Identification of an Avirulence Gene in the Fungus *Magnaporthe grisea* Corresponding to a Resistance Gene at the *Pik* Locus

N. Yasuda, M. T. Noguchi, and Y. Fujita

First author: National Agricultural Research Center, 3-1-1 Kannondai, Tsukuba 305-8666, Japan; second author: National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai, Tsukuba 305-8604, Japan; and third author: Akita Agricultural Experiment Station, Aikawa, Yuwa, Akita 010-1231, Japan.

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ABSTRACT

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A rice isolate of *Magnaporthe grisea* collected from China was avirulent on rice cvs. Hattan 3 and 13 other Japanese rice cultivars. The rice cv. Hattan 3 is susceptible to almost all Japanese blast fungus isolates from rice. The genetic basis of avirulence in the Chinese isolate on Japanese rice cultivars was studied using a cross between the Chinese isolate and a laboratory isolate. The segregation of avirulence or virulence was studied in 185 progeny from the cross, and monogenic control was demonstrated

for avirulence to the 14 rice cultivars. The resistance gene that corresponds to the avirulence gene (*Avr-Hattan 3*) is thought to be located at the *Pik* locus. Resistance and susceptibility in response to the Chinese isolate in F_3 lines of a cross of resistant and susceptible rice cultivars were very similar to the *Pik* tester isolate, Ken54-20. Random amplified polymorphic DNA markers and restriction fragment length polymorphism markers from genetic maps of the fungus were used to construct a partial genetic map of *Avr-Hattan 3*. We obtained several flanking markers and one co-segregated marker of *Avr-Hattan 3* in the 144 mapping population.

Additional keywords: rice blast.

Blast caused by *Magnaporthe grisea* (Hebert) Barr (27) is one of the most economically devastating diseases of rice worldwide. The extensive use of blast-resistant rice cultivars has led to the development of new blast fungus races virulent to the resistant cultivars and subsequent breakdown of their resistance to the disease.

Race-cultivar specificity in the rice blast pathosystem complies with the gene-for-gene interaction (6,29), in which the pathogen carries a gene for avirulence that corresponds to each resistance gene in the host. Knowledge about the molecular evolution of these individual avirulence genes in the rice blast fungus may provide further insights into how the blast fungus overcomes resistance in rice cultivars.

To date, more than 40 major rice blast resistance gene loci, denoted *Pi* genes, are known (28), and an increasing number of corresponding avirulence genes in the rice blast fungus have been analyzed (25). Among these, at least four avirulence genes, *PWL2*, *AVR1-CO39*, *AVR-Pita*, and *ACE1*, have been isolated so far (1,5,26,30). *AVR-Pita* encodes a putative zinc-dependent metalloprotease (26). *ACE1* encodes a putative hybrid between a polyketide synthase and a nonribosomal peptide synthetase (1). Direct interaction between *AVR-Pita* product and the corresponding resistance gene, *Pita*, product was demonstrated by yeast two-hybrid and in vitro experiments (10). With respect to the mechanism of the generation of new races, deletion, insertion, or point mutations have been found at the avirulence locus in virulent isolates (4,11,12,21,26). However, little is known about the selection pressures on these avirulence genes that have led to new blast fungus races and subsequent epidemics. An important question is, what conditions enhance the selection pressure on the avirulence gene?

One of our laboratory blast fungus isolates, Y90-71, isolated from rice in Yunnan Province in China, is avirulent on Japanese cultivars such as Hattan 3, Kadunowase, Zenkoujimochi, and Nakateshinriki. These four cultivars are known to be susceptible to almost all Japanese blast fungus isolates (15). Therefore, this Chinese isolate may possess an avirulence gene that is not found in Japanese isolates. Why the Japanese isolates do not have a functional avirulence gene against these cultivars, while the Chinese isolate does, is an intriguing question.

