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S. Fukuoka · K. Okuno

QTL analysis and mapping of *pi21*, a recessive gene for field resistance to rice blast in Japanese upland rice

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Abstract Field resistance is defined as the resistance that allows effective control of a parasite under natural field conditions and is durable when exposed to new races of that parasite. To identify the genes for field resistance to rice blast, quantitative trait loci (QTLs) conferring field resistance to rice blast in Japanese upland rice were detected and mapped using RFLP and SSR markers. QTL analysis was carried out in F_4 progeny lines from the cross between Nipponbare (moderately susceptible, lowland) and Owarihatamochi (resistant, upland). Two QTLs were detected on chromosome 4 and one QTL was detected on each of chromosomes 9 and 12. The phenotypic variation explained by each QTL ranged from 7.9 to 45.7% and the four QTLs explained 66.3% of the total phenotypic variation. Backcrossed progeny lines were developed to transfer the QTL with largest effect using the susceptible cultivar Aichiasahi as a recurrent parent. Among 82 F_3 lines derived from the backcross, resistance segregated in the expected ratio of resistant 1 : heterozygous 2 : susceptible 1. The average score for blast resistance measured in the field was 4.2 ± 0.67, 7.5 ± 0.51and 8.2 ± 0.66, for resistant, heterozygous and susceptible groups, respectively. The resistance gene, designated *pi21*, was mapped on chromosome 4 as a single recessive gene between RFLP marker loci *G271* and *G317* at a distance of 5.0 cM and 8.5 cM, respectively. The relationship to previously reported major genes and QTLs conferring resistance to blasts, and the significance of marker-assisted selection to improve field resistance, are discussed.

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S. Fukuoka·K. Okuno (✉)
Department of Genetic Resources I,
National Institute of Agrobiological Resources,
Kannondai 2-1-2, Tsukuba, Ibaraki, 305-8602 Japan
e-mail: fukusan@abr.affrc.go.jp

Present address:
K. Okuno, Department of Low-Temperature Sciences,
Hokkaido National Agricultural Experiment Station,
Hitsujigaoka 1,Toyohira, Sapporo, Hokkaido, 062-0045 Japan

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Introduction

Rice blast, caused by *Pyricularia grisea* Sacc. [formerly *Pyricularia oryzae* Cav.] is a destructive disease of rice in most rice growing areas worldwide (Ou 1985). Breeding efforts are aimed to introduce genes for blast resistance into desirable genetic backgrounds. The genetics of rice blast have been extensively studied and two types of resistance have been described, complete (true) and field resistance (Ezuka 1972; Parlevliet 1979). Complete resistance is a hypersensitive reaction, often a complete form of resistance, and is characterized by a resistant infection type. At least 20 loci for complete blast resistance have been identified (McCouch et al. 1994). Although cultivars with complete resistance have been developed, they have rapidly been overcome by compatible races of the pathogen (Kiyosawa 1982). Field resistance, incomplete and usually polygenic, is a susceptible infection type, that allows effective control of a parasite under natural field conditions and is considered to be durable when exposed to new races of blast.

Genetic studies of field resistance have been conducted using Japanese upland rice cultivars since the 1970s. Most studies have suggested that field resistance to blast is under complex genetic control and multiple genes are responsible for the expression of field resistance (Higashi and Kushibuchi 1978; Maruyama et al. 1983; Higashi and Saito 1985). Previous studies, however, did not identify the genes involved in field resistance or their chromosomal locations. To characterize field resistance genes, genome map information for each putative gene and the materials with a gene in a well-characterized genetic background are necessary.

Recent progress in QTL analysis using DNA markers allows a better understanding of the genetics of the traits that are controlled by multiple genes. A large number of QTLs for morphological and physiological traits have

been reported in rice and were reviewed by Yano and Sasaki (1997). They suggested a strategy to develop backcrossed progeny lines for each QTLs by marker-assisted selection and to analyze QTL as single Mendelian factors. Such a strategy using fine-scale mapping makes it possible to characterize each QTL.

QTLs for blast resistance have been reported by Wang et al. (1994). They analyzed resistance to rice blast using recombinant inbred lines between the resistant West African *japonica* cultivar Moroberekan and susceptible *indica* cultivar CO39. They found two complete resistance genes and ten chromosomal regions conferring partial resistance, which is an analogous type of resistance to field resistance, in Moroberekan. Although the precise map locations of the ten QTLs have not been determined, the relationship between the chromosomal regions for partial resistance and those in other resistant cultivars is relevant for understanding the genetic control and evolution of field resistance in rice.

