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Development and Characterization of Blast Resistance Using Differential Varieties in Rice

Edited by
Yoshimichi Fukuta, Casiana M. Vera Cruz
and Nobuya Kobayashi

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Forwards

Japan International Research Center for Agricultural Sciences (JIRCAS) is conducting a collaborative study on "Blast Research Network for Stable Rice Production" with the International Rice Research Institute (IRRI) in the Philippines, one of international organizations of Consultative Group on International Agricultural Research (CGIAR) center, since 2006. Main objective of this project is to develop universal differential system for blast pathogen and resistance genes in rice varieties as a first step of building up the durable resistant system for blast which is the most serious rice disease in all over the world, having participation of NARES partners from Korea, China, Vietnam, Philippines, Indonesia, and Laos. A special workshop on "Development and characterization of blast resistance using differential varieties in rice" of this research project was held at Changsha, Hunan, China, in October 2007 as a satellite meeting of the International Rice Blast Congress. In the workshop, some application research with differential system, monitoring blast races in Japan, conventional genetic analysis for blast resistance, characterization of monogenic lines and near isogenic lines for blast resistance gene as the differential varieties, new designation system using monogenic lines for developing the system, and identification of resistance genes using DNA markers, were presented. These results and achievements are arranged and demonstrated as the protocols and manuals for pathological studies and conventional genetic analyses for blast resistance based on the differential system in this working report. The resistance genes and their selection DNA markers have been reported by many scientists. Those information were also reviewed for clearifing the effectiveness, limitations, and problems in this report to understand the research situation of resistance genes and to apply them for marker aided selection (MAS) of resistance genes in rice breeding. The reviews and reports in this JIRCAS working report will help in understanding output of blast studies for improvement of blast resistance in rice varieties. I would like to share these information among all scientists, and hope our collaboration based on the differential system will contributed for the developing a durable protection and stable production methods in rice.

Kenji Iiyama
President
Japan International Research Center for Agricultural Sciences (JIRCAS)

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

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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 different 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).

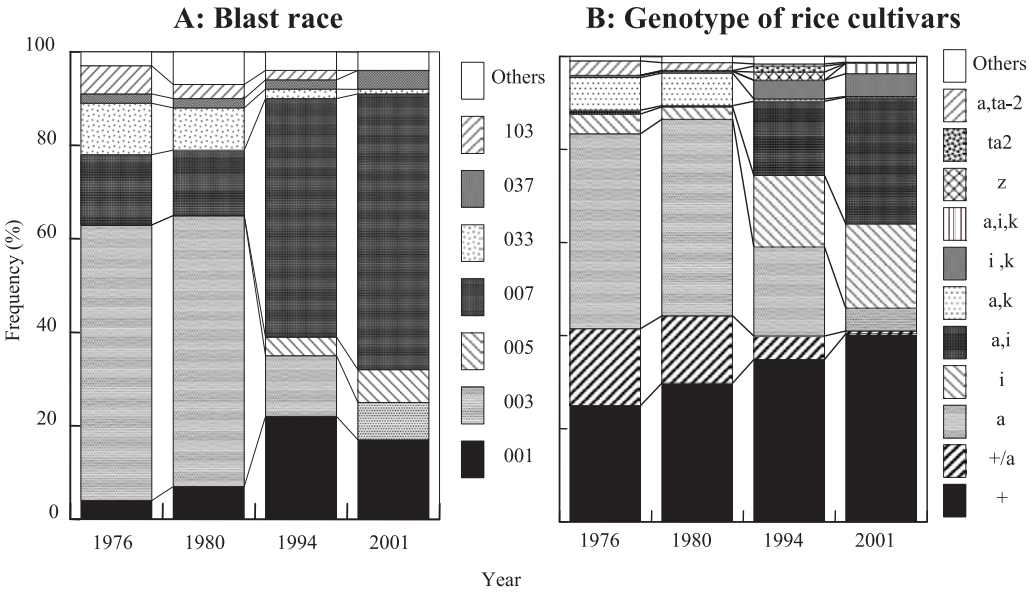

Fig. 1. Changes in frequencies of predominant blast races and percentages of blast resistance genotypes in cultivated rice cultivars in Japan

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.

