Characterization of Rice Blast Resistance Genes in the *Pik* Cluster and Fine Mapping of the *Pik-p* Locus

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ABSTRACT

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Pik-p is carried by cv. K60, which is one of the Japanese differentials widely used in both Japan and China since the 1980s. Its utility and specificity was evaluated with a total of 612 isolates of *Magnaporthe oryzae* collected from various regions in China in combination with 16 main resistance genes being used in the breeding programs. *Pik-p* is an independently and dominantly acting gene in the *Pik* cluster, which conditions differential reactions against many isolates and contains higher resistance in Guangdong, Jiangsu, and Sichuan provinces, China, indicating that this gene could be still used in these regions. A high-resolution genetic map of *Pik-p* was constructed using genomic position-ready

markers. A set of 47 recombinants out of 681 F₂ plants derived from the crosses cv. K60 (resistant) × cv. AS20-1 (susceptible) and × cv. Kasalath (susceptible) was identified in the genetic interval defined by the markers RM5926 and K37 which flank the *Pik* gene cluster. This set was then genotyped with seven markers known to reside within the interval. The closest markers to *Pik-p* were K28 (≈0.60 centimorgans [cM]) and K39 (≈0.07 cM). A further four markers in the K28–K39 interval were developed from an *in silico* analysis based on the cv. Nipponbare genome sequence, and these all co-segregated with *Pik-p*. This 0.67-cM region is equivalent to a physical separation in cv. Nipponbare of ≈126 kb, plus an as-yet-unfilled genomic gap of unknown length. Four nucleotide-binding site leucine-rich repeat-type resistance genes are present in this interval, and these represent good candidates for *Pik-p*.

Rice blast, caused by the fungus *Magnaporthe oryzae*, is one of the most devastating diseases of rice worldwide (10). The deployment of genetic resistance and, in particular, the breeding of cultivars protected by a spectrum of resistance (*R*) genes, remains the most economical, effective, and environmentally sound disease control strategy. Currently, >80 major blast *R* genes have been documented, approximately half of which have been genetically mapped (4,37), with 9 having been isolated using a map-based cloning approach (2,5,9,14,19,20,28,33,35,40). Many plant *R* genes are known to be organized into gene clusters (3,25). On rice chromosome 11, some 201 *R*-like genes, representing 4.5% of the total number of predicted genes on this chromosome, have been identified from the cv. Nipponbare genome sequence (32). Of these, approximately one-third belongs to the nucleotide-binding site leucine-rich repeat (NBS-LRR) type. The distal region of the long arm of chromosome 11 houses a cluster of at least eight characterized rice blast *R* genes (1,6,7,13,18,29,31,36,39), along with a number of bacterial blight *R* genes (30,33,37).

The rice blast pathosystem largely follows the classical gene-for-gene concept (16). Host recognition of the presence of the pathogen, conditioned by the interaction between a specific *R* gene and its matching pathogen avirulence (*Avr*) gene, leads to the activation of the defense response which, in turn, prevents colonization of the host by the pathogen. Recent findings support a more elaborate model in which the *R* proteins detect the modification of other host proteins by pathogen effectors, rather than recognizing the effectors themselves (11). Although several *R* genes and *Avr* genes have been isolated to date, the molecular

mechanisms underlying the *R*–*Avr* interaction have been elucidated largely using the *Pita*–*AvrPita* system (16). A more general understanding of these molecular events clearly requires a diversification of the pathosystem gene pairs which can be studied at this level.

Pik-p is carried by cv. K60, which is a Japanese differential cultivar widely used in both Japan and China since the 1980s (34). The race specificity of K60 is monogenic and the corresponding pathogen *Avr* gene, *AvrPik-p*, which may be identical to *AvrPi7*, and is being isolated in our laboratory (12). The objective of this study was to evaluate the utility and specificity of the *Pik-p* gene in China, and to develop co-segregating markers linked to the *Pik-p* locus with a view to its marker-aided selection in the Chinese resistance breeding programs. Achieving this would provide a starting point for marker-aided gene isolation and could be a good alternative to the *Pita*–*AvrPita* pathosystem for the molecular dissection of the mechanisms underlying the host resistance response.

