Identification of a Novel Locus *Rmo2* Conditioning Resistance in Barley to Host-Specific Subgroups of *Magnaporthe oryzae*

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ABSTRACT

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Barley cultivars show various patterns of resistance against isolates of *Magnaporthe oryzae* and *M. grisea*. Genetic mechanisms of the resistance of five representative barley cultivars were examined using a highly susceptible barley cultivar, 'Nigrata', as a common parent of genetic crosses. The resistance of the five cultivars against *Setaria*, *Oryza*, *Eleusine*, and *Triticum* isolates of *M. oryzae* was all attributed to a single locus, designated as *Rmo2*. Nevertheless, the *Rmo2* locus in each cultivar was effective against a different range of isolates. Genetic analyses of

pathogenicity suggested that each cultivar carries an allele at the *Rmo2* locus that recognizes a different range of avirulence genes. One allele, *Rmo2.a*, corresponded to *PWT1*, which conditioned the avirulence of *Setaria* and *Oryza* isolates on wheat, in a gene-for-gene manner. The other alleles, *Rmo2.b*, *Rmo2.c*, and *Rmo2.d*, corresponded to more than one avirulence gene. On the other hand, the resistance of those cultivars to another species, *M. grisea*, was conditioned by another locus, designated as *Rmo3*. These results suggest that *Rmo2* is effective against a broad range of blast isolates but is specific to *M. oryzae*. Molecular mapping revealed that *Rmo2* is located on the 7H chromosome.

Additional keywords: *Hordeum vulgare*.

Blast diseases of gramineous plants are caused by several species belonging to the genera *Pyricularia* (in anamorph) or *Magnaporthe* (in teleomorph) (10). *Magnaporthe oryzae* (4) is the most common species among them, and is found on various crops (e.g., rice, foxtail millet, finger millet, and wheat). However, isolates from each crop are almost exclusively pathogenic on their original host genus (13,19), indicating that *M. oryzae* is composed of host genus-specific subgroups. Members of each subgroup have been called *Oryza* isolates, *Setaria* isolates, *Eleusine* isolates, *Triticum* isolates, and so on, using the host genus names. *M. grisea* is another member of the causal agents of blast diseases but is found only on *Digitaria* spp., including crabgrass (13); in other words, *M. grisea* consists of only one host-genus specific subgroup, *Digitaria* isolates.

Tosa and his coworkers (19,20) performed genetic analyses of the subgroup-genus specificity using wheat as a model crop. Murakami et al. (16) found that the avirulence of a *Setaria* isolate (GFS11-7-2) on wheat was conditioned by two genes, *PWT1* and *PWT2*. These genes were also involved in the avirulence of an *Oryza* isolate (PO12-7301-2) on wheat (20). Similarly, Takabayashi et al. (17) found that the avirulence of an *Avena* isolate (Br58) on wheat was conditioned by two genes, *PWT3* and *PWT4*. Interestingly, common wheat 'Chinese Spring' did not recognize *PWT4*. By using this cultivar as a susceptible parent, they successfully identified a resistance gene corresponding to *PWT4* and desig-

nated it as *Rwt4*. This was direct evidence that gene-for-gene interactions underlay the subgroup-genus specificity. We also tried to identify *Rwt1*, a putative resistance gene corresponding to *PWT1*, in common wheat, but failed in the identification because all hexaploid wheat lines tested recognized *PWT1* (3).

Barley is another member of the family Triticeae that shares syntenic 7 chromosomes with wheat. Genetic analyses of blast resistance in barley have been performed in some laboratories. Yaegashi (22) reported that a half of his test cultivars were resistant to an isolate from barley while the others were susceptible. By using this specificity, he identified a blast resistance gene in barley and designated it as *PHR-1*. Inukai et al. (11) identified a resistance gene against a rice isolate in barley, and designated it as *Rmo1*. In our preliminary screening, we found that the interaction between barley and *M. oryzae* included various types, from nonhost-like immune responses through host-like compatible responses. These findings suggested that the barley population had a lot of polymorphisms in terms of resistance or susceptibility to *M. oryzae* and *M. grisea* subgroups. We hypothesized that such a high polymorphism might make it possible to identify resistance genes involved in host species specificity. The key material for such an analysis is an exceptional cultivar that is highly susceptible to any subgroup of *M. oryzae* and *M. grisea*. We found that barley 'Nigrata' had such an exceptional characteristic. Using this cultivar, we successfully identified a core locus conditioning the resistance of barley to various subgroups of *M. oryzae*.

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MATERIALS AND METHODS

Plant and fungal materials. The plant materials used were six barley (*Hordeum vulgare* L.) cultivars and a common wheat

(*Triticum aestivum* L.) cultivar, ‘Norin 4’ (N4) (Table 1). The barley and wheat cultivars were provided by Dr. U. Hiura, an emeritus professor at Okayama University, and Dr. K. Tsunewaki, an emeritus professor at Kyoto University, respectively.

Fungal isolates used were *M. oryzae* GFSI1-7-2 (Si-1J) isolated from foxtail millet (*Setaria italica*), PO12-7301-2 (O-23IN) isolated from rice (*Oryza sativa*), MZ5-1-6 (Ec-3J) isolated from finger millet (*Eleusine coracana*), Br48 (T-4B) isolated from common wheat (*T. aestivum*), and *M. grisea* Dig41 (Dsa-1J) isolated from crabgrass (*Digitaria sanguinalis*) (Table 1). The species of these isolates were identified by Hirata et al. (10) using genealogical concordance phylogenetic species recognition (18). In addition to these five isolates, *M. oryzae* Br116.5 (T-7B), isolated from common wheat, was used as a mating partner of an *MAT1-1* culture. For segregation analyses of pathogenicity, the F₁ cultures derived from Si-1J × T-4B (16) and O-23IN × T-4B (20) were employed. These isolates or cultures have been maintained on barley seed media in the Laboratory of Plant Pathology, Kobe University, as described previously (10). They were transferred to a potato dextrose agar slant just before use and grown at room temperature. For the experiments, 1-week-old to 3-month-old cultures were used.