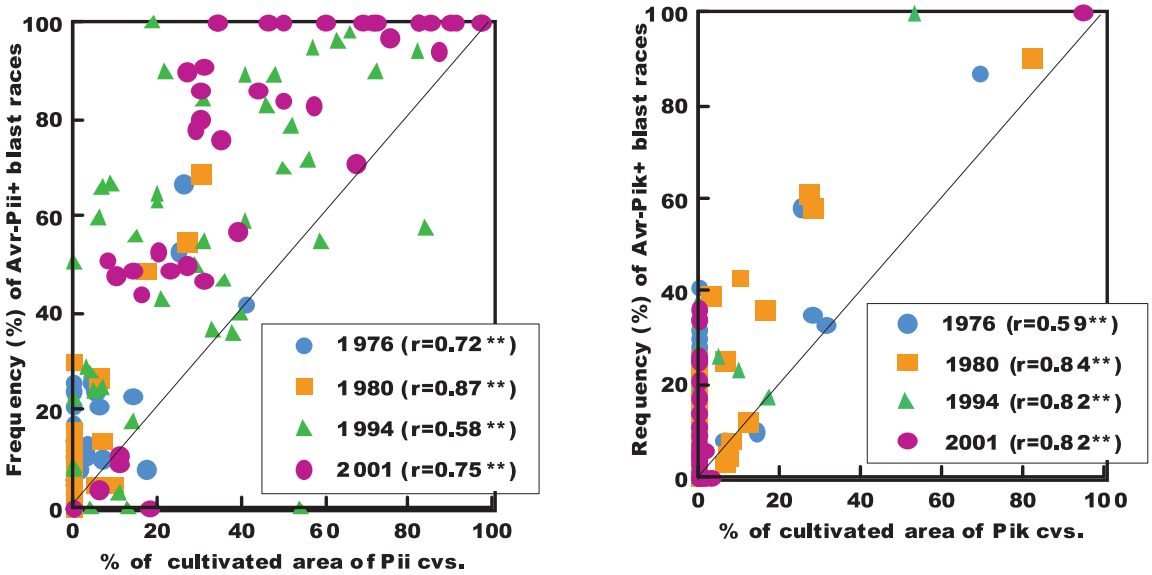

Fig. 2. Relationship between percentages of cultivated areas of rice cultivars carrying *Pii/Pik* and isolated frequencies of *Avr-Pii*$^+$/*Avr-Pik*$^+$ blast races in Japan. Each symbol represents values of the percentage and frequency in a prefecture in a monitoring year

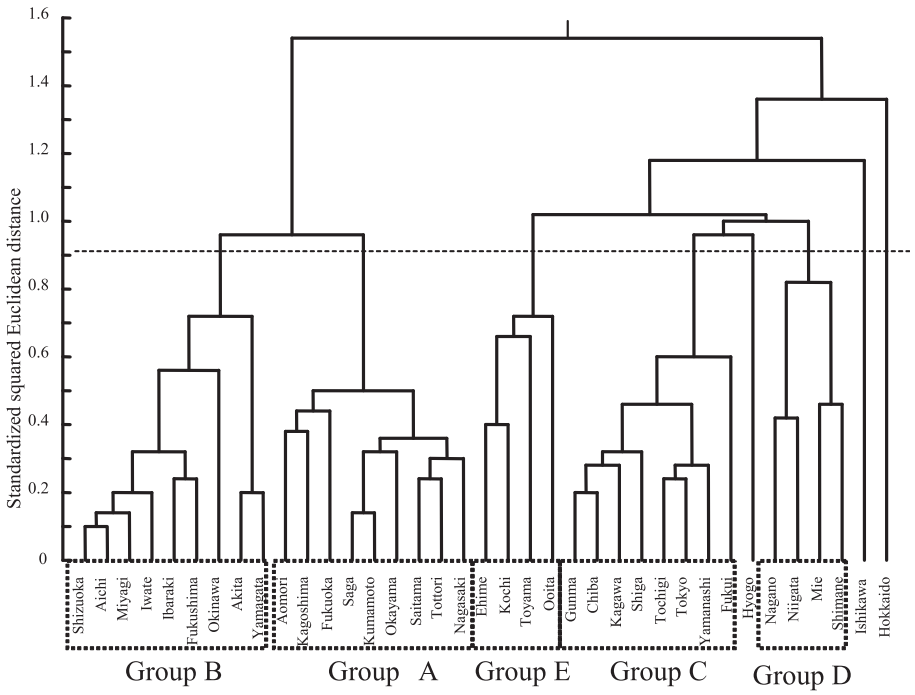

Fig.3. Cluster analysis using data sets of isolation frequencies of blast races virulent to *Pia*, *Pii*, *Piz* and *Pita* in 1976, 1980, 1994 and 2001, divided 37 prefectures in Japan