The objective of this study was to determine the chromosomal regions responsible for field resistance to blast in the Japanese upland rice cultivar Owarihatamochi. In addition, genetic analysis was conducted in advanced backcrossed lines, with marker-assisted selection and map-based cloning of the gene conferring the greatest effect on the expression of field resistance.

Materials and methods

Plant material

A total of 146 F_4 lines were developed from a cross between two *japonica* rice cultivars, the lowland cultivar Nipponbare and the upland cultivar Owarihatamochi. Nipponbare and Owarihatamochi have no genes for complete resistance to rice blast, based on screening using seven differential blast races (Abe et al. 1976). In field tests Nipponbare is susceptible while Owarihatamochi is highly resistant to rice blast. Backcrossed lines (BC_1F_3) derived from two F_3 plants of the cross between Nipponbare and Owarihatamochi were developed to locate a field resistance gene on rice chromosomes. Aichiasahi, a highly susceptible cultivar, was used as the recurrent parent.

Field resistance test

Field resistance in F_4 lines was assessed at the Aichi-ken Agricultural Research Center, Mountainous Region Institute, Aichi, Japan, in 1995. All F_4 lines and parents of the cross were grown in upland rice nursery beds with two replications. The disease severity of 40–50 day old plants was scored using a rating from 0 (highly resistant: no symptoms) to 10 (highly susceptible: leaves totally dead) based on the diseased leaf area, following the method of Asaga (1976). The field resistance in backcrossed lines was assessed using the same procedures as described above with three replications in 1998.

DNA analysis

Total DNA was extracted using the CTAB method described by Murray and Thompson (1980). For RFLP analysis DNA samples were digested with nine restriction enzymes (*Apa*I, *Kpn*I, *Dra*I, *Hind*III, *Bam*HI, *Bgl*II, *Eco*RI *Eco*RV and *Hha*I) and, after electrophoresis, blotted onto a positively charged nylon membrane Biodyne B (Pall, US) according to the manufacturer's instruction. Hybridization and signal detection were conducted using the ECL direct nucleic acid labeling and detection kit (Amersham Pharmacia Biotech, U.K.). Three hundred RFLP probes from two published RFLP linkage maps (Saito et al. 1991, Kurata et al. 1994) were surveyed for probe-enzyme combinations polymorphic in the parents. Selected markers distributed on the 12 rice chromosomes were used for segregation analysis in F_4 and backcrossed lines.

Five SSR (simple sequence repeat) markers on chromosomes 9 and 12 were also mapped (Akagi et al. 1996; Chien et al. 1997). The amplified products of the mapping population were separated on a 8–10% polyacrylamide gel in 1 × TBE buffer. The patterns were visualized using either ethidium bromide or a silver staining kit (Daiichi Pure Chemicals, Japan)

Data analysis

The program MAPMAKER/EXP 3.0 (Lander et al. 1987) based on the Kosambi function was used to build an RFLP linkage map. The PROC GLM program in the Statistical Analysis Systems package (SAS Institute 1989) was used to determine the association between RFLP markers and field resistance. MAPMAKER/QTL ver. 1.0 (Lander and Botstein 1989) was used to calculate LOD scores for significant marker loci and to obtain estimates of the percentage of total phenotypic variance explained by each QTL. Putative QTLs were identified using a LOD threshold of 2.0.

Results

Field resistance in F_4 lines

The frequency distribution of field resistance to rice blast based on disease severity in F_4 lines is presented in Fig. 1. The distribution was continuous and the score ranged from 1 to 10. Owarihatamochi and Nipponbare, used as parents in this study, differed significantly in the blast resistance scores which were 2.8 and 8.0, respectively.

