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ORIGINAL PAPER

Identification and fine mapping of *AvrPi15*, a novel avirulence gene of *Magnaporthe grisea*

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Abstract Avirulence of *Magnaporthe grisea* isolate CHL346 on rice cultivar GA25 was studied with 242 ascospore progenies derived from the cross CHL346 × CHL42. Segregation analysis of the avirulence in the progeny population was in agreement with the existence of a single avirulence (*Avr*) gene, designated as *AvrPi15*. For mapping the *Avr* gene, we developed a total of 121 microsatellite DNA markers [simple sequence repeat (SSR)], which evenly distributed in the whole-genome of *M. grisea* through bioinformatics analysis (BIA) using the publicly available sequence. Linkage analysis of the *AvrPi15* gene with these SSR markers showed that six markers on chromosome 6, MS6-1, MS6-2, MS6-3, MS6-7, MS6-8 and MS6-10, were linked to the *AvrPi15* locus. To further define the chromosomal location of the *AvrPi15* locus, two additional markers, MS6-17 and STS6-6, which were developed based on the sequences of telomeric region 11 (TEL11), were subjected to linkage analysis. The results showed that MS6-17 and STS6-6 were associated with the locus by 3.3 and 0.8 cM, respectively. To finely map the *Avr* gene, two additional candidate avirulence gene (CAG) markers, CAG6-1 and CAG6-2, were developed based on the gene annotation of the sequence of TEL 11. Linkage analysis of the *Avr* gene with these two markers revealed that both of them completely cosegregated with the *AvrPi15* locus. Finally, this locus was physically mapped into ~ 7.2-kb interval of the TEL11 by BIA using these sequence-ready markers. This is the key step toward positional cloning of the *AvrPi15* gene.

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Introduction

The filamentous ascomycete fungus *Magnaporthe grisea* causes rice blast (Rossman et al. 1990), which is one of the most devastating fungal diseases of rice worldwide (Ou 1985). Using resistant cultivars is an effective approach to eliminate the use of pesticides and minimize crop losses due to this disease. However, the blast resistant cultivars of rice often succumb to the disease after 2–3 years of cultivation, due either to the emergence of new pathogen races, or selection of a rare component of the pathogen population that is already virulent (Bonman 1992; Chauhan et al. 2002; Kiyosawa 1989; Ou 1980). Thus, the utility of resistance genes (*R* genes) in controlling rice blast diseases has been limited by variability of the pathogen. Nevertheless, little is known about the mechanism of interactions of the rice cultivars with the fungus races.

M. grisea is a model organism of filamentous fungi for studying various aspects of host–pathogen interaction (Martin et al. 2002; Valent 1990). Unfortunately, genetic analysis of the blast pathogen had been hindered by the absence of the perfect stage for making crosses among the isolates interested. Hebert (1971) first produced the perfect states of *M. grisea*, in vitro, and demonstrated that the fungus is heterothallic. Later

on, many attempts were made to identify fertile rice isolates mainly in Japan, America, France, and China (Notteghem et al. 1994). However, only a few avirulence genes (*Avr* gene) have been identified until 1990s, because of the low fertility of isolates pathogenic to rice and because these isolates often behave only as males in a cross (Itoi et al. 1983; Kolmer and Ellingboe 1988; Leung and Williams 1985; Notteghem et al. 1994).

Identification of Guy11, a hermaphroditic isolate pathogenic to rice, has made it possible to make crosses with isolates that are pathogenic to rice (Leung et al. 1988; Notteghem et al. 1994). Moreover, several hermaphroditic isolates with high fertility were later identified in China, especially in Yunnan province, where is recognized as a center for genetic diversity in Asian cultivated rice (Hayashi et al. 1997). By using these excellent isolates, more than 40 *Avr* genes have been identified at the key laboratories in China, America, Japan, and France (Notteghem et al. 1994; Yasuda et al. 2004; Hirayae et al. 1999; Li et al. 2000; Liu et al. 2001; Wang et al. 2002; Lin et al. 2002; Luo et al. 2004; Pan QH, unpublished data). Among them, five *Avr* genes *PWL1*, *PWL2*, *Avr1-CO39*, *Avr-Pita* and *ACE1* have been cloned by positional cloning approach (Böhnert et al. 2004; Farman and Leong 1998; Kang et al. 1995; Orbach et al. 2000; Sweigard et al. 1995).