MATERIALS AND METHODS

Resistance characterization. To compare the specific reaction patterns and resistance frequencies among four *R* genes at the *Pik* locus (17), Kusabue (*Pik*), K60 (*Pik-p*), Shin 2 (*Pik-s*), and Tusuyake (*Pik-m*) were selected for resistance evaluation, with a total of 612 isolates collected from Guangdong (GD, 60 isolates), Fujian (FJ, 40 isolates), and Hunan (HN, 40 isolates) in the southern region; Guizhou (GZ, 60 isolates), Yunnan (YN, 43 isolates), and Sichuan (SC, 66 isolates) in the southwestern region; Jiangsu (JS, 72 isolates) in the eastern region; and Liaoning (LN, 108 isolates), Jilin (JL, 60 isolates), and Heilongjiang (HLJ, 63 isolates) in the northeastern region in China (Table 1). The resistance of each allele was evaluated as higher (<90%), intermediate (89 to 80%), and lower (>80%) according to the

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resistance frequencies in the respective populations. For determining how to effectively use the resistance of the *Pik-p* in the rice resistance breeding programs in China, the further resistance comparison was focused on 13 cultivars (lines), including the parents of those carrying the major *R* genes being employed in the programs (Table 2).

Mapping population construction. For solely tagging *Pik-p*, a progeny isolate, M028, which carries *AvrPik-p* that was selected from a monogenic segregation population for *AvrPik-p* (12), was used for constructing mapping populations for the *R* gene, *Pik-p*. The two test populations were 340 F₂ plants derived from the cross cv. K60 (resistant) × cv. AS20-1 (population A), and 341 F₂ progeny from the cross cv. K60 × cv. Kasalath (population B) (Table 3). Blast inoculation and disease evaluation were performed according to Pan et al. (26). Briefly, seedlings were grown in a greenhouse at 20 to 35°C for ≈3 weeks before inoculation. At the four- to five-leaf stage, seedlings were transferred into an inoculation chamber and inoculated by spraying with spore suspension at 1 × 10⁵ conidia/ml. After inoculation, the seedlings were kept in the chamber at 25°C with saturated humidity for 20 h, and then transferred to a moist vinyl tunnel at 20 to 35°C. Disease reactions were scored for ≈7 days after inoculation by use of a 0-to-5 disease scale, where the seedlings of scores 0 to 2 were referred to as resistant and those of scores 3 to 5 were referred to as susceptible.

Molecular marker development. Because *Pik-p* was thought to be either an allele of or at least tightly linked to *Pik-m*, which has been successfully mapped by Li et al. (18), it was possible to exploit all the molecular markers used for the mapping of *Pik-m*. A number of additional *Pik-p*-specific markers were developed by a strategy based on an in silico analysis, as has described elsewhere (8,18,21). An overview of the polymerase chain reaction (PCR)-based markers used in the current study is given in Table 4.

Genetic map construction. A bulk segregant analysis (BSA) was carried out to identify molecular makers linked to *Pik-p* (8,21). Similar linkage relationships emerging from the two F₂ populations enabled their combination into a single mapping population of 681 plants (8,18). A genetic linkage map was constructed on the basis of recombination frequencies, as described previously (22,26).

In silico physical map construction. Based on the genetic map of *Pik-p*, an in silico localized physical map was constructed, using the reference sequence of cv. Nipponbare (8,18,21,22). First, primer sequences of the linked molecular markers were matched to the bacterial artificial chromosome (BAC) of cv. Nipponbare released by the International Rice Genome Sequencing Project (IRGSP) (<http://rgp.dna.affrc.go.jp/IRGSP>) through BLASTN analysis. Second, sequences of the matched clones were aligned using another bio-software tool, Pairwise BLAST. The physical map covering the *R* gene locus was consequently constructed based on these aligned sequences of cv. Nipponbare.

RESULTS

Characterization of the *Pik-p* gene. Numerous differential reactions were identified among the four *R* genes in the various populations tested, indicating that the *Pik-p* could be distinguished from the other three alleles at the *Pik* locus with these reactions (Table 1). In the FJ, YN, JS, and HLJ populations, the resistance frequencies in decreasing order among the four *R* genes were *Pik-m* > *Pik* > *Pik-p* > *Pik-s*, which is in agreement with the supposition described by Kiyosawa (17). In the other populations, the resistance frequency is not ordered as *Pik-m* > *Pik* > *Pik-p* > *Pik-s*. Because the *Pik-p* gene contains higher resistance in GD (96.7%), JS (90.9%), and SC (90.9%), it means that the *Pik-p* gene expresses province-specific resistance in the various rice-growing regions in China, and that could be effectively used in

GD, JS, and SC provinces. In addition, numerous complementary reactions were identified in the various populations compared with other main *R* genes being used in the Chinese resistance breeding programs (Table 2), indicating that the *Pik-p* gene could be incorporated in elite cultivars with other *R* genes such as *Pita*, *Pita-2*, *Piz*, *Piz-t*, *Pib*, and *Pi9*, which have been finely mapped and even isolated.