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

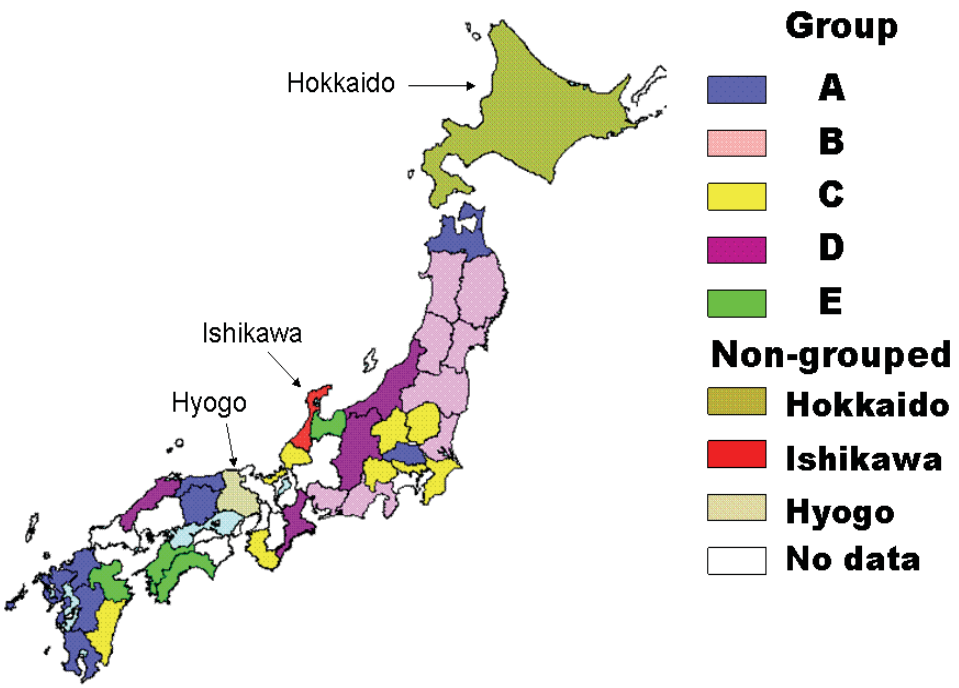

Fig. 4. Geographical classification of Japanese prefectures with cluster analysis based on frequencies of *Avr-Pia*$^{+}$, *Avr-Pii*$^{+}$, *Avr-Piz*$^{+}$ and *Avr-Pita*$^{+}$ blast races in 1976, 1980, 1994 and 2001

