

Monitoring of blast races to ensure durability of blast resistance in Japanese rice cultivars

Shinzo Koizumi

Lowland Crop Rotation Research Team, National Agricultural Research Center
for Tohoku Region, Yotsuya, Daisen, Akita, 014-0102 Japan
E-mail: skoizumi@affrc.go.jp

Abstract

The breakdown of complete resistance to blast in rice cultivars in the 1960s led to genetic analyses of blast resistant cultivars in Japan, and currently, partially and completely resistant genotypes of most Japanese rice cultivars are known. Differential systems of blast races have also been established, and monitoring of blast races has been undertaken throughout Japan in 1976, 1980, 1994 and 2001. The results of monitoring blast races have been used for the analyses of virulence genes in the pathogen population (Kiyosawa, 1985). The proportions of components in three released Japanese multilines for rice blast control, consisting of three to four near-isogenic lines with different genes to confer complete resistance, were also based on the monitoring results. Moreover, the results accelerated development of rice cultivars with high levels of partial resistance to blast on the basis of stability of the partially resistant genotypes. Monitoring of blast races in areas where the genotypes of the cultivated rice cultivars that are completely resistant to blast are not well known, is also useful for the selection of effective complete resistance genes for blast control. Inoculation with the isolated races has revealed promising rice cultivars for blast control with some showing partial resistance. Although monitoring of blast races has been conducted for rice blast control in several countries, sufficient quantitative analyses of blast race epidemics, which are necessary for the durability of blast resistant cultivars, have not been carried out. This is because monitoring of blast races is very laborious and reliable epidemiological data on population interaction between host and pathogen is lacking. For durability of blast resistance in rice, it is therefore necessary to develop methods that make it easy to monitor blast races, such as DNA markers that are tightly linked to avirulence genes in the fungus. By accumulating epidemiological data on population interactions between host and pathogen, it will be possible to construct reliable epidemiological models that can simulate increases in blast races in host populations with different degrees of resistance.

Keywords: monitoring, blast races, durability of resistance, rice

Introduction

Monitoring of blast races has been conducted using various differential varieties in a number of countries (Kobayashi et al. 2007). However, the durability of blast resistance in rice cultivars around the world is only empirically known because partially and completely resistant genotypes of the cultivated rice varieties have not yet been determined. In contrast, in Japan, after the breakdown of complete resistance to blast in rice cultivars in the 1960s, partially and completely resistant genotypes of most cultivated rice varieties had been determined and monitoring of blast races carried out. However, the effects of prolonged use of blast resistant cultivars is unknown because sufficient quantitative analyses of changes in blast races in host populations with differ-

ent degrees of resistance have not been performed to date. This paper describes the durability of blast resistant rice cultivars by monitoring blast races.

Differential systems in Japan

After the first discovery of blast races by Sasaki (1922) and the breakdown of complete resistance in many rice cultivars in the 1960s, several differential systems for the classification of blast races were developed and distributions of blast races have been monitored in Japan. Goto et al. (1964) first constructed a differential system, and then cooperative research between the US and Japan developed an international differential system in 1967 (Goto et al. 1967). However, these differential systems were not based on gene-for-gene relationships between host

and pathogen. In 1974, Kiyosawa (1974) performed gene analyses of blast resistance and found many resistance genes in rice cultivars. Following the gene analyses and identification of gene-for-gene relationships between resistance in rice and avirulence in blast fungus, Yamada et al. (1976) and Kiyosawa (1984) developed new differential systems and these systems are currently being used for the monitoring of blast races in Japan.

Monitoring blast races in Japan

The distribution of blast races has been surveyed throughout Japan (1976, 1980, 1994 and 2001) using the differential systems (Yamada et al. 1979; Yamada 1985; Naito et al. 1999; Koizumi et al. 2007). The procedure for blast race monitoring, based on that of Yamada et al. (1979), involves the analyses of distribution data of both blast races and cultivated rice cultivars in Niigata prefecture located in central Japan.

The procedure comprises the following steps. First, a paddy field of approximately 1,000 ha is selected or 4,000 ha of paddy fields are subjected to randomized systematic sampling. Second, one blast lesion per 1000 ha field or four lesions per 4,000 ha fields is (are) collected. Third, blast races of the isolates are identified by spray-inoculation using Japanese differentials after mono-conidial isolation.