Recombinant screening and mapping population construction. Distinct reactions were observed among three parental cultivars and their F₂ progenies when challenged with the progeny isolate M028 (only parental cultivar reactions shown in Table 2). The segregation of blast resistance in both F₂ populations fit a monogenic ratio (Table 3), suggesting that *Pik-p* behaves as a single dominant gene controlling blast resistance. To verify that the resistance was determined by a gene on chromosome 11, the five simple-sequence repeat (SSR) markers (RM254, RM456C, RM224, RM7443, and RM5926) which map to the distal region of the long arm (23) were applied to test for polymorphism

TABLE 1. Comparison of specific reaction patterns and resistance frequencies among resistance genes *Pik-p*, *Pik-s*, *Pik*, and *Pik-m* located at the *Pik* cluster in the 10 *Magnaporthe oryzae* populations collected in China

Population ^a	Cultivar (gene) ^b			
	Shin 2 (<i>Pik-s</i>)	K60 (<i>Pik-p</i>)	Kusabue (<i>Pik</i>)	Tsuyuaque (<i>Pik-m</i>)
GD isolates				
CHL1968	S	R	R	S
CHL2007	S	R	R	S
Frequency (%)	21.7	96.7	100	93.3
FJ isolates				
CHL487	R	S	R	R
CHL518	S	S	R	R
Frequency (%)	5	45	89.8	95
HN isolates				
CHL412	S	S	R	R
CHL479	S	S	R	R
Frequency (%)	10	80	97.5	97.5
YN isolates				
CHL207	S	S	S	R
CHL250	R	S	R	R
Frequency (%)	14	53.4	67.4	86
GZ isolates				
CHL583	S	S	R	R
CHL590	S	R	R	S
Frequency (%)	13.3	73.3	86.7	81.7
SC isolates				
CHL871	S	R	R	S
CHL901	S	R	S	S
Frequency (%)	54.5	90.9	97	95.5
JS isolates				
CHL335	S	S	R	R
CHL359	R	S	R	R
Frequency (%)	2.8	90.9	94.4	95.5
LN isolates				
EL0401	S	R	R	S
EL0466	S	S	S	S
Frequency (%)	0.9	38.9	52.8	38.9
JL isolates				
CHL703	S	S	R	R
CHL762	S	R	S	S
Frequency (%)	3.3	23.3	21.7	26.7
HLJ isolates				
CHL775	S	R	S	S
CHL777	S	S	S	S
Frequency (%)	15.9	17.5	20.6	25.4

^a Populations: GD (Guangdong, 60 isolates), FJ (Fujian, 40 isolates), and HN (Hunan, 40 isolates) in the southern region; YN (Yunnan, 43 isolates), GZ (Guizhou, 60 isolates), and SC (Sichuan, 66 isolates) in the southwestern region; JS (Jiangsu, 72 isolates) in the eastern region; and LN (Liaoning, 108 isolates), JL (Jilin, 60 isolates), and HLJ (Heilongjiang, 63 isolates) in the northeastern region in China. Frequency = resistance frequency.

^b Specific reaction patterns selected in the respective populations: R = resistant and S = susceptible.

between the resistant and susceptible parental lines and BSA bulks. Only RM5926, the most telomeric of these, identified such a polymorphism (data not shown), and delivered 28 recombinants with resistance in population A (equivalent to ≈ 4.1 centimorgans [cM]), but only 14 in population B (≈ 2.1 cM) (Table 3). The second most centromeric marker, K37 (18), was informative in both crosses and, therefore, was used to identify recombinants on the centromeric side of *Pik-p*. Applying this assay to the two segregating populations resulted in the detection of three and two recombinants, respectively (Table 3), equivalent to map distances of ≈ 0.4 and ≈ 0.3 cM, respectively. Thus, in the pooled population of 681 individuals, *Pik-p* was flanked on the telomeric side by RM5926 and on the centromeric side by K37, with its location defined by 47 recombinants (Table 3; Fig. 1).

