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Genetics of Durable Resistance to Rice Panicle Blast Derived from an Indica Rice Variety Modan

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ABSTRACT

Rice blast resistance of a quantitative nature with a weak expression for leaf blast and a strong one for panicle blast was found in Japanese rice cultivars 'Tsukinohikari' and 'Asanohikari' harboring the *Rice stripe virus* (RSV) resistance gene, *Stvb-i*, derived from an Indica cultivar 'Modan'. These cultivars and their progeny cultivars have not shown any breakdown of resistance to rice panicle blast in farmers' paddy fields located in different areas of Japan for more than 20 years from the dissemination of the cultivars thus far. A novel major gene, *Pb1*, conferred the "panicle blast resistance" in RSV-resistant cultivars. The *Pb1* locus was mapped in the Modan-derived chromosomal region in the middle part of the long arm of chromosome 11. *Pb1* and *Stvb-i* are linked to each other with a recombination value of 5.2 ±1.5%. These two genes had been incorporated into Japanese cultivars from 'Modan'. Based on the linkage and graphical genotyping analyses revealed that RFLP marker S723 was the closest marker to *Pb1* gene among the tested markers. The *Pb1* gene does not confer any complete resistance with hypersensitive reactions, and the protective ability of the gene against rice panicle blast is sufficient for commercial rice production in Japan except in environments highly conducive to the disease. Since *Pb1* is considered to be a gene conferring durable adult resistance, it is useful in rice breeding and is also an important gene in plant protection.

Keywords: adult resistance, disease resistance, DNA marker, durability, *Magnaporthe grisea*, major gene, marker-assisted selection (MAS), *Oryza sativa*, partial (field) resistance, *Pb1*, protective effect, RFLP

Abbreviations: AARC, Aichi Prefectural Agricultural Research Center; **MAS**, marker-assisted selection; **NIL(s)**, Near-isogenic line(s); **RFLP**, restriction fragment length polymorphism; **RIL(s)**, recombinant inbred line(s); **RSV**, *Rice stripe virus*

CONTENTS

INTRODUCTION

Rice blast is a fungal disease caused by *Magnaporthe grisea* (*Pyricularia grisea*). It is one of the most widespread and destructive diseases that afflict rice. The disease is classified into two forms: leaf blast (**Fig. 1A**) and panicle blast (**Fig. 1B**). Leaf blast occurs from infection between the seedling and maximum tillering stages (**Table 1**). Panicle blast occurs after heading (**Table 1**), and directly reduces the number of mature panicles, the weight of individual grains and the quality of brown rice. It consequently causes severe damage under blast-conducive conditions (climatic, nutritional, geographical and epidemic) (Torres and Teng 1993). In Japan, most of the leading rice cultivars (e.g. 'Koshihikari', 'Hitomebore', 'Hinohikari', 'Akitakomachi' and 'Kinuhikari') with excellent eating quality are susceptible to panicle blast (Fujii *et al*. 2005b) and many kinds of fungicide (e.g. carpropamid, ferimzone, fthalide, iprobenfos, isoprothiolane, kasugamycine, probenazole, pyroquilon, tricyclazole) have been applied for commercial rice production to control blast disease. Therefore, growing resistant rice cultivars is one of the most effective measures for controlling this disease and for reducing the use of agricultural chemicals.

TWO TYPES OF BLAST RESISTANCE: COMPLETE RESISTANCE AND PARTIAL RESISTANCE

Many studies in the world clarified that there are two types of blast resistance in rice plants: complete (true) resistance

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Invited Review

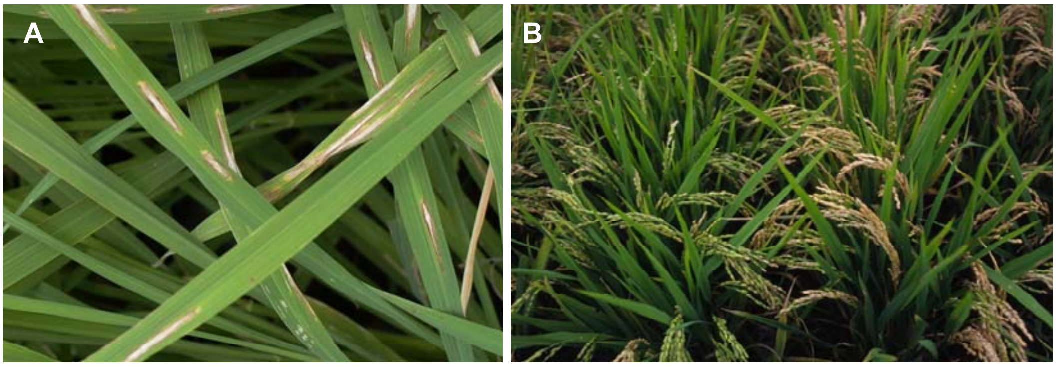

Fig. 1 Typical symptoms of rice blast. Leaf blast with spindle-shaped lesions (**A**) and Panicle blast with neck or branch rot (**B**). NIL of rice with *Pb1* gene confers panicle blast resistance of a quantitative nature is shown on the *left* side and susceptible sib-line without *Pb1* gene is shown on the *right* side (**B**).

Table 1 Growth stages of rice plants in the central areas of Japan.