Genetic crosses of fungal cultures. Fungal cultures were crossed on oatmeal agar medium (20 g of oatmeal, 10 g of agar, and 2.5 g of sucrose in 500 ml of water) as described by Murakami et al. (16). In a cross between Ec-3J and T-4B, all ascospores were isolated from each ascus, and sister progenies were identified by fingerprinting with MGR583 (9,12). A set of tetrads was established by choosing one culture from each sister progenies arbitrarily.

Bioassay. Barley and wheat seedlings were grown at 22°C for 7 days and inoculated with a conidial suspension (1 to 2 × 10⁵ spores/ml) as described previously (16). After incubation in a dark, humid box at 22°C for 24 to 42 h, they were returned to a growth chamber controlled at 22°C and incubated further. Five days after inoculation, infection types were determined based on the color (B, brown; G, green) and the size of lesions (0, no visible evidence of infection; 1, pinpoint spots; 2, small lesions <1.5 mm in diameter; 3, scattered lesions with an intermediate to large size; 4, large, typical lesions; and 5, complete blighting of leaf blades). For example, infection type 1B represents brown lesions with size 1. Each infection assay was repeated three to five times.

To compare segregation patterns of resistance to different isolates, F₂ seedlings were sequentially inoculated with two isolates in various combinations with an interval of 24 h. The first isolate was sprayed on primary leaves of the F₂ seedlings, whose bottom half was covered with aluminum foil. After 24 h of incubation in a dark, humid box, the second isolate was sprayed on the same leaves, whose upper half was covered with aluminum foil. After incubation in the dark, humid box for additional 24 h, they were transferred to a growth chamber with a 12-h photoperiod. Disease intensity was evaluated 4 days after the second inoculation (5 days after the first inoculation).

Molecular mapping. Twenty seeds were retrieved from each of 91 F₃ lines derived from a cross between resistant and susceptible cultivars, sown in vermiculite in a seedling case, and grown at 22°C in a controlled-environment room with a 12-h photoperiod of fluorescent lighting for 7 days. Genomic DNA was extracted from 20 leaves following the procedure of Appels and Moran (1), with the modification by Koebner et al. (15), and used as a sample equivalent to the genomic DNA of the parental F₂ plant.

Out of simple-sequence repeat (SSR) loci reported by Varshney et al. (21), 11, 11, 11, 9, 13, 10, and 11 loci were chosen from the 1H, 2H, 3H, 4H, 5H, 6H, and 7H chromosomes, respectively, so that they were distributed evenly on the chromosomes. They were amplified from genomic DNA of 10 “all resistant” and 10 “all susceptible” F₃ lines. Polymerase chain reaction (PCR) products were fractionated by electrophoresis through 6% denaturing polyacrylamide gels and stained using SILVER SEQUENCE DNA Staining Reagents (Promega Corp., Madison, WI).

After the target gene was found to be linked to an SSR marker located on 7H, additional SSR loci were arbitrarily chosen from

Fig. 1. Symptoms on primary leaves of parental cultivars that were sequentially inoculated with two isolates, 4 days after the second inoculation. The top half was first inoculated with Si-1J and incubated for 24 h; then, the bottom half was inoculated with T-4B. Infection types with Si-1J and T-4B are shown at the top and bottom of the panel.

TABLE 1. Infection types of barley cultivars with *Magnaporthe* isolates

Cultivar (abbreviation)	Infection type with ^a				
	GFSI1-7-2 (Si-1J)	PO12-7301-2 (O-23IN)	MZ5-1-6 (Ec-3J)	Br48 (T-4B)	Dig41 (Dsa-1J)
H.E.S.4 (H4)	0*	0*	5G	5G	5G
Nakaizumi-zairai (NZ)	0*	0*	5G	5G	5G
Nepal (Npl)	0*	0*	5G	5G	2B*
Russian 74 (R74)	0*	0*	2B*	4-5G	4G
Russian 81 (R81)	0*	3BG*	2B*	2B*	2B*
Nigrate (Ngt)	5G	4G	5G	5G	5G

^a For code names of isolates, letters before hyphen represent original hosts: Si, *Setaria italica*; O, *Oryza sativa*; Ec, *Eleusine coracana*; T, *Triticum aestivum*; Dsa, *Digitaria sanguinalis*. An asterisk (*) indicates an incompatible interaction.

those on 7H (21), and amplified from genomic DNA of the 91 F₃ lines. PCR products were fractionated as described above. Segregation data were analyzed using MAPMAKER Macintosh V2.0 with the F₂ intercross program. Thresholds of linkage for map construction were a minimum log of the likelihood ratio of 4.0 and a maximum recombination fraction of 0.4. The Kosambi mapping function was employed to compute map distances in centimorgans.