Construction of a high-resolution genetic map. Initially, the seven markers RM144, K25, K15-2, K22, K28, K34, and K39, all located in the interval flanked by markers RM5926 and K37, were used to genotype the 47 recombinants between RM5926 and K37 (Table 4; Fig. 1). Three of these (RM144, K25, and K15-2) were informative in population A but not in population B (Fig. 1C). The analysis identified 17, 9, and 8 recombinants, respectively, with respect to RM5926 in population A. K22 and K28, mapping on the telomeric side of *Pik-p*, produced 12 and 8 recombinants, respectively, with respect to RM5926, equivalent to a map distance between *Pik-p* and K28 of 0.6 cM (Fig. 1). On the centromeric side, there were two and one recombinants, respectively, between K34, K39, and *Pik-p*, giving an estimate of 0.07 cM for the genetic interval between K39 and *Pik-p* (Fig. 1). For a second round of mapping, markers K33, K40, K41, and K42 were developed. The focus for marker development was the region defined by K28 and K39. Complete linkage was obtained between *Pik-p* and all four of these markers (Fig. 1). Thus, the

location of *Pik-p* was narrowed to a 0.67-cM region defined by K28 and K39.

Construction of an in silico physical map. Because the same delimiting markers (RM5926 and K37) were also used for the analysis of *Pik-m* (18), the *Pik-m* contig and physical maps were directly utilizable for the analysis of *Pik-p*. The *Pik-p* anchor markers were aligned to the cv. Nipponbare sequence and, based on these positions, a physical map covering *Pik-p* was assembled (Fig. 1B). The K39 to K28 segment covers ≈ 126 kb, plus a genomic gap of unknown length separating BAC clones OSJNBa0047M04 and OSJNBa0036K13. Critically, *Pik-p* is distinct from *Pik-m*, because there are eight recombinants between K28 and *Pik-p* but none between the same marker and *Pik-m* (18) (Fig. 1).

DISCUSSION

We have constructed a high-resolution genetic map in the region of *Pik-p*, which will be of particular use for the selection of closely linked markers for blast *R* gene stacking (15). *Pik-p* is associated with a higher level of resistance against pathogen populations originating from GD, JS, and SC provinces and with an intermediate resistance against those from HN and GZ provinces, but with a lower level against those from YN, FJ, LN, JL, and HLJ provinces. This implies that the gene should be beneficial for blast resistance breeding programs in the former three provinces, and we are currently initiating a joint project with rice breeders in these regions to exploit the genetic linkages.

On the basis of resistance spectra against Japanese isolates, Kiyosawa (17) inferred semi-fine structures of the four alleles at the *Pik* locus, in which *Pik*, *Pik-p*, and *Pik-s* all form part of the larger or stronger allele, *Pik-m* (Fig. 2). That is, the resistance

TABLE 2. Differential reactions of 3 parental rice cultivars and 11 cultivars carrying the main resistance genes being used in the Chinese breeding programs to the progeny isolate M028 and 15 isolates of *Magnaporthe oryzae* selected from Guangdong (GD), Sichuan (SC), Jiangsu (JS), Jilin (JL), and Heilongjiang (HLJ) provinces, China^a

Cultivar ^b	Gene	GD isolates			SC isolates			JS isolates			JL isolates			HLJ isolates			M028 ^c
		CHL1880	CHL1946	CHL2089	CHL842	CHL867	CHL930	CHL333	CHL350	CHL409	CHL678	CHL684	CHL693	CHL768	CHL772	CHL776	
Set A																	
K60	<i>Pik-p</i>	R	R	S	R	R	R	R	R	R	S	S	S	S	S	S	R
Kasalath	<i>Pi36</i>	R	R	S	R	R	S	S	R	R	S	S	S	R	R	S	S
AS20-1	<i>Pi26</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Set B																	
K1	<i>Pita</i>	R	R	R	S	R	S	S	S	S	S	S	S	S	R	S	R
PiNo.4	<i>Pita-2</i>	R	S	R	S	S	R	S	S	S	S	S	S	S	R	R	R
Fukunishiki	<i>Piz</i>	R	R	R	R	S	R	R	R	R	S	S	R	S	R	R	R
Toride 1	<i>Piz-t</i>	S	R	S	S	S	S	R	R	R	R	R	R	R	R	R	R
BL1	<i>Pib</i>	S	R	S	S	S	S	R	S	S	R	R	R	R	R	R	R
C101LAC	<i>Pi1</i>	R	R	R	R	R	R	R	R	R	S	S	S	R	S	R	R
C101A51	<i>Pi2</i>	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S
C104PKT	<i>Pi3</i>	S	R	S	S	S	S	R	R	R	S	S	S	S	S	S	R
C101PKT	<i>Pi4</i>	S	R	S	S	R	S	S	R	R	R	S	S	S	S	R	S
IRBL20	<i>Pi5</i>	R	S	R	S	S	S	R	R	R	S	S	S	S	S	S	ND
IRBL22	<i>Pi9</i>	R	S	R	R	R	S	R	R	R	R	S	S	R	R	S	ND

^a R = resistant, S = susceptible, and ND = not determined.