Map-based cloning is an effective method to isolate the target genes, especially when no expression products and functional information of target genes can be available (Peter et al. 2003). As for fungal pathogens, the diversity and lack of sequence similarity or conservation in the fungal *Avr* gene products were demonstrated by comparison of *Avr* genes identified in *M. grisea*, *Cladosporium fulvum* and *Rhynchosporium* (Dean et al. 2005). However, this way was tediously hampered by the quick identification of anchor markers for chromosome walking to the target locus. Fortunately, the emergence of genomic sequences of the fungus pathogen (http://www.fungalgenomics.ncsu.edu; http://www.genome.wi.mit.edu) offers powerful tools to increase the density of markers in the genetic map and to assemble the contiguous clones (contig) in the physical map, where a target gene was located (Chen et al. 2005, 2006; Dean et al. 2005; Liu et al. 2005; McCouch et al. 2002; Schular 1998). This has made map-based cloning performed in *M. grisea* much more efficiently. Doubtlessly, construction of sequence-based map of the target gene is a crucial step for map-based cloning.

It is worth noting that many *Avr* genes in *M. grisea* having key roles in pathogen–host interactions were located in the telomeric regions (Orbach et al. 2000; Valent and Chumley 1994). This suggested that the location of these genes in highly dynamic telomere region might confer an adaptive advantage so that pathogen could evade the host recognition (Farman and Kim 2005; Gao et al. 2002; Orbach et al. 2000; Valent and Chumley 1994). However, if the target gene was located on the telomeric region, it is difficult to identify co-segregated markers. There was additional difficulty to finely map the target gene at the telomeric region, if sequence of the telomere is not available. Fortunately, 14 telomeres of *M. grisea* have recently been achieved and then integrated into the scaffold by Farman and Kim (2005, http://www.genome.kbrin.uky.edu/fungi_tel/index.html). The telomere commonly contains tandem repeats of five to eight nucleotides that are highly conserved in eukaryotes (Zakian 1989), thereby, many telomeric fragments had been achieved on the basis of the conserved repeat motif (TTAGGG)*n* (Dean et al. 2005; Orbach et al. 2000; Yang et al. 2005).

In the present study, we identified a novel *Avr* gene *AvrPi15* in a progeny population derived from a cross between two rice field isolates CHL346 and CHL42, which were collected from Jiangsu and Yunnan provinces, China, respectively; and genetically mapped it at the telomeric region of chromosome 6, TEL11, using PCR-based markers including simple sequence repeat (SSR), sequence-tagged site (STS) and candidate avirulence gene (CAG) markers; and then physically mapped it to ≈ 7.2-kb interval through bioinformatics analysis (BIA) using these sequence-ready markers.

Materials and methods

Fungal isolates and mapping population construction

The above-mentioned parental isolates CHL346 (*MAT1-1*) and CHL42 (*MAT1-2*) were made a cross after determining their mating types as well as fertilities with standard isolates on oatmeal agar medium (0.5% sucrose, 3% ground oatmeal and 1.5% agar) in a 9 cm glass petri plate. They were incubated for 7 days at 25°C and thereafter at 20°C under continuous illumination with fluorescent light. Single ascospores were isolated at random with glass pin under microscope and then stocked on filter paper. A total of 242 progeny isolates were used for establishing a mapping population for the target gene.

Plant materials

The host rice cultivar GA25 carrying *R* gene *Pi15*, and a susceptible check cultivar Sariceltik, as well as other 66 cultivars with the respective resistance genotypes

(data will be presented elsewhere), were used in this study. Seeds of these cultivars were sown in a plastic pot (57×30 cm^2) as described before (Pan et al. 2003). Five plants per cultivar were planted in a row. In all experiments, nitrogen was applied three times to keep all the plants healthy and dark green. Seedlings were grown in a greenhouse at 15–30°C under natural light for about three weeks before inoculation.

