Identification and Isolation of Blast Resistance Genes in Three *indica*-type Rice Varieties

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Summary

Two methods were used to identify blast resistance genes in *indica*-type varieties and isolate the genes found: one was an inoculation of F₃ lines between *indica*-type and *japonica* varieties (Suweon 262 × Reimei) with a common fungus strain (Ken 54-20) and a fungus strain having many virulence genes (Ao 14-20) and selection of F₃ lines showing different reactions to the two fungus strains, and the other was selection of F₃ lines showing a 3 : 1 segregation ratio in the hybrid (Norin 29 × Hokuriku 129 and Norin 22 × Sifeng 43) for Ken 54-20. The former method was used to identify and to select the resistance gene breakdown by Ao 14-20 having several number of virulence genes. The latter was to identify and isolate all major genes included in the resistant variety. It was demonstrated that Suweon 262 had *Pi-i* and an unidentified major gene and at least a minor one, and Hokuriku 129 had two major genes, *Pi-i* and *Pi-b*; Sifeng 43 had one major gene *Pi-b* and at least one minor gene. Minor genes disturbed the isolation of major genes in cross combinations of Suweon 262 × Reimei and Norin 29 × Hokuriku 129. It was considered that Hokuriku 129 is heterogeneous at least for *Pi-i* and *Pi-b*.

Key Words : rice blast resistance, inheritance, *indica*, Hokuriku 129, Suweon 262, Sifeng 43, cumulative frequency distribution curve.

Introduction

To make strategies for using efficiently resistance genes to disease, genetic studies of resistance are essential. In rice, gene analysis for blast resistance was completed for many of Japanese lowland rice varieties. There are many problems in genetic analysis of rice varieties in countries other than Japan. Especially, it is considered that *indica* rice varieties have many genes for blast resistance, but have not yet been analyzed, except for some varieties. Some *indica* varieties have at least three or more resistance genes, but their identification is very difficult. To date, genes, *Pi-a*, *Pi-i*, *Pi-k^h*, *Pi-k^p*, *Pi-z*, *Pi-z^t*, *Pi-ta*, *Pi-ta²*, *Pi-b*, and *Pi-t* in

varieties other than *japonica* have been identified (Kiyosawa 1981). In these cases, these genes were identified after isolation of single genes was carried out. Many of them were isolated by backcross breeding to introduce resistance from *indica* varieties in Japanese varieties and then identified by gene analyses. In them, *Pi-ta*, *Pi-ta²*, *Pi-b*, *Pi-t* and *Pi-z^t* were introduced from *indica* varieties and *Pi-z* from *javanica* variety. Recently, Inukai *et al.* (1994) found four resistance genes [*Pi-1* (*t*), *Pi-2* (*t*), *Pi-3* (*t*) and *Pi-4* (*t*)] in near-isogenic lines of *indica* varieties for blast resistance in IRRI. Out of them, at least two [*Pi-1* (*t*) and *Pi-3* (*t*)] are considered to be new genes.

Attempts to isolate single genes from *indica*-type varieties were carried out in some tests. The genes, *Pi-k^p* and *Pi-k^h*, were identified in such attempts (Kiyosawa 1969, Kiyosawa and Murty 1969). However, these genes are epistatic genes in original varieties. Attempts to isolate hypostatic or all genes included in original varieties failed. As for methods in which all major genes including hypostatic genes can be isolated, two have been proposed. One is selection of resistant plants in lines showing a 3 : 1 segregation in F₃ generation from a hybrid of a susceptible variety and a resistant variety. This method was already used for isolating a gene *Pi-t* from BL 7 (Kiyosawa 1972). The other is selection of resistant plants in 1 : 1 segregating lines derived from B₁F₂ plants obtained by backcrossing the F₁ plant between the susceptible variety and resistant variety to the susceptible variety (Toriyama *et al.* 1983).

In this paper, attempts of isolation of single blast resistance genes from *indica*-type and *indica* varieties, Suweon 262, Hokuriku 129 and Sifeng 43, will be reported. It has been known that Hokuriku 129 and Sifeng 43 have two and one major genes, respectively (Purba *et al.* 1994).

