, S15628, which were located in the target region, were adopted from IRMI and Rice Genome Sequence Program, Japan (RGP) Web site (http://rgp.dna.affrc.go.jp). Among them, the two markers RM543 and RM319, where 10 and 23 recombinants from those identified at RM128 and RM486 loci, respectively, were detected, indicating that the *Pi37*(t) locus was further defined by the markers RM543 and RM319 on both sides with 0.7 and 1.6 cM, respectively. The rest of the three markers, RM302, RM212 and S15628, were completely co-segregating with the *Pi37*(t) locus (Table 3; Fig. 1a, b).

Table 3 Genotypes at fourteen marker loci of 200 plants with a recombination point near the *Pi37*(t) locus

Marker	93 plants	10 plants	1 plants	2 plant	20 plants	74 plants
RM128	M	M	m	m	m	m
RM543	m	M	m	m	m	m
RM302	m	m	m	m	m	m
FPSM4	m	m	m	m	m	m
RM212	m	m	m	m	m	m
FSTS4	m	m	m	m	m	m
S15628	m	m	m	m	m	m
FSTS1	m	m	m	m	m	m
FSTS3	m	m	m	m	m	m
FPSM1	m	m	M	m	m	m
FPSM2	m	m	M	M	m	m
FSTS2	m	m	M	M	m	m
RM319	m	m	M	M	M	m
RM486	m	m	M	M	M	M

M represents homozygous for St. No. 1 or heterozygous for both parents, *m* represents homozygous for susceptible parents

For further fine mapping of the *Pi37*(t) locus, four FPSM markers and four FSTS markers were developed in the smaller region based on the reference sequence of cv. Nipponbare by BIA (Table 2). Among the eight new PCR-based markers developed, all but one, FPSM3, show monomorphic to the parents (Table 2). Of the seven polymorphic markers tested, two and one recombinants derived from RM486 were detected at FSTS2 and FPSM1 loci, respectively, and no recombinant was detected at the other loci (Table 3). It, therefore, revealed that a total of seven markers, RM302, FPSM4, RM212, FSTS4, S15628, FSTS1 and FSTS3, co-segregated with the *Pi37*(t) locus (Table 3; Fig. 1b). The genetic region spanning the *Pi37*(t) locus was estimated ≈ 0.8 cM in length.

Physical map construction in silico

All the anchor markers used in the chromosome walking to the *Pi37*(t) locus were landed on the reference sequence of cv. Nipponbare by BIA using software tool, BLASTN (http://www.ncbi.nlm.nih.gov/blast/index.html). Sequences matching RM128 and RM486 were found in the sequences AP003218.5 of BAC clone OSJNBb0021A09 and AP003446.3 of OJ1529_G03, respectively, on chromosome 1 (Fig. 1c). Likewise, RM543, RM302, FPM4, RM212, FSTS4, S15628, FSTS1, FSTS3, FPM1, FPM2, FSTS2 and RM319, which were flanked by RM128 and RM486, identified matching sequences in the respective clones as shown in Fig. 1c. The BAC/PAC clones identified were downloaded from the RGP Web site and then aligned as a contig map covering the *Pi37*(t) locus through Pairwise BLAST analysis. The physical distance between markers