Growth stage		Blast form	Approximate days from germination		
			Early maturity cultivar[a]	Medium maturity cultivar[b]	Late maturity cultivar[c]
Germination			0	0	0
Seedling or transplanting			25-30	20-25	20-25
Tillering		Leaf	40-45	35-40	35-40
Maximum tillering		blast	70-75	60-65	60-65
Stem elongation			80-85	70-75	65-70
Booting[d]			95-100	85-90	80-85
Heading			115-120	105-110	100-105
Flowering			115-120	105-110	100-105
Ripening	Milk stage	Panicle	125-130	116-121	115-120
	Dough stage	blast	135-140	128-133	130-135
Mature grain			145-150	140-145	145-150

[a] When transplanted in late April.
[b] When transplanted in late May.
[c] When transplanted in middle June.
[d] Beginning with panicle initiation.

and partial (field) resistance. Complete resistance to rice blast is a qualitative nature, and conferred by a major gene. Hence it is comparatively easy to breed rice cultivars harboring complete resistance to blast. However, complete resistance to blast often breaks down within a few years after the dissemination of the rice cultivars with the complete resistance gene (Ujihara *et al*. 1955; Kiyosawa 1972; Ou 1979). This is due to the occurrence of blast fungal race(s) that have adapted to the complete resistance gene (Kiyosawa 1982; Bonman *et al*. 1986). To avoid breakdown of resistance to rice blast, the use of multilines have been studied (Koizumi *et al*. 2004; Kojima *et al*. 2004). Multilines are mixtures of several near-isogenic lines (NILs) with nearly the same genetic backgrounds but with different resistance genes. It has been reported that the use of multilines with different complete resistance genes is effective in controlling blast disease and to prevent breakdown of resistance (van der Plank 1984). However, developing multiline cultivars and producing their seed are more laborious than in ordinary cultivars.

On the other hand, partial resistance is a quantitative nature, and usually conferred by several partial resistance genes (Kiyosawa 1970; Chen *et al*. 2000; Fukuoka *et al*. 2004). Rice cultivars with partial resistance genes often show durable resistance to blast (Bonman and Leung 2004). The durability of resistance is associated with partial resistance that is quantitatively inherited. Hence, it is not easy to incorporate several minor blast resistance genes into one elite rice cultivar by conventional breeding methods.

DISCOVERY OF PANICLE BLAST RESISTANCE IN RSV-RESISTANT RICE CULTIVARS

Genetic studies on panicle blast resistance had been hampered by the inaccuracy and difficulty in evaluating the level of resistance because the disease incidence is not uniform in experiment fields and fluctuate every year. In the plain region of the central areas of Japan, panicle blast is usually not severe. Touyama *et al*. (1993) developed a reliable method to assess the degree of panicle blast resistance in these regions of the central areas of Japan. An outline of the field experiment method is as follows; late transplanting (in late June), heavy fertilizing (0.7 nitrogen kg/a as basal fertilizer, 1.0-1.4 nitrogen kg/a as top dressing), transplanting blast-diseased seedlings in early July in the test paddy field as spreaders, sprinkling water for about an hour every morning, afternoon and evening from late July (e.g. at the end the rainy season) to late September (e.g. maturing stage of tested cultivars/lines) to maintain a moist environment. Panicle blast in the experimental paddy field plots developed sufficiently to evaluate the disease resistance of almost all early to medium maturing rice cultivars/lines under this experimental condition, although high temperatures in August somewhat reduced the disease severity of panicle blast. Consequently, blast resistance of a quantitative nature with a weak expression for leaf blast and strong one for panicle blast, was found in Japanese cultivars 'Tsukinohikari' and 'Asanohikari' harboring the *Rice stripe virus* (RSV) resistance gene, *Stvb-i*, derived from an Indica cultivar 'Modan' (Koumura *et al*. 1985; Fujii *et al*. 1988; Koumura *et al*. 1988; Fujii *et al*. 1999a).

CHARACTERISTICS, DURABILITY AND ORIGIN OF THE PANICLE BLAST RESISTANCE

Fujii *et al*. (1999a) conducted a series of field experiments of panicle blast resistance using the improved method (Touyama *et al*. 1993) in order to analyze the characteristics, durability and origin of the panicle blast resistance of a rice cultivar 'Tsukinohikari' and its two related cultivars. 'Tsukinohikari', a Japonica rice cultivar also resistant to RSV, was developed at Aichi Prefectural Agricultural Research Center (AARC) in Japan in 1985. The experiments had been carried out in Nagakute and Inabu (Toyota) located in the central part of Japan for 11 years from 1984 to 1993 and 1996. The genotype of complete resistance to blast of 'Tsukinohikari', 'Asanohikari' and 'Aichi 67', which are common genotypes in Japan were *Pii*, *Pia Pii* and *Pia/+*, respectively. Races 007 and 037 of the blast fungus were the prevalent races in the test field. Both of these races were virulent to all the cultivars used in the tests of blast resistance. Partial resistance to leaf blast of 'Tsukinohikari', 'Asanohikari' and 'Aichi 67' was comparatively low. 'Tsukinohikari' and 'Aichi 67' were found to exhibit a moderate to intermediate resistance to leaf blast, and 'Asanohikari' an intermediate resistance (Fujii *et al*. 1999a; **Table 2**). On the other hand, in the field, 'Tsukinohikari' and 'Aichi 67' were estimated to be highly resistant or resistant to panicle blast, while 'Asanohikari' were resistant (Fujii *et al*. 1999a; **Table 2**). These results showed that the three rice cultivars expressed a significantly stronger resistance to panicle blast than to leaf blast.

'Tsukinohikari' showed a durable resistance to panicle blast that persisted during an experimental period of 11 years in environments conducive to virulent disease both in

Table 2 Partial (field) resistance to rice blast of Japanese rice cultivar 'Tsukinohikari' and its sister lines[a].