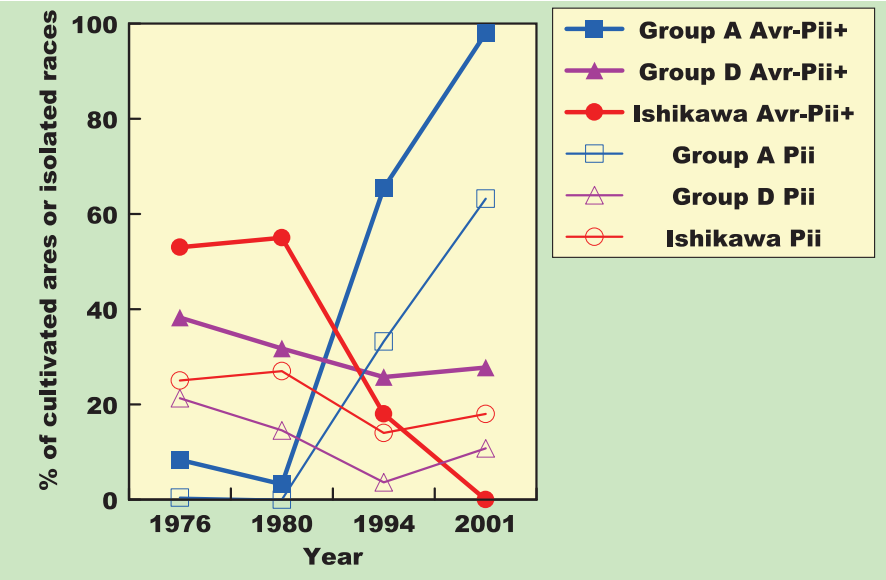

Fig. 5. Frequencies of *Avr-Pii*$^{+}$ blast races and percentages of cultivated areas of *Pii* in each group from 1976 to 2001 in Japan

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-

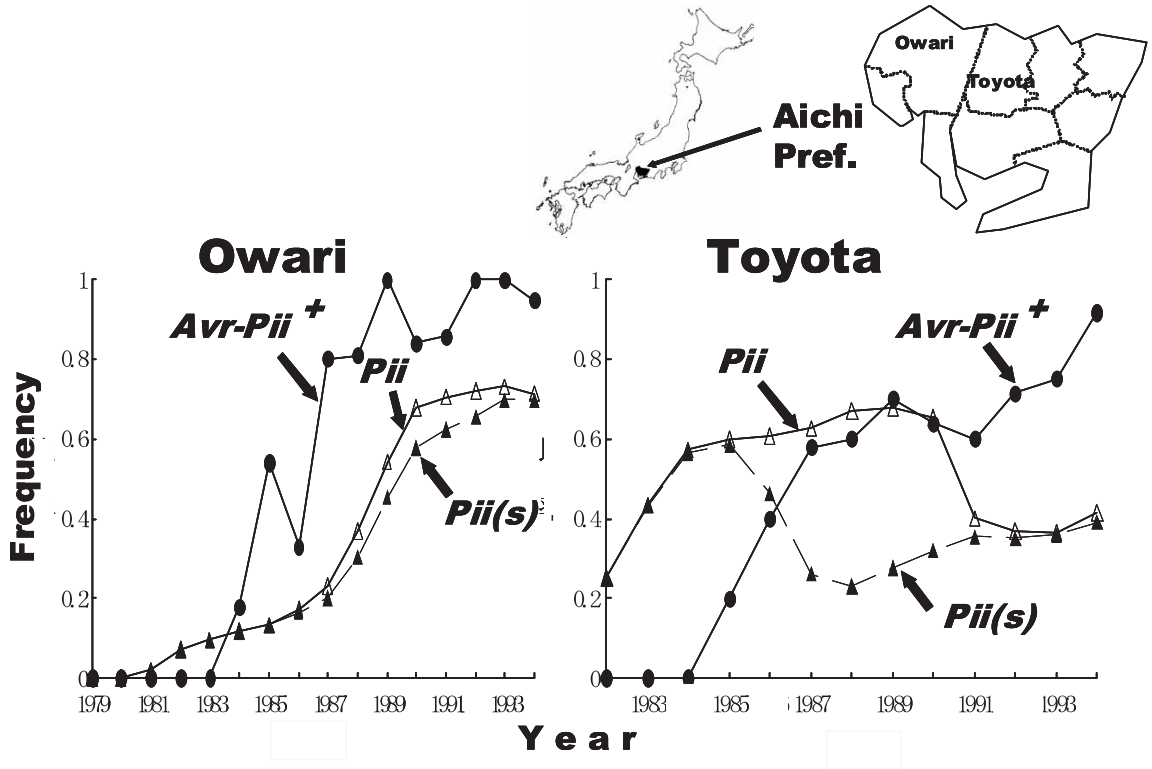

Fig. 6. Frequencies of *Avr-Pii* races, *Pii* genotype and *Pii* genotype with low levels of partial resistance in two regions of Aichi prefecture from 1981 to 1994

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 (*Avr-Pia*$^+$)	25.8	9.8×10^3
033 (*Avr-Pia*$^+$, *Avr-Pik*$^+$)	21.9**	8.3×10^3*

* 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 BLASTMUL, 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 *AVRPi34* 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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Proposal for a new international system of differentiating races of blast (*Pyricularia oryzae* Cavara) by using LTH monogenic lines in rice (*Oryza sativa* L.)