Inoculation and infection type investigation

Inoculation was carried out according to Pan et al. (2003). After inoculation, the seedlings were kept in the inoculation incubator at 24–28°C with saturated humidity for 18–24 h, and then transferred to a moist vinyl tunnel at 20–30°C to facilitate producing lesions. Ratings for infection types were recorded ≈ 6 days after inoculation by use of a 6-class scale (Pan et al. 1996). According to this scale, an isolate was regarded as virulent when it caused lesions on the host cultivar with scores 3–5, i.e., the sporulating lesions, whereas 0–2 scores correspond to avirulent isolates. The experiments were conducted at least twice.

Mycelia culture and DNA extraction

Five agar scraps colonized by the isolates of *M. grisea* were inoculated in 250 ml of liquid complete medium (glucose 1%, yeast extract 0.3%) in 500 ml Erlenmeyer flasks. The flasks were incubated at 25°C with shaking at 170 rpm in a shaker (HAQ-F160, Donglian Electronic Technology Development Co., Ltd., Harbin, China) for 3–4 days. Before producing dark pigments in the flasks, mycelia were harvested by filtration through filter paper and gauze meshes with a vacuum pump (Welch Vacuum, THOMAS®, IL, USA). After several hours of pre-freeze at − 80°C in a deep freezer, they were frozen and dried in lyophile apparatus (Free zone 64, Labconco, USA). About 100 mg lyophilized mycelia were quickly frozen in liquid nitrogen, and grinded into a fine powder. Total DNA was extracted by fungal DNA kit (E.Z.N.A.® Fungal DNA Kit, Omega, USA) following the manufacturer's instructions. The DNA isolated was dissolved in $1 \times$ TE buffer (10 mM Tris, pH 7.5, and 0.5 mM EDTA) or double distilled water, and then electrophoresed on 0.8% agarose gel to detect its quality and concentration. The rest was kept at − 20°C in refrigerator until use.

Markers development and analysis

Only PCR-based markers such as SSR, STS and CAG were used in the current study. Firstly, sequence-ready markers (or locus-specific markers), SSRs were developed for mapping the *Avr* gene targeted based on the whole-genome sequence of *M. grisea* released by the International Rice Blast Genome Project (IRBGP, http://www.riceblast.org; Dean et al. 2005) through BIA using the appropriate software tools. That is, primer pair for SSR marker was designed to the flanking region of the SSR using software tools SSRIT (http://www.gramene.org/microsat/ssrtool) and Primer Premier 5.0 (http://www.primerbiosoft.com) with parameters essentially as described by Chen et al. (2002). A total of 121 SSR markers were developed from the whole genome of *M. grisea* at intervals as even as possible (data not shown). For further narrowing down the chromosomal location of the target gene, additional SSR and STS markers were developed in the region defined by the first round survey with the 121 SSR markers. The last round survey was carried out with the CAG markers, which were developed based on the sequence of the candidate genes predicted using the gene annotation system, Softberry FGENESH (http://www.softberry.com). All primers designed were synthesized by SBS Genetech Co., Ltd. (Beijing, China). The primer sequences, marker positions, PCR conditions and detection procedures were shown in Table 1.

Genetic mapping of the *Avr* gene

According to pathogenicity test, two contrasting bulks were prepared, each containing DNA from six avirulent or virulent isolates toward the rice cultivar GA25. The concentration of each DNA examples was adjusted to be equal before pooling. Mapping of the target gene to a particular *M. grisea* chromosome was achieved by three steps (Chen et al. 2005, 2006; Liu et al. 2005). First, bulked-segregant analysis (BSA, Michelmore et al. 1991) was performed to screen polymorphic markers for the target gene. Then, these markers were determined as candidate markers for the target gene by testing individual isolates consisted of both pools. Third, candidate markers were confirmed as linkage markers for the target gene by testing all the isolates other than those included in both pools in the mapping population.