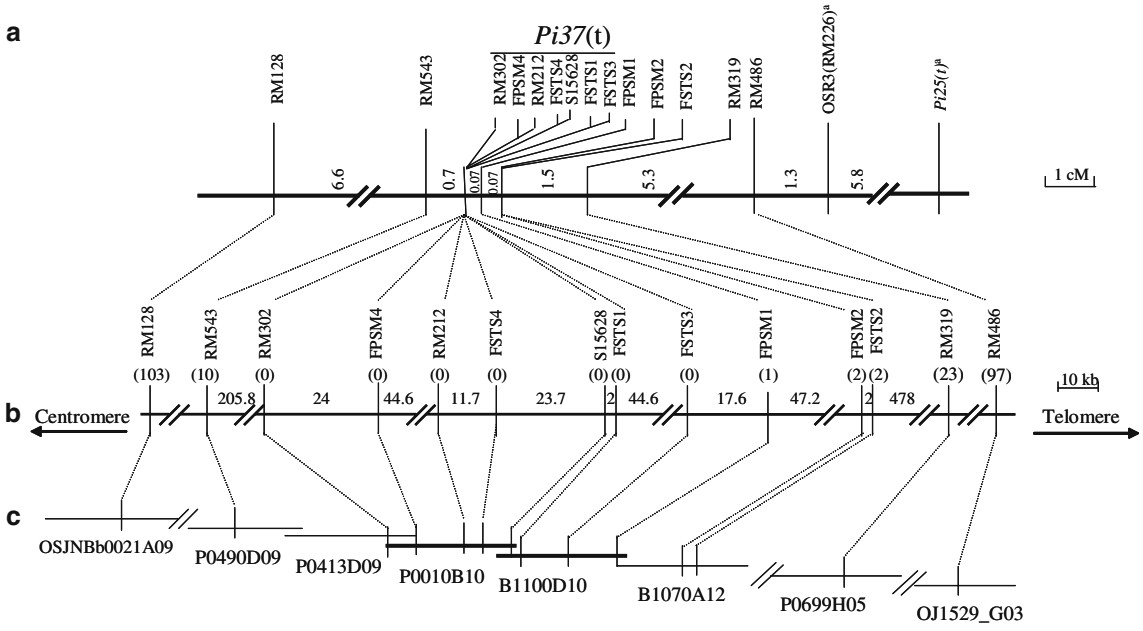

Fig. 1 **a** Genetic map of the resistance gene, *Pi37*(t), which confers resistance to blast on the long arm of rice chromosome 1. The location of gene *Pi25*(t) identified in the present research was estimated based on the data from Yang et al. (2001). Map distances are in centimorgans (cM). **b**. Physical map of the *Pi37*(t) locus. The STS marker S15628 was derived from rice RGP public data. Three position-specific microsatellite markers and four STS markers were developed in this study. The *numbers in parenthesis* are the recombination events in the mapping populations. The *digits* between markers are physical distances. **c**. The *short horizontal lines* represent BAC/PAC clones of cv. Nipponbare, which were released by IRGSP and assembled by the corresponding markers linked to the *Pi37*(t) locus. The *vertical lines* denote the position of the respective marker. The *dashed lines* designate the relative positions of the corresponding markers

RM543 and FPSM1 is 374 kb on the RGP BAC/PAC contig (Fig. 1c).

Discussion

The availability and utilization of the sequence information for the rice whole-genome of the two subspecies, especially the *japonica* cv. Nipponbare released by IRGSP, have made map-based cloning in rice easier and more efficient (Jiang and Wang 2002; Jeon et al. 2003; Gu et al. 2004; Liu et al. 2005). In the present study, we used a more efficient method, which consists of three approaches, BSA, RCA and BIA, for establishing a high-resolution map of the *Pi37*(t) locus without construction of artificial chromosome library from the donor cv. St. No. 1 and utilization of any molecular marker other than PCR-based markers. First, SSR markers released by IRMI were adopted for roughly mapping the resistance gene. Second, additional known SSR and STS markers located in the target region were searched for finely mapping the resistance gene. Third, the resistance gene-specific markers, FPSM and FSTS, which were newly developed in the smaller region according to the BIA, were used to complete chromosome walking to the resistance gene locus. Fourth, the anchor markers used in chromosome walking were used to assemble the RGP BAC/PAC clones for establishing the physical map of the resistance locus through BIA.

Although blast resistance in many cvs is short-lived, relatively durable or broad- spectrum resistance has been observed in certain cvs in certain environments (Bonman 1992; Wang et al. 1994; Ahn et al. 2000; Tabien et al. 2000; Jeon et al. 2003; Liu et al. 2004). The genetic basis of durable resistance or broad- spectrum resistance is, however, not well understood, the rising evidences showed that it may be governed by single genes or multiple genes with cumulative effects (Johnson 1981; Wang et al. 1994; Jeon et al. 2003). In the former case, the resistance genes *Pi1, Pi2* and *Pi9* have been recognized to confer broad-spectrum resistance to many isolates collected from various countries (Chen et al. 1996; Liu et al. 2002). In the latter case, five resistance genes have been identified in African cv. Moroberekan (Wang et al. 1994; Inukai et al. 1996; Naqvi and Chattoo 1996; Chen et al. 1999), four resistance genes in Korean cv. Suweon 365 (Ahn et al. 2000), three resistance genes in Chinese cv. Teqing (Tabien et al. 2000) and three resistance genes in Chinese cv. Sanhuangzhan 2 (Liu et al. 2004), those are well known as durably resistant cvs in certain regions. The St. No. 1 was developed from the cross of "Modan/6*Norin8" in Chugoku National Agriculture Experiment Station, Japan, and is recognized as the representative of cvs rendering partial resistance (termed 'field resistance' by Japanese researchers) in Japan since 1960s (Ezuka et al. 1969a, b; Yunoki et al. 1970; Shinoda et al. 1971; Hayano-Saito et al. 1998; Fujii et al. 2000; Zenbayashi et al. 2002). As to Chinese isolates, St. No. 1 expressed