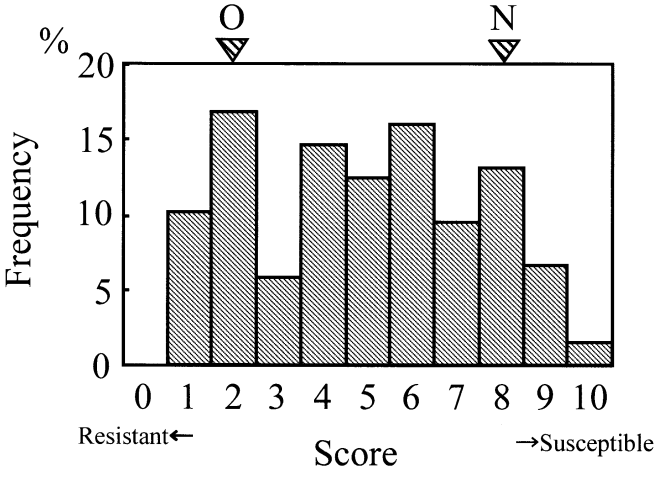

Fig. 1 Frequency distribution of the scores for field resistance in F_4 lines of the cross between Nipponbare and Owarihatamochi. *N* and *O* indicate the score of the parents Nipponbare and Owarihatamochi, respectively

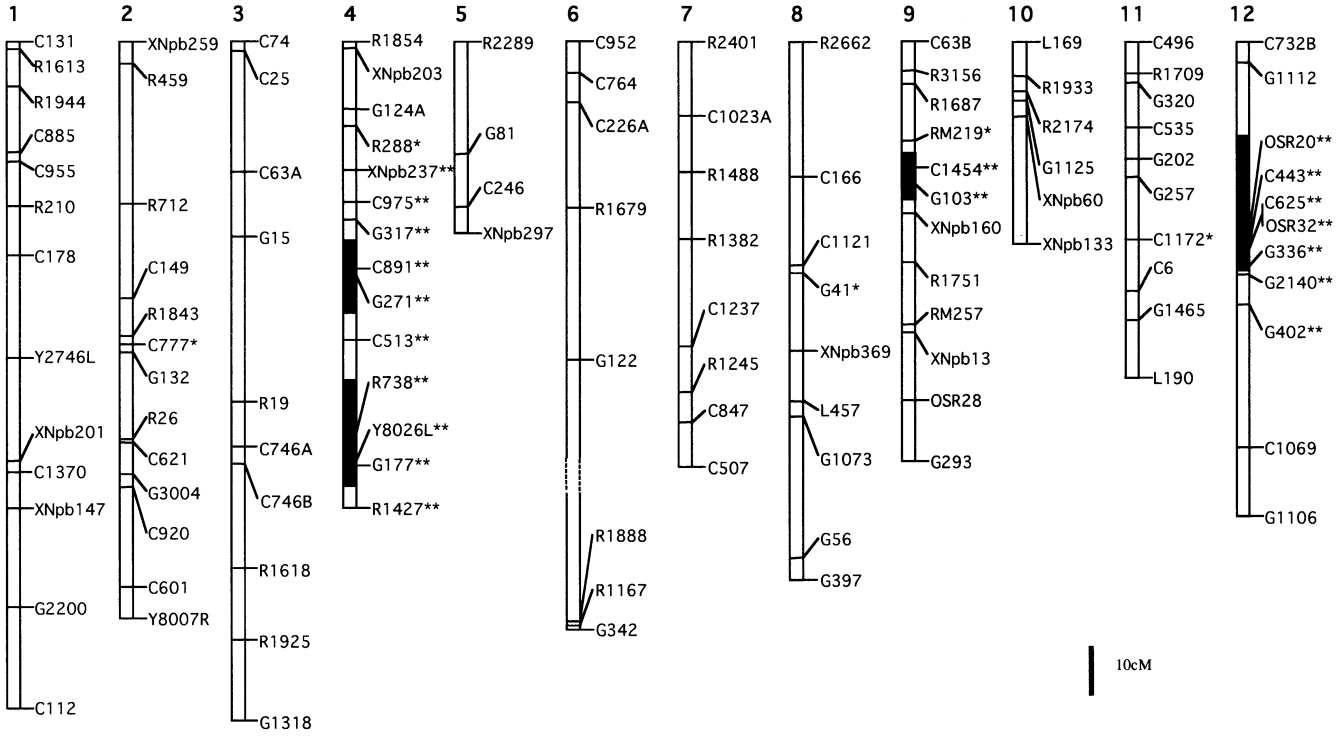

Fig. 2 Linkage map and the positions of QTLs for field resistance. *Black bars* represent putative region of QTLs with a LOD value more than 2.0. Markers that are significant at the 0.05 and 0.01 probability level are marked with * and **, respectively

