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Genetic and physical mapping of *AvrPi7*, a novel avirulence gene of *Magnaporthe oryzae* using physical position-ready markers

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Rice blast, caused by the fungal pathogen *Magnaporthe oryzae*, is one of the most devastating crop diseases worldwide. The avirulence gene corresponding to rice blast resistance gene *Pi7* in field isolate CHL346 was inherited as a single gene, designated *AvrPi7*, in a segregating population consisting of 189 ascospore progenies derived from a cross between field isolates CHL346 and CHL42. In order to determine the chromosomal location of the *AvrPi7* locus, a total of 121 simple sequence repeat (SSR) markers were developed based on the whole-genome sequence of reference isolate 70-15 of *M. oryzae*. Linkage analysis of the locus with these SSR markers showed that eight SSR markers on chromosome 1 were linked to the locus, among which the closest flanking markers MS1-9 and MS1-15 were 3.2 and 16.4 cM from the locus, respectively. For fine mapping, additional PCR-based makers including eight SSR markers and three candidate avirulence gene (CAG) markers were developed in the region flanking both markers. The *AvrPi7* locus was genetically delimited within a 1.6-cM region flanked by markers MS1-21 and MS1-22, and co-segregated with the marker CAG2. To construct a physical map of the *AvrPi7* locus, molecular markers linked to the *Avr* gene were mapped on the supercontigs of the reference isolate 70-15 through bioinformation analysis (BIA). Consequently, the *AvrPi7* locus was delimited to a 75-kb interval flanked by markers MS1-21 and MS1-22 based on the reference sequence. Merodiploids observed in this study are also discussed.

Magnaporthe oryzae, avirulence gene, high-resolution map, merodiploid

Rice blast, caused by the filamentous ascomycete *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*), is one of the most widespread and devastating diseases of rice[1]. Disease control consists of the use of resistant cultivars and application of fungicides (International Rice Blast Genome Project, IRBGP, http://www.riceblast.org)[2]. Undoubtedly, use of resistant cultivars is the most effective, economical and environmentally friendly strategy to eliminate the use of fungicides and to reduce yield loss due to this disease. However, newly developed resistant rice cultivars are often overcome after only a few years in field due to the emergence of new pathogenic races or to the selection of a race component of the pathogen population that is already virulent[3]. Thus, the creation and use of rice cultivars with durable resistance will require a better understanding of pathogenic variation of *M. oryzae*.

The rice blast system is a classical gene-for-gene system in which avirulence (*Avr*) genes in the pathogen show a functional correspondence with particular resistance (*R*) genes in rice[4]. Blast resistance in rice is de-

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pendent on the presence of a corresponding cognate *Avr* gene in *M. oryzae*. Therefore, it is essential to characterize the molecular genetic mechanism of *Avr* genes in order to understand how the pathogen overcomes host resistance.

In the past, genetic analysis of the blast pathogen had been hindered because an appropriate stage for making crosses among the isolates of interest had yet to be discovered. Hebert first found such a suitable stage of *M. oryzae in vitro* and demonstrated that the fungus is heterothallic[5]. Later, many researchers focused their attention on identifying fertile rice isolates, mainly in Japan, America, France, and China[6]. However, only a few *Avr* genes were identified because of the low fertility of isolates pathogenic to rice and because these isolates often behave only as males in crosses[6,7].

Identification and use of Guy11, a hermaphroditic isolate pathogenic to rice, have allowed for crosses with isolates pathogenic to rice[6,8]. By using this key isolate, Leung et al. identified *POS1* (pathogenicity on *Oryza sativa*) and *POS2* in a cross between Guy11 and laboratory isolate 2359, and *POS3* and *POS4* in a cross between Guy11 and rice field isolate CH104-3[8]. Leong and colleagues identified *avrCO39* (later, *AVR1-CO39*) in a cross between Guy11 and the improved rice isolate 2359[9,10]. Lau et al.[11] identified *P11* and *P12* in a cross between Guy11 and the improved rice isolate 66.10. By segregation analyses, Silué et al.[12] identified *Avr1-PiNo4* in a cross between Guy11 and the improved rice isolate ML25, and *AVR1-CIC6*, *AVR1-Ku86* and *AVR1-IRT7* (later, *ACE1*) in a cross between Guy11 and rice isolate 2/0/3, which is a sib-progeny from the cross of Guy11 and ML25. Later, Dioh et al.[13] mapped *Avr1-IRT7*, *Avr1-Ku86*, and *Avr1-MedNoï* on their respective chromosomes with RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) markers. Wang et al.[14] identified *Avr-Pi1, Avr-Pi2* and *Avr-Pi4a* in a cross between Guy11 and isolate 81278ZB15 collected from Fujian Province, China.