In this study, we crossed the Chinese isolate with a laboratory isolate. We then tested the pathogenicity of subsequent individual progeny on Japanese rice cultivars and found phenotypes consistent with a single dominant avirulence gene in this Chinese isolate, here named *Avr-Hattan 3*. We also genetically analyzed the resistance and susceptibility in the F_3 progeny lines from a cross between resistant and susceptible Japanese rice cultivars to help determine which resistance gene contributes to the resistance against this Chinese isolate. *Avr-Hattan 3* was shown to correspond to a resistance gene at the *Pik* locus, which is known to have several blast resistance genes (2). We then constructed a partial linkage map for *Avr-Hattan 3*.

MATERIALS AND METHODS

***M. grisea* isolates.** *M. grisea* isolates Y90-71 (*Mat 1-1*, race 102) and 3514-R-2 (*Mat 1-2*, race 136) are hermaphroditic, female fertile, and pathogenic to rice. Y90-71 is a field isolate collected from Yunnan Province in China in 1990, and 3514-R-2 is a laboratory isolate kindly provided by N. Hayashi. These two isolates were used as the parental isolates for a cross to genetically analyze avirulence in *M. grisea*. Isolate Ken54-20 was used to confirm resistance genes in rice cultivars because Kiyosawa (34) originally used these isolates to identify the resistance gene *Pik*.

Crosses of blast fungus isolates. *M. grisea* isolates were crossed according to the procedure described by Yaegashi and

Corresponding author: N. Yasuda; E-mail address: yasuda@affrc.go.jp

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Nishihara (33). Each parental isolate was placed on oatmeal agar (1 liter of distilled water, 15 g of agar, 20 g of sucrose, and 50 g of ground oatmeal) a few centimeters apart in a 9-cm petri dish and then grown under continuous illumination with fluorescent light (18 W) at 22°C. Perithecia formed at the intersection of mycelial growth of the two isolates and matured within 20 to 30 days after inoculation. Ascospores were released from mature perithecia in distilled water and then placed on 2% water agar. After 5 h of incubation at 26°C, germinated ascospores were transferred individually to potato sucrose agar (1 liter of boiled extract from 250 g of potato tubers, 20 g of sucrose, and 15 g of agar) and incubated at 26°C for 5 days. The progeny isolates were placed onto oatmeal agar plates with small pieces of no. 2 filter paper (ADVANTEC, Tokyo) scattered on the surface and incubated at 26°C for 10 days. The filter papers with mycelia were picked from the plates and dried for storage at -80°C.

Plant materials. For genetic analysis of avirulence in *M. grisea*, six seed of each of 15 cultivars (Norin 3, Hattan 3, Kadunowase, Zenkougimochi, Nakateshinriki, Yamato 3, Yamatowase, Ishikarishiroke, Hokuriku 12, Suehiromochi, Fujisaka 5, Gohyakumangoku, Kanto 51, Mangetsumochi, and K3) were sown in plastic trays (15 by 5 by 10 cm; five rows with six seeds per row).

To identify resistance genes in the rice cultivars, Kanto 51 was crossed with rice cv. Norin 3, which had no resistance gene to any blast fungus isolates used in this study. F₁ seed from the cross were grown in the greenhouse to obtain F₂ populations. F₂ seed were planted in the paddy field. Each F₂ individual was self-pollinated to generate F₃ line seed, and ≈30 seed of each F₃ line were sown in a plastic tray. Plants were grown in the greenhouse at a temperature of 20 to 30°C.

Inoculation and disease rating. For inoculum, *M. grisea* isolates were grown on oatmeal agar at 26°C for 11 or 12 days, and then aerial mycelia of the agar culture were gently washed off with a water-soaked paintbrush. They were placed at 21°C for 3 to 4 days under continuous illumination with fluorescent light (18 W) to induce sporulation. To make a conidial suspension, the mycelia were scraped and flooded with water containing 0.01% Tween 20. The conidial suspension was filtered through a gauze mesh and adjusted to 1 to 5 × 10⁵ spores/ml.