The numbers of blast races differentiated each year in the monitoring from 1976 to 2001 with the nine Japanese differential varieties are shown in Table 1. In 1979 and 1980, the predominant race was 003 (virulent to the resistance gene *Pia*), followed by races 007, 033, 001 and 103. In contrast, in 1994 and 2001, race 007 (virulent to the gene *Pii* and *Pia*) predominated, followed by races 001, 003, 005, 037 and 033 (Fig. 1).

The monitoring results indicated that the prevalence of the blast races was affected by frequencies of corresponding complete resistance genes in cultivated rice cultivars (Fig. 1) and showed that statistically significant positive correlations existed between isolation frequencies of blast races virulent to *Pia*, *Pii*, *Pik*, *Pita*, *Piz* and frequencies of the genes in cultivated rice cultivars (Fig. 2).

Cluster analysis, which was carried out using data sets of isolation frequencies of blast races virulent to *Pia*, *Pii*, *Piz* and *Pita* in 1976, 1980, 1994 and 2001, divided 37 prefectures and the full data set divided 47 prefectures across Japan into five groups and three prefectures (Fig. 3, 4). Each of the groups or prefectures had distinctive characteristics in terms of changes in the frequencies of the blast races. Variations in isolation frequencies of the blast races virulent to the genes in each of the groups or prefectures corresponded to those of cultivated percentages of rice cultivars carrying the genes (Fig. 5).

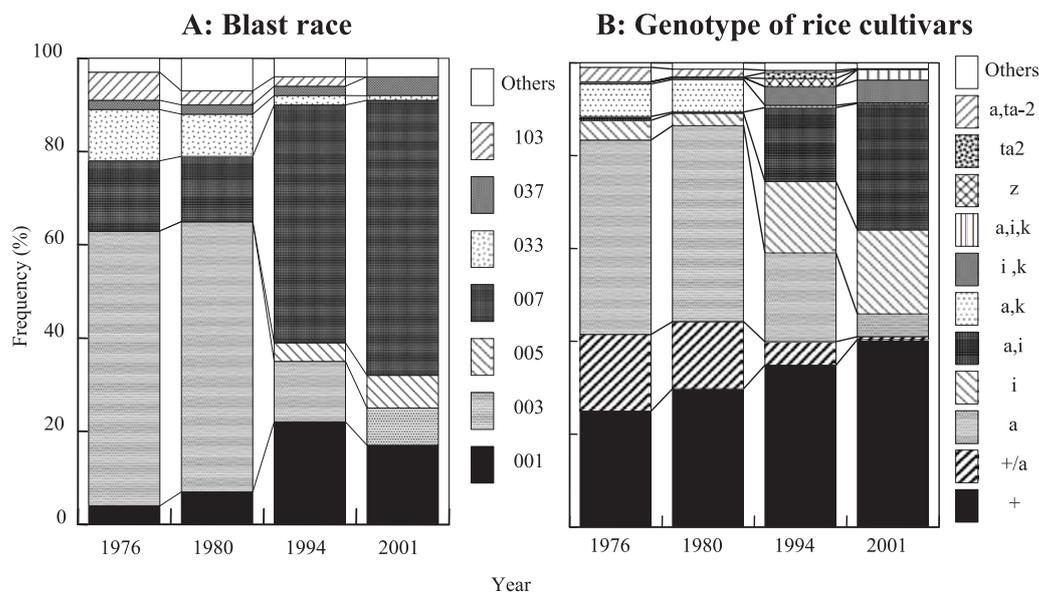


Fig. 1. Changes in frequencies of predominant blast races and percentages of blast resistance genotypes in cultivated rice cultivars in Japan