RESULTS

Infection types on barley cultivars caused by subgroups of *M. oryzae* and *M. grisea*. The six barley cultivars were inoculated with a *Setaria* isolate (Si-1J), an *Oryza* isolate (O-23IN), an *Eleusine* isolate (Ec-3J), a *Triticum* isolate (T-4B), and a *Digitaria* isolate (Dsa-1J). Infection types were classified into two categories: 0-3BG (resistant or avirulent) and 3G-5G (susceptible or virulent). 3BG was classified into the resistant group because green blast lesions in this type were surrounded by brown tissues derived from hypersensitive reaction (HR) (data not shown). 'H.E.S.4' (H4) and 'Nakaizumi-zairai' (NZ) showed the same pattern of reactions; resistant to Si-1J and O-23IN but susceptible to the others (Table 1). 'Nepal' (Npl) and 'Russian 74' (R74) were resistant to Dsa-1J and Ec-3J, respectively, in addition to Si-1J and O-23IN. 'Russian 81' (R81) was resistant to all isolates, although its resistance to O-23IN was very weak (3BG). On the other hand, Nigra (Ngt) was susceptible to all isolates tested (Table 1).

Identification of resistance genes. Against Si-1J, all cultivars except Ngt showed strong resistance with no visible symptoms

(infection type 0) (Table 1). When F₂ populations derived from crosses between the five resistant cultivars and Ngt were inoculated with Si-1J, resistant and susceptible seedlings segregated in a 3:1 ratio (Table 2; Supplemental Figure 1), suggesting that the resistance of each cultivar to Si-1J is controlled by a single major gene. Crosses between the resistant cultivars yielded no susceptible seedlings (Table 2), suggesting that the resistance genes in these cultivars are located at the same locus.

Against O-23IN, four cultivars (H4, NZ, Npl, and R74) were resistant while Ngt was susceptible (Table 1). When F₂ populations derived from crosses between the four cultivars and Ngt were inoculated with O-23IN, resistant and susceptible seedlings segregated in a 3:1 ratio (Table 2), suggesting that the resistance of each cultivar to O-23IN is controlled by a single major gene. Crosses between the resistant cultivars yielded no susceptible seedlings (Table 2), suggesting that the resistance genes in these cultivars are located at the same locus. The resistance of R81 to O-23IN was weak (infection type 3BG) (Table 1). When an F₂ population from R81 × Ngt was inoculated with O-23IN, the distribution of infection types was not discontinuous (data not shown). Therefore, this interaction (between R81 and O-23IN) was excluded from the further analysis.

Against Ec-3J, two cultivars (R74 and R81) were resistant while the others were susceptible (Table 1). When F₂ populations derived from R74 × Ngt and R81 × Ngt were inoculated with Ec-3J, resistant and susceptible seedlings segregated in a 3:1 ratio (Table 2). However, a cross between R74 and R81 yielded no susceptible seedlings (Table 2). These results suggest that the resistance of R74 and R81 to Ec-3J is controlled by a single gene located at the same locus.

TABLE 2. Distribution of infection types with *Magnaporthe* isolates in F₂ populations derived from crosses between barley cultivars

Isolate, cross	Number of F ₂ seedlings								Total	Resistant ^a	Susceptible ^b	χ^2 (3:1)	P
	Infection type												
	0	1B	2B	2BG	3B/3BG	3G	4G	5G					
Si-1J													
H4 × Ngt	127	21	32	0	0	0	23	40	243	180	63	0.11	0.73
NZ × Ngt	96	32	52	0	0	0	26	29	235	180	55	0.31	0.57
Npl × Ngt	44	13	31	2	0	0	9	19	118	90	28	0.10	0.75
R74 × Ngt	123	2	3	0	0	0	3	34	165	128	37	0.58	0.44
R81 × Ngt	177	7	0	0	0	0	6	46	236	184	52	1.11	0.29
H4 × NZ	296	0	0	0	0	0	0	0	296	296	0	...	...
H4 × Npl	258	0	0	0	0	0	0	0	258	258	0	...	...
H4 × R74	311	0	0	0	0	0	0	0	311	311	0	...	...
H4 × R81	282	0	0	0	0	0	0	0	282	282	0	...	...
R74 × Npl	287	0	0	0	0	0	0	0	287	287	0	...	...
R74 × R81	300	0	0	0	0	0	0	0	300	300	0	...	...
R81 × NZ	306	0	0	0	0	0	0	0	306	306	0	...	...
R81 × Npl	235	0	0	0	0	0	0	0	235	235	0	...	...
O-23IN													
H4 × Ngt	183	0	0	0	0	0	3	57	243	183	60	0.01	0.91
NZ × Ngt	185	0	2	0	0	0	3	59	249	187	62	0.00	0.97
Npl × Ngt	163	0	0	0	0	0	1	49	213	163	50	0.26	0.60
R74 × Ngt	181	0	1	0	0	0	1	64	247	182	65	0.22	0.63
H4 × NZ	286	0	0	0	0	0	0	0	286	286	0	...	...
H4 × Npl	262	0	0	0	0	0	0	0	262	262	0	...	...
H4 × R74	296	0	0	0	0	0	0	0	296	296	0	...	...
R74 × Npl	274	0	0	0	0	0	0	0	274	274	0	...	...
Ec-3J													
R74 × Ngt	14	14	31	65	0	0	0	47	171	124	47	0.56	0.45
R81 × Ngt	99	82	78	0	0	0	0	85	344	259	85	0.01	0.90
R74 × R81	135	20	58	0	0	0	0	0	213	213	0	...	...
T-4B													
R81 × Ngt	0	1	47	94	0	0	1	48	191	142	49	0.04	0.84
Dsa-1J													
Npl × Ngt	26	13	16	25	0	0	5	19	104	80	24	0.20	0.65
R81 × Ngt	9	30	99	24	0	0	0	61	223	162	61	0.66	0.42
R81 × Npl	133	29	40	0	0	0	0	0	202	202	0	...	...