^b Set A indicates the parental cultivars used for constructing mapping populations and Set B indicates the famous cultivars or lines which carry the main resistance genes employed in Chinese breeding programs.

^c Progeny isolate M028 was selected from a mapping population for *AvrPik-p* that is identical to *AvrPi7* (12).

TABLE 3. F₂ segregation of *Pik-p* from the crosses cv. K60 (resistant) $\times$ cv. AS20-1 (susceptible) and $\times$ cv. Kasalath (susceptible), and linkage analysis of *Pik-p* with respect to the flanking markers K37 and RM5926

Population code	Cross	Isolate ^a	No. of F ₂ plants			χ^2 ^b	Size ^c	Recombinants	
			Resistant	Susceptible	Total			K37	RM5926
A	K60 $\times$ AS20-1	M028	1,038	316	1,354	1.9	340	3	28
B	K60 $\times$ Kasalath	M028	1,041	310	1,351	2.3	341	2	14

^a Test isolate carries the avirulence gene *AvrPik-p*.

^b For 3:1 ratio.

^c Population size. Viable susceptible F₂ plants along with a random selection of resistant F₂ plants.

TABLE 4. Polymerase chain reaction (PCR)-based markers used for genetic mapping of *Pik-p*

Marker ^a	Type	Primer sequence (5'-3')	T _m (°C) ^b	Enzyme ^c
Recombinant screening				
RM5926	SSR	F: ATATACTGTAGGTCCATCCA R: AGATAGTATAGCGTAGCAGC	52	n/a
K37	CAPS	F: CGATTGTCTCTTCACTTTGG R: CTTTCTGTAGGCTTGGTGTG	58	<i>Mva</i> I
High-resolution map				
RM144	SSR	F: TGCCCTGGCGCAAATTTGATCC R: GCTAGAGGAGATCAGATGGTAGTGCATG	52	n/a
K25	CAPS	F: TGA ACA TCG TGC CCG AGT GC R: CAC GTA TTG CTG CTC CCG AC	62	<i>Bgl</i> II
K15-2	CAPS	F: GATCATCCGTGTCGCCAAGG R: GCTCGAGAAGAAGGGGGTTG	60	<i>Hha</i> I
K22	CAPS	F: TCCTCTTCTGT TCTTCTCG R: GGGCACAACAGTATCCAAC	60	<i>Hae</i> III
K28	CAPS	F: TCCCTTACGACGCGGATATG R: GTATCGTAGGAAGTAGGGTC	60	<i>Dra</i> I and <i>Eco</i> RI
K34	STS	F: ACCTTGGCTCCTGACTGTTG R: CCAGGCAGGCTGTTGGTTCA	52.7	n/a
K39	CAPS	F: TGATTCCAACGTGGCAGTG R: ACGTGGTGGCCTCTTTCATC	58	<i>Pvu</i> II
<i>Pik-p</i> locus				
K33	CAPS	F: TTTGGTGCTCCTCTTACGGG R: TCGGAGTTCACAGACCAAG	62	<i>Hin</i> I
K40	CAPS	F: TAGCTAGCAATCCGCTGCAACC R: GCACGTACGGTGTGGACTAAAG	62	<i>Bgl</i> II
K41	CAPS	F: CAGTAGAGGTCGGTGACAGAG R: ACCTTGTCGTGGAGACGGATG	60	<i>Pst</i> I
K42	SSR	F: ATCGAGAATAGTCCACG R: ATTAATGAACCGCCTCGTCG	54	n/a

^a Markers used for recombinant screening or high-resolution map, or cosegregated with *Pik-p* locus. Markers prefixed with RM (i.e., RM5926 and RM224) were adopted from McCouch et al. (23) and those prefixed with K were developed in our laboratory.

^b Annealing temperature.