Materials and Methods

Three *indica*-type and *indica* varieties, Suweon 262, Hokuriku 129 and Sifeng 43, were used for analyses. Genealogical relations of Suweon 262 and Hokuriku 129 are shown in Fig. 1 (E. J. Lee, personal communication, Kobayashi *et al.* 1990). These F₃ lines of Suweon 262 × Reimei and Norin 29 × Hokuriku 129 were prepared at

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Fig. 1. Genealogical relationships of Suweon 262 and Hokuriku 129.

the National Agriculture Research Center and Hokuriku National Agricultural Experiment Station, respectively. Gene analyses of the two materials were begun in 1984 and 1989, respectively. Crossing and F_3 tests of Norin 22 $\times$ Sifeng 43 were carried out in 1986 and 1989, respectively.

These studies were carried out at the National Institute of Agrobiological Resources during 1984 to 1989, at the Tsukuba Research Laboratories, Kyowa Hakko Co. during 1989 to 1992, and at the Tsukuba International Agricultural Training Centre during 1992 to 1994. F_2 tests were repeated twice or thrice in the final two years. The number of F_2 plants in each test was at least 2720 for one cross combination and the number of F_2 families derived from the different F_1 seeds in the same combination of varieties was three to six in many cases.

Immune plants and plants having b type lesions (brown spot) were classified as resistant (r) and plants with bg type lesions (small gray lesion with brown margin) as moderately resistant (m), and plants having bG (large gray lesion with brown margin) and pG type lesions (large gray lesion with purple or no margin) as susceptible (s) by the method of Kiyosawa (1974). Genetic analysis in F_3 generation was carried out by cumulative frequency distribution curve method (Kiyosawa 1970, 1974, 1976).

Results and Discussion

Identification of known genes in Hokuriku 129 by crossing with differential varieties

Hokuriku 129 was crossed with varieties having known resistance genes (Fig. 2): Aichi Asahi ($Pi-a$), Inabawase ($Pi-i$), Kusabue ($Pi-k$), Tsuyuake ($Pi-k^m$), Fukunishiki ($Pi-z$), Toride 1 ($Pi-z^t$), Yashiromochi ($Pi-ta$), Pi No. 4 ($Pi-ta^2$), BL 1 ($Pi-b$) and K 59 ($Pi-t$) (Kiyosawa 1984).

The hybrid of Hokuriku 129 with BL 1 having the $Pi-b$ gene showed no segregation. This means that Hokuriku 129 has at least one resistance gene $Pi-b$ or its resistance allele.

A 15 : 1 segregation ratio was found in hybrids of Hokuriku 129 with Inabawase (having $Pi-i$), Kusabue

Fig. 2. Selection from F_3 of Suweon 262 $\times$ Reimei.

($Pi-k$), Fukunishiki ($Pi-z$) and K 59 ($Pi-t$). This indicates that Hokuriku 129 has only one gene other than these three genes. The ratio 63 : 1 was observed in hybrids of Hokuriku 129 with Tsuyuake ($Pi-k^m$), Yashiromochi ($Pi-ta$), Pi No. 4 ($Pi-ta^2$) and Toride 1 ($Pi-z^t$). Pi No. 4 and Yashiromochi have been known to have their genes in the same locus, $Pi-ta$. Accordingly, a 63 : 1 ratio can be expected only when there is a close linkage in repulsion between the gene in Hokuriku 129 and $Pi-ta$ locus. However, Tsuyuake and Kusabue, and Fukunishiki and Toride 1 which have allelic resistance genes in the same loci $Pi-k$ and $Pi-z$, respectively, have different segregations in the hybrids with Hokuriku 129. This cannot be expected when Hokuriku 129 is a homogeneous variety for blast resistance. Therefore, Hokuriku 129 is considered to be heterogeneous in a few genes: plants which were crossed with Kusabue and Fukunishiki have single resistance genes and plants which were crossed with Tsuyuake and Yashiromochi have two genes.

The hybrids of Hokuriku 129 with Aichi Asahi and Yashiromochi showed 1 : 0 and 63 : 1 segregations with Ina 72 or Ina 168 which are avirulent to $Pi-a$ varieties. This means that Hokuriku 129 has $Pi-a$ and another gene other than $Pi-ta$.

These facts suggest that Hokuriku 129 is heterogeneous for blast resistance.

Selection from hybrid families

Resistance of Suweon 262, Reimei, Hokuriku 129 and Norin 29 to 11 fungus strains is shown in Table 1. It has been known that Sifeng 43 shows high resistance to all of Japanese fungus strains used and susceptibility to all Philippine fungus strains used (Kiyosawa and Ling 1984). The *indica*-type and *indica* varieties show generally high resistance to all Japanese fungus strains used.