^a Infection type 0 to 3BG.

^b Infection type 3G to 5G.

Against T-4B, R81 was resistant while the others were susceptible (Table 1). When an F₂ population derived from R81 × NgT was inoculated with T-4B, resistant and susceptible seedling segregated in a 3:1 ratio (Table 2), suggesting that the resistance of R81 to T-4B is controlled by a single gene.

Against Dsa-1J, two cultivars (Npl and R81) were resistant while the others were susceptible (Table 1). Crosses between Npl or R81 and NgT produced resistant and susceptible F₂ seedling in a 3:1 ratio but a cross between Npl and R81 yielded no susceptible seedlings (Table 2). These results suggest that the resistance of Npl and R81 to Dsa-1J is controlled by a single gene located at the same locus.

Allelic relationships of the resistance genes. The segregation analyses mentioned above revealed that the resistance of the barley cultivars to each isolate was controlled by the same gene. To determine whether the resistance of each cultivar to different isolates was controlled by the same gene, we performed “double inoculation” in which seedlings were sequentially inoculated with two different isolates with a 24-h interval. When the six parental cultivars were first inoculated with Si-1J (virulent on a cultivar and avirulent on the others) and then with T-4B (avirulent on a cultivar and virulent on the others), they showed the same infection types as those inoculated with Si-1J alone and T-4B alone, respectively (Fig. 1; Table 1). This result indicates that infection types with the second inoculum are not affected by the first inoculation; or, in other words, that the effect of induced resistance can be neglected in this system.

H4, NZ, Npl, and R74 were resistant to both Si-1J and O-23IN. F₂ seedlings from H4 × NgT, NZ × NgT, Npl × NgT, and R74 × NgT were inoculated first with Si-1J and then with O-23IN. In all F₂ populations, seedlings resistant to Si-1J were also resistant to O-23IN while those susceptible to Si-1J were also susceptible to O-23IN (Table 3). This result suggests that the resistance genes against Si-1J and O-23IN identified in these cultivars are located at the same locus.

R74 was resistant not only to Si-1J and O-23IN but also to Ec-3J (Table 1). The segregation patterns against these three isolates

were perfectly concordant in F₂ seedlings from R74 × NgT (Table 3), suggesting that the resistance genes against the three isolates are located at the same locus in R74.

R81 was resistant not only to Si-1J and Ec-3J but also to T-4B (Table 1). The segregation patterns against these three isolates were perfectly concordant in F₂ seedlings from R81 × NgT (Table 3), suggesting that the resistance genes against the three isolates are located at the same locus in R81. To confirm this conclusion directly, F₃ populations derived from R81 × NgT were inoculated with Si-1J, Ec-3J, and T-4B. With all three isolates, all resistant, segregating, and susceptible populations segregated in a 1:2:1 ratio (Table 4). The segregation patterns with the three isolates were perfectly concordant (Table 4). These results support the conclusion drawn from the double inoculation of F₂ seedlings.

Taken together, the resistance of all cultivars to all *M. oryzae* isolates tested in the present study was attributed to a single locus. This locus was designated as *Rmo2*, following the designation proposed by Inukai et al. (11). On the other hand, the resistance (of Npl and R81) to *M. grisea* (Dsa-1J) was controlled by another locus. When F₂ seedlings from Npl × NgT and R81 × NgT were inoculated first with *M. oryzae* (O-23IN, Si-1J, Ec-3J, or T-4B) and then with *M. grisea* (Dsa-1J), recombinants were observed (Table 3). However, the segregation of RpRq (resistant to both), RpSq (resistant to the former and susceptible to the latter), SpRq (susceptible to the former and resistant to the latter), and SpSq (susceptible to both) was significantly deviated from a ratio which was expected from independent inheritance (Table 3). These results suggest that the resistance of Npl and R81 to Dsa-1J is controlled by a locus that is distinct from but linked to *Rmo2*. This locus (and gene) against *M. grisea* was designated as *Rmo3*.

Identification of avirulence genes. The results mentioned above suggest that the resistance of those cultivars against *M. oryzae* is conditioned by a single gene at the *Rmo2* locus. Nevertheless, these cultivars are resistant to various ranges of *M. oryzae* subgroups. One possible explanation is that they carry different

TABLE 3. Segregation of reactions to two *Magnaporthe* isolates in F₂ populations derived from crosses between resistant and susceptible barley cultivars

Cross	Isolates		Number of F ₂ seedlings ^a				Total	χ^2 (linkage)	P
	p (1st)	q (2nd)	R _p R _q	R _p S _q	S _p R _q	S _p S _q			
H4 × NgT	Si-1J	O-23IN	147	0	0	51	198	...	...
NZ × NgT	Si-1J	O-23IN	150	0	0	54	204	...	...
Npl × NgT	Si-1J	O-23IN	146	0	0	50	196	...	...
R74 × NgT	Si-1J	O-23IN	177	0	0	64	241	...	...
R74 × NgT	Si-1J	Ec-3J	89	0	0	29	118	...	...
R74 × NgT	O-23IN	Ec-3J	81	0	0	27	108	...	...
R81 × NgT	Si-1J	Ec-3J	61	0	0	23	84	...	...
R81 × NgT	Si-1J	T-4B	75	0	0	30	105	...	...
R81 × NgT	Ec-3J	T-4B	60	0	0	22	82	...	...
Npl × NgT	O-23IN	Dsa-1J	72	15	15	12	114	7.89	<0.01
R81 × NgT	Si-1J	Dsa-1J	66	9	5	16	96	32.67	<0.01
R81 × NgT	Ec-3J	Dsa-1J	47	12	11	11	81	8.13	<0.01
R81 × NgT	T-4B	Dsa-1J	39	8	9	9	65	8.14	<0.01

^a R = resistant (infection type 0 to 3BG) and S = susceptible (infection type 3G to 5G). For example, R_pS_q represents seedlings which are resistant to p and susceptible to q.