Cultivars and lines	Genotype of complete (true) resistance	Number of susceptible lesions on upper three leaves	Partial resistance to leaf blast[b]	Heading date	Percentage of diseased grains (%)	Partial resistance to panicle blast[c]
Tsukinohikari	*Pii*	1.9	MR[d]	Aug. 29	7.7	HR/R[d]
Asanohikari	*Pia Pii*	2.7	M	Aug. 27	12.3	R
Aichi 67	*Pia*/+	2.0	MR	Aug. 31	6.7	HR/R
Koganenishiki	+	0.9	R	Sep. 6	15.0	R
Akibare	*Pia*	1.1	R	Aug. 30	27.7	MR
Nipponbare	*Pia*/+	2.8	M	Aug. 30	41.3	M
Koganebare	*Pia pii*	4.6	S	Aug. 30	63.3	MS
Wakamizu	*Pii*	4.4	S	Aug. 28	83.3	S
Kijumochi	+	5.1	S/HS	Aug. 30	89.0	S/HS

[a] Average of three year's field test from 1986 to 1988 in Aichi Agricultural Research Center, Aichi, Japan
[b] Partial resistance to leaf blast was estimated from the number of susceptible-type lesions on upper three leaves of each plant of the cultivar/line, as compared with the differential cultivars listed below the dotted line.
[c] Partial resistance to panicle blast of each cultivar/line was estimated from the percentage of blast-diseased grains, as compared with the differential cultivars listed below the dotted line.
[d] HR: highly resistant in a quantitative sense, R: resistant, MR: moderately resistant, M: intermediate, MS: moderately susceptible, S: susceptible, HS: highly susceptible.
(Reprinted from Fujii *et al.* (1999a) *Breeding Research* **1**, 69-76, with kind permission from Japanese Society of Breeding (JSB))

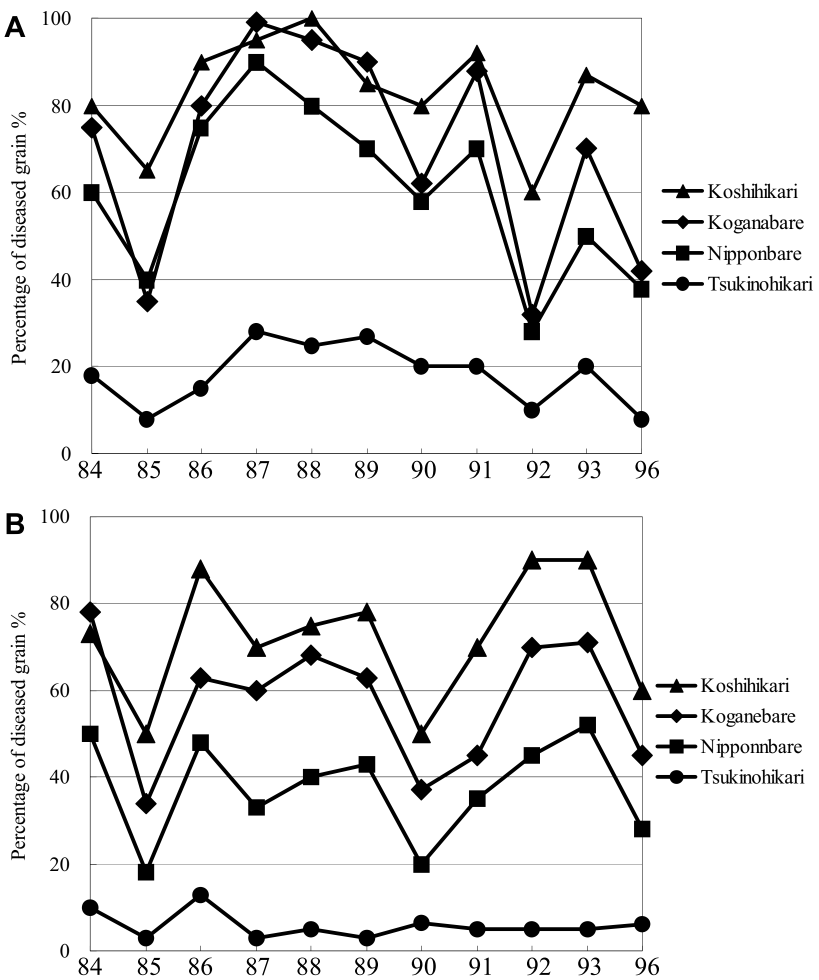

Fig. 2 Fluctuation of the percentage of blast-diseased grains of rice cultivar 'Tsukinohikari' in blast-conducive experimental paddy fields in Inabu (Toyota City) (A) and Nagakute (B), located in semi-mountainous and plain region of the central area of Japan, respectively, for eleven years from 1984 to 1993 and 1996. Judgments of partial (field) resistance to panicle blast of three differential cultivars, 'Koshihikari', 'Koganebare' and 'Nipponbare' are susceptible, moderately susceptible and intermediate, respectively. (Reprinted from Fujii *et al.* (1999a) *Breeding Research* **1**, 69-76, with kind permission from the Japanese Society of Breeding (JSB))

test fields in Nagakute and Inabu (Fujii *et al.* 1999a; **Fig. 2**). Furthermore, these cultivars and all of the progeny cultivars from 'Tsukinohikari' with RSV resistance (e.g. 'Asanohikari', 'Matsuribare', 'Aichinokaori-SBL', 'Akanezora', 'Aoinokaze', 'Daichinokaze', 'Sainokagayaki', 'Goropikari' and 'Koshihikari-Aichi-SBL') have not shown any breakdown of resistance to rice panicle blast in farmers' paddy fields located in different areas of Japan for more than 20 years from the dissemination of the cultivars thus far.