Suweon 262

For Suweon 262 $\times$ Reimei, each of 58 F_3 lines was divided into two, and one was inoculated with Ken 54-20

Table 1. Reaction of parents to 11 fungus strains

Variety	Fungus strain (race number)									
	P-2b	Ken	Ina 72	Hoku 1	Ken	231-1	Kaminoho	24-11-1-1	Niigatawase	Myojin
	(303.1)	53-33 (137.1)	(031.0)	(007.0)	54-20 (003.0)	(033.3)	33 (301.0)	(173.3)	2-18 (047.0)	D 27 (073.1)
Suweon 262	R ^h	R ^h	R ^h	R ^h	R ^h	R	R ^h	MR	R	R ^h
Reimei	S	S	R ^h	MS	S	S	R ^h	S	S	S
Norin 29	S	S	S	S	S	S	S	S	S	S
Hokuriku 129	R	R ^h	R ^h	R ^h	R ^h	R	R ^h	R	R	R ^h

Inoculated at 5.9 plant stage on 19 May.

Fig. 3. Selection from F₃ of Norin 29 × Hokuriku 129.

(race number 003.0) and the other with Ao 14-20 (337.3). Difference in segregations for two fungus strains (Fig. 3) indicates that Suweon 262 has one gene of *Pi-a*, *Pi-i*, *Pi-k*, *Pi-ta* and *Pi-b* loci. To identify and isolate this gene, resistant plants were individually selected from some lines showing different segregations for the two fungus strains. Finally, three lines were selected from them. Individual selection of resistant plants was continued over four years to get homozygous plants and homogeneous lines for blast resistance (Fig. 3). Line A was selected to identify a resistance gene which controls different responses to both fungus strains Ken 54-20 and Ao 14-20. Line B was for getting line resistant to Ken 54-20 and susceptible to Ao 14-20. Line C was selected under the expectation that a gene differing from those expected in Lines A and B might be obtained, as reaction to Ao 14-20 differs from Line A.

Hokuriku 129 and Sifeng 43.

For each of Norin 29 × Hokuriku 129 and Norin 22 × Sifeng 43, 116 F₃ lines were used for one test with two parents which were distributed in two of twelve boxes used.

To isolate major genes which have been found in Hokuriku 129 and Sifeng 43 (Purba *et al.* 1994), resistant plants were individually selected from lines showing a 3 : 1 segregation ratio in F₃ generation for three years as shown in Figs. 4 and 5, from Norin 29 ×

Fig. 4. Selection from F₃ of Norin 29 × Sifeng 43.

Fig. 5. Segregations in F₂ between Hokuriku 129 and differential varieties inoculated with Ken 54-20.

Hokuriku 129 and Norin 22 × Sifeng 43.

Identification of *Pi-a* gene in derivatives from hybrids of Suweon 262, Hokuriku 129 and Sifeng 43

Derivatives from the families of Suweon 262 × Reimei, Norin 29 × Hokuriku 129 and Norin 22 × Sifeng 43 were crossed with Aichi Asahi having *Pi-a*. Their F₂ populations were inoculated with Ina 168 avirulent to *Pi-a*. F₂ populations showing no segregations were not found (Fig. 6). In them, eleven and five families derived from the hybrids of Suweon 262 × Reimei and of Norin 29 × Hokuriku 129 were included, respectively. This indicates that *Pi-a* was not present in these families.

Derivatives from Suweon 262 × Reimei did not show the presence of *Pi-a* in eleven families derived from single F₁ seeds. Since Reimei has *Pi-a* gene (Kiyosawa 1967; Shimoyama *et al.* 1972), if Suweon 262 has *Pi-a*, all plants should have *Pi-a* in the hybrid with Reimei.

The presence of *Pi-a* in Hokuriku 129 cannot be determined by this analysis, because we cannot eliminate the possibility that the *Pi-a* gene in Hokuriku 129 might be removed at the selection of these five derivatives from the hybrid of Hokuriku 129 $\times$ Norin 29. In derivatives from a hybrid of Norin 22 $\times$ Sifeng 43, only one hybrid was obtained with Aichi Asahi (*Pi-a*). Although a 15 : 1 segregation ratio was observed, the presence of *Pi-a* in Sifeng 43 could not be determined.

The derivatives were crossed with differential varieties for pathogenicity test of blast fungus and other derivatives. The number of obtained cross combinations was 236. F₂ populations obtained were tested with Ken 54-20 to see segregation for blast resistance.