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Abstract

A new systematic, expandable method that allows easy understanding of the relationships between races and resistance genes is proposed for building up an international standard designation and classification of blast races. Blast races were characterized by reactions to 26 Lijian-xintuan-heigu (LTH) monogenic lines for targeting 23 resistance genes, which were divided into five groups, (1) LTH, IRBLa-A, IRBLsh-S, IRBLb-B, and IRBLt-K59, (2) 3 lines of *Pii* locus region, (3) 7 lines of *Pik* region, (4) 4 lines of *Piz* region, and (5) 7 lines of *Pita* region. Each group consists of 1 to 3 variety unit(s), which were allocated with 3 differential lines (genes) in each and applied codes, 1, 2, and 4, for compatible reactions of blast isolates, respectively. A blast race is characterized by the sum of codes in combinations of three varieties' reactions in each unit with the Gilmour method. In the case of all compatible reactions to differential lines, the race No., 73-i7-k177-z17-ta733, will be designated. This designation method will contribute diversity research for blast races and rice resistance, and pathological, genetic, and breeding studies of blast disease and rice varieties, to build up the durable protection system for blast disease, through enhancement of communication among pathologists and breeders at the grobal level.

Keywords: IRRI, differential variety, Gilmour method, compatible reaction, pathogen

Introduction

Rice blast is severe in uplands and rainfed lowlands in tropical areas and also in irrigated lowlands in temperate areas. The use of resistance rice varieties has been one promised way to control blast disease caused by *Pyricularia oryzae* Cavara, but there is little information on genotype of Indica-type resistance varieties and on pathogenic races of the blast fungus distributed in tropical areas. Yamada et al. (1976) selected and proposed the nine differential varieties Aichi Asahi for *Pia*, Ishikari-shiroke for *Pii*, Kanto 51 for *Pik*, Tsuyuake for *Pik-m*, Shin 2 for *Pik-s*, Yashiro-mochi for *Pita* (*Pi4*(t)), Pi No. 4 for *Pita-2*, Fukunishiki for *Piz*, and Toride 1 for *Piz-t*. Kiyosawa (1984) selected and rearranged a differential variety set consisting of 12 varieties for targeting 12 resistance genes: Aichi Asahi for *Pia*, BL1 for *Pib*, Fujisaka 5 for *Pii*, Kusabue for *Pik*, Tsuyuake for *Pik-m*, K60 for *Pik-p*, Shin 2 for *Pik-s*, K59 for *Pit*, K1 for *Pita*, Pi No. 4 for *Pita-2*, Fukunishiki for *Piz*, and Toride 1 for *Piz-t*. Using these differential varieties, a designation system for blast races was developed in Japan, and surveys of distribution of pathogenic races of rice blast fungus have been performed by Yamada et al. (1976), Yamada et al. (1985), Naito et al. (1999), and Koizumi et al. (2006).

Every differential variety represents each dominant resistance gene identified by Kiyosawa and others. Therefore, both differentials have a higher ability for differentiating blast races. These differential variety sets and the designation system have been used in Japan and several other countries. However, the system was not fully functioning for the international comparison of blast isolates among these countries because these were unknown genetic factors (resistance genes) in these backgrounds, and they showed an unexpected reaction to blast isolates from tropical areas. A new gene, *Pish*, was identified in these differentials and by using the race, it

appeared in the newly released variety, Reiho, in Kyushu (Imbe and Matsumoto, 1985). *Pish* was included in seven of 12 differentials. This fact is against the principle that a differential should have one resistance gene. Actually, sometimes shown is that the differential system has insufficient ability to identify the races of the fungus isolates in other countries such as race 000.0, because the isolates may have an avirulence gene corresponding to *Pish*. Furthermore, differential varieties having multiallelic genes in *Pik* and *Piz* loci disperses to a different group. These indicate that it is difficult to use the Japanese differential system as an international common tool.