Parental survey of DNA markers and QTL analysis in F_4 lines

With the 300 RFLP probes tested, 111 were polymorphic between Nipponbare and Owarihatamochi. The ratio of RFLPs found between these two cultivars (present study) with respect to the RFLPs detected in the cross Nipponbare/Kasalath (Kurata et al. 1994) varied among the 12 chromosomes from 22.2 to 64.3%, with an average of 37.0%. QTL analysis was carried out using these RFLP markers and the five SSR markers. For a total of 118 DNA markers, 24 markers on chromosomes 2, 4, 8, 9, 11 and 12 showed a significant correlation to the blast resistance score at the 5% level, while 19 markers on chromosomes 4, 9 and 12 were significant at the 1% level following the analysis performed with SAS/GLM. The analysis with MAPMAKER/QTL revealed four QTLs located on these three chromosomes with LOD scores higher than 2.0. The map positions of these QTLs are shown in Fig. 2. The resistant allele on chromosome 9 is derived from Nipponbare while the resistant alleles on chromosomes 4 and 12 come from Owarihatamochi. The two QTLs on chromosomes 9 and 12 explained 7.9 and 13.7% of the phenotypic variation, respectively, while the two QTLs on chromosome 4, close to RFLP marker loci *G271* and *G177*, explained 45.7% and 29.4% of the total phenotypic variation, respectively (Table 1). All together, the four QTLs detected in this study explained 66.3% of the tota l phenotypic variation.

Table 1 Putative QTLs for field resistance to rice blast

NML[a]	Chr.	LOD	Var. exp. (%)[b]	AE	DPE[d]
G271	4	19.3	45.7	1.8[c]	O
G177	4	11.0	29.4	1.5	O
C625	12	4.7	13.7	1.1	O
G103	9	2.6	7.9	–0.6	N
Total			66.3[e]		

[a] Nearest marker locus of putative QTLs
[b] Phenotypic variation explained by each QTL
[c] Additive effects (1/2 weight) of the Owarihatamochi allele by the field resistance score
[d] Direction of phenotypic effect; O and N indicate that the Owarihatamochi and Nipponbare alleles increase the values, respectively
[e] Estimates obtained from a multiple QTL model

Field resistance in backcrossed lines

Two backcrossed plants (BC_1F_1) with the resistance allele from Owarihatamochi near the RFLP marker locus *G271* on chromosome 4 were obtained. In these plants, the other three alleles were replaced by the alleles from the susceptible cultivar Aichiasahi. One plant, designated 96BC131, possessed alleles of Owarihatamochi in the chromosomal region between RFLP marker loci *G271* and *XNpb237*. The other plant, designated 96BC122, possessed the chromosoma l segment between RFLP marker loci *C513* and *G271*. Sixteen and eleven percent, respectively, of the alleles were from Owarihatamochi in 96BC131 and in 96BC122. Eighty two and seventy six BC_1F_3 lines derived from 96BC131 and from 96BC122, respectively, were developed and were subjected to field resistance tests. The frequency distribution of field resis-

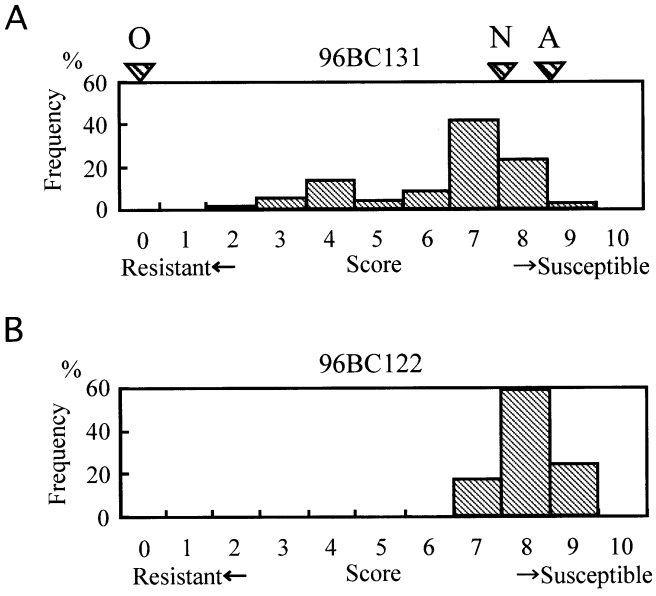

Fig. 3 Frequency distribution of the score for field resistance in two BC_1 F_3 lines, 96BC131 (**A**) and 96BC122 (**B**). *N, O* and *A* indicate the score for the cultivars Nipponbare, Owarihatamochi and Aichiasahi, respectively