Derivatives from Suweon 262 $\times$ Reimei were crossed with differential varieties having different resistance genes. F₂ populations were inoculated with Ken 54-20. These results are shown in Fig. 7. The ratio of 1 : 0 was observed in cross combinations of Fujisaka 5 with 29 K 107, 29 K 33, 29 K 41 and 29 K 77, 63 : 1 segregations were frequently obtained in some combinations. This indicates that at least some of these derivatives have the *Pi-i* gene or its allele, and some have two resistance genes.

The derivatives from a hybrid of Norin 29 and Hokuriku 129 were crossed with differential varieties. The results of F₂ analyses (Fig. 8) show that some derivatives (30 K 5, 30 K 6 and 30 K 8) have the *Pi-b* gene or its resistance allele, and some plants in 30 K 3 and 30 K 4 have no *Pi-i* gene or its resistance allele.

Five derivatives of the hybrid of Norin 22 $\times$ Sifeng 43

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were crossed with differential varieties. The results of these F₂ analyses are also shown in Fig. 8. In only one derivative 30 K 12 tested, *Pi-b* or its resistance allele was found.

Relationship among derivatives from Suweon 262, Hokuriku 129 and Sifeng 43 hybrids

In Fig. 9, relationships among derivatives from Suweon 262, Hokuriku 129 and Sifeng 43 hybrids are shown. There are some discrepancies: 1 : 0 segregation ratios are found between 29 K 107 and 30 K 4, and between 29 K 107 and 29 K 41, but not between 30 K 4 and 29 K 41. Similar discrepancies are found among 29 K 107, 30 K 3 and 29 K 33. These discrepancies can be explained by the assumption that 30 K 3 and 30 K 4 are heterogeneous as shown in Tables 2 and 3, and that some

plants in both lines have the *Pi-i*.

Test for resistance of derivatives and gene analyses

In the tests of F₂ populations above, the existence of *Pi-i* or *Pi-b* or these resistance alleles in some lines was suggested. However, these analyses do not always indicate that these genes are the same genes as *Pi-i* or *Pi-b*. To ascertain these relationships, fungus strains overcoming *Pi-b* or *Pi-i*, 15-11-1 (103.2) and 83-7 (007.0), were inoculated to these derivatives with fungus strain Ken 54-20 which is avirulent to both genes (Tables 2 and 3).

If derivatives mentioned above have *Pi-i* or *Pi-b*, it is expected that these lines are attacked by one or two (15-11-2 and 83-7) of three fungus strains used. The results of the tests were more or less different from

Table 2. Segregation of r, m and s plants in lines selected from Suweon 262 × Reimei and Norin 29 × Hokuriku 129 to four blast fungus strains (1), (2)

Line	Fungus strain (1)			(2)	Gene (<i>Pi</i> —) presumed from reaction
	Ken 54 - 20 (003.0)	15 - 11 - 2 (103.2)	83 - 7 (007.0)	Ao 14 - 2 (337.3)	
29 K 1	15, 0, 0 (r, m, s)	16, 0, 0	14, 0, 0	9, 4, 5	x ; i, x ; U, x
29 K 2	17, 0, 0	16, 0, 0	17, 0, 0	12, 2, 3	x ; i, x ; U, x
29 K 3	17, 0, 0	16, 0, 0	13, 3, 1 ^a	12, 2, 1	i, x
29 K 4	17, 0, 0	17, 0, 0	9, 6, 2 ^a	15, 0, 2	i, x
29 K 5	17, 0, 0	17, 0, 0	5, 12, 0	10, 4, 5	i, x
29 K 6	15, 1, 0	17, 0, 0	9, 8, 0	8, 0, 7	i, x
29 K 7	17, 0, 0	16, 0, 0	9, 7, 1 ^a	11, 3, 3	i, x
29 K 10	14, 0, 0	15, 0, 0	8, 3, 3 ^a	14, 0, 2	i, x
29 K 14	17, 0, 0	14, 0, 0	11, 3, 2 ^a	6, 0, 10	i, x
29 K 17	14, 0, 0	15, 0, 0	10, 6, 0 ^a	6, 0, 11	i, x
29 K 33	14, 0, 0	16, 0, 0	13, 2, 0 ^a	3, 0, 14	i, x
29 K 34	13, 3, 1	14, 0, 0	6, 9, 1	10, 0, 7	i, x
29 K 35	15, 0, 0	12, 0, 0	9, 5, 3 ^a	9, 2, 6	i, x
29 K 36	14, 0, 0	12, 0, 0	11, 2, 0 ^a	4, 0, 13	
29 K 37	14, 3, 0	17, 0, 0	6, 11, 0	3, 0, 13	i, x
29 K 39	15, 0, 1	14, 0, 0	2, 7, 3	8, 0, 9	i, x
29 K 40	15, 0, 0	14, 0, 0	16, 1, 0 ^a	7, 0, 11	i, x
29 K 41	16, 1, 0	17, 0, 0	11, 5, 1 ^a	13, 0, 4	i, x
29 K 42	16, 0, 0	16, 0, 0	10, 5, 1 ^a	11, 4, 2	i, x
29 K 43	15, 0, 0	17, 0, 0	9, 6, 1 ^a	6, 1, 6	i, x
29 K 44	16, 0, 0	15, 0, 0	0, 11, 4	8, 0, 9	i, x
29 K 48	10, 0, 0	13, 0, 0	14, 2, 0 ^a	15, 0, 1	
29 K 54	16, 0, 0	17, 0, 0	15, 2, 0 ^a	11, 1, 2	
30 K 1	1, 10, 6	10, 0, 7	2, 1, 14	10, 0, 5	
30 K 2	16, 0, 0	7, 2, 7 ^b	14, 1, 0	9, 1, 1	b, y
30 K 3	7, 3, 6	10, 0, 6	5, 1, 11	10, 0, 0	i, x
30 K 4	10, 2, 3	14, 1, 1	4, 1, 7	14, 0, 1	i, x
30 K 5	17, 0, 0	8, 1, 4 ^b	16, 0, 0	4, 1, 9	b, y
30 K 6	17, 0, 0	6, 2, 9 ^b	17, 0, 0	7, 1, 7	b, y
30 K 7	16, 0, 0	12, 0, 5 ^b	17, 0, 0	3, 0, 9	b, y