Table 1. Blast races isolated from Japan in 1976, 1980, 1994 and 2001

Race	1976	1980	1994	2001
001	3.8*	7.2	22.5	16.6
002		2.4		
003	58.7	57.6	12.7	8.3
005	0.04	0.04	3.8	7.6
007	14.5	14.5	50.9	57.9
011			0.1	
013	0.2		0.5	0.2
015			0.1	
017	0.2	0.04	0.8	1
031	0.8	0.8	0.3	0.1
033	10.9	8.8	2.2	1.3
035	0.2		0.3	0.7
037		2.3	2.2	4.7
041		0.04	1.4	0.5
043		0.2	0.2	
045			0.1	0.1
047		0.3	0.7	0.6
073		0.3		
101	0.5	0.1	0.3	0.4
102	0.04	0.9	0.1	
103	5.9	3.1	0.3	0.1
105			0.1	
107	0.4	0.1	0.3	
113	0.04			
131	0.1			
133		0.2		
137	0.04	0.1		
301			0.2	
303	1.5	0.8		
307	0.1			
331	0.04			
333	0.04	0.1		
337		0.04		
401	0.04			
403	0.04			
407			0.1	
Number of isolates	2,245	2,376	1,526	1,050
Number of races	23	22	23	15
Shannon index (Hw)	1.35	1.51	1.53	1.41
Rice cultivated area per isolate (ha)	1,221	989	1,442	1,590
Rice cultivated area (ha)	2,741,000	2,350,000	2,200,051	1,699,645

* Isolation frequency (%) of the race.

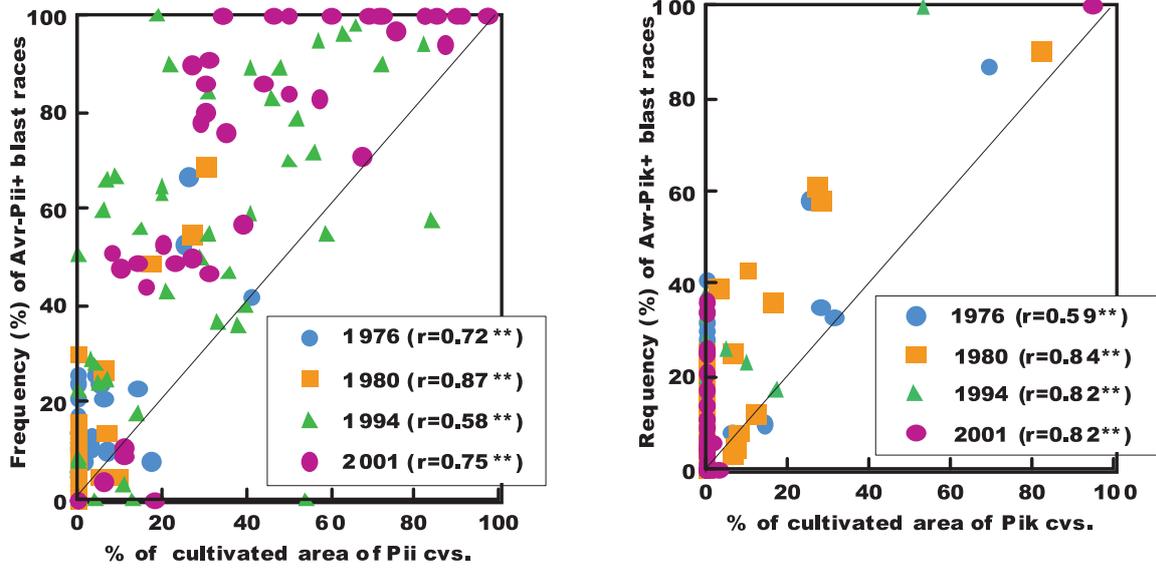


Fig. 2. Relationship between percentages of cultivated areas of rice cultivars carrying *Pii/Pik* and isolated frequencies of *Avr-Pii*⁺/*Avr-Pik*⁺ blast races in Japan. Each symbol represents values of the percentage and frequency in a prefecture in a monitoring year