Following the identification of Guy11, several hermaphroditic isolates with high fertility were identified in China[3,15−17]. By using the following Chinese field isolates, a number of *Avr* genes have been identified. Valent and colleagues[18] identified *AVR1-CO39*, *AVR1-M201* and *AVR1-YAMO* in a cross between Chinese rice field isolate O-135 and the weeping lovegrass pathogen 4091-5-8, and *Avr2-YAMO* (later, *AVR-Pita*), *AVR1-MARA*, *AVR1-TSUY* and *AVR1-MINE* in a cross between isolates 4224-7-8 and 6043 (both are laboratory isolates pathogenic to rice and improved by Chinese isolate O-137 and Guy11, respectively)[19]. Yasuda et al.[20] identified *Avr-Hattan3* in a cross between field isolate Y90-71 and laboratory isolate 3514-R-2, and *Avr-Pia*, *Avr-Pii* and *Avr-Pit* in a cross between rice field isolates Y93-164a-1 and Y93-165g-1[21]. Li et al.[15] identified *Avr-Xiu* in a cross between rice field isolates 94-84c and 95-23-4a. Liu et al.[22] identified *Avr-Pik*^{m} in a cross between rice field isolates S159 and S1522. Lin et al.[16] identified *Avr2-Pish* and *Avr2-Pit* in a cross between isolates CHL124 and CHL125. Luo et al.[3] identified *AvrPik*, *AvrPiz*, *AvrPiz-t* in a cross between field isolates 84R-62B and Y93-245C-2. Thus, over 40 genes conditioning avirulence on rice cultivars have been reported, although only three *Avr* genes, *AVR1-CO39*[9], *AVR-Pita*[23] and *ACE1*[24], conferring cultivar-specificities, have been cloned.

Isolation of *Avr* genes largely depends on a map-based cloning strategy[25]. For map-based cloning, construction of a high-resolution map including genetic and physical information for the target gene locus is absolutely necessary. However, this method depends on the availability of markers closely linked to the target gene. The whole-genome sequence of reference isolate 70-15, released by IRBGP (http://www.broad.mit.edu/annotation/genome/magnaporthe_grisea), provides enough sequence information for the development of physical position-ready markers to increase the density of markers in the genetic map and to assemble contiguous clones in the physical map, allowing chromosome walking to the target locus[2]. Although different isolates of *M. oryzae* have high sequence diversity, it is feasible to clone a gene from the other isolates based on the sequence information of the reference isolate because of the synteny and conservation of genome sequence among the different isolates of the same species.

In the present study we analyzed the inheritance pattern of a novel *Avr* gene, *AvrPi7* of *M. oryzae*, in a progeny population derived from a cross between two rice field isolates, CHL346 and CHL42. We mapped the gene to an interval of 1.6 cM on chromosome 1 with a physical distance of about 75-kb using physical position-ready markers developed from the reference sequence.

1 Materials and methods

1.1 Genetic cross, ascospore isolate production and fungal DNA extraction

The parental isolates CHL346 (*MAT1-1*, male) and CHL42 (*MAT1-2*, hermaphrodite) were obtained from diseased rice plants in fields in Jiangsu and Yunnan provinces, China, respectively. Genetic cross and ascospore isolate production were performed as previously described[26]. For long-term storage of the progeny isolates, two agar pieces with mycelia of each progeny isolate were placed onto a piece of sterile filter paper which covered the surface of a plate of rice polish agar medium (20 g of rice polish powder, 2 g of yeast extract, and 11 g of agar per liter of distilled water, RPA[27]), and incubated at 26℃ for 10 d. Next, the filter papers with mycelia growth were removed from the plates and transferred into a vacuum drier with desiccator for about three weeks. The dried filter papers were cut into small pieces (about 25 mm^2) and vacuum-packed for storage at –20℃. Mycelia culture and fungal DNA extraction were performed as previously described[26].