(1) Inoculated at plant stage 4.1 on 1 December.

(2) Inoculated at plant stage 6.1 on 23 December.

Both were sown on the same date.

a : Mean of frequencies of r plant in lines marked by a was used as functional value of genotype ++++CC in Fig. 12.

b : Mean of frequencies of (r+m) plants in line marked by b was used as functional value of genotype ++++C+ in Fig. 13.

x and y mean minor genes in 29 K lines 30 K lines, respectively.

y is unknown major gene.

Table 3. Reaction patterns of lines selected from Suweon 262 × Reimei and Norin 29 × Hokuriku 129 to three blast fungus strains (3)

Line	Fungus strain			Gene (<i>Pi</i> —) presumed from reaction
	Ken 54-20 (003.0)	15-11-2 (103.2)	83-7 (007.0)	
29 K 57	17,0,0(r, m, s)	16,0,0	12,4,1 ^a	<i>i</i> , <i>x</i>
29 K 58	17,0,0	12,0,0	11,2,2 ^a	<i>i</i> , <i>x</i>
29 K 59	17,0,0	14,0,0	11,2,1 ^a	<i>i</i> , <i>x</i>
29 K 61	11,1,0	10,0,0	11,2,0 ^a	
29 K 79	17,0,0	12,0,0	8,6,0 ^a	<i>i</i>
29 K 103	16,0,0	17,0,0	11,3,1 ^a	<i>i</i>
29 K 107	16,0,0	16,0,0	17,0,0	<i>x</i> ; <i>i</i> , <i>x</i> ; <i>U</i> , <i>x</i>
30 K 9	12,1,0	17,0,0	9,3,4	<i>i</i> , <i>y</i>
30 K 10	14,0,0	5,0,0	11,0,0	<i>y</i> or <i>b</i> , <i>y</i>
30 K 11	12,0,0	5,3,2	15,0,0	<i>b</i>
30 K 12	17,0,0	13,0,3	17,0,0	<i>b</i> , <i>y</i>
30 K 15	13,0,2	1,0,3	3,3,7	<i>b</i> or <i>i</i>
30 K 16	11,1,0	3,0,7	11,0,0	<i>b</i>
Shin 2	0,0,17	0,0,15	0,0,17	<i>sh</i>
Aichi Asahi	0,2,15	1,0,15	1,0,16	<i>a</i>
Fujisaka 5	16,1,0	17,0,0	0,2,14	<i>i</i>
Kusabue	16,0,0	17,0,0	17,0,0	<i>k</i>
Tsuyuaake	16,0,0	15,0,0	16,0,0	<i>k^m</i>
Fukunishiki	17,0,0	14,1,0	8,5,2	<i>z</i>
K 1	16,0,0	17,0,0	16,1,0	<i>ta</i>
Pi No.4	17,0,0	17,0,0	17,0,0	<i>td²</i>
Toride 1	17,0,0	17,0,0	17,0,0	<i>z¹</i>
K 60	17,0,0	17,0,0	15,0,0	<i>k^p</i>
BL 1	17,0,0	3,0,12	17,0,0	<i>b</i>
K 59	11,3,3	16,0,1	9,4,3	<i>t</i>
Suweon 262	17,0,0	16,0,1	15,0,0	
Hokuriku 129	16,0,0	5,0,11	14,0,2	

Inoculated at the plant stage 5.1 on 20 December.
(See Table 2 for explanation)

our expectation.