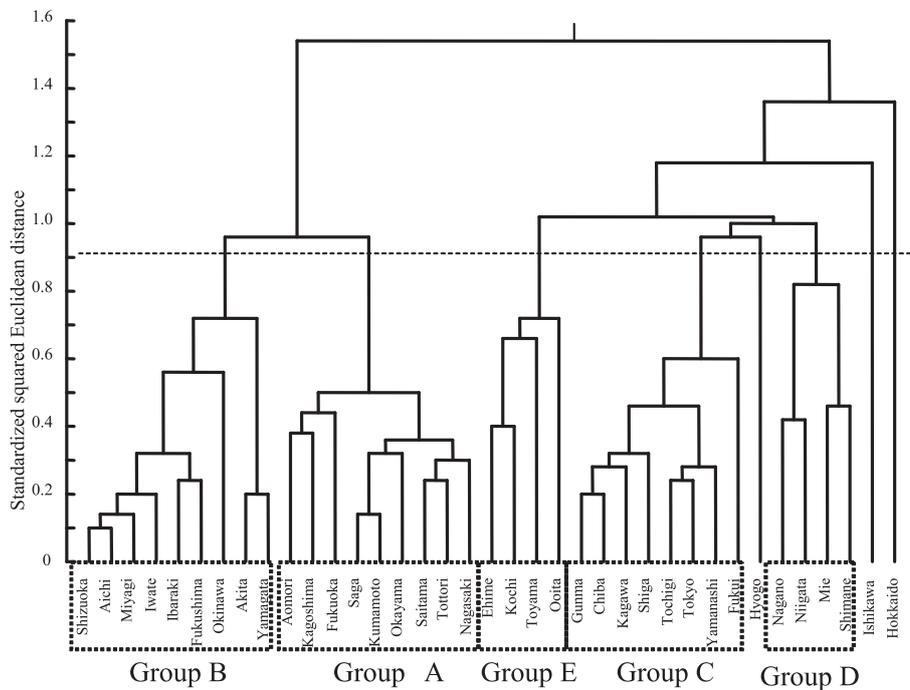


Fig.3. Cluster analysis using data sets of isolation frequencies of blast races virulent to *Pia*, *Pii*, *Piz* and *Pita* in 1976, 1980, 1994 and 2001, divided 37 prefectures in Japan

Cluster analysis was conducted with Ward's method using standardized Euclidean distances.

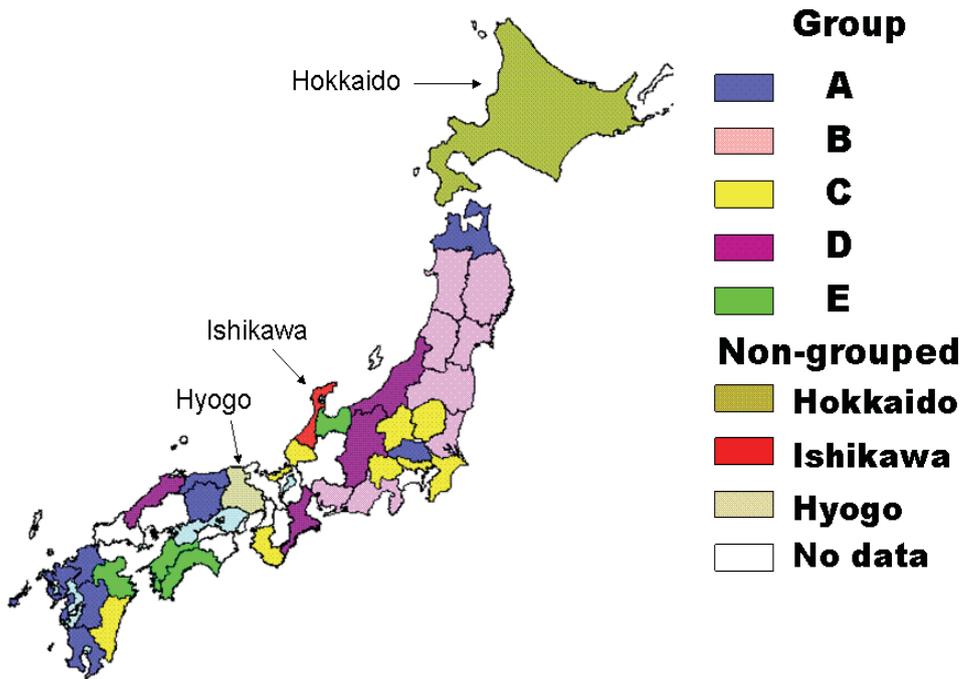


Fig. 4. Geographical classification of Japanese prefectures with cluster analysis based on frequencies of *Avr-Pia*⁺, *Avr-Pii*⁺, *Avr-Piz*⁺ and *Avr-Pita*⁺ blast races in 1976, 1980, 1994 and 2001