A genealogical analysis of 'Tsukinohikari' showed that among its ancestral cultivars, 'Aoisora', 'Aichi 6', 'St.No.1' and Indica donor variety 'Modan' exhibited strong partial resistance to panicle blast. All of them also were RSV-resistant. Besides, most of the progeny cultivars of 'Tsukinohikari' or 'Aoisora', such as 'Aoinokaze', 'Akanezora', 'Aichi 86', 'Matsuribare', 'Aichi 89' and 'Hoshinohikari' showed high resistance to panicle blast of a quantitative nature. They were also resistant to RSV (Fujii *et al.* 1999a).

GENE ANALYSIS OF THE PANICLE BLAST RESISTANCE OF A QUANTITATIVE NATURE

The mode of inheritance of panicle blast resistance of a quantitative nature in cultivar 'Tsukinohikari' and related cultivar 'Asanohikari' was investigated (Fujii *et al.* 1999b). Both cultivars are also resistant to RSV. The experiments for panicle blast resistance had been carried out using the F_1, F_3 lines and F_4 lines in environments conducive to the occurrence of severe blast disease. The percentage of grains infected with blast in the F_1 plants derived from the cross between the panicle blast resistant cultivar 'Tsukinohikari' and the susceptible breeding line 'Akei-ta 494' was not significantly different from that of 'Tsukinohikari'. Therefore, the panicle blast resistance of a quantitative nature was considered to be a dominant character.

The segregation of panicle blast resistance phenotypes in 119 lines of the F_3 generation derived from the cross between the resistant cultivar 'Asanohikari' and susceptible cultivar 'Koshihikari', fitted the expected ratio of 1:2:1, resistant ('Asanohikari' type), segregating type and susceptible ('Koshihikari' type). Also, the segregation of the panicle blast resistance phenotypes in 60 lines of the F_4 generation derived from the cross between the resistant cultivar 'Tsukinohikari' and susceptible cultivar 'Koganebare', fitted the expected ratio of 3:2:3, resistant ('Tsukinohikari' type), segregating type and susceptible ('Koganebare' type). These results indicated that a novel major gene controls the panicle blast resistance of a quantitative nature. Fujii *et al.* (1999b) designated this novel resistance gene as *Pb1* (**P**anicle **b**last resistance-**1**). This gene symbol was registered in 1999 (Nagato and Yoshimura).

LINKAGE ANALYSIS BETWEEN THE PANICLE BLAST RESISTANCE GENE AND RSV RESISTANCE GENE

Fujii *et al.* (1999b) also performed a linkage analysis between the panicle blast resistance gene *Pb1* and a RSV resistance gene *Stvb-i* using the 119 F_3 lines derived from the cross between cultivar 'Asanohikari' (resistant to both panicle blast and RSV) and cultivar 'Koshihikari' (susceptible to both panicle blast and RSV). The results showed that the two resistance genes are linked to each other with a recombination value of 5.2 ± 1.5%. Linkage analysis using 60 F_4 lines derived from the cross between cultivar 'Tsukinohikari' (resistant to both panicle blast and RSV) and cultivar 'Koganebare' (susceptible to both panicle blast and RSV) confirmed the linkage relationship between the two genes. Besides, among the six full-related lines in the F_7 generation, all derived from the cross between 'Tsukinohikari' and 'Koshihikari', three lines with RSV resistance showed strong partial resistance (R/MR) to panicle blast, while three lines without RSV resistance showed weak resistance (S/MS) to panicle blast. Partial resistance to panicle blast of these lines were judged into seven grades (HR, R, MR, M, MS, S, HS, see **Table 2**) in accordance with percentage of blast-diseased grains of rice plants in the experimental paddy field. These findings indicated the presence of a linkage relationship between the panicle blast resistance gene *Pb1* and the RSV resistance gene *Stvb-i*.

The current method for evaluating panicle blast resistance of a quantitative nature in paddy fields is laborious, and can be applied only once a year. Hence, the breeders at AARC were using RSV resistance as a marker for indirect selection of the panicle blast resistance conferred by *Pb1* gene in the rice-breeding program at AARC (Fujii *et al.* 1993, 1999b). This is because they have developed a reliable mass screening method of RSV resistance, enabling the screening of several thousands entries throughout the year. However, in some cases, RSV-resistant rice cultivars without panicle blast resistance have been developed (Ohya *et al.* 1996; Fujii *et al.* 1999a), because the genetic distance of the two loci is not close enough. Therefore, marker-assisted selection (MAS) (McCouch and Tanksley 1991) using molecular marker(s) tightly linked to the *Pb1* gene should be an accurate method for indirect selection of the panicle blast resistance conferred by *Pb1*.