^c Restriction enzyme used to detect polymorphism between the resistant and susceptible parental lines of the mapping populations; n/a = amplicon size polymorphism, not requiring post-PCR restriction digestion.

Fig. 1. Rice chromosome 11 genetic and physical maps in the region of *Pik-p*. **A**, Integrated genetic map of the six resistance genes mapping to the end of rice chromosome 11. Map positions extracted from (a) McCouch et al. (23), (b) Wang et al. (33), (c) Yang et al. (38), (d) Li et al. (18), (e) Sharma et al. (29), (f) Sun et al. (30), and (g) Xu et al. (36). **B**, Physical map of *Pik-p*, constructed from the cv. Nipponbare genomic sequence. The long black horizontal line represents rice chromosome 11, the shaded bars represent the *Pik-p* region, and the short horizontal bars represent overlapping cv. Nipponbare bacterial artificial chromosome (BAC) clones. The positions of the markers on the BAC clones are indicated by vertical dotted lines. **C**, Recombinants between marker loci and *Pik-p*. The upper figure corresponds to mapping population A, and the lower to mapping population B; (g) indicates noninformative markers in population B.

spectra of *Pik-m* might cover those of *Pik*, *Pik-p*, and *Pik-s*. As to Chinese isolates, however, we have identified many isolates which are virulent to *Pik-m* but avirulent to *Pik* and *Pik-p* in the various populations (Table 1). Resistance spectra were ordered as *Pik-m* > *Pik* > *Pik-p* > *Pik-s* in FJ, YN, JS, and HLJ populations but this was not the case for the remaining six populations tested, where some resistance frequencies of *Pik* and even *Pik-p* were higher than those of *Pik-m* (Table 1). The results from the present study indicated that the alleles *Pik-m*, *Pik*, *Pik-p*, and *Pik-s* at the *Pik* locus are, indeed, independent *R* genes that condition respective reactions against various isolates. This further indicates that these *R* genes in a cluster could also be stacked in elite cultivars through marker-aided selection approach.

The integrated genetic and physical map provides some further insight into the organization and evolution of the chromosome 11 *R* gene cluster. Currently, some six functional *R* genes—specifically, *Pik-h* (36), *Pik-m* (18), and *Pik-p*, along with *Xa4* (30), *Xa22* (23) and *Xa26* (38)—together with at least 30 *R*-like genes of unknown function, have been located within the 110- to 119-cM region of chromosome 11 (32). Similarly, conventional allelism tests concluded that *Pik*, *Pik-s*, *Pik-m*, *Pik-p*, *Pik-h*, and *Pik-g* are all alleles at a single locus (27); the present genetic data show conclusively that marker K28 lies between *Pik-p* and *Pik-m* and, thus, that these two *R* genes must be, by definition, non-allelic. Furthermore, the most promising candidate genes of both *R* genes are different: the former is predicted as NBS-LRR and the latter is protein kinase (18). *R* genes within a single cluster can follow strikingly distinct evolutionary trajectories, as has been suggested by McDowell and Simon (24).

As has been commonly documented (18,30,33,38), the ratio between the physical and the genetic distance across an *R* gene cluster can be very uneven. Variation in this ratio appears to depend not only on the genomic context but also on the genetic background (Table 3; Fig. 1C). In the K37–K40 interval, the rate of recombination in the two populations was highly similar, but this was not the case for the RM5926–K33 interval, where fewer recombinants emerged in population B.

A physical map is a prerequisite for attempting map-based cloning (19,21). *Pik-p* has now been delimited to a 126-kb interval plus a genomic gap bounded by markers K39 and K28 and including four co-segregating markers (Fig. 1). In this region, a

Fig. 2. Presumed semifine structures of resistance (*R*) genes at *Pik* locus and their corresponding avirulence (*Avr*) genes at *AvrPik* locus (adopted from Kiyosawa [17]). The black horizontal line represents active sites and the dotted line represents inactive sites. Uppercase letters represent an active site in *R* gene and lowercase letters represent an active site in its corresponding *Avr* gene.

bioinformatic analysis has identified a maximum of four NBS-LRR-type genes (data not shown). A new BAC contig (consisting of clones AC129339, AC135189, and AC131246) has recently been mapped into the genomic gap mentioned above; however, no *R* gene homologue could be identified in this >300-kb contig. We are currently targeting the four candidate NBS-LRR genes to determine which, if any, of these represent *Pik-p*.

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