1.2 Plant materials, inoculation and disease evaluation

A monogenic line with the *R* gene *Pi7*, IRBL7-M[28], a susceptible check cultivar, Sariceltik, and other 66 cultivars with the respective resistance genotypes (data will be presented elsewhere), were used in this study. Seedling growth and inoculum preparation were carried out as previously described[27]. Disease reactions were scored for about 6 d after inoculation using a 6-class scale described by Pan et al.[29]. An isolate was regarded as virulent when it caused lesions on the host cultivar (score 3－5), whereas 0－2 scores corresponded to avirulent isolates. Each isolate was tested at least twice. Segregation of avirulence in the progeny population was examined with a Chi-square test for goodness of fit to 1A∶1V and/or 3A∶1V (avirulent/virulent) ratios, indicating that avirulence is controlled by one gene and/or two genes, respectively, in the haploid pathogen.

1.3 Marker development and genetic mapping of the *Avr* gene locus

For rapid mapping of the targeted *Avr* gene, SSR and CAG (candidate avirulence gene) markers were developed based on the sequence information of the reference isolate 70-15 released by IRBGP. In the first round of linkage analysis, SSR markers were developed for mapping the *Avr* gene based on the whole-genome sequence of *M. oryzae* through bioinformatics analysis (BIA) using the appropriate software as described eslewhere[26]. In the second round of linkage analysis, additional SSR markers were developed in the region defined by the first-round SSR markers to narrow down the chromosomal location of the target gene. In the third round of linkage analysis, an allele specific polymorphic (ASP) marker, ASP-CO39, developed from the *AVR1-CO39* locus[30], was used for a molecular allelism test of the *AVR1-CO39* locus near the *AvrPi7* locus. In the last round of linkage analysis, CAG markers were developed for fine mapping of the *Avr* gene locus based on the sequence of the candidate genes predicted using the gene annotation system Softberry FGENESH (http://www.softberry.com). All designed primers were custom synthesized by SBS Genetech Co., Ltd. (Beijing, China). The primer sequences, marker positions, PCR conditions and detection procedures of the polymorphic markers used are shown in Table 1.

Two contrasting bulks were prepared according to a pathogenicity test. Each bulk consisted of DNA from 8 progeny isolates that were avirulent or virulent toward the rice monogenetic line IRBL7-M. The concentration of DNA from each progeny of a given bulk was equalized before pooling. Mapping of the target gene to a particular *M. oryzae* chromosome was achieved as described previously[26,31－33]. As the fungus is haploid, recombination frequency is simply calculated by the ratio $r=N_r/N_T$, where N_r is the number of the recombinants and N_T is the total number of indivdiduals in the mapping population. The recombination frequency was transformed into centimorgans (cM) as described elsewhere[27].

1.4 *In silico* physical mapping of the *Avr* gene locus

Since the genetic map of the *Avr* gene locus was constructed using physical position-ready markers, the *Avr* gene-linked markers were placed on the respective supercontig of the reference isolate 70-15 through BIA using the software BLASTN (http://www.broad.mit.edu/cgi-bin/annotation/magnaporthe/blast). Consequently, the physical map of the *Avr* gene locus was constructed *in silico* based on the available reference sequence as described previously[26,31－33].

Table 1 Description of the polymorphic microsatellite (SSR), allele-specific polymorphic (ASP) and candidate avirulence genes (CAG) markers used in the present study

Marker	Primer (5′−3′)[a)]	Marker type	SSR Motif[b)]	Supercontig[c)]	PCR[d)]	Gel[e)]	Expected length (bp)
MS1-3	gctgtccaaccctcactgct (F) gttgttcgtcacacccttg (R)	SSR	$(CA/GT)_{25}$	2	A	I	295
MS1-5	tcgtagagtagtttccagtc (F) cgctcgaggatagtcgtgtt(R)	SSR	$(TA/AT)_{22}$	2	A	I	185
MS1-6	gactcccatagcggtgtttg (F) caagacctccacaccatccag (R)	SSR	$(CAG/GTC)_{11}$	2	B	I	177
MS1-7	cagtgcttggttcttgggag (F) ctaacctcccctgtcgctct (R)	SSR	$(TG/AC)_{20}$	2	A	I	259
MS1-8	ttagaccgtcatcgccatcag (F) caacggaaatggtgaggtat (R)	SSR	$(CT/GA)_{18}$	13	B	I	229
MS1-9	ccaagaaaccaagtccatcc (F) gactcctgtgttgattgcgg (R)	SSR	$(AC/TG)_{19}$	13	A	I	256
MS1-13	tggtggaagcacgacggaat (F) gcttgtgctctcccgactc (R)	SSR	$(CT/GA)_{11}$	16	A	I	224
MS1-15	caggcaagactatggcaagg (F) ctggactgctgggtggttc (R)	SSR	$(CA/GT)_{24}$	17	B	I	240
MS1-21	ggtcgcgtcagtgaactaac (F) gagaaccgcatggctgagag (R)	SSR	$(TAC/ATG)_{9}$	17	C	I	334
MS1-22	gtcatggttgagccctagcc (F) cgtttgagccaggaggagtc (R)	SSR	$(CA/GT)_{11}$	13	C	I	324
MS1-25	cggtagctccgctccaatag (F) cccagggtagtaatcaacgg (R)	SSR	$(TAGG/ATCC)_{18}$	17	C	I	288
CAG2	ggaattgttgaagggccgtag (F) gtaggagattacagcagcgag (R)	CAG	——	17	D	II	1100
ASP-CO39	aactgcgctgagctacc (F) aatagactagcttccgt (R)	ASP	——	1534	E	II	1200