Linkage relationship was determined by testing segregation mode generated by gene pairs combined, using a χ^2 test (Table 2). When the χ^2 for linkage was significant at less than 5% level, recombination frequency of gene pair was calculated by the maximum likelihood method (Allard 1956), and converted to map distance in centimorgans (cM) by Kosambi's function (Kosambi 1944).

Table 1 Primer sequences, PCR conditions, and detection procedures of microsatellite (SSR), sequence-tagged site (STS) and candidate avirulence gene (CAG) markers developed in this study

Marker	Forward primer	Reverse primer	Marker types	Repeat[a]	Location[b]	PCR[c]	Gel[d]	Expected size (bp)
MS6-1	gttgcatgcggtgatcgtcc	gcaatagttgagtgtgacggc	SSR	$(CA/GT)_{14}$	194	A	I	284
MS6-2	cgtggtagatggcagtcgttg	ctcattacagccctgcacgc	SSR	$(TGC/ACG)_{11}$	194	A	I	234
MS6-3	ccacatcgcagcctcacttg	ccagtcggttccaggtcttg	SSR	$(GCT/CGA)_{10}$	194	A	I	197
MS6-7	ccaaggcaccagcacagacc	cagaagatgcctcggagatc	SSR	$(AC/TG)_{24}$	194	A	I	307
MS6-8	ggagcttgatgcatgcaggtg	ccctataacgcccaggtgag	SSR	$(AT/TA)_{21}$	194	A	I	254
MS6-10	caacatgtggtgtctgatcc	gcagggcccactacacatgt	SSR	$(ATG/TAC)_{11}$	194	A	I	344
MS6-17	caagaatgcagagaaccacag	gttttccgctgtgtaaataga	SSR	$(TAG/ATC)_{8}$	TEL11	A	I	159
STS6-6	ggacatttcaggcaggacc	gcgtcgctaaatcaaaatccg	STS	–	TEL11	A	I	482
CAG6-1	ccctaacgaatacaacgcact	ttccgaacggcgacatacg	CAG	–	TEL11	C	II	1,951
CAG6-2	Gccactcgtacctactaccatt	cggacggtgggtagaggtggc	CAG	–	TEL11	C	II	1,937

[a] The microsatellite motif and its complement is followed by the number of times the motif is repeated in the original contig

[b] The supercontig or telomere numbers (http://www.riceblast.org)

[c] 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, 35 PCR cycles (30 s at 94°C, 30 s at 62°C, 2 min at 72°C), followed by 7 min at 72°C; C: after preheating 4 min at 94°C, 35 PCR cycles (30 s at 94°C, 3 min at 68°C), followed by 10 min at 72°C

[d] I = 6% acrylamide and II = 1% agarose

Physical mapping of the *Avr* gene, *in silico*

Since the genetic map of the *Avr* gene locus was established by sequence-ready markers, i.e., SSR, STS and CAG markers, the *Avr* gene-linked markers were landed on the respective bacterial artificial chromosome (BAC) clones of the reference isolate 70-15 released by IRBGP through BIA using the software tool, 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 accessible reference sequence (Chen et al. 2005, 2006; Liu et al. 2005).

Results

Segregation analysis of the *Avr* gene

Two hundred and forty two progeny isolates derived from a cross of an avirulent isolate CHL346 with a virulent isolate CHL42, were tested their pathogenicities on the rice cultivar GA25. The results showed that each progeny isolate performed distinguishable reactions on the host plants in all the experiments. Segregation ratio of 1:1 (avirulence/virulence) was observed in the mapping population (121 avr:121 vir, $\chi^2 = 0.04$, $P > 0.8$), suggesting that avirulence on GA25 in the isolate CHL346 was controlled by a single gene. This result further suggested that the mapping population used was consisted of random ascospore isolates (Table 2). This *Avr* gene corresponding to the *R* gene *Pi15* was, therefore, designated as *AvrPi15*.