GENETIC MAPPING OF THE PANICLE BLAST RESISTANCE GENE, *Pb1*, AND IDENTIFICATION OF A RFLP MARKER TIGHTLY LINKED TO THE GENE

The *Stvb-i* gene has been mapped on the long arm of rice chromosome 11 (Hayano-Saito *et al.* 1998). The linkage relationship between *Pb1* and *Stvb-i* suggests that the *Pb1* gene is located near the *Stvb-i* gene on chromosome 11. Fujii *et al.* (2000) precisely mapped the *Pb1* locus for the panicle blast resistance of a quantitative nature on rice chromosome 11 using restriction fragment length polymorphism (RFLP) markers. Based on the cosegregation of the resistant genotypes of *Pb1* and a RSV resistance gene, *Stvb-i*, derived from an Indica variety 'Modan', they examined the linkage relationships between *Pb1* and 13 *Stvb-i*-linked RFLP markers located on the long arm of chromosome 11. As a result, the *Pb1* locus was mapped in the 'Modan'-derived chromosomal region in the middle part of the long arm of chromosome 11. *Pb1* was located on the telomeric side in relation to the *Stvb-i* locus (Fujii *et al.* 2000; **Fig. 3**).

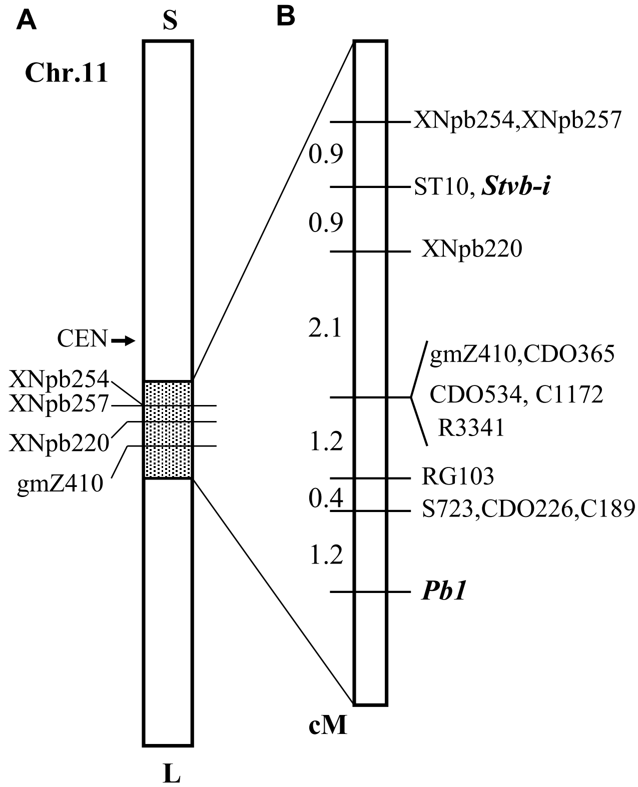

Fig. 3 RFLP map around *Pb1* gene. (**A**) Graphical genotype of rice chromosome 11 in 'Asanohikari' (from Hayano-Saito *et al.* 1998). The *shaded portion* indicates the segment derived from 'Modan', the Indica donor cultivar. CEN: centromere. S and L: short and long arm, respectively. (**B**) Integrated RFLP map of the genomic region around the panicle blast resistance gene, *Pb1*, on rice chromosome 11. The RFLP maker ST10 indicated the *Stvb-i* locus (Hayano-Saito *et al.* 1998). (Reprinted from Fujii *et al.* 2000, *Breeding Science* **50**, 183-188, with kind permission from JSB)

Consequently, the *Pb1* gene was closely located at 1.2 cM from three RFLP markers: S723, CDO226 and C189. Then, they examined the graphical genotypes of 34 'Modan'-derived RSV-resistant cultivars with or without panicle blast resistance, and 12 susceptible Japonica cultivars, using 21 RFLP markers. Among them, cultivars with panicle blast resistance were classified into four types: A, B, C and D, and those without it into six types: E, F, G, H, I and J. In all of the panicle blast-resistant cultivars, the 'Modan'-type bands were observed in S723, CDO226 and C189. On the other hand, in all the panicle blast-susceptible cultivars, the Japonica-type band was observed in S723, whereas the Modan-type bands were noticed in CDO226 and C189 in Type E cultivars. Consequently, only the genotypes of S723 locus completely coincided with the genotypes of the *Pb1* locus. Although, it was determined, based on the linkage analysis, that S723, CDO226 and C189 were located at the same locus, graphical genotyping analysis, using many progeny cultivars derived from 'Modan', revealed that S723