a) F, Forward; R, reverse. b) The SSR motif and its complement are followed by the number of times the motif is repeated in the original contig. c) The supercontig numbers (http://www.riceblast.org). d) A, After preheating 4 min at 94℃, 30 PCR cycles (30 s at 94℃, 45 s at 60℃, 1 min at 72℃), followed by 7 min at 72℃; B, after preheating 4 min at 94℃, 30 PCR cycles (30 s at 94℃, 30 s at 56℃, 1 min at 72℃), followed by 7 min at 72℃; C, after preheating 4 min at 94℃, 30 PCR cycles (30 s at 94℃, 30 s at 62℃, 1 min at 72℃), followed by 7 min at 72℃; D, after preheating 4 min at 94℃, 35 PCR cycles (30 s at 94℃, 30 s at 64℃, 2 min at 72℃), followed by 7 min at 72℃; E, after preheating 4 min at 94℃, 35 PCR cycles (30 s at 94℃, 30 s at 52℃, 2 min at 72℃), followed by 7 min at 72℃. e) I, 6% polyacrylamide; II, 3% polyacrylamide.

2 Results

2.1 Genetic analysis of an *Avr* gene in the progeny population

The pathogenicities of the parental isolates were tested on the monogenic line IRBL7-M and on Sariceltik. The results show that CHL346 and CHL42 were avirulent and virulent on the cultivar IRBL7-M, respectively, and that both isolates were virulent on the control cultivar Sariceltik. Profuse perithecia were observed at the intersection of mycelia during pathogen growth. A total of 189 progeny isolates were independently tested under the same conditions. Each isolate showed distinguishable reactions on the host plants of IRBL7-M in all experiments. Segregation of avirulence and virulence occurred at a ratio of 91∶98, fitting a ratio of 1∶1 (χ^2=0.19, *P* value=0.5−0.75). This is consistent with the hypothesis that CHL346 carries a single dominant gene conferring avirulence to the monogenic line IRBL7-M. The progeny population was, therefore, considered a suitable mapping population for the *Avr* gene corresponding to the *R* gene *Pi7*, called *AvrPi7*.

2.2 Primary mapping of the *AvrPi7* locus

To determine the chromosomal position of the *AvrPi7* locus, a total of 121 SSR markers distributed evenly on all seven chromosomes of *M. oryzae* were used for screening via the BSA (bulked-segregant analysis)[34] approach. Out of 15 evenly positioned markers on chromosome 1, 8 markers (MS1-3, MS1-5, MS1-6, MS1-7, MS1-8, MS1-9, MS1-13 and MS1-15) showed consistent polymorphism for the parents and the pools, indicating that these markers were linked to the *AvrPi7* locus. The progeny isolates in the avirulent and virulent pools were then tested individually for confirmation. The results suggest that all candidate markers were