Genetic mapping of the *Avr* gene

To define chromosomal location of the *AvrPi15*, a total of 121 SSR markers covering the majority of the genome of *M. grisea*, were screened by BSA. The results showed that six markers, MS6-1, MS6-2, MS6-3, MS6-7, MS6-8 and MS6-10, which located on chromosome 6, were polymorphic for the two bulks. To confirm the polymorphic markers, the isolates consisted of the both pools were tested, individually. The results showed that these six markers were linked to the *Avr* gene. For linkage analysis of the *Avr* gene with these six markers, the rest isolates in the mapping population, i.e., 230 isolates, were tested. The above-mentioned markers showed linkage with the *Avr* gene locus with genetic distances of 11.4, 11.4, 11.4, 17.1, 17.1 and 19.1 cM, respectively (Table 2).

Since the closest SSR marker MS6-1was localized at the distal of supercontig 194 (formerly contig 201) on the TEL 11 side, four additional markers were developed based on the sequence of TEL 11 that was recently released by the Farman group (Farman and Kim 2005), for further narrowing down the genetic region of the *Avr* gene locus (Table 1 and Fig. 1a, b). The results showed that the polymorphic markers SSR6-17 and STS6-6 linked to the *Avr* gene with genetic distance of 3.3 and 0.8 cM, respectively (Table 2 and Fig. 1a). To finely map this locus, two CAG markers, CAG6-1 and CAG6-2, were developed

Table 2 Linkage analysis of the avirulence gene *AvrPi15* of *Magnaporthe grisea* in a mapping population derived from a cross between the avirulent isolate CHL346 and the virulent isolate CHL42 using microsatellite (SSR), sequence-tagged site (STS) and candidate avirulence gene (CAG) markers

Gene pair	Segregation mode[a]				χ^2 for linkage	$r \pm$ SE (%)[b]	Map distance (cM)
	AM	Am	VM	Vm			
AvrPi15–CAG6-1	121	0	121	0	242.0***	0.0	0.0
AvrPi15–CAG6-2	121	0	121	0	242.0***	0.0	0.0
AvrPi15–STS6-6	119	2	121	0	234.1***	0.8 ± 0.6	0.8 ± 0.6
AvrPi15–MS6-17	119	2	115	6	211.1***	3.3 ± 1.1	3.3 ± 1.1
AvrPi15–MS6-1	110	11	16	105	146.1***	11.2 ± 2.0	11.4 ± 2.1
AvrPi15–MS6-2	110	11	16	105	146.1***	11.2 ± 2.0	11.4 ± 2.1
AvrPi15–MS6-3	110	11	16	105	146.1***	11.2 ± 2.0	11.4 ± 2.1
AvrPi15–MS6-7	103	18	22	99	108.5***	16.5 ± 2.4	17.1 ± 2.7
AvrPi15–MS6-8	103	18	22	99	108.5***	16.5 ± 2.4	17.1 ± 2.7
AvrPi15–MS6-10	100	21	23	98	98.1***	18.2 ± 2.5	19.1 ± 2.9
	M_1M_2	M_1m_2	m_1M_2	m_1m_2			
CAG6-1–CAG6-2	121	0	0	121	242.0***	0.0	0.0
CAG6-1–STS6-6	119	2	0	121	234.1***	0.8 ± 0.6	0.8 ± 0.6
CAG6-1–MS6-17	119	2	6	115	211.1***	3.3 ± 1.1	3.3 ± 1.1
CAG6-1–STS6-3	110	11	16	105	146.1***	11.2 ± 2.0	11.4 ± 2.1
CAG6-1–MS6-7	103	18	22	99	108.5***	16.5 ± 2.4	17.1 ± 2.7
CAG6-1–MS6-10	100	21	23	98	98.1***	18.2 ± 2.5	19.1 ± 2.9
STS6-6–MS6-17	121	0	6	115	218.7***	2.5 ± 1.0	2.5 ± 1.0
STS6-6–MS6-7	105	16	22	99	114.3***	11.6 ± 2.1	11.8 ± 2.2
STS6-6–MS6-10	102	19	23	98	103.2***	17.4 ± 2.4	17.8 ± 2.7
MS6-17–STS6-3	112	9	10	111	172.0***	7.9 ± 1.7	8.0 ± 1.7
MS6-17–MS6-7	105	16	16	105	130.9***	13.2 ± 2.2	13.5 ± 2.4
MS6-17–MS6-10	102	19	17	104	119.5***	14.9 ± 2.3	15.4 ± 2.5
MS6-3–MS6-1	126	0	0	116	242.2***	0.0	0.0
MS6-3–MS6-2	126	0	0	116	242.2***	0.0	0.0
MS6-3–MS6-7	110	7	6	113	192.9***	5.4 ± 1.5	5.4 ± 1.5
MS6-3–MS6-8	110	7	6	113	192.9***	5.4 ± 1.5	5.4 ± 1.5
MS6-3–MS6-10	113	10	8	111	175.4***	7.4 ± 1.7	7.5 ± 1.7
MS6-7–MS6-8	125	0	0	117	242.2***	0.0	0.0
MS6-7–MS6-10	119	3	2	118	222.4***	2.1 ± 0.9	2.1 ± 0.9
MS6-8–MS6-10	119	3	2	118	222.4***	2.1 ± 0.9	2.1 ± 0.9