TABLE 4. Segregation of reactions to *Magnaporthe oryzae* isolates in F₃ populations derived from R81 × NgT

Isolate	Number of F ₃ populations ^a				χ^2 (1:2:1)	P
	All resistant	Segregating	All susceptible	Total		
Si-1J	13	24	13	50	0.08	0.96
Ec-3J	13 ^b	24 ^c	13 ^d	50	0.08	0.96
T-4B	13 ^b	24 ^c	13 ^d	50	0.08	0.96

^a The same 50 populations were used for all isolates. Twenty seeds were retrieved from each population for infection assay with each isolate.

^b The 13 populations that were all resistant to Si-1J.

^c The 24 populations that were segregating to Si-1J.

^d The 13 populations that were all susceptible to Si-1J.

alleles at the *Rmo2* locus that correspond to different (ranges of) avirulence genes. To examine whether this is the case, segregation analyses were performed using hybrid populations derived from crosses between the *M. oryzae* isolates.

The avirulence of Si-1J on the five barley cultivars (H4, NZ, Npl, R74, and R81) was analyzed using the F₁ population between Si-1J (GFSI1-7-2) and T-4B (Br48) produced by Murakami et al. (16). This F₁ population was composed of 10 tetrads (40 cultures). Of the 40 cultures, 5 mutants that lost the basic capacity of penetration were excluded, and the remaining 35 were sprayed on wheat N4, which had been used for the identification of *PWT1* (associated with the brown lesion color and HR) and *PWT2* (associated with the lesion number and papilla formation). Murakami et al. (16) recognized four phenotypes on N4 inoculated with the F₁ population: B- (≈0 to 2B), B+ (≈4 to 5B), G- (≈3G), and G+ (≈4 to 5G). In the present study, 4B or 5B were not observed due to the lower temperature (21 to 22°C) compared with 25°C used by Murakami et al. (16). The lower temperature made it easier to recognize the segregation of *PWT1*. In the present condition, *PWT1* and *pwt1* carriers showed infection types 0 to 3B and 3G to 5G, respectively, on N4. When these two categories of infection types were assumed to be avirulent and virulent, their segregation fitted a 1:1 ratio, as expected (Table 5). The segregation of *PWT2* seemed to be reflected to the peak at

3G among the *pwt1* carriers (Table 5). However, the lesion number was affected by a fluctuation of concentration and viability of inocula. To simplify the analysis, we focused on the segregation of *PWT1* playing the primary role in the avirulence of Si-1J.

When H4 and NZ were inoculated with the F₁ population derived from Si-1J × T-4B, avirulent and virulent cultures also segregated in a 1:1 ratio (Table 5). The segregation patterns on these barley cultivars were perfectly concordant with that on N4 (Table 6), suggesting that H4 and NZ recognize *PWT1*. When Npl and R74 were inoculated with the F₁ population, avirulent and virulent cultures segregated in a 3:1 ratio (Table 5), suggesting that two genes are involved in the avirulence of Si-1J on each of these cultivars. If one of the two genes is *PWT1* detected on N4, the combined segregation on N4 and Npl or R74 should fit a 2:0:1:1 ratio (Supplemental Figure 2). If none of the two genes is *PWT1*, the combined segregation should fit a 3:1:3:1 ratio. When the segregation data on N4 and Npl or R74 were combined, there were no cultures that were avirulent on N4 and virulent on Npl or R74 (Table 6); in other words, the *PWT1* carriers were all avirulent on Npl and R74. The segregation of ApAq (avirulent on both N4 and Npl/R74), VpAq (virulent on N4 and avirulent on Npl/R74), and VpVq (virulent on both) fitted in a 2:1:1 ratio. These results suggest that one of the two genes detected on Npl

TABLE 5. Segregation of virulence on wheat and barley cultivars in F₁ populations derived from crosses between field isolates of *Magnaporthe oryzae*

Cross, cultivar	Number of F ₁ cultures									χ^2 for ^c	
	Infection type										
	0/1B	2B/2BG	3B/3BG	3G	4G	5G	Avirulent ^a	Virulent ^b	Total	1:1	3:1
Si-1J × T-4B											
N4 ^d	10	9	1	8	2	5	20	15	35	0.71	5.95*
H4	7	12	1	1	5	9	20	15	35	0.71	5.95*
NZ	9	9	2	2	3	10	20	15	35	0.71	5.95*
Npl	10	11	4	0	5	5	25	10	35	6.42*	0.29
R74	13	13	1	2	2	4	27	8	35	10.31**	0.09
R81	12	18	3	0	0	2	33	2	35	27.45**	6.94**
O-23IN × T-4B											
H4	2	11	5	3	4	11	18	18	36	0.00	12.00**
NZ	2	9	7	3	6	9	18	18	36	0.00	12.00**
Npl	12	4	7	3	4	6	23	13	36	2.78	2.37
R74	16	9	2	0	1	8	27	9	36	9.00**	0.00
Ec-3J × T-4B											
R74	2	17	0	0	5	16	19	21	40	0.10	16.14**
R81	3	27	0	0	5	5	30	10	40	10.00**	0.00

^a Infection type 0 to 3BG.