Recently, 29 monogenic lines, each containing one of 24 resistance genes, *Pia*, *Pib*, *Pii*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pish*, *Pit*, *Pita*, *Pita-2*, *Piz*, *Piz-t*, *Piz-5*, *Pi1*, *Pi3*(t), *Pi5*(t), *Pi7*(t), *Pi9*, *Pi11*(t), *Pi12*(t), *Pi19*(t), and *Pi20*(t), were developed through collaboration between the Japan International Research Center for Agricultural Sciences (JIRCAS) and the International Rice Research Institute (IRRI) as a new differential variety set (Tsunematsu et al., 2000, Kobayashi et al., 2007). A Chinese Japonica-type rice variety Lijiangxintuanheigu (LTH), which has no major resistance gene for rice blast, was used as a recurrent parent in these monogenic lines. The development of these monogenic lines has allowed us to differentiate the race of blast fungus isolate to a high level of precision. Telebanco-Yanoria et al.(2008) could characterize the pathogenecities of blast isolates from the Philippines and selected a set of standard differential blast isolates. These materials will be useful for the classification and characterization of blast isolates, but the number of resistance genes targeted in the variety set is two times more in comparison with those of previous one (Kiyosawa, 1984). The designation system using the differential variety set is insufficient to distinguish and characterize in detail of blast isolates, when using those of Telebanco-Yanoria et al. (2008).

An aim of this research is to develop a new international classification and designation system for blast races, which can be commonly used by breeders and pathologists from tropical and temperate areas. In the meantime, we would like to propose a tentative system, which will enhance discussion, understanding, and comparison of blast races from tropical and temperate areas using the new differential variety set by Telebanco-Yanoria et al. (2008). In this study, the characters of the new designation system will be explained and discussed in comparison with the previous designation system.

Materials and methods

Evaluation and selection of monogenic lines using standard differential blast isolates

First, the responses of each LTH monogenic line to more than 50 fungal isolates including standard strains of blast fungus in Japan and isolates from a different ecosystem in the whole world were recorded according to infection type (generally, 0-2 resistant; 3-5 susceptible) in accordance with the IRRI *Standard Evaluation System* (SES, 1996) (Table 1).

Classifications

To clearly delineate the relationship between race code number and resistance gene, the system of nomenclature of blast fungus race employed Gilmour's method (Gilmour, 1973). This nomenclature method is excellent in regularity and flexibility, and suitable for a differential system composed of many varieties. The selected LTH monogenic lines are divided into groups with 3 lines, and each of the 3 lines is given the code number 1, 2, 4, respectively. The total of the code numbers of the three LTH monogenic lines, which are compatible to blast fungus, show the race number.

Results and discussion

Selection of representative differential varieties

The monogenic lines that had the same reaction to all blast fungus were eliminated except one. As a result we chose 26 representative lines for 23 resistance genes, *Pia*, *Pib*, *Pii*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pish*, *Pit*, *Pita*, *Pita-2*, *Piz*, *Piz-t*, *Piz-5*, *Pi1*, *Pi3*(t), *Pi5*(t), *Pi7*(t), *Pi9*, *Pi12*(t), *Pi19*(t), and *Pi20*(t), including LTH as a susceptible check variety. The reactions of monogenic lines harboring multiallelic genes in *Pik* locus to blast fungus isolates are shown in Table 2.

Grouping of differential variety

Many multiallelic blast resistance genes are known in rice. In total, twenty of the LTH monogenic lines are multiallelic genes in four genetic loci. The LTH monogenic lines having genes presumed to be multiallelic or close link in *Pii*, *Pik*, *Piz*, and *Pita* locus were divided into the group, respectively, and arranged in accordance with the Gilmour method.

In this study, a new international system for differentiating races of *Pyricularia oryzae* Cavara using LTH monogenic lines is proposed as shown in Table 3. Each race code number has the following five parts divided by a hyphen (i.e., 1st-2nd-3rd-4th-5th). The 1st part of the race code number has a two-digit number and is composed of LTH, and IRBLa-A in the ones place, and IRBLsh-S, IRBLb-B and IRBLt-K59 in the tens place. The 2nd part has a one digit number and is composed IRBLi-F5, IRBL3-CP4, and IRBL5-M, which are multiallelic or closely linked. The 3rd part has a three-digit number and is composed of IRBLk-Ka, IRBLkp-K60 and IRBL7-M in the ones place, IRBLkm-Ts, IRBL1-CL and IRBLkh-K3 in the tens place, and IRBLks-S, in the hundreds place, which are multiallelic or closely linked. The 4th part has a two-digit number and is composed IRBLz-Fu, IRBLz5-CA, and IRBLzt-T in the ones place, and IRBL9-W in the tens place, which are multiallelic or closely linked. The 5th part has a three-digit number and