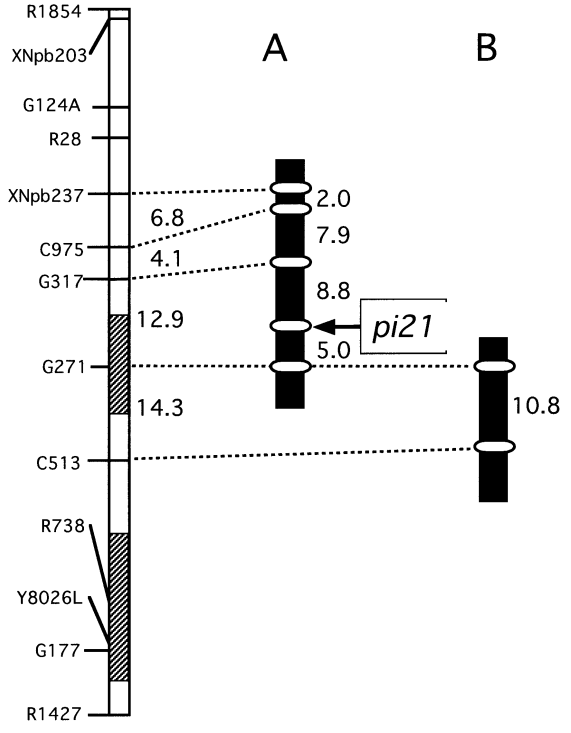

Fig. 4 The introgressions on chromosome 4 from Owarihatamochi in two BC_1 F_1 plants, 96BC131 (**A**) and 96BC122 (**B**), respectively. The locations of RFLP marker loci and a gene for field resistance, *pi21*, are indicated with an *open ellipse*. Genetic distances among them in BC_1 F_3 populations are shown right on right in centimorgans. The map of rice chromosome 4 in Fig. 2 is shown on the left, indicating the location of putative QTLs with *shaded boxes*. The locus C891, monomorphic in two backcrossed populations, is not indicated on this map

tance in these sets of BC_1F_3 lines is presented in Fig. 3. The score for the cultivars Owarihatamochi (O) and Aichiasahi (A) were 0.7 and 9.2, respectively. The score of the lines derived from 96BC122 ranged from 7.5 to 9.7, while that of the lines from 96BC131 ranged from 2.5 to 9.2 and showed a bimodal distribution.

Mapping of a gene for field resistance

The BC_1F_3 lines derived from backcrossed plant 96BC131 were used to map one gene for rice blast field resistance. The lines were divided into three classes based on field resistance: class 1 were lines with a score of lower than 6.0, while classes 2 and 3 included the lines with a score higher than 6.0 and were discriminated by the presence or absence of segregation for a diseased leaf area among plants within line. With respect to the genotype of the field resistance gene, the first class was homozygous for the resistant allele and the second class showing no segregation was homozygous for the susceptible allele. The third class that included segregating lines, was considered to be heterozygous at this locus. The average score for the classes 1, 2 and 3 were 4.2 ± 0.67, 8.2 ± 0.66 and 7.5 ± 0.51, respectively. The locus conferring field resistance, designated *pi2l*, was mapped between RFLP marker loci *G271* and *G317* at a distance of 5.0 cM and 8.3 cM, respectively.

Discussion

Japanese upland rice cultivars are potential gene donors for field resistance to rice blast. The present study was carried out to identify chromosomal regions for field resistance to rice blast in Japanese upland rice using DNA markers, and one of the QTLs identified for this resistance was mapped as a single recessive gene using backcrossed progeny lines.

Among F_4 lines the score for disease severity assessed in the field ranged from 1 to 10. Lines with a higher resistance than Owarihatamochi, as well as lines more susceptible than Nipponbare, were observed. This suggested multigenic inheritance of field resistance that was confirmed by QTL analysis in this study. The Owarihatamochi allele increased the resistance to blast in three out of four QTLs. Two QTLs on chromosome 4 explained 45.7 and 29.4% of the phenotypic variation, indicating that two genes on chromosome 4 play a major role in the expression of field resistance to blast. Previous studies suggested five to eight genes involved in this resistance based on diallel analysis or analysis using phenotypic markers (Higashi and Kushibuchi 1978; Higashi and Saito 1985). One explanation for the difference in the number of resistance genes estimated might be due to the difference in genetic markers used for linkage analysis. The use of molecular markers allows genetic dissection in detail and the precise localization of QTLs on rice chromosomes. The other reason, which

might be more important, is due to environmental changes in the expression of resistance and the race-specific reaction to blast in the field (Yunoki et al. 1960; Ikehashi and Kiyosawa 1981; Koide et al. 1987; Koizumi and Fuji 1995). Upland rice in Japan has maintained long-term durability of resistance to blast. However, there is still little information on race-specific interaction and environmental factors in relation to field resistance to blast. Further studies on these topics are necessary.