^b Infection type 3G to 5G.

^c Significant at the 5% (*) and 1% (**) levels.

^d Wheat 'Norin 4' on which *PWT1* was identified.

TABLE 6. Combined segregation of virulence on two wheat/barley cultivars in the F₁ populations derived from crosses between field isolates of *Magnaporthe oryzae*

Cross	Cultivar		Number of F ₁ cultures ^a				χ^2 for ^b	
	p	q	A _p A _q	A _p V _q	V _p A _q	V _p V _q	Total	2:(0:)1:1
Si-1J × T-4B	N4 ^c	H4	20	0	0	15	35	...
	N4	NZ	20	0	0	15	35	...
	N4	Npl	20	0	5	10	35	2.14
	N4	R74	20	0	7	8	35	0.77
	N4	R81	20	0	13	2	35	7.63*
	Npl	R74	23	2	4	6	35	...
O-23IN × T-4B	H4	NZ	18	0	0	18	36	...
	H4	Npl	18	0	5	13	36	3.56
	H4	R74	17	1	10	8	36	...
	Npl	R74	22	1	5	8	36	...
Ec-3J × T-4B	R74	R81	19	0	11	10	40	0.01

^a A = avirulent (infection type 0 to 3BG) and V = virulent (infection type 3G to 5G). For example, A_pV_q represents cultures which are avirulent on p and virulent on q.

^b An asterisk (*) indicates a value significant at the 5% level.

^c Wheat 'Norin 4' on which *PWT1* was identified.

and R74 is *PWT1*. When the segregation patterns of Npl and R74 were combined, both recombinants (ApVq and VpAq) were observed (Table 6), indicating that different genes are involved in the avirulence on Npl and R74 in addition to the common gene, *PWT1*. The genes specifically detected on Npl and R74 were tentatively designated as *AVR-Xs* and *AVR-Ys*, respectively (“s” indicates derived from a *Setaria* isolate). R81 was resistant to both parents of the F₁ population (Table 1). When R81 was inoculated with the F₁ population, two virulent cultures were observed (Table 5), indicating that the avirulence of Si-1J and T-4B are conditioned by different genes. However, the segregation of avirulent and virulent cultures did not fit a 3:1 ratio (Table 5), suggesting that those genes are linked or that more than two genes are involved.

The avirulence of O-23IN on the four barley cultivars (H4, NZ, Npl, and R74) was analyzed using the F₁ population derived from the cross between O-23IN (PO12-7301-2) and T-4B (20). Of the 14 tetrads produced by Tosa et al. (20), 9 tetrads were chosen

which did not contain mutant cultures lacking the basic capacity of penetration. When H4 and NZ were inoculated with the 36 cultures (= 4 × 9), avirulent and virulent cultures segregated in a 1:1 ratio (Table 5). The segregation patterns on these cultivars were perfectly concordant (Table 6). These results suggest that the avirulence of O-23IN on H4 and NZ is controlled by the same, single gene as in the case of the avirulence of Si-1J on these cultivars. This gene was considered to be *PWT1* because O-23IN had been already reported to carry *PWT1* (20). On Npl, the segregation of avirulent and virulent cultures was not significantly different from 1:1 or 3:1 ratios (Table 5). However, the *PWT1* carriers (cultures avirulent on H4) were all avirulent on Npl whereas the *pwt1* carriers (cultures virulent on H4) were composed of avirulent and virulent cultures on Npl (Table 6). These results suggest that, in addition to *PWT1*, one gene is involved in the avirulence on Npl. This gene was tentatively designated as *AVR-Xo* (“o” indicates derived from an *Oryza* isolate). On R74, avirulent and virulent cultures segregated in a 3:1 ratio (Table 5), suggesting that two genes are involved. One of the two genes should be *PWT1* because R74 recognized *PWT1* derived from Si-1J. When the segregation patterns on H4 and R74 were combined, the cultures avirulent on H4 were also avirulent on R74, with one exception (Table 6). On the other hand, the cultures virulent on H4 were avirulent or virulent on R74 in a 1:1 ratio (10:8). This gene was tentatively designated as *AVR-Yo*.

To analyze the avirulence of Ec-3J on R74 and R81, Ec-3J was crossed with T-4B, and 10 tetrads were isolated (see Materials and Methods). R74 was resistant to Ec-3J but susceptible to T-4B (Table 1). When R74 was inoculated with the 40 F₁ cultures, avirulent and virulent cultures segregated in a 1:1 ratio (Table 5), suggesting that one gene is involved in the avirulence of Ec-3J on R74. This gene was designated as *PBY1* (for “pathogenicity on barley”). On the other hand, R81 was resistant to both parental isolates (Table 1). When R81 was inoculated with the 40 F₁ cultures, the segregation fitted a 3:1 ratio (Table 5), suggesting that this F₁ population contains two avirulence genes, one from Ec-3J and the other from T-4B. When the segregation patterns on R74 and R81 were combined, all of the cultures avirulent on R74 were also avirulent on R81 while the cultures virulent on R74 were split into two groups, (i.e., avirulent and virulent on R81) in a 1:1 ratio (Table 6). These results suggest that the avirulence gene from Ec-3J detected on R81 is *PBY1*. The other gene (from T-4B) detected on R81 was designated as *PBY2*.