Rice seedlings were inoculated at the 4.5- to 4.9-leaf stages by spraying 50 ml of the spore suspension of each of the blast fungus isolates on a separate plastic tray. The inoculated seedlings were immediately placed in a dark chamber with a moisture-saturated atmosphere at 25°C for 20 h and then transferred to the greenhouse at 20 to 28°C. At ≈7 days after inoculation, the plants were scored for resistance to the blast fungus isolates according to the classification of Hayashi et al. (8), where 0 = no visible evidence of infection, 1 = brown specks less than 0.5 mm in diameter, 2 = roundish lesions smaller than the interval between thin veins or less than 1 mm in diameter surrounded by brown margins, 3 = small eyespot lesions with a diameter less than one and a half times the distance between thin veins or less than 1.5 mm in diameter with dark brown margins, 4 = intermediate-size eyespot lesions with a diameter less than twice the distance between thin veins or less than 2 mm in diameter with necrotic gray centers and water-soaked or brown margins, and 5 = large eyespot lesions with a diameter more than twice the interval between thin veins or more than 2 mm in diameter. Plants with infection types 0 and 1 were classified as resistant, those with types 2 and 3 were classified as intermediate, and those with types 4 and 5 were classified as susceptible. The experiments were conducted at least twice.

Genomic DNA isolation. Each *M. grisea* isolate was grown in 36 ml of complete medium (CM; 0.6% yeast extract, 0.6% casein hydrolysate, and 1% sucrose) together with 8 to 10 glass beads (12 mm in diameter) in 100-ml flasks and grown for 5 days at 26°C with gentle shaking by hand once a day for maceration. The entire culture was transferred into 150 ml of fresh CM and incubated for two additional days at 26°C with constant shaking

(66 rpm). Fungal mycelia were harvested by filtration through no. 1 filter paper (ADVANTEC).

DNA was extracted by the cetyltrimethylammonium bromide (CTAB) method (22) with modifications. Lyophilized mycelium and two zirconia balls (YTZ ball, 5 mm in diameter, Tosoh, Tokyo) were put into a 2-ml centrifuge tube together. They were frozen at -80°C for 1 h and mixed vigorously with a vortex for ≈30 s. The mycelial powder was mixed with 0.7 ml of hot (70°C) lysis buffer (1.5% CTAB; 1.05 M NaCl; 75 mM Tris-HCl, pH 8.0; 15 mM EDTA) and incubated for 30 min at 56°C with gentle agitation (15 rpm). The solution was extracted twice with chloroform/isoamyl alcohol (24:1, vol/vol). The supernatant was mixed with 0.7 ml of precipitation buffer (1% CTAB; 50 mM Tris-HCl, pH 8.0; 7.5 mM EDTA) and centrifuged at 18,700 × g at 20°C for 10 min. The resulting pellet was suspended in 0.3 ml of 1 M NaCl with RNase at 10 µg/ml and incubated at 56°C for 30 min. A DNA pellet was obtained by isopropanol precipitation. After a wash with 70% ethanol, the pellet was dried and dissolved in Tris-EDTA buffer (pH 8.0).

Random amplified polymorphic DNA analysis. A total of 800 primers (Kit A to Z and Kit AA to AN; Operon Technologies Inc., Alameda, CA) was used in random amplified polymorphic DNA (RAPD) analysis. Polymerase chain reaction (PCR) was performed in a 25-µl reaction containing 1 unit of TaKaRa *Taq* DNA polymerase (TaKaRa, Ohtsu, Japan), 1× PCR buffer provided by the manufacturer, 100 µM each dNTP, 0.4 µM primer, and 20 ng of template DNA. Amplification was carried out in a model 480 thermocycler (Perkin-Elmer, Norwalk, CT) programmed for 45 cycles of 1 min at 94°C, 1 min 30 s at 36°C, and 2 min at 72°C, followed by 7 min at 72°C. Amplification products were separated by electrophoresis.