linked with the *Avr* gene (data not shown). For linkage analysis of the *Avr* gene with these markers, a total of 189 individuals, including those of the two pools, were tested. The results show that four recombination breakpoints were detected on chromosome 1 covered by the eight markers mentioned above (Figure 1). At the first breakpoint there is a marker cluster (Cluster I) consisting of MS1-3, MS1-5, MS1-6, and MS1-7, where 12 recombinants were detected. This point (cluster) is ~6.4 cM away from the locus (Figure 1). At the second breakpoint there is another marker cluster (Cluster II) including MS1-8 and MS1-13 where seven recombinants were detected, ~3.7 cM from the locus. The third recombinant breakpoint was identified at the marker locus MS1-9, where six recombinants were detected, located at ~3.2 cM from the *Avr* locus. The fourth recombination breakpoint was identified at the marker locus MS1-15, where 31 recombinants were identified ~16.4 cM away from the *Avr* locus. Recombinants detected at the MS1-15 locus were different from those found at loci from MS1-3 to MS1-9, suggesting that the *AvrPi7* locus is flanked by marker loci from MS1-3 to MS1-9 on the TEL1 side and MS1-15 on the TEL2 side (Figure 1). Therefore, chromosome walking to the locus was started from both MS1-9 and MS1-15 with a total of 37 recombinants (Table 2; Figures 1 and 2).

Table 2 Genotypes of 43 isolates at 13 marker loci with a recombination point near the *AvrPi7* locus[a)]

Marker	1 Isolate	26 Isolates	4 Isolates	5 Isolates	1 Isolate	4 Isolates	2 Isolates
MS1-3	m	m	m	M	M	M	M
MS1-7	m	m	m	M	M	M	M
MS1-5	m	m	m	M	M	M	M
MS1-6	m	m	m	M	M	M	M
MS1-13	m	m	m	m	M	M	M
MS1-8	m	m	m	m	M	M	M
MS1-9	m	m	m	m	m	M	M
ASP-CO39[b)]	m	m	m	m	m	m	H
MS1-22	m	m	m	m	m	m	H
CAG2	m	m	m	m	m	m	m
MS1-21	M	m	m	m	m	m	m
MS1-25	M	M	m	m	m	m	H
MS1-15	M	M	M	m	m	m	m

a) m, Homozygous genotypes for the parental isolates CHL346 or CHL42; M, normal recombinant types; H, heterozygous genotype for both parental isolates (abnormal recombinant types). b) This marker was directly developed at the *AVR1-CO39* locus, for distinguishing its genetic position from that of the *AvrPi7* locus (Figure 1).

2.3 Fine mapping of the *AvrPi7* locus

In order to fine map this locus, additional SSR markers

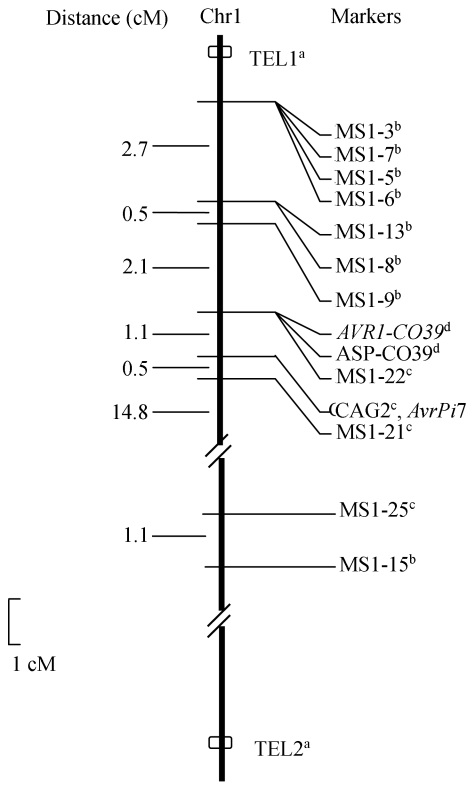

Figure 1 An genetic map of the *Avr* genes *AvrPi7* on chromosome 1. The genetic distances on the left side of the map were calculated based on the data from the present study. The mapping data were integrated from the following article: a, Nitta et al.[45]; b, the first-round markers developed in the present study; c, the second-round markers developed in the present study; d, Farman et al.[30]. MS, Microsatellite/SSR markers; CAG, candidate avirulence gene marker; TEL, telomere.

were developed in the region flanked by MS1-9 and MS1-15. Out of the seven new SSR markers developed, three (MS1-21, MS1-22 and MS1-25) showed polymorphism between the parental isolates. Linkage analysis with the 37 recombinants showed that 29 and one recombinant(s) were detected at the MS1-25 and MS1-21 loci, respectively, indicating that these two markers were 15.3 and 0.5 cM away from the *Avr* locus, respectively, on the TEL2 side (Table 2; Figure 1). On the TEL1 side, only two recombinants originating from the MS1-9 locus were identified at the MS1-22 locus, 1.1 cM away from the *Avr* locus. Consequently, the *AvrPi7* locus was further restricted to a 1.6 cM interval flanked by MS1-22 and MS1-21 (Table 2; Figures 1 and 2).