Table 1. Rice blast fungus isolates used in the study

Isolates	Old race No.[1]	Rice variety isolated	Organ infected	Location collected	Isolated year	MAFF No.[2]	Remarks
Mu-95	001.2	Shin2	panicle	NARC Kyushu Okinawa, Japan	1993	101505	standard strain
Kyu89-246	003.0	Nangoku-mochi	panicle	Miyakonojou, Miyazaki Pref. Japan	1989	101506	standard strain
Ken54-04	003.0	Shinriki11	leaf	Gifu, Gifu Pref. Japan	1954	101507	standard strain
Ken54-20	003.0	Kairyou-aikoku	leaf	Oouchimachi, Yamaguchi Pref. Japan	1954	101508	standard strain
95Mu-29	003.2	BL1	leaf	NARC Kyushu Okinawa, Japan	1995	101509	standard strain
Shin83-34	005.0	unknown	panicle	Kamibayashimura, Niigata Pref. Japan	1983	101510	standard strain
Ina86-137	007.0	Koganenishiki	-	Inabu, Aichi Pref. Japan	1986	101511	standard strain
Hoku1	007.0	Norin20	node	Sapporo, Hokkaido, Japan	1948	101512	standard strain
31-4-151-11-1	007.2	BL1	leaf	NIAS	1991	101513	standard strain
Kyu92-22	017.1	Hinohikari	leaf	Uekimachi, Kumamoto Pref. Japan	1992	101514	standard strain
1804-4	031.1	unknown	leaf	Miwamura, Niigata Pref. Japan	1976	101515	standard strain
Ina72	031.1	Kanto37	neck	Nagano, Nagano Pref. Japan	1957	101516	standard strain
TH68-126	033.1	unknown	-	NARC Tohoku Oomagari, Japan	1968	101517	standard strain
TH68-140	035.1	Dewaminori	-	Yamagata Pref. Japan	1968	101518	standard strain
24-22-1-1	037.1	unknown	panicle	Igamachi, Mie Pref. Japan	1994	101519	standard strain
Ai79-142	037.3	Hama-asahi	neck	Inabu, Aichi Pref. Japan	1979	101520	standard strain
Kyu9439013	047.0	Natsuhikari	leaf	Toyomachi, Kochi Pref. Japan	1994	101521	standard strain
TH69-8	071.1	Fukunishiki	-	Fukushima Pref. Japan	1969	101522	standard strain
Sasamori121	077.1	Akiyutaka	-	Mogamimachi, Yamagata Pref. Japan	1990	101523	standard strain
Ina168	101.0	Suzuhara-mochi	leaf	Inabu, Aichi Pref. Japan	1958	101524	standard strain
Ken53-33	137.1	Kanto51	neck	Inabu, Aichi Pref. Japan	1953	101525	standard strain
Ina93-3	301.0	Yamahikari	spikelet	Kikukawamachi, Yamaguchi Pref. Japan	1993	101526	standard strain
GFOS8-1-1	303.0	Yamahikari	neck	Ena, Gifu Pef. Japan	1993	101527	standard strain
P-2b	303.1	-	-	-	1957	101528	standard strain
0528-2	333.1	Hatsunishiki	-	Minasemura, Akita Pref. Japan	1976	101529	standard strain
Ao92-06-2	337.1	unknown	-	Towada, Aomori Pref. Japan	1992	101530	standard strain
Mu-183	337.3	BL1	-	NARC Kyushu Okinawa, Japan	1995	101531	standard strain
IW81-04	437.1	Toride1	leaf	Oomagari, Akita Pref. Japan	1981	101532	standard strain
Ai74-134	477.1	unknown	-	Inabu, Aichi Pref. Japan	1974	101533	standard strain
4209-R-39	-	-	-	-	-	-	progeny strain from 4132-R-12 and CHNOS59-9-1
CHNOS58-3-1	000.0		neck	Yunnan, China	1992	101267	upland
CHNOS66-2-1	013.7		leaf	Yunnan, China	1992	101308	upland
CHNOS102-2A-1	115.5		neck	Baoshan, Yunnan, China	1994	-	upland
CHNOS122-3-3	017.5		leaf	Wenshan, Yunnan, China	1995	-	upland
CHNOS125-3-3	137.5		leaf	Wenshan, Yunnan, China	1995	-	upland
FR2	106.4		leaf	Combi, Guiana	1978	-	-
H02-58-1	117.1		leaf	Muse, Shan, Myanmar	2002	-	lowland
H02-75-1	712.0		leaf	Hsipaw, Shan, Myanmar	2002	-	-
H05-67-1	013.0	Sensho	leaf	Ibaraki, Japan	2005	-	upland
H05-72-1	031.1		leaf	Ibaraki, Japan	2005	-	upland
H05-99-1	014.0		leaf	Ibaraki, Japan	2005	-	upland
H05-100-1	413.0		leaf	Ibaraki, Japan	2005	-	upland
H06-35-1	000.0		neck	Cabanatuan, Philippines	2006	-	lowland
H06-36-1	000.0		neck	Cabanatuan, Philippines	2006	-	lowland
H07-107-1	002.0		leaf	Kalaw, Shan, Myanmar	2007	-	lowland
H07-198-1	000.0		leaf	Muang Xay, Laos	2007	-	Rainfed lowland
IBOS8-2-1	010.0		neck	Ibaraki, Japan	1988	-	upland
IDOOS6-1-1	102.4	GH 126	leaf	North Sumatera, Indonesia	1985	-	-