Suweon 262

For example, 29 K 35 shows a 15 : 0 : 0 (*r* : *m* : *s*) segregation ratio or variation for fungus strain Ken 54-20 and 9 : 5 : 3 for 83-7. The segregation or variation in this line for 83-7 cannot be explained by the assumption that this line should be genetically fixed for a major gene. This phenomenon can be explained by considering the presence of two genes : one is the dominant gene *Pi-i*, and the other is an unknown recessive gene. In such a case, the F_3 line should show a 13 : 3 segregation ratio. In this case, we cannot differentiate 3 : 1 from 13 : 3. If this line includes heterozygously two genes, we should get seven resistant genotypes in the following generation: AABB, AAB+, AA ++, A+BB, A +B+, A+++ and +++++. Five of them should show no phenotypic segregation in the following generation. If gene A controls incomplete resistance, AA=*r*, A+=*m*, BB=*r*, B+=++=*s*, and *r* response is epistatic over *m* or *s* response, we get 7 : 6 : 3. This is near the 9 : 5 : 3 above. To verify this assumption, means of frequencies of *r*, *m* and *s* were calculated from lines marked with "a"

Fig. 10. Comparison of observed frequency and cumulative frequency distribution curves (B and D) and expected ones (A, C and E) from one, two and three major dominant genes, respectively.

Fig. 11. Segregation of *r* type plants in F_3 of Suweon 262 × Reimei inoculated with Ken 54-20.
(Inoculated on 19 June at 4.8 plant stage in leaf number)

in Tables 2 and 3. Using mean frequencies obtained, 0.7006, 0.2361 and 0.0633 for *r*, *m* and *s*, frequency and cumulative frequency distribution curves were drawn by Kiyosawa's method (Kiyosawa 1970, 1974, 1976). As shown in Fig. 10, observed distribution curves of *r* type resistance in F_3 of Suweon 262 × Reimei to Ken 54-20 (B in Fig. 10) and Ao 14-20 (D) were at first compared with distribution curves calculated for *r* type resistance which was expected from contributions of one (A), two (C) or three (E) major genes. This figure shows that the

distribution curves (D) obtained by inoculation with Ken 54-20 located between those made from two (C) and three (E) major genes, and one (B) inoculated with Ao 14-20 located between one (A) and two (C) major genes. This suggests that the observed curves (B and D) can be explained by one and two major genes, respectively, with some minor genes. The difference between the two observed curves (B and D) suggests that it corresponds to the difference of one major gene.

For the minor gene, mean frequency of r plants 0.7006 was used for functional values of genotypes, ++ ++ CC, as shown in Fig. 11. The functional value of genotype ++++C+ was selected by calculation in computer to minimize the maximum difference between expected curve and observed curve. After all, expected curves were obtained by giving functional values shown in Fig. 11. The difference between expected and observed curves was not significant. A similar result was obtained for ($r+m$) type resistance when Ken 54-20 was inoculated (figure is not shown).

In the same way, expected curves for segregation of F_3 lines inoculated with Ao 14-20 were drawn in Fig. 12 for r resistance type, with nonsignificant differences. In this case, only one major gene was considered, indicating that another gene in Suweon 262 was broken down by Ao 14-20. A similar result was obtained for ($r+m$) type resistance (figure is not shown). This suggests that one gene in Suweon 262 is one of *Pi-i*, *Pi-k*, *Pi-ta*, or *Pi-b* which control different reactions to two fungus strains inoculated. In Fig. 11, frequency and cumulative frequency distribution curves were drawn by adding a minor gene having functional values 0.7006 for genotype ++++CC and 0.3529 for genotype ++++C+ to two major genes (A and B) having functional values, 1 and 1, respectively. For ($r+m$) resistance (figure is not shown), 0.9376 and 0.7059 were given to ++++CC and ++++C+ with giving 1 to all other genotypes to get frequency and cumulative frequency distribution curves fitting to those for observed curves. After all, frequency and cumulative frequency distribution curves for $r : (m+s)$ and ($r+m$) : s segregations can be explained by assuming two major genes and one minor gene in Suweon 262. One of major genes is *Pi-i*.