Farman et al.[30] reported that *AVR1-CO39* was located in the same region as *AvrPi7*. To define the physical relationship between the *AVR1-CO39* and *AvrPi7* loci, ASP-CO39, which was directly developed from the *AVR1-CO39* locus, was subjected to the allelism test

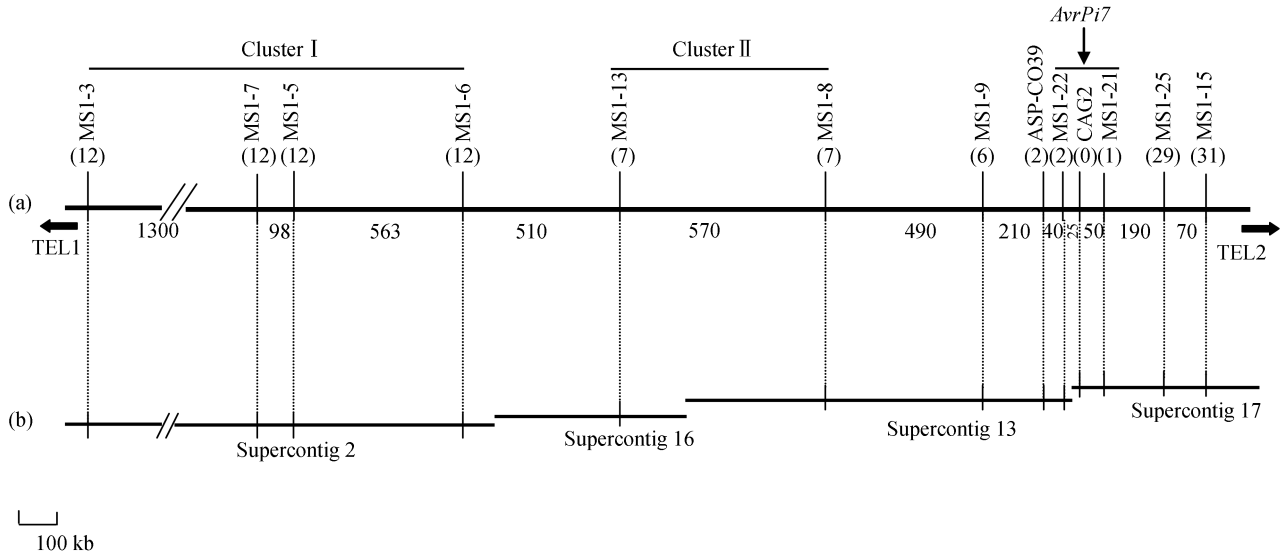

Figure 2 (a) A physical map of the *AvrPi7* locus. The *long horizontal line* indicates the physical region containing the locus. The *short vertical line* indicates the positions of markers linked to the *AvrPi7* locus. The *numbers in parentheses* are recombination events detected at the corresponding marker loci. The *numbers below* the *long line* are relative physical distance in kilobase (kb) estimated based on the physical positions of markers developed on the reference sequence released by the International Rice Blast Genome Project (IRBGP, http://www. riceblast.org). Types and resources of markers are described in Figure 1. (b) A supercontig map of the *AvrPi7* locus constructed based on the FGL database (http://www.fungal.genomics.ncsu.edu). The *short horizontal lines* represent supercontig adopted from the IRBGP database. The *dashed lines* designate the relative positions of the corresponding markers.