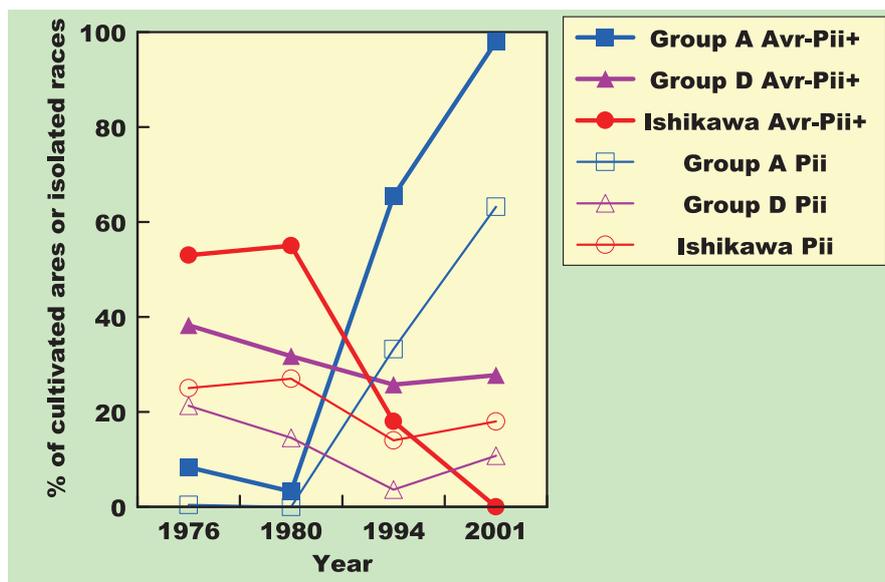


Fig. 5. Frequencies of *Avr-Pii*⁺ blast races and percentages of cultivated areas of *Pii* in each group from 1976 to 2001 in Japan

Factors changing prevalence of blast races

Aside from the complete resistance genotypes of cultivated rice cultivars described above, several factors affecting the prevalence of blast races have been reported. These include levels of partial resistance in the host; changes in rice cultivars with different blast resistance in scale and time; fitness costs concerning virulence, variation in virulence, genetic drift and gene flow of the blast fungus; environmental conditions; cropping system; crop management and others (Mundt 2002).

Fig. 6 shows that high levels of partial resistance to blast in cultivated rice cultivars reduced the prevalence of blast races virulent to *Pii* in small districts in Aichi prefecture in central Japan. The aggressiveness of blast races also affects their prevalence. We examined the

aggressiveness of the blast races 003 and 033, which were collected by Yamada et al. (1979) during blast race monitoring that was carried out throughout Japan in 1976. The race 003 cannot attack *Pik*, whereas 033 can. Race 003 on Norin 29, which has no complete resistance to most Japanese isolates, is more aggressive than race 033 (Table 2; Koizumi 1982). In Niigata prefecture, the isolation frequencies of race 033 were reduced after the decrease of cultivated areas with rice cultivars containing *Pik* in the 1970s (Yaoita et al. 1977). The lower aggressiveness of race 033 probably affected the decrease in race isolation frequencies.

Mutation rates of the rice blast fungus for virulence are estimated to be 10^{-3} to 10^{-5} (Kiyosawa 1976). Point mutation, deletion, transposon insertion and parasexual recombination are considered to cause variations in viru-