In Tables 2 and 3, $r : m : s$ ratio in 29 K 5, 29 K 34 and 29 K 44 differing from the ratio mentioned above is considered to show that these lines are heterogeneous on this minor gene. If the major gene is an important one, we could isolate the major gene from such a heterozygous line for a minor gene.

Hokuriku 129 and Sifeng 43

Eight lines, 30 K 1 to 30 K 9, are derivatives from the hybrid of Norin 29 $\times$ Hokuriku 129. By a similar consideration to that mentioned above, 30 K 1, 30 K 3, and 30 K 9 are considered to have the *Pi-i*. Resistant plants remaining after inoculation of 15-11-2 (race 103.2) are considered to depend upon minor gene(s).

Reactions of four lines, 30 K 2, 30 K 5, 30 K 6 and

Fig. 12. Segregation of r type plants in F_3 of Suweon 262 $\times$ Reimei inoculated with Ao 14-20.

(See Fig. 11 for explanatory note)

Fig. 13. Segregation of r type plants in F_3 of Norin 29 $\times$ Hokuriku 129 inoculated with Ken 54-20.

(See Fig. 11 for explanatory note)

Functional value of genotype ++++CC was the mean of frequencies of r plants in lines marked by b in Table 2.

(Inoculated on 29 June at 6.2 plant stage in leaf number)

30 K 7, to fungus strains Ken 54-20 and 15-11-1 indicate that these lines have the *Pi-b*.

Previously Purba *et al.* (1994) reported that Hokuriku 129 has only two major genes. At that time, Hokuriku 129 showed high resistance (immune type). In the present gene analyses, the same derivatives of Norin 129 $\times$ Hokuriku 129 showed resistance having brown spots (b type lesions) in many cases. Here, the same data of F_3 lines were reanalyzed by the method described in Method. Thus, the presence of two major genes and one minor gene was suggested.

Derivatives from the F_3 lines of Norin 29 $\times$ Hokuriku 129 were tested with three fungus strains at plant stage 4.1 in leaf number counted from incomplete leaf and a fungus strain attacking *Pi-i* and *Pi-b* genes (Ao 14-20) at plant stage 6.1 (Tables 2 and 3). Some lines included plants showing susceptible reaction to strain 15-11-2 attacking the *Pi-b*, and some other lines included plants showing susceptible reactions to strain 83-7 attacking the *Pi-i*. These lines were considered to have the *Pi-b* and *Pi-i* genes, respectively. In these lines, some resist-

ant plants were found. This suggests the presence of a minor gene in these lines.

The same lines were inoculated with Ao 14-20 virulent to both of the *Pi-b* and *Pi-i* at 6.1 plant stage. In almost all lines inoculated, susceptible plants were found, although there were a smaller number of susceptible plants in comparison with the test at 4.1 plant stage. These results show that all these lines have the *Pi-b* or *Pi-i* gene. The increase of resistant plants in comparison with inoculation at 4.1 leaf stage is considered to be due to increase of field resistance by a minor gene with progress of stage.

The reactions of Hokuriku 129 to three fungus strains are shown in Table 3. Hokuriku 129 included susceptible plants to two fungus strains, 15-11-2 and 83-7. For 15-11-2, 11/16 plants showed susceptible reaction. This cannot be expected when Hokuriku 129 has homogeneously two genes *Pi-b* and *Pi-i*. It is thought that for 15-11-2, resistance controlled by *Pi-b* gene was overcome by a virulence gene, *Av-b*⁺ in 15-11-2 and remaining resistant reaction is due to the *Pi-i* gene heterogeneously present or minor gene. Similarly for 83-7, the presence of susceptible plants suggests the heterogeneous presence of the *Pi-b* or minor gene, except the *Pi-i* gene which was overcome. Again evidence that Hokuriku 129 is the mixture of the *Pi-i*⁺ *Pi-b*, *Pi-i* *Pi-b*⁺ and probably *Pi-i* *Pi-b* was shown.