Southern-hybridization analysis. Restriction fragment length polymorphism (RFLP) markers for mapping were provided by N. Hayashi, T. Teraoka, and S. A. Leong. The markers provided by Hayashi and Teraoka were 091, 079, 084, 180, and 613-1-16. The markers provided by Leong's group were 2-9-G, 11, G34R, 16-1-B, CH2-90H, 4-78, 46-12-C, 31-6-G, 37-10-G, CH5-176H, 22-9-C, 55, 4-14, CH3-123H, 42-10-C, CH3-67H, 28-6-E, cos58, TRP1, CH3-108H, 4-178, CH3-44H, CH4-121H, CH2-57H, G131R, CH5-75H, CH2-32H, and 40-12-G. Genomic DNA (5 µg) from parental isolates Y90-71 and 3514-R-2 or from progeny were digested by 25 units of restriction enzymes *Bam*HI, *Dra*I, *Eco*RI, *Hind*III, *Pst*I, *Sal*I, *Sma*I, and *Xho*I (TaKaRa), separated by 0.8% agarose gel electrophoresis, and transferred to a Hybond N+ membrane (Amersham Pharmacia Biotech Inc., Piscataway, NJ). Southern analyses were carried out with the 33 labeled RFLP markers as probes. A nonradioactive method (ECL system: Amersham Pharmacia Biotech) was used for labeling and detection.

Analysis of linkage. Segregation data were analyzed using MAPMAKER (18) (version 2 for Macintosh; S. Tingley, E. I. Dupont). Parameters for genetic linkage analysis were a minimum log of the likelihood ratio score of 3.0 and a maximum theta of 0.4. The Kosambi function (17) was used to compute recombination distances in centimorgans (cM).

RESULTS

Genetic analysis of avirulence in *M. grisea*. Pathogenicity of the parental isolates on the 15 rice cultivars is shown in Table 1. The cross between Y90-71 and 3514-R-2 produced 185 progeny. All of the parental isolates and progeny from the cross were pathogenic on the rice cv. Norin 3. Y90-71 was avirulent to 14 rice cultivars, Hattan 3, Kadunowase, Zenkougimochi, Nakateshinriki, Yamato 3, Yamatowase, Ishikarishiroke, Hokuriku 12, Suehiromochi, Fujisaka 5, Gohyakumangoku, Kanto 51, Mangetsumochi, and K3, whereas 3514-R-2 was virulent on these 14 cultivars. The progeny were classified into two different types according to their pathogenicity on these 14 cultivars. All progeny

in type A were avirulent on Hattan 3 as well as on the other 13 rice cultivars (Kadunowase, Zenkoujimochi, Nakateshinriki, Yamato 3, Yamatowase, Ishikarishiroke, Hokuriku 12, Suehiromochi, Fujisaka 5, Gohyakumangoku, Kanto 51, Mangetsumochi, and K3). The other progeny, classified as type B, were virulent on all 14 cultivars. Avirulence or virulence of each progeny was clear and no intermediate infection type was seen. All progeny from the cross belonged to either type A or B, with no progeny of other type. The ratio of avirulence to virulence in the progeny on the 14 cultivars was 95:90, fitting a 1:1 ratio (Table 1) and indicating that an avirulence gene interacts with the same single resistance gene in these 14 cultivars. Thus, the avirulence gene in Y90-71 against these 14 cultivars was named *Avr-Hattan 3*.

Genetic analysis of resistance in rice cultivars. Although 12 dominant resistance genes effective to Japanese blast fungus isolates have been identified in Japanese rice cultivars (*Pik-h*, *Pia*, *Pii*, *Pik*, *Pik-m*, *Piz*, *Pita*, *Pita-2*, *Piz-t*, *Pik-p*, *Pib*, and *Pit*) (14), *Avr-Hattan 3* doesn't interact with any of these genes. This is evident because 4 of the 14 cultivars on which Y90-71 is avirulent (Hattan 3, Kadunowase, Zenkoujimochi, and Nakateshinriki) belong to the Shin 2 group which has none of the 12 identified resistance genes. On the other hand, Shin 2 has resistance gene *Pik-s* against a Philippine isolate, KenPh-03 (13). Kiyosawa (13) reported that rice cvs. Ishikarishiroke, Fujisaka 5, and Hokuriku 12 also have *Pik-s*. These cultivars are thought to also have the resistance gene that corresponds to *Avr-Hattan 3* (Table 1). These facts led us to hypothesize that *Avr-Hattan 3* corresponds to *Pik-s*.