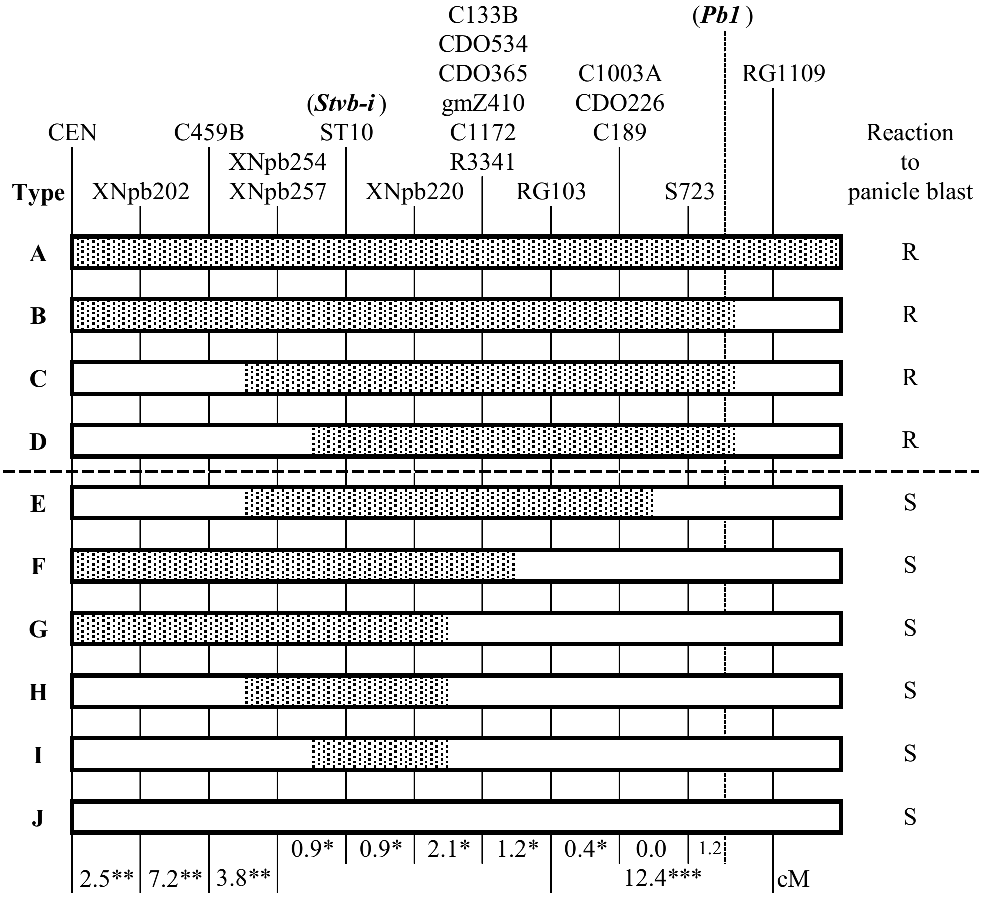

Fig. 4 Schematic representation of ten graphical genotypes of the long arm of rice chromosome 11 among 46 cultivars with or without panicle blast resistance. The designation of the graphical genotypes is listed on the *left* side. The disease reaction to panicle blast is shown on the *right* side: R, resistant in a quantitative sense; S, susceptible in a quantitative sense. Marker designations are placed on *top*, and the genetic distance is given in cM at the bottom: *, from **Fig. 2B** and Hayano-Saito *et al.* (1998); **, from Harushima *et al.* (1998); ***, from Chen *et al.* (1997). The approximate position of *Pb1* is shown schematically. CEN: centromere. The *shaded areas* represent the introgressed segment from the Indica donor cultivar 'Modan'. The *white areas* represent the Japonica regions. (Reprinted from Fujii *et al.* 2000, *Breeding Science* **50**, 183-188, with kind permission from JSB)

(Kurata *et al.* 1994) was the closest marker to *Pb1* gene among the three RFLP markers (Fujii *et al.* 2000; **Fig. 4**).

The linkage and graphical genotyping analyses in this study revealed that the *Pb1* gene had been incorporated into Japanese cultivars with RSV resistance gene, *Stvb-i*, from the Indica cultivar 'Modan'. 'St. No.1' (Toriyama *et al.* 1966) and 'Chugoku 31' (Toriyama *et al.* 1968), which were developed from the same cross ('Modan'/6*'Norin 8') and classified as Type A, are the first Japanese breeding lines to be resistant to both panicle blast and RSV. Most of the advanced RSV-resistant progeny cultivars from 'St. No.1' are also resistant to panicle blast (Fujii *et al.* 1999a) and mostly classified as Type C. While, all of the three RSV-resistant progenies from 'Chugoku 31' are susceptible to panicle blast (Fujii *et al.* 2000) and classified as Types F and G. This genealogical difference in panicle blast resistance can be attributed to the difference in the genotype around the S723 locus associated with *Pb1* gene (Fujii *et al.* 2000; **Fig. 4**).

The rice breeders at AARC confirmed that the S723 probe was useful for MAS of the panicle blast resistance conferred by *Pb1* in a rice breeding program (Izawa *et al.* 2001), and developed a couple of STS markers and a CAPS marker for practical use based on the genomic sequence in and around the cDNA clone S723 (Touyama *et al.* 1998). 'Koshihikari-Aichi-SBL' was the first near-isogenic resistant cultivar of Koshihikari for *Pb1* and *Stvb-i* selected by MAS (Sugiura *et al.* 2004). These molecular markers are currently used to develop rice cultivars with panicle blast resistance of a quantitative nature conferred by *Pb1* and near-isogenic lines for this gene in seventeen rice breeding laboratories located in different areas of Japan. MAS can be very efficient for identifying the *Pb1*, since the expression of the gene is influenced by the environment and is phenotypically difficult to evaluate. Furthermore, in a backcross-breeding program, we can select desired $BCnF_1$ individuals for use for further backcrossing before flowering by MAS. On the other hand, we could not choose them before flowering by the conventional field assay method, because the panicle blast resistance was evaluated at the ripening stage of each plant. We can also identify resistant heterozygous $BCnF_1$ plants accurately using MAS even in the off-season of field assays for panicle blast resistance. These advantages enable backcross cycles to be performed efficiently. In addition, since the conventional evaluation of panicle blast resistance in the field is still laborious, MAS may enable us to solve this problem as well.