Using the frequencies of *r* and (*r*+*m*) plants in lines marked with *b* in Table 2 as functional values of heterozygous genotype for the minor gene (*C*⁺), frequency and cumulative frequencies were again calculated from original data of *F*₃ of Norin 29 × Hokuriku 129 which were used to get frequency and cumulative frequency distribution curves by Purba *et al.* (1994). The results were drawn in Fig. 13. A good fit was obtained between observed (original data) and expected curves (calculated data based on the assumption above). This fact suggests that there is at least no large discrepancy in the assumption that there is a minor gene having high functional value.

For five derivatives from Sifeng 43, at least two lines (30 K 15 and 30 K 16) indicated to have the gene *Pi-b* with minor gene(s) (Table 3).

General Discussion

These studies were carried out to find a method to identify and isolate new resistance genes in host plants. The number of major resistance genes in varieties, Suweon 262, Hokuriku 129 and Sifeng 43 is two, two and one, respectively. Additionally, at least one minor gene was found in Suweon 262, Hokuriku 129 and Sifeng 43 in this study. The presence of minor genes in Sifeng 43 has never been known (Purba *et al.* 1994).

Identification of resistance genes in these varieties was carried out by two methods, gene analyses by crossing with susceptible varieties and differential varieties (allelic test) and resistance tests with some different

genotypes for virulence. The latter was based on gene-for-gene theory. Based on these tests, the *Pi-i* was identified in Suweon 262 and Hokuriku 129, and the *Pi-b* in Hokuriku 129 and Sifeng 43. Another major gene in Suweon 262 has not yet been identified. Resistance pattern of Hokuriku 129 to several fungus strains with different genotypes for avirulence cannot be explained by only two major genes. The minor gene found in this variety in these analyses is considered to play an important role in a wide range of resistance of Hokuriku 129. As lines demonstrated to have this minor gene showed susceptibility to fungus strains attacking the *Pi-b* or *Pi-i* genes in winter, expression of this minor gene is thought to be very variable.

After all, *Pi-b* and *Pi-i* genes were identified in Hokuriku 129. However, it was shown that this variety is heterogeneous in resistance genes in the tests of resistance with various fungus strains having different virulence patterns. When Hokuriku 129 was crossed with Inabawase (*Pi-i*), segregation of susceptible plants (absence of *Pi-i*) was observed and analyses of derivatives from Norin 29 × Hokuriku 129 showed the presence of *Pi-i* in Hokuriku 129, because the partner of the crosses, Norin 29, has been demonstrated to have no *Pi-i* gene. The fact that two- and three-genic segregations were observed when Hokuriku 129 was directly crossed with differential varieties possessing single genes also suggests the possibility. This means that the genotype for blast resistance of Habataki which was selected from Hokuriku 129 and recently registered cannot be determined without further investigations.

Takanari, which is a sister variety of Hokuriku 129, was analyzed by Yamaguchi *et al.* (1993). They identified two genes, *Pi-a* and *Pi-b*, with two major unknown genes in Takanari. Similarity of gene constitution between both varieties is expected as both are sister varieties. In consideration of heterogeneity of Hokuriku 129 on blast resistance, however, the results in Hokuriku 129 cannot always be applied to gene constitution of Takanari.

The attempt to isolate single genes in resistant varieties was not always successful, although one gene could be removed from three genes in Suweon 262. A recessive minor or major gene with resistance in a wide range disturbed the isolation of single genes in this study. Selection of resistant plants in lines which showed 3 : 1 segregations was used to obtain lines with single genes. A 3 : 1 ratio cannot, however, be differentiated from a 13 : 3 ratio, expected when one dominant gene and one recessive gene are present. Devices to remove minor genes which have a wide range of function or recessive gene are necessary.

In this study, heterogeneity of Hokuriku 129 was shown for blast resistance. Heterogeneity of varieties having plural resistance genes has also been observed in Tongil (Korean variety) (Kiyosawa and Cho 1980; Kiyosawa *et al.* 1983) and Hama Asahi (Kiyosawa *et al.* 1984). In such cases, the homogeneity test by selfing

cannot be available for hypostatic genes. In this study, only one F_1 plant (only one pollen and only one egg cell) was used for the F_3 test. Therefore, we cannot get a general conclusion of gene constitution of Hokuriku 129 when gene isolation is carried out in one F_3 progeny. To come to a general conclusion on gene constitution, F_3 tests from many F_1 's are necessary. When B_1F_2 proposed or used by Toriyama *et al.* (1983) and Yamaguchi *et al.* (1993) are employed, many F_1 's obtained from many plants of a resistant variety are used for back-crossing, and many B_1F_2 's or B_1F_3 's must be separately analyzed.

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