TABLE 1. Pathogenicity of the parental isolates of *Magnaporthe grisea* and progeny from the cross Y90-71 × 3514-R-2 on 15 rice cultivars

Genes, cultivars ^a	Parental isolates		Progeny ^b	
	Y90-71	3514-R-2	Type A	Type B
<i>None</i>				
Norin 3	V	V	V	V
Hattan 3	AV	V	AV	V
Kadunowase	AV	V	AV	V
Zenkoujimochi	AV	V	AV	V
Nakateshinriki	AV	V	AV	V
<i>Pia</i>				
Yamato 3	AV	V	AV	V
Yamatowase	AV	V	AV	V
<i>Pii</i>				
Ishikarishiroke	AV	V	AV	V
Hokuriku 12	AV	V	AV	V
Suehiromochi	AV	V	AV	V
Fujisaka 5	AV	V	AV	V
Gohyakumangoku	AV	V	AV	V
<i>Pik</i>				
Kanto 51	AV	V	AV	V
Mangetsumochi	AV	V	AV	V
<i>Pik-h</i>				
K3	AV	V	AV	V
No. of progeny	...	...	95	90

^a Genes identified for resistance to Japanese blast isolates; *None* = no resistance gene to Japanese isolates was found.

^b V = virulent, AV = avirulent, expected ratio = 1:1, $\chi^2 = 0.05$, and *P* value = 0.8 to 0.9.

Unfortunately, the blast fungus isolate, KenPh-03, which was used to identify *Pik-s*, is now extinct. Instead, we employed Ken54-20, a tester isolate for *Pik* (34), to investigate the relationship between *Pik* and the resistance gene that corresponds to *Avr-Hattan 3* because *Pik-s* is thought to be closely linked to but distinct from *Pik* (13). Rice cv. Kanto 51 is known to have resistance gene *Pik* to Ken54-20 (34) and also was shown to have another resistance gene to Y90-71 (Table 1). We compared the reactions of the F₃ lines from the cross between Kanto 51 (resistant to Y90-71 and Ken54-20) and Norin 3 (susceptible to Y90-71 and Ken54-20). One hundred F₃ lines from the cross were inoculated with either Y90-71 or Ken54-20. With Ken54-20, the segregation in the F₃ lines for resistant/segregating/susceptible was 27:51:22 (Table 2), consistent according to a χ^2 test with a 1:2:1 ratio. This data confirmed single gene control for resistance in Kanto 51 to Ken54-20. In the inoculation test with Y90-71, except for one line, reactions of all F₃ lines were identical to those with Ken54-20 (Table 2), showing that the resistance gene in Kanto 51 to Y90-71 is tightly linked to the *Pik* gene.

Genetic mapping of *Avr-Hattan 3*. RAPD markers linked to *Avr-Hattan 3* were identified. Of 800 primers tested, 62 primers (7.8%) produced polymorphic fragments between two parental isolates. The 62 primers were tested with 14 progeny to determine the patterns of inheritance of the RAPD markers. Five markers that had fewer than two recombinants in the 14-progeny sample were further tested with 144 progeny to determine the degree of linkage. By means of segregation analysis with 144 progeny, five RAPD markers with linkages to *Avr-Hattan 3* were found.