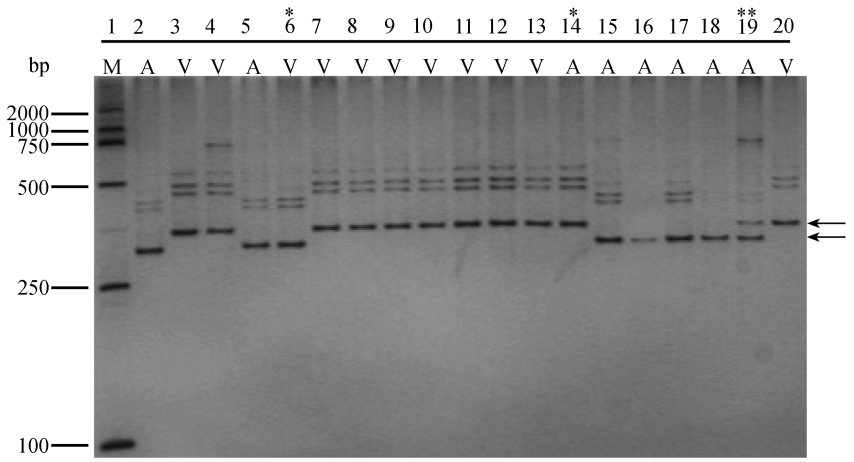

Figure 3 Segregation of microsatellite (SSR) marker MS1-25 in a part of ascospore isolates in the mapping population for the *AvrPi7* gene. Lane 1, Molecular standard marker, DL2000; lane 2, the avirulent parent CHL346; lane 3, the virulent parent CHL42; lanes 4－20, the progenies. Arrows indicate SSR alleles of the marker MS1-25. A, Avirulent; V, virulent; *, recombinant progeny; **, merodiploid progeny (M063).

with the marker genotypes. Two recombinants were identified from linkage analysis at the ASP-CO39 locus, suggesting that the *AVR1-CO39* gene is 1.1 cM away from the *AvrPi7* gene (Table 2; Figure 1).

It is notable that two progeny isolates, M063 and M087, were recognized as normal recombinants at loci from MS1-3 to MS1-9 and as abnormal recombinants (heterozygous genotypes) at the three loci MS1-22, ASP-CO39 and MS1-25 (Table 2; and one of them shown in Figure 3). These results were observed after several modifications to PCR conditions.

For further fine-scale mapping of the *AvrPi7* locus, CAG markers were directly developed based on the predicted genes in the target region flanked by the markers MS1-21 and MS1-22. Of three CAG markers developed, only CAG2 showed polymorphism in the two parental isolates. Linkage analysis with recombinants detected at both MS1-21 and MS1-22 loci showed that this marker completely co-segregated with the *AvrPi7* locus. That is, no recombinant was detected at the CAG2 locus (Table

2; Figures 1 and 2). A total of 43 recombinants detected at 13 marker loci flanking the *AvrPi7* locus (Table 2) were used to construct a high-resolution genetic map of the *AvrPi7* locus (Figure 1).

2.4 Physical mapping of the *AvrPi7* locus

Since a genetic map of the *AvrPi7* locus had been constructed by the physical position-ready markers, the physical map of the locus could be established based on the reference sequence aligned with the anchor markers by BIA. As a result, the *AvrPi7* locus was delimited to a 75.0-kb interval at the junction between supercontigs 13 and 17 (Figure 2(a) and (b)). Three candidate genes for *AvrPi7*, all of which encode hypothetical proteins, were predicted in this region by the gene annotation system, Softberry FGENESH (data not shown).

3 Discussion

Map-based cloning or positional cloning is an approach for gene isolation that does not require prior knowledge of the gene product(s) but does require a high-resolution map including genetic and physical information for a target gene[35]. Through this approach, an increasing number of *Avr* genes in several plant pathogenic fungi such as *Cladosporium fulvum*[36], *M. oryzae*[9,23,24,37], *Phytophthora sojae*[38] and *Leptosphaeria maculans*[39] have been successfully cloned. We have recently constructed several high-resolution maps for the rice blast *R* genes *Pi36*[32] and *Pi37*[31], the *Avr* gene *AvrPi15*[26], and the rice brown planthopper *R* gene *bph19*[33]. In the present study, we have adopted an efficient strategy for genetic and physical mapping of a novel *Avr* gene, *AvrPi7*. Initially, a total of 121 SSR markers distributed evenly on the seven chromosomes of *M. oryzae* were developed based on the entire sequence of the reference isolate 70-15. These markers were used to roughly map the *Avr* gene identified in the progeny population. Additional SSR and CAG markers were then developed in the smaller region for fine-scale mapping of the target locus. Finally, the target gene-linked markers were used to assemble the supercontigs of the reference isolate to construct the physical map of the target locus by BIA. That is, a high-resolution map of the *AvrPi7* was constructed using only PCR-based markers derived from the reference isolate sequence data without the need to construct an artificial library from the donor isolate CHL346. This strategy saved time and labor because construction and assembly of an artificial library for establishing a contig map spanning the target gene is a daunting task.