QUANTITATIVE EVALUATION OF THE PROTECTIVE EFFECTS OF THE *Pb1* GENE

To evaluate quantitatively the protective effects of the *Pb1* gene, which confers strong partial resistance to rice panicle blast, tests for blast resistance were conducted in paddy fields over a period of two years at two test sites using three pairs of near-isogenic lines (NILs) for the *Pb1* locus selected from about 2000 recombinant inbred lines (RILs) which were developed from a cross between the Japanese rice cultivars 'Koganebare' (susceptible to rice blast without the *Pb1* gene) and 'Tsukinohikari' (resistant to the disease with *Pb1*) (Fujii *et al.* 2005a). A NIL pair of cultivar 'Koshihikari' for *Pb1* and *Stvb-i*, a gene conferring resistance to RSV, was also used for the evaluation, in which the protective effect was given as follows:

Protective effect
= (rate of infected part without *Pb1* - rate of infected part with *Pb1*) / (rate of infected part without *Pb1*) × 100

The protective effect of the *Pb1* gene against rice blast increased with the progression of the growth stages of the rice plants; leaf (vegetative growth) stage < flag leaf stage < panicle (reproductive growth) stage. Therefore, the *Pb1* gene conferred the so-called adult resistance to rice blast. The average protective value of the *Pb1* gene for the percentage of diseased grains was quite high (93) for each year,

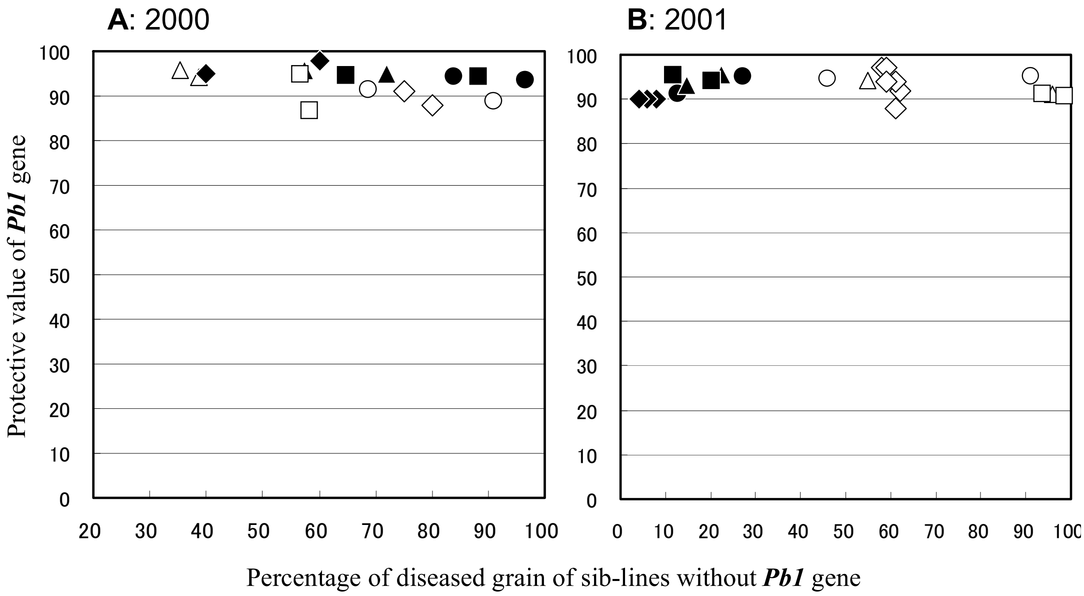

Fig. 5 Distribution of the protective values of *Pb1* gene against rice panicle blast using four near-isogenic line pairs (NILP). (**A**) 2000, (**B**) 2001. Average protective values for 2000 (**A**) and 2001 (**B**) were 93.3+/-1.6 and 93.1+/-1.1, respectively. ●: NILP178-Nagakute, ○: NILP178-Inabu, ▲: NILP422-Nagakute, △: NILP422-Inabu, ■: NILP563-Nagakute, □: NILP563-Inabu, ♦: NILP "Koshihikari"/"Koshihikari Aichi SBL"-Nagakute, ◊: "Koshihikari"/"Koshihikari Aichi SBL"-Inabu. (Reprinted from Fujii *et al.* (2005) *Breeding Research* **7**, 75-85, with kind permission from JSB)

Table 3 Comparison of rice grain yield and its components between NIL pairs with and without *Pb1* gene under blast-conducive environments[a].

Year	Test site	Climatic environment	Comparison between NIL pairs	Winnowed rough rice yield[b]	Head brown rice ratio[c]	Head brown rice yield[b]	1000- kernel weight (g)	Protein content of brown rice[d]
2000	Nagakute	Conducive to blast	NILP-*Pb1*[e]	650	86.5	469	21.2	9.8
			NILP-+[f]	283	59.3	138	20.2	10.3
			Significance	*P*<0.001	*P*<0.01	*P*<0.001	*P*<0.05	*P*<0.01
			NIL-*Pb1*/NIL-+	2.30	1.46	3.40	1.05	0.95
	Inabu	Conducive to blast	NILP-*Pb1*	629	81.7	429	20.1	7.7
			NILP-+	261	47.9	106	18.8	8.1
			Significance	*P*<0.01	*P*<0.01	*P*<0.01	*P*<0.01	*P*<0.05
			NIL-*Pb1*/NIL-+	2.41	1.71	4.07	1.07	0.94
2001	Nagakute	Less conducive to blast	NILP-*Pb1*	653	95.8	513	21.9	9.2
			NILP-+	649	93.3	489	21.7	9.1
			Significance	N. S.	N. S.	N. S.	N. S.	N. S.
			NIL-*Pb1*/NIL-+	1.01	1.03	1.05	1.01	1.01
	Inabu	Highly conducive to blast	NILP-*Pb1*	790	91.0	620	21.0	9.7
			NILP-+	230	55.9	122	20.0	10.7
			Significance	*P*<0.01	*P*<0.01	*P*<0.001	*P*<0.01	*P*<00.1
			NIL-*Pb1*/NIL-+	3.44	1.63	5.09	1.05	0.91

[a] Average of three near-isogenic line pairs selected from recombinant inbred lines developed from a cross between 'Koganebare' without *Pb1* and 'Tsukinohikari' with *Pb1*
[b] g m^{-2}
[c] %, Head brown rice weight / Gross brown rice weight
[d] %, 15% water content
[e] Near-isogenic lines with *Pb1* gene
[f] Sib-lines without *Pb1* gene
(Reprinted from Fujii *et al.* 2005b, *Breeding Research* **7**, 75-85, with kind permission from JSB)

which showed that *Pb1* could reduce the percentage of diseased grains to less than 1/10, compared with sib-lines without the *Pb1* gene (Fujii *et al.* 2005a; **Fig. 5**).