The race-specificity of a single QTL (gene) for field resistance is a crucial point, but has not been studied. Backcrossed inbred lines or near-isogenic lines with a single QTL are useful to evaluate the effect of each QTL (gene) underlying resistance to a spectrum of blast races (Inukai et al. 1996; Yu et al. 1996; Chen DH et al. 1999). Such materials are useful for investigating the mechanism of field resistance. The field resistance gene mapped in detail in this study is recessive, while most reported disease resistance genes are dominant. A detailed survey of this gene might be a useful approach to understanding the mechanism of defense response in plants.

DNA polymorphism is one of the limiting factors for the detailed genetic dissection of chromosomal regions of interest and for positional cloning. The frequency of polymorphisms between Japanese lowland and upland rice was not as high as for the *indica*/*japonica* crosses. In this study 38% of RFLP markers tested were polymorphic between the two cultivars, although the degree of genetic variation varied among the 12 chromosomes and was highest on chromosomes 4 and 12. The use of substitution lines with the chromosomal segment from *indica* rice in susceptible *japonica* background is one strategy to obtain a higher mapping resolution. Highly polymorphic mapping populations are now being developed with the objective of cloning *pi21*.

This study suggested that resistance genes to blast are restricted to several particular regions of the rice genome (Causse et al. 1994). The clusters of previously reported genes for complete resistance and QTLs for partial resistance support this observation to some extent (McCouch et al. 1994; Wang et al. 1994). One of four QTLs found in this study which held the largest effect on field resistance was located in analogous chromosomal regions to the complete resistance gene, *Pi5(t)*, involved in Moroberekan (Wang et al. 1994). The other complete resistance gene, *Pi ?*, was reported in the chromosomal region harboring the QTL with the second largest effect on field resistance in this study (Causse et al. 1994). Several resistance genes for bacterial leaf blight have also been reported in this chromosomal region (Yamada and Horino 1981; Yoshimura et al. 1994). Furthermore, the QTL on chromosome 12 was located in the same region as previously reported for several complete resistance genes and one QTL for partial resistance (Causse et al. 1994; Wang et al. 1994; Inukai et al. 1996; Yu et al. 1996). Although blast resistance genes have not been found in the region near the QTL on chromosome 9, preliminary results suggested that some resistance genes might be located in this chromosomal region (Chen D et al. 1997). Clustered disease resistance genes and their multi-allelic nature have been reported in other crop species and were reviewed by Hammond-Kosck and Jones (1997) and by Michelmore and Mayers (1998). Based on accumulated sequence data of isolated resistance genes, these reviews proposed a hypothesis that interallelic recombination, unequal crossing-over and gene conversion are involved in the rapid evolution of resistance genes. The chromosomal regions highlighted in the present study are relevant in respect of the molecular evolution of the genes involved in disease resistance and may be used to confirm their hypothesis. Further genetic studies of these chromosomal regions are needed to enhance our understanding of the evolutionary processes involved in the genetic differentiation of disease resistance.

Although Japanese upland rice cultivars have been extensively used in breeding since the 1920s, the undesirable characters of upland rice cultivars limit the introduction of genes for blast field resistance to elite rice cultivars (Morimoto 1980). Only some part of field resistance in upland rice has been introduced to commercial cultivars (Higashi 1995). In this study the two QTLs with the greatest effect were found on chromosome 4. The progenies of the cross with the two resistant alleles from upland rice should have a large part of chromosome 4 from upland rice since the recombination per chromosome per plant was 2.5-times on chromosome 4 (Harushima et al. 1998). This assumption may explain in part why most resistant progenies from the upland/lowland cross have undesirable characters (Morimoto 1980). Therefore, building up QTLs using near-isogenic lines for each QTL in a desirable genetic background is one strategy for the effective use of these QTLs. DNA markers are powerful tools in the selection of QTLs for field resistance to blast in rice breeding.

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