Construction of a high-resolution map with sequence-ready markers will reveal the exact physical distances relative to genetic intervals, allowing identification of "hot" and "cold" spots of meiotic recombination[40]. In the present study we observed dramatically unequal recombination on chromosome 1, a result also described by Farman and Leong[9]. "Cold" spots were detected in the intervals from MS1-3 to MS1-6 (Cluster I) and from MS1-13 to MS1-8 (Cluster II), and "hot" spots in the intervals from MS1-9 to CAG2 and from CAG2 to MS1-25 (Figure 2(a)). In the hot spot on the TEL1 side, 6 recombinants were detected in the 275-kb interval with recombinant events occurring every 45.8-kb, on average. In the hot spot on the TEL2 side, 29 recombinants were identified in the 240-kb interval, indicating the occurrence of recombinant events every 8.3-kb, on average. In contrast, no recombination was observed in the cold spots spanning 670 kb (Cluster I) and 2 Mb (Cluster II) regions, even though there are three and four polymorphic markers present in these regions, respectively. Suppression of recombination is generally caused by proximity to the centromere[41] and lack of sequence homology between the parental genotypes[42]. The former phenomenon has been observed in *Neurospora crassa*[41] and yeast[43], and may apply to the cold spot, cluster II, identified in the present research that was also assigned as the centromere of chromosome 1 in the previous study[9]. The cold spot in Cluster I may be due to a lack of homologous sequence between the two parental isolates. We have revealed that there is high genetic diversity and specificity between two populations from Jiangsu and Yunnan provinces, China, through DNA fingerprinting and pathotype analyses[44]. Different ecological and genetic selection pressure on the two populations may result in divergent genomic structures of the parental isolates. Fortunately, the *AvrPi7* locus sits in the recombination-active region. It is reasonable to consider that a high frequency of recombination may partly be the cause for emergence of new *Avr* genes corresponding to new *R* genes introduced into the host[45].

The genome of *M. oryzae* is haploid, consisting of seven chromosomes[2,46]. Therefore, it would seem to be impossible that a heterozygous genotype formed in the *M. oryzae* genome. However, 2 out of 189 ascospore progeny in the mapping population, M063 and M087, formed heterozygous genotypes at the MS1-22 and

MS1-25 loci (Table 2; Figure 3). At first glance, these heterozygous genotypes may be caused by contamination with those derived from the different progeny with complementary genotypes. But this possibility could be excluded with the mapping of another *Avr* gene, *AvrPi15*, using the same mapping population in our laboratory[26]. That is, there is no contamination found in all 189 ascospore isolates. The most plausible explanation is that the genomes of both progenies are merodiploid caused by the presence of supernumerary chromosome.

Merodiploids, also called partial diploids, are usually observed in bacterial cells containing an F′ plasmid. In this type of cell, the genome carries two copies of a particular region, thus genes/markers located in that region will appear to have two alleles[47]. Similar to the F′ plasmid, supernumerary chromosomes are small, extra chromosomes composed primarily of DNA that is not present in all isolates of a species in fungi. Some research has shown that supernumerary chromosomes are found in the majority of *M. oryzae* isolates originating from *Oryza sativa* and *Seteria italica*[48−51]. Recently, Luo et al.[3] reported that a 1.6-Mb extra chromosome was present in the parental isolate 84R-62B compared with the matching parental isolate Y93-245c-2. Furthermore, they demonstrated that this extra chromosome is actually a fragment of chromosome 1 that arose when the large chromosome was broken. In the present study, both progeny isolates M063 and M087 may have an extra chromosome derived from a fragment of chromosome 1 due to reciprocal recombination between homologues of unequal length of two parental isolates. Supernumerary chromosomes may also arise from the rearrangement of chromosomes[52]. Heterozygous genotypes may form in the progeny isolates with merodiploid of genomes in which both an extra chromosome and its complete chromosome have matched sequence for the marker loci. We are now testing the hypotheses described above[3,51].

The results from this study further demonstrate that use of the genomic sequence of *M. oryzae* greatly accelerates the fine mapping of genes of interest. The high-resolution genetic and physical map provides valuable insight into the genomic basis for pathogenic variability in *M. grisea*. These maps serve as a solid foundation for genetic dissection of the target gene. The cloning and genetic transformation of the *Avr* gene is under way in our laboratory.

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