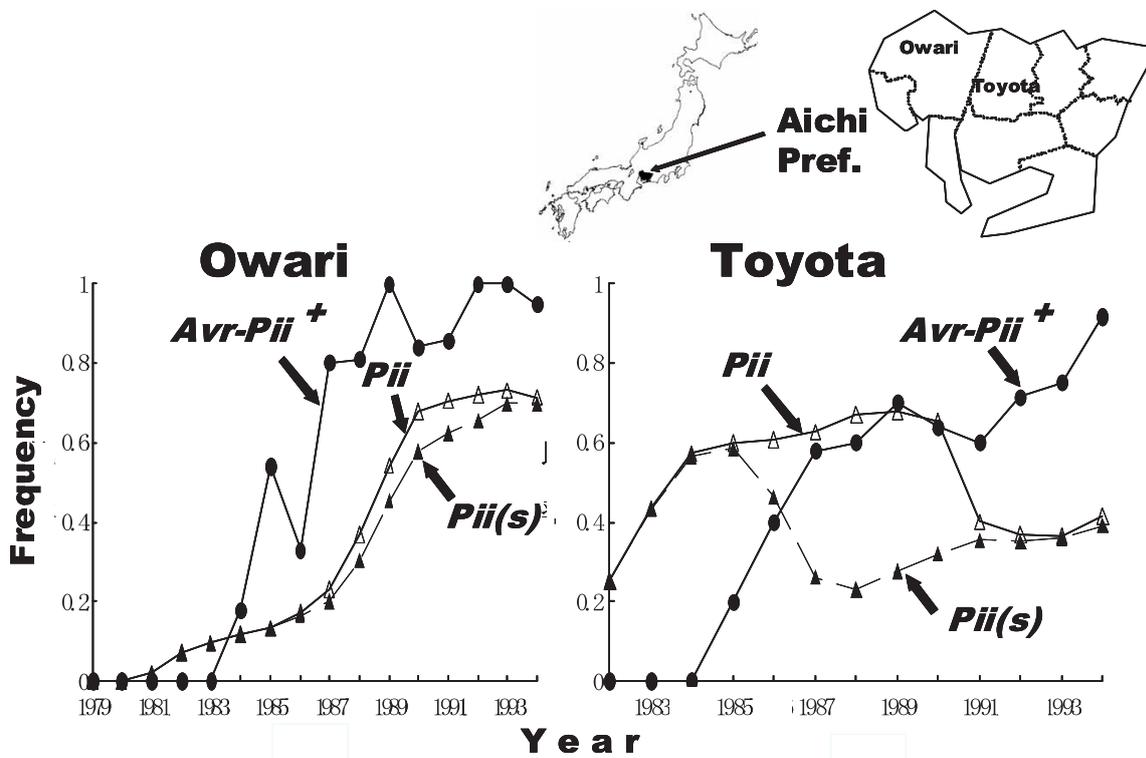


Fig. 6. Frequencies of *Avr-Pii* races, *Pii* genotype and *Pii* genotype with low levels of partial resistance in two regions of Aichi prefecture from 1981 to 1994

Table 2. Difference in aggressiveness of blast races 003 and 033 on Norin 29

Race	Lesion area (mm ²)	Sporulation ability (No. of formed spores/lesion)
003 (<i>Avr-Pia</i> ⁺)	25.8	9.8×10^3
033 (<i>Avr-Pia</i> ⁺ , <i>Avr-Pik</i> ⁺)	21.9**	$8.3 \times 10^3^*$

* and ** represent statistically significance at $P < 0.01$ and $P < 0.05$. Values denote means of 22 isolates belonging to the respective races.

lence (Koizumi 2007; Noguchi et al. 2006), and virulence mutants of the blast fungus are commonly found to have less fitness than the original isolates. However, Fujita et al. (2007) showed that the fitness of variants formed by mutations differed between the variants and the existence of stabilizing selection had not yet been proven. Further studies are necessary to ascertain the existence of any common features amongst blast races for fitness or other factors affecting prevalence.

Durability of blast resistance by monitoring of blast races

Kiyosawa (1985) proposed several methods to ensure the durability of blast resistance. The methods include mixtures of cultivars or near-isogenic lines with different types of complete resistance; gene rotation of complete resistance; pyramiding of complete resistance genes; use of partial resistance; and accumulation of both complete resistance and partial resistance. Kiyosawa (1985) carried out theoretical comparisons among the methods to predict their resistance durability. However, the best methods for ensuring durable blast resistance remain unclear because sufficient data on the prevalence of blast races in host populations with different resistance are not yet available. Epidemiological data on blast race prevalence is necessary to interpret the quantitative analyses of the changes in blast race frequencies. To gather such data, methods that make blast race monitoring easy, including seedling traps, need to be developed because the conventional method of blast race identification is laborious for mono-conidial isolation, inoculation and phenotype evaluation.