***Indicates significance at $P = 0.001$ level for a χ^2

[a] A/V and M_1/m_1 or M_2/m_2 indicate alleles of avirulence, and first and second marker loci, respectively

[b] Recombination frequency (percent) ± standard error

downstream of STS6-6. The results showed that both markers co-segregated with the *AvrPi15* locus (Table 2 and Fig. 1a).

Physical mapping of the *Avr* gene, *in silico*

To physically map the *Avr* gene locus, all the *AvrPi15*-linked markers were anchored on the reference sequence by BIA using the software tool, BLASTN. The contigs anchored were downloaded from the IRBGP and the Comparative Genomics of Telomeres in Pathogenic and Saprophytic Fungi Web sites above-mentioned, and then aligned as a contig map spanning the *AvrPi15* locus, *in silico*, by BIA (Chen et al. 2005; Liu et al. 2005). Based on the reference sequence, this locus was defined in a ~ 7.2-kb interval in touch with the TEL 11 repeat motif (Fig. 1b, c). Only two candidate genes for the *AvrPi15* were predicted in this region by the gene annotation system, Softberry FGENESH, and one is highly homologous to the telomere-linked helicase (*TLH*), and another is an unknown gene.

Discussion

Recently, we have developed a powerful strategy for genetic and physical mapping of the functional genes of rice using the publicly available genetic resources as well as various software tools for BIA (Chen et al. 2005, 2006; Liu et al. 2005). In the present study, we have adopted this strategy to finely define genetic and