Allelic relationships of the avirulence genes detected in Si-1J and O-23IN. Si-1J carried *AVR-Xs* and *AVR-Ys* in addition to *PWT1* while O-23IN carried *AVR-Xo* and *AVR-Yo* in addition to *PWT1*. *AVR-Xs* and *AVR-Xo* were specifically detected on Npl while *AVR-Ys* and *AVR-Yo* were specifically detected on R74. A simple hypothesis is that *AVR-Xs* and *AVR-Xo* are the same gene while *AVR-Ys* and *AVR-Yo* are the same gene. This hypothesis was tested as illustrated in Figure 2.

OR59, an F₁ culture derived from Si-1J × T-4B, was virulent on H4 but avirulent on Npl and R74 and, therefore, was considered to carry *AVR-Xs* and *AVR-Ys* but not *PWT1* (Fig. 2). This culture

Fig. 2. Genetic crosses for checking allelic relationships among *AVR-Xs*, *AVR-Ys*, *AVR-Xo*, and *AVR-Yo*.

TABLE 7. Segregation of virulence on barley cultivars in populations derived from crosses involving *AVR-X* and *AVR-Y* carriers

Cross	Cultivar	Number of progeny cultures			χ^2 for 1:1
		Avirulent ^a	Virulent ^b	Total	
OR59 × T-4B	Npl	20	19	39	0.03 ^c
	R74	20	19	39	0.03 ^c
49N1 × T-7B	Npl	20	20	40	0.00 ^c
	R74	21	19	40	0.10 ^c
OR59 × 49N1	Npl	59	0	59	...
	R74	59	0	59	...

^a Infection type 0 to 3BG.
^b Infection type 3G to 5G.
^c Not significant at the 5% level.

was interfertile with T-4B because it carried the *MAT1-2* allele at the mating type locus that was opposite to the *MAT1-1* allele in T-4B. 0R59 was backcrossed with T-4B, and 10 tetrads were obtained. Among a total of 40 cultures, 1 culture had lost the basic capacity of penetration. When Npl and R74 were inoculated with the remaining 39 cultures, avirulent and virulent cultures segregated in a 1:1 ratio on both cultivars, as expected (Table 7). When the segregation patterns on Npl and R74 were combined, recombinants (ApVq and VpAq) were observed but much fewer than those expected from independent inheritance; the segregation of ApAq:ApVq:VpAq:VpVq was significantly deviated from a 1:1:1:1 ratio (Table 8). These results suggest that *AVR-Xs* and *AVR-Ys* are linked.

The F₁ population derived from O-23IN × T-4B also contained several cultures that were virulent on H4 but avirulent on Npl and R74. They were considered to carry *AVR-Xo* and *AVR-Yo* but not *PWT1*. Out of these cultures, 49N1 carrying the *MAT1-1* mating type was chosen, and crossed with T-7B carrying the *MAT1-2* mating type (Fig. 2). T-7B (Br116.5), a *Triticum* isolate collected in Brazil, was virulent on all of H4, Npl, and R74 as T-4B, and was also considered to carry none of *PWT1*, *AVR-Xo*, and *AVR-Yo*. From this cross, 10 tetrads (in total, 40 cultures) were obtained. When Npl and R74 were inoculated with the 40 cultures, avirulent and virulent cultures segregated in a 1:1 ratio on both cultivars, as expected (Table 7). When the segregation patterns on Npl and R74 were combined, recombinants (ApVq and VpAq) were observed but, again, much fewer than those expected from independent inheritance (Table 8). These results suggest that *AVR-Xo* and *AVR-Yo* are linked.

Finally, 0R59 (*MAT1-2*) was crossed with 49N1 (*MAT1-1*). In this cross, one ascospore was arbitrarily chosen from ascospores in each ascus and isolated. The resulting hybrid population was composed of 59 random cultures derived from 59 asci (59 meiotic events) (Fig. 2). When Npl and R74 were inoculated with the 59 cultures, no virulent cultures were observed (Table 7). This result suggests that the hypothesis is correct: *AVR-Xs* and *AVR-Xo* are the same gene while *AVR-Ys* and *AVR-Yo* are the same gene. Here, we designate *AVR-Xs/Xo* and *AVR-Ys/Yo* as *PBY3* and *PBY4*, respectively.

Molecular mapping of the *Rmo2* locus. To map the *Rmo2* locus, 91 F₂ plants derived from the cross NZ × NgT were grown in a field and selfed. From each of the resulting 91 F₃ lines, a portion (20 seeds) was retrieved and used for inoculation with O-23IN. All resistant, segregating, and susceptible F₃ lines were 23, 50, and 18, fitting a 1:2:1 ratio ($\chi^2 = 1.44$, $0.1 < P < 0.5$), as expected. Based on these F₃ reactions, genotypes of the F₂ plants at the *Rmo2* locus were determined.

Another portion (20 seeds) was retrieved from each of the 91 F₃ lines, grown for 7 days, and pooled for DNA extraction. Selected SSR loci (see Materials and Methods) were amplified from these DNA samples. Genotypes of the F₂ plants at each SSR locus were determined based on banding patterns of the amplicons on polyacrylamide gel electrophoresis gels. When these molecular data were combined with the inoculation data, the *Rmo2* locus was mapped on the chromosome 7H (Fig. 3).

DISCUSSION

Inukai et al. (11) identified a gene for resistance to an *Oryza* isolate (Ken54-20) of *M. oryzae* in a barley cultivar, and designated it as *Rmo1*. *Rmo1* was located on the 1H chromosome. *Rmo2*, a locus for resistance to *M. oryzae* identified in the present study, is apparently distinct from *Rmo1* because *Rmo2* is located on the 7H chromosome. *Rmo3*, a locus for resistance to *M. grisea*, should also be distinct from *Rmo1* because *Rmo3* is linked to *Rmo2*.