Many avirulence genes, including cloned *Avr-Pita*, *Avr-Co39* and *ACE1*, have been found in the rice blast fungus and DNA markers linked to the avirulence genes have been identified (Koizumi 2007). Development of DNA marker sets tightly linked to the avirulence genes in the blast fungus are useful for easy monitoring of blast races.

Epidemiological model

To simulate leaf blast epidemics in multilines over a short term, Ashizawa et al. (2005) developed BLAST-MUL, an epidemiological computer simulation model based on BLASTL, a systems analytical model for leaf blast epidemics. BLSTMUL can mimic leaf blast epidemics in 'Sasanishiki' and 'Koshihikari' multilines. The model includes parameters on virulence mutation rates, fitness cost, and degrees of partial resistance, and can quantitatively simulate epidemics of respective blast races after setting initial and incorporated amounts of inocula of the blast races. The model is thought to be able to simulate blast epidemics in rice populations with different blast resistant varieties as well as multilines.

Hence, the model will likely contribute to the understanding of the durability of blast resistance after more reliable parameters based on accumulated epidemiological data are introduced into it and it is combined with other long-term models.

Kiyosawa (1972) and Kiyosawa (1976) carried out theoretical comparisons among the different usage types of blast resistance to clarify their durability using mathematical models. Additionally, Sasaki (2002) mathematically analyzed host-parasite co-evolution in a multilocus gene-for-gene system. It is conceivable that in the future, with increased empirical data, such mathematical models will be able to contribute to the durability of blast resistance over the long term.

New differential system

The IRRI-Japan Collaborative Research Project recently developed new differential varieties, composed of LTH monogenic lines with 24 single complete resistance genes (Kobayashi et al. 2007). Hayashi and Fukuta (2007) have proposed a new international system for classifying blast races using these new differentials. Some Japanese differential varieties have genes such as Pish in addition to the target genes, and they distort the effect of the target genes when introduced to rice cultivars, particularly those from tropical regions. However, the new differential varieties developed by the IRRI-Japan Project do not contain these additional genes. Therefore, the system will provide more information on the effective and stable use of blast resistant cultivars globally by accumulating monitoring data of blast races.

Monitoring of completely and partially resistant rice cultivars

Complete resistance genotypes and degrees of partial resistance of most rice cultivars in Japan have already been determined. However, the resistance of rice cultivars in many other countries is limited, despite this information being necessary for epidemiological analyses for the prevalence of blast races. The standard blast isolates to detect complete resistance genotypes of rice cultivars and development of methods that make it easy to detect complete resistance genes, such as DNA markers tightly linked to complete resistance genes, are urgently needed for these countries.

Partial resistance also affects the prevalence of blast races and monitoring of partial resistance levels is important for the durability of blast resistance. Recent quantitative trait loci (QTL) analyses in rice cultivars have identified many partial resistance blast genes, such as the cloned *pi 21*. These analyses also revealed the putative chromosomal locations of the resistance genes and allowed the development of DNA markers linked to the genes (Koizumi 2007). Tightly linked DNA markers can detect the partial resistance genes easily and estimate

degrees of partial resistance in rice cultivars. This information can improve the accuracy in analyses of prevalence of blast races.

Partial resistance to blast is generally non-race specific. However, Zenbayashi et al. (2005) recently found a new gene-for-gene relationship between the isolate-specific partial resistance gene *Pi34* and the aggressiveness gene *AVR*Pi34** in the blast pathogen by crossing the host and the pathogen. Partial resistance with similar characteristics to *Pi34* must be examined to ensure that resistance is durable.

Conclusion

New differential varieties developed by the IRRI-Japan Collaborative Research Project made global surveys of blast race distribution and estimation of complete resistance genotypes of rice cultivars possible. However, the best approach to ensuring durable blast resistance is not yet clear since sufficient quantitative analyses of changes in blast race frequencies, which are required to ensure such durability, are not yet available. The development of methods that make it easy to monitor blast races as well as resistance genotypes of rice cultivars using DNA markers tightly linked to avirulence or resistance genes can accelerate the accumulation of epidemiological data on population interaction between host and pathogen. The analyses of the accumulated data will allow reliable epidemiological simulation models to be constructed and these can guide the use of blast resistant cultivars through simulations of prevalence of blast races in host populations with different resistance.

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