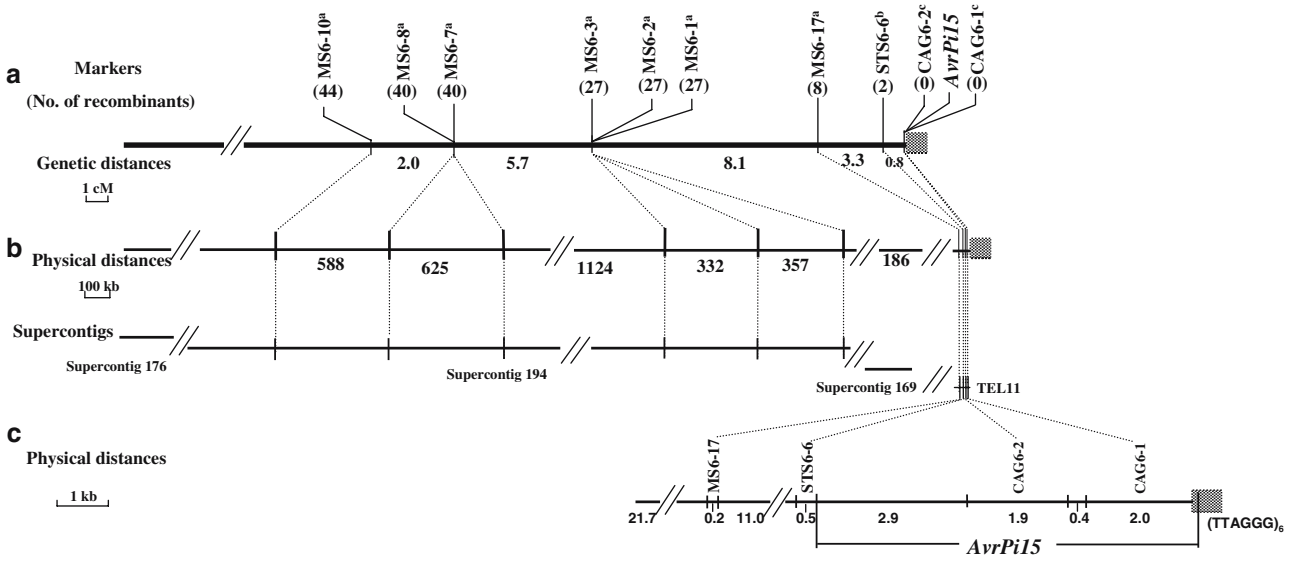

Fig 1 **a** Genetic map of the *AvrPi15* gene of *Magnaporthe grisea* on chromosome 6. Linkage analysis was conducted to determine the map position of the *AvrPi15* locus using 242 ascospore isolates. *Value below the horizontal line* indicates genetic distance in centimorgans (cM). The markers, which linked to the locus, are listed above the map, and the *numbers in parentheses* are recombinants occurred at the corresponding marker loci. The telomere repeat motif of chromosome 6 of *M. grisea* represented by the shaded box. [a]The simple sequence repeat (microsatellite, SSR) markers; [b]The sequence-tagged site (STS) marker; [c]The candidate avirulence gene (CAG) marker. **b** A physical map of the *AvrPi15* locus. The *long horizontal line* indicates the genomic region containing the locus. The *numbers below* the map are relative physical distances in kilobase (kb) estimated based on the reference sequence released by International Rice Blast Genome Project (IRBGP, http://www.riceblast.org). The *vertical lines* denote the positions of the respective markers. The *dashed lines* designate the relative positions of the corresponding markers. **c** A physical map of the telomere 11 (TEL11) of chromosome 6. The *numbers below* the map are relative physical distances in kb estimated based on the reference sequence of TEL 11 released by the Comparative Genomics of Telomeres in Pathogenic and Saprophytic Fungi (http://www.genome.kbrin.uky.edu/fungi_tel/index.html)

physical positions of the novel *Avr* gene *AvrPi15* in the corresponding pathogen *M. grisea*. First, the entire genome sequence-based SSR markers were developed by BIA for the first round linkage analysis. Secondly, additional PCR-based markers were developed by BIA for further narrow down the target region. Thirdly, CAG markers were developed by BIA for identifying candidate genes for the target gene. Finally, physical map of the target gene was constructed by BIA with the reference sequences, those anchored by the linkage markers. The results from this study indicated that the availability and utilization of the sequence information for the *M. grisea* whole-genome have largely facilitated the generation of a high-resolution map and, ultimately, the isolation of the target gene. This may be the first report that a novel *Avr* gene was identified at the new telomere of *M. grisea* by such strategy mentioned above.