higher resistance in the four populations, which were collected from South China including GD, Fujian, Hunan and Yunan provinces (data not shown). It was noteworthy that the resistance frequency of St. No. 1 in GD population, which consists of 357 isolates collected from early season (from March to July) in 2000 to late season (from July to November) in 2002 is 87.7%, those of other cvs carrying important resistance genes were 75.2% (*Pi1*), 91.2% (*Pi2*), 81.9% (*Piz*), 70.4% (*Piz*^{t}) and 76.8% (*Pita*^{2}) (Q. H. Pan, unpublished data). This, together with the new resistance gene identified in the present study, led to the hypothesis that the durable resistance of the St. No. 1 is, in fact, controlled by not only the *Pif* gene, but also *Pi37*(t) and even others. Indeed, we have initially been in an effort to map the *Pif* gene on chromosome 11, and not any linkage relationship between the *Pif* gene and the molecular markers mapped on chromosome 11 was found in each of the three F_2 populations developed in the current research. Based on this finding, we have checked all the rest 11 chromosomes and identified another new resistance gene *Pi37*(t) on chromosome 1. Since the *Pi37*(t) gene conveys high resistance complementing to those controlled by the important resistance genes, such as *Pi1, Pi2*, *Piz, Piz*^{t}, and *Pita*^{2} (data not shown), it is therefore considered that this gene should be an expectative partner with these resistance genes in rice improving projects in China.

Four other genes for blast resistance, *Pit* (Kaji et al. 1997), *Pish* (Imbe et al. 1985), *Pi25*(t) (Yang et al. 2001) and *Pi27*(t) (Zhu et al. 2004), were also mapped on chromosome 1. According to the comprehensive map of the four resistance genes previously constructed (Zhu et al. 2004), as well as the rice SSR map (McCouch et al. 2002), it, therefore, elucidated that *Pit, Pish* and *Pi27*(t) were mapped on the short arm, whereas *Pi25*(t) and *Pi37*(t) on the long arm of chromosome 1, and the *Pi37*(t) locus is away from the *Pi25*(t) locus by ≈14 cM (Fig. 1a).

Recently, linkage disequilibriums have frequently been identified in the resistance gene-containing regions (Jiang and Wang 2002; Chauhan et al. 2002; Jeon et al. 2003; Sun et al. 2003; Yang et al. 2003; Palaise et al. 2004). In the current study, the *Pi37*(t) gene was physically defined by RM543 and RM319 in the middle region of the long arm of chromosome 1 (Fig. 1; McCouch et al. 2002). In ≈ 900 kb interval, crossover hot spots were detected in the outer parts flanked by RM543 and RM302 as well as FSTS3 and RM319, respectively, and cold spot in the inner part flanking RM302 and FSTS3 (Fig. 1b). In the hot spots, 10 and 23 recombinants were detected in the 205.8 and 544.8-kb regions, meaning recombinant events occurred every 20.6 and 23.7 kb intervals, on average, respectively. In contrast, no recombination breakpoint was observed in the cold spot spanning ≈ 150 kb in length, even though seven polymorphic markers presented in this region. Suppression of recombination has also been observed in the other resistance gene-covering regions, such as the *Mi* (van-Daelen et al. 1993), *Mla* (Wei et al. 1999), *Pita*^{2} (Nakamura et al. 1997), *Pi-CO39*(t) (Chauhan et al. 2002), and *Pi5* (Jeon et al. 2003) loci. Interestingly, these loci were introgressed from their subspecies or wild resources. It is, therefore, conceivable, but not ascertained, that the region spanning the *Pi37*(t) locus, especially the 150-kb interval, is a divergent and recombination-suppressed segment introgressed from its donor parent, the *indica* cv. Modan.

Although we fail to further narrow down the *Pi37*(t)-covering region in the 150-kb interval, we are successful to find only four candidate genes carrying resistance gene-specific structure, NBS-LRR (Dangl and Jones 2001), in ≈ 60 kb region flanking FSTS1 and FPSM1 through BLASTX analysis using the reference sequence. We are undertaking to isolate and identify these four candidate genes by using long-range PCR approach in combination with *Agrobacterium*-mediated transformation technology (Feuillet et al. 2003; Horvath et al. 2003; Song et al. 2003).

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