The head brown rice yield ratios of NILs with *Pb1* to the sib-lines without *Pb1* were 2.40:1-16.2:1 under conducive conditions for blast disease, revealing a very high protective effect on yield loss associated with panicle blast infection, while 0.78:1-1.29:1 ((1.07±0.18):1) under conditions less conducive to the disease. The values of the head/ gross brown rice rate and thousand kernel weight in brown rice of the NILs with *Pb1* under disease-conducive conditions were significantly higher, while the protein content was significantly lower than the values of the sib-lines without *Pb1*, respectively. Thus, the *Pb1* gene also showed a secondary protective effect on rice quality under the blast fungus-conducive environment (Fujii *et al.* 2005a; **Table 3**).

GENE PYRAMIDING EFFECT

Gene pyramiding effect of *Pb1* and other partial resistance gene *pi21* (Fukuoka and Okuno 2001) or *Pi39*(t) (Terashima *et al.* 2006) were studied using rice RILs developed from the crosses Iwanan 3/Touhoku 176, Matsuribare//Inakei-IL946/2*Koshihikari, and Chubu 111/Inakei 978. Gene combination of *Pb1* and *pi21* significantly decreased disease severity of leaf blast as well as of panicle blast compared with single use of *Pb1* or *pi21* in Iwate Prefecture located in Tohoku region of Japan (Abe *et al.* 2007). Gene combination effects of *Pb1*+*pi21* and *Pb1*+*Pi39*(t) were also tested under highly blast-conducive environment in Inabu, semi-mountainous region of Aichi Prefecture located in the central area of Japan. Under highly blast-conducive condition, gene combination between *Pb1* and *pi21* significantly decreased disease severity of leaf blast as well as of panicle blast compared with single use of *Pb1*. The same results were obtained for gene combination of *Pb1* and *Pi39* compared with single use of *Pb1* (Saka *et al*. 2007). Consequently, positive effects of gene combination between *Pb1* and *pi21* as well as *Pb1* and *Pi39* to control leaf blast and panicle blast were observed in both test sites in Japan.

CONCLUDING REMARKS

The *Pb1* gene derived from an Indica donor variety Modan does not confer any complete resistance with hypersensitive reactions, and the protective ability of the gene against rice panicle blast is sufficient for commercial rice production in Japan except in environments highly conducive to the disease (Fujii *et al*. 1999a, 1999b). Complete resistance to blast controlled by a major gene often breaks down within a few years after the dissemination of cultivars with the complete resistance gene (Ujihara *et al*. 1955; Kiyosawa 1972; Ou 1979), due to the occurrence of compatible blast fungal race(s) adapted to the resistance gene (Kiyosawa 1982; Bonman *et al*. 1986). Although a major gene, *Pb1*, also confers strong partial resistance to panicle blast, the breakdown of this resistance has not been reported for more than 20 years since the beginning of commercial cultivation of rice cultivars with *Pb1* in Japan (Fujii *et al*. 1999a, 2005b). Therefore, since *Pb1* is considered to be a gene conferring durable adult resistance, it is useful in rice breeding and is also an important gene in plant protection. The isolation of the gene would be essential for studies on the gene function and gene-pathogen relationship. Further studies to detect flanking markers and more precise mapping of the *Pb1* locus has been conducting by N. Hayashi *et al*. in order to isolate the *Pb1* gene (Hayashi *et al*. 2004, 2005, 2006).

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JAPANESE ABSTRACT

いもち病は *Magnaporthe grisea* を病原菌とするイネの最重要病害である。なかでも穂いもちの発生は収量減と品質低下に直結する。日本においてはいもち病に感受性の「コシヒカリ」および「コシヒカリ」近縁の良食味品種に作付けが集約しており、穂いもち被害を防ぐため、殺菌剤が予防的に広く使用されている。こうした現状のなか、品種の抵抗性を高めることがいもち病の被害を軽減し、農薬の使用を低減できる最も有効な方法である。この総説では、インド型イネ「Modan」からイネ縞葉枯ウイルス（RSV）抵抗性を導入した日本型品種（「月の光」、「朝の光」）が示す特異な穂いもち抵抗性に着目し、その抵抗性の特性，系譜，遺伝，安定性，穂いもち発病抑制効果についての解明を通じて穂いもち抵抗性遺伝子の育種利用への実用性を評価する。さらに分子遺伝学的手法を用いて抵抗性遺伝子の染色体上座乗位置を明らかにし、密接に連鎖する DNA マーカーを特定した最近の研究知見を紹介する。最後に、穂いもち圃場抵抗性育種を効率的に行うための DNA マーカー選抜（MAS）システムの実用化と、実際に MAS により「コシヒカリ」に穂いもち抵抗性を付与した準同質遺伝子系統（NIL）「コシヒカリ愛知 SBL」を開発した例を紹介する。