We then used the RFLP markers from the genetic maps constructed using crosses between other *M. grisea* isolates. In the genetic map of Chinese blast fungus isolates (CHNOS37-1-1 × CHNOS32-2-4) constructed by Hayashi (7), an avirulence gene that determines specificity of the blast fungus to rice cv. Shin 2 is located on linkage group 2 (7; N. Hayashi, *personal communication*). Shin 2 is known to be susceptible to most Japanese blast fungus isolates, just as is Hattan 3. We examined five single-copy markers on linkage group 2 of their map. All the markers were polymorphic between parental isolates of our cross and turned out to be linked to *Avr-Hattan 3* in the segregating population of 144 progeny. Another map created by the cross between Guy11 and 2539 contains 203 markers (24). We used 28 RFLP markers distributed over seven chromosomes in the Guy11 × 2539 map. Of 28 markers, 10 were polymorphic between parental isolates of our cross. Two markers, 4-178 and 28-6-E, on chromosome 5 and one marker, 22-9-C, on chromosome 4 were linked to *Avr-Hattan 3* in the segregation analyses of 14 progeny. Probe 22-9-C hybridizes to two bands that are polymorphic between parental isolates, both of which were linked to *Avr-Hattan 3*. Further analysis of 144 progeny confirmed that these markers were linked to *Avr-Hattan 3*.

The predicted order of the markers and *Avr-Hattan 3*, as determined by MAPMAKER, is shown in Figure 1. Four RFLP markers, 180, 079, 084, and 091, from the map of Chinese blast fungus isolates were closely linked to *Avr-Hattan 3*. In particular, marker 180 was inseparable from *Avr-Hattan 3*. The RAPD marker *OPR-17* was amplified from parent 3514-R-2 that is virulent to Hattan 3 and was mapped at 1 cM from *Avr-Hattan 3*.

TABLE 2. Segregation for resistance in the F₃ lines from the cross Norin 3 × Kanto 51 to two *Magnaporthe grisea* isolates, Y90-71 and Ken54-20

Reaction to Ken54-20	Reaction to Y90-71 ^a				<i>P</i> value
	Res	Seg	Sus	Total	
Res	27	0	0	27	...
Seg	1	50	0	51	(1:2:1)
Sus	0	0	22	22	0.70–0.80
Total	28	50	22	(100)	...
<i>P</i> value		(1:2:1)	0.50–0.70	...	...

^a Number of F₃ lines classified by reaction to Ken54-20 or Y90-71. Res or Sus = all individual plants in a given F₃ line had resistant or susceptible reactions, respectively, to the blast isolate used. Seg = host reaction to the blast isolate segregated depending on individual plant of the same rice line.

DISCUSSION

In this study, we identified an avirulence gene, *Avr-Hattan 3*, from a Chinese isolate. The gene is responsible for the resistance response of 14 Japanese rice cultivars. According to the genetic study of resistance, *Avr-Hattan 3* corresponds to one of the resistance genes at the *Pik* locus.

The *Pik* locus is known to have several blast resistance genes, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pi1*, *Pi7(t)*, *Pi18(t)*, and *Pialm2* (2,9,16,23,31). A focus of this work was to begin to answer the question, what is the particular gene that corresponds to *Avr-Hattan 3*? *Avr-Hattan 3* determines avirulence to the 14 rice cultivars tested in this report, including Hattan 3, Kadunowase, Zenkoujimochi, and Nakateshinriki. Resistance is not determined by *Pik*, *Pik-h*, *Pik-m*, and *Pik-p* because these genes are not present in Hattan 3, Kadunowase, Zenkoujimochi, and Nakateshinriki, which contain no major resistance genes against most Japanese isolates (15). Thus, *Avr-Hattan 3* must correspond to either one of the other resistance genes in the *Pik* cluster—*Pik-s*, *Pi1*, *Pi7(t)*, *Pi18(t)*, or *Pialm2*—or to an unidentified resistance gene at the *Pik* locus. We have no information about inheritance of *Pi1*, *Pi7(t)*, *Pi18(t)*, or *Pialm2* in Japanese rice cultivars. On the other hand, Kiyosawa (13) showed that rice cvs. Ishikarishiroke, Fujisaka 5, and Hokuriku 12 have *Pik-s*. These three cultivars also were presumed to have the resistance gene that corresponds to *Avr-Hattan 3* in this study. It is possible that *Avr-Hattan 3* is identical to *AvrPik-s*. However, further study of the relationship between *AvrPik-s* and *Avr-Hattan 3* was difficult because the *Pik-s* tester isolate, KenPh-03, is now extinct. A Chinese blast fungus isolate, CHNOS60-2-3, previously was identified to have *AvrPik-s* (8). Because a sequence-tagged site marker that was closely linked to *AvrPik-s* (K. Hirayae, unpublished data) co-segregated with *Avr-Hattan 3*, it is likely that they are the same gene.