The resistance of the five barley cultivars to the four isolates of *M. oryzae* was all attributed to the *Rmo2* locus. On the other hand, the resistance of those cultivars to another species, *M. grisea*, was conditioned by another locus, *Rmo3*. These results suggest that *Rmo2* is effective against a broad range of blast isolates but is specific to *M. oryzae*. Interestingly, the *Rmo2* genes in those cultivars were effective against different ranges of *M. oryzae* isolates (or subgroups). The segregation analyses of pathogenicity revealed that this is because the *Rmo2* locus in each cultivar recognizes a different range of avirulence genes. Here, we assume that each cultivar carries an allele at the *Rmo2* locus. Interactions of these alleles with the avirulence genes are summarized in Table 9. The allele in H4 and NZ corresponds to *PWT1* alone, and is designated as *Rmo2.a*. The allele in Npl corresponds to *PWT1* and *PBY3*, and is designated as *Rmo2.b*. The allele in R74 corresponds to *PWT1*, *PBY4*, and *PBY1*, and is designated as *Rmo2.c*. The allele in R81 corresponds to at least *PBY1* and *PBY2*, and is designated as *Rmo2.d*. With respect to the interaction between *Rmo2.c* and *PWT1*, some data were difficult to interpret: the F₁ population derived from O-23 × T-4B contained one exceptional culture that was avirulent on H4 and virulent on R74. Further research is necessary to test this unique culture.

Chuma et al. (3) showed that *PWT1* was recognized by H4, NZ, and Npl using 63 BC₁F₁ cultures isolated randomly. The present study showed that the recognition was conditioned by *Rmo2.a* in H4/NZ and *Rmo2.b* in Npl. It should be noted that *Rmo2.a* corresponded to *PWT1* in a gene-for-gene manner in barley-*M. oryzae* interactions. *PWT1* was first identified as a gene con-

TABLE 8. Combined segregation of virulence on two barley cultivars in the populations derived from crosses involving *AVR-X* and *AVR-Y* carriers

Cross	Cultivar		Number of progeny cultures ^a				Total	χ^2 for 1:1:1:1 ^b
	p	q	A _p A _q	A _p V _q	V _p A _q	V _p V _q		
0R59 × T-4B	Npl	R74	18	2	2	17	39	24.69**
49N1 × T-7B	Npl	R74	17	3	4	16	40	17.00**

^a A = avirulent (infection type 0 to 3BG) and V = virulent (infection type 3G to 5G). For example, A_pV_q represents cultures which are avirulent on p and virulent on q.

^b Asterisks (**) indicate significant at the 1% level.

TABLE 9. Interactions between alleles at the *Rmo2* locus in barley cultivars and avirulence genes in *Magnaporthe oryzae*

Allele	Carrier	Interaction with genes ^a				
		<i>PWT1</i> (Si-1J, O-23IN)	<i>PBY3</i> (Si-1J, O-23IN)	<i>PBY4</i> (Si-1J, O-23IN)	<i>PBY1</i> (Ec-3J)	<i>PBY2</i> (T-4B)
<i>Rmo2.a</i>	H4, NZ	+	—	—	—	—
<i>Rmo2.b</i>	Npl	+	+	—	—	—
<i>Rmo2.c</i>	R74	+	—	+	+	—
<i>Rmo2.d</i>	R81	ND	ND	ND	+	+

^a Avirulence genes are in italics and carriers of the avirulence gene are in parentheses; + = yes, — = no, and ND = not determined.

trolling the avirulence of Si-1J on wheat cultivars (16). To identify *Rwt1* (a hypothetical resistance gene corresponding to *PWT1*), Chuma et al. (3) screened common wheat cultivars for a non-carrier of *Rwt1* using a BC₃F₁ population in which *PWT1* segregated. Unfortunately, all common wheat cultivars tested recognized *PWT1* and, therefore, were presumed to be *Rwt1* carriers. In the present study, we found a barley cultivar (Ngt) that did not recognize *PWT1*, which made it possible to identify *Rmo2.a* corresponding to *PWT1* in a gene-for-gene manner. *Rmo2* might possibly be a barley ortholog of *Rwt1*.

Interestingly, *Rmo2.b*, *Rmo2.c*, and *Rmo2.d* corresponded to more than one avirulence gene. If each of these alleles is actually composed of a single gene, it is a deviation from the gene-for-gene theory (6). Interactions between one resistance gene and more than one avirulence gene have been reported in bacterial (2,5,7,14) and oomycete (8) systems. Alternatively, each of the alleles at the *Rmo2* locus may be composed of more than one gene at the molecular level. To clone the *Rmo2* locus, a fine mapping is under way.

The identification of *Rmo2* and *Rmo3* is primarily attributable to the finding of the universal susceptible, Ngt. However, it raises the question of why Ngt is so susceptible to blast isolates. When Ngt is grown in a field, its heading is much earlier than that of the other barley cultivars. Due to this trait, Ngt may be harvested before *Magnaporthe* isolates start their infection cycles in natural fields. It should be noted that, when incubated at lower temperature (<20°C) after inoculation with Si-1J or O-23IN, Ngt sometimes produces pale brown flecks. Ngt might have had the *Rmo2* gene but have gradually lost its function through accumulation of mutations because its function is unnecessary due to the ecological escape.

Fig. 3. Linkage map of the 7H chromosome constructed using an F₂ population derived from the cross ‘Nakaizumi-zairai’ (NZ) × ‘Nigrate’ (Ngt). Genotypes of the F₂ plants were determined using F₃ seedlings.

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