The rice blast pathogen, *M. grisea*, is notorious for its variability in pathogenicity (Kiyosawa 1982; Ou 1980). It is mainly due to asexual recombination and/or spontaneous mutation (Kiyosawa 1982; Valent and Chumley 1991). Recent progresses in genetic mapping and molecular characterization of *Avr* genes indicate that the chromosomal position or organization is relative to their variability (Dioh et al. 2000; Gao et al. 2002; Luo et al. 2005). Interestingly, five known *Avr* genes of *M. grisea*, *Avr-Pita*, *Avr1-TSUY*, *Avr1-Ku86*, *Avr1-MedNoï*, and *PWL1* were also mapped at the respective telomeres (Dioh et al. 2000; Gao et al. 2002; Kang et al. 1995; Sweigard et al. 1993; Valent and Chumley 1991). The presence of these genes in highly dynamic chromosome ends may provide a selective advantage to *M. grisea* by allowing them to rapidly adapt to new *R* genes in host plants (Gao et al. 2002; Orbach et al. 2000; Valent and Chumley 1994). It is intriguing to consider that the instability of *Avr* genes may be an effect of its telomeric location (Farman and Leong 1995; Orbach et al. 2000), even though some alternative explanations for the high variability of *Avr* genes in *M. grisea* cannot be ruled out. The full influence of telomeric location of *Avr* genes on the dynamics of rice blast disease in the field remains to be in question. Thus, molecular identification and cloning of the *Avr* genes will provide the basis for the detailed understanding of their variability.

Another interesting phenomenon, uneven physical/genetic (*P*/*G*) ratios, was observed on the majority of

the chromosome 6 of *M. grisea* (Fig. 1). In the Supercontig 194, there are only 17 recombination events occurred in ~ 3,026 kb interval, giving a *P*/*G* ratio of 393 (3,026/7.7) kb/cM. This is much higher than the average *P*/*G* ratio of ~ 33.5 kb/cM estimated for the whole genome of *M. grisea* (Hamer et al. 1989). In contrast, *P*/*G* ratios estimated for Supercontig 169 and TEL 11 were ~ 23.0 (186/8.1) kb/cM and ~ 4.7 (18.5/4.1) kb/cM, respectively (Fig. 1). The regional difference in this ratio varied dramatically on the chromosome 6 for more than 80-fold. As to the recombination-suppressed region, it could be due to the proximity of this region to the centromere, called "centromere effect" (Chauhan et al. 2002; Farman and Leong 1998). The region flaking markers MS6-8 and MS6-7 is most likely a centromere region of the chromosome 6, because no recombinant was detected in this region spanning ~ 625 kb in length, and the intervals around this region, i.e., the regions between markers MS6-10 and MS6-8, and MS6-7 and MS6-3, were also recombination suppressed (Fig. 1b). Another likely interpretation is that the two parents have chromosome inversions or rearrangements relative to each other, which could result in lack of sequence homology between the parental genotypes (Chauhan et al. 2002; Jeon et al. 2003). It is most likely the cause of the recombination-suppressed region flanked by markers MS6-3 and MS6-1, because its adjacent regions on the centromere side and that on the telomere side were recognized as recombination-suppressed and recombination-active regions, respectively. As to the recombination-active region, many researches showed that subtelomeric regions have been regarded as gene-rich regions (reviewed by Barry et al. 2003; Bishop et al. 2000; Bringaud et al. 2002; Riethman et al. 2001; Scherf et al. 2001). It is reasonable to consider that a high frequency of recombination may be another important factor for generation of new *Avr* genes confronting with new *R* genes introduced in the new host cultivars.

Evidence is accumulating that the rice blast is a typical gene-for-gene pathosystem (Flor 1971; Jia et al. 2000; Silué et al. 1992). However, only one matched *Avr/R* gene pair, *Avr-Pita*/*Pita*, has been analyzed in the system, so far (Jia et al. 2000). As to this gene pair, *Avr-Pita* functions as an elicitor molecule, which physically interacts with the product of the *Pita*, and then triggers a signal transduction cascade leading to resistance (Jia et al. 2000). To deeply and exactly understand this system, more cases (gene pairs) should be studied. Since the corresponding *R* gene *Pi15* is being isolated in our laboratory (Pan et al. 2003, and unpublished data), it is necessary to move forward our program in cloning the *AvrPi15* gene based on this study.

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