Although *Avr-Hattan 3* was identified in a Chinese blast fungus isolate from rice, most Japanese blast fungus isolates are thought to lack a functional *Avr-Hattan 3* because most of them are virulent to rice cv. Hattan 3 (15). In contrast, the fact that some blast fungus isolates collected from Yunnan province in China were avirulent on cvs. Shin 2, Ishikarishiroke, and Kanto 51 (32; unpublished data) indicates that some Chinese isolates have functional *Avr-Hattan 3*. An important question is, why does *Avr-Hattan 3* survive in China while most Japanese isolates do not have a functional *Avr-Hattan 3*? The isolate Y90-71, which has *Avr-Hattan 3*, was collected in Yunnan Province in China. Yunnan Province is a center for genetic diversity in Asian cultivated rice (3). Therefore, survival of *Avr-Hattan 3* in Yunnan Province might be a consequence of the genetic diversity within rice cultivars. The diversity of rice cultivars in Yunnan province might include some that lack resistance to *Avr-Hattan 3*, allowing its maintenance in the blast populations. The distribution of *Avr-Hattan 3* in Yunnan isolates and the resistance gene, which corresponds to *Avr-Hattan 3* in Japan and Yunnan cultivars, should be examined. However, it should be noted that the possibility that no blast fungus isolates with a functional *Avr-Hattan 3* have come to Japan by pure chance could not be ruled out. An investigation of the structural diversity of the virulent locus of *Avr-Hattan 3* in the isolates in Japan and Yunnan may give us some clues as to its origin.

We constructed a partial genetic map of *Avr-Hattan 3* using 144 random progeny. Two RFLP markers (4-178 and 28-6-E) on chromosome 5 and one marker (22-9-C) on chromosome 4 in the genetic map created by the cross between blast fungus isolates Guy11 and 2539 (24) were linked to *Avr-Hattan 3*. Location of *Avr-Hattan 3* in the Guy11 × 2539 map was not identified because there were few polymorphic markers from the Guy11 × 2539 map. Although *Avr-Hattan 3* seemed to be located on linkage group 2 of the CHNOS37-1-1 × CHNOS32-2-4 map, the relationship between the Guy11 × 2539 map and the CHNOS37-1-1 × CHNOS32-2-4 map have not yet been examined. The genetic

markers closely linked to *Avr-Hattan 3* are now being used for positional cloning.

The other avirulence genes that correspond to the resistance genes at the *Pik* locus have been examined. *AvrPik* was identified and a partial linkage map of *AvrPik* was constructed by Luo et al. (19). Moreover, *AvrPik-m* was presumed to be linked to *AvrPik* from the segregation ratio of avirulence or virulence in the progeny of the cross used for the *AvrPik* mapping (20). On the other hand, tightly linked blast resistance genes at the *Pik* locus were presumed to have evolved from the same ancestral gene. Future work regarding the function and evolution of the avirulence genes that correspond to the resistance genes at the *Pik* locus will help reveal part of the history of rice cultivar–blast fungus arms race.

Fig. 1. Genetic maps of the region in the *Magnaporthe grisea* genome containing *Avr-Hattan 3*. Map was constructed using Mapmaker 2.0 with a minimum log of the likelihood ratio score of 3.0 and a maximum theta of 0.4. Kosambi centimorgan (cM) units are shown on the left, and the *Avr* locus and markers are listed on the right.

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