1: Determined by Japanese 12 differential system

2: National Institute of Agrobiological Sciences Genebank

Table 2. Infection type of LTH monogenic lines *Pik* locus genes to blast fungus isolates

Monogenic line and susceptible variety	Resistance gene	Blast fungus isolate: 1804-4 (Japan, J031.1)	H05-72-1 (Japan, J031.1)	FR2 (Guiana, J106.4)	H02-58-1 (Myanmar: J117.1)	IBOS8-2-1 (Japan, J010.0)	H05-99-1 (Japan, J014.0)	H05-100-1 (Japan, J413.0)	H05-67-1 (Japan, J003.0)	H06-35-1 (Philippines, J000.0)
IRBLks-S	*Pik-s*	5	5S	5S	5	5S	5S	5S	5S	0
IRBLk-Ka	*Pik*	5	5S	5S	4'	4	5	5S	0	5
IRBLkp-K60	*Pik-p*	5	4'	5S	5	1	2s	2s	1	0
IRBL7-M	*Pi7*	5	5	5'	5S	2s	2L-3'	2L	0	5S
IRBLkm-Ts	*Pik-m*	5	5	2s	0	1	1'	2L	0	5S
IRBL1-CL	*Pi1*	5	4'	2L	2s	2L	2s	5	0	5S
IRBLkh-K3	*Pik-h*	5	2L	2s	1	1	1	1	1	5S
LTH	+	5	5S	5S	5	5S	5S	5S	5S	5S

0-2(2s, 2L): Resistant reaction, 3-5(5S): Susceptible reaction

Table 3. New designation system for blast races based on the reaction of monogenic line with LTH genetic background

Group	I		II	III			IV		V		
Locus	-		*Pii*	*Pik*			*Piz*		*Pita*		
Target resistance gene	*Pish*	+	*Pii*	*Pik-s*	*Pik*-m	*Pik*	*Pi9*	*Piz*	*Pita-2*	*Pita*	*Pi19*(t)
	Pib	*Pia*	*Pi3*(t)	-	*Pi1*	*Pik-p*	-	*Piz-5*	*Pita-2*	*Pita*	*Pi20*(t)
	Pit	-	*Pi5*(t)	-	*Pik-h*	*Pi7*(t)	-	*Piz-t*	*Pi12*(t)	-	-
Monogenic line (IRBL)	sh-S	LTH	i-F5	ks-S	km-Ts	k-Ka	9-W	z-Fu	ta2-Pi	ta-K1	19-A
	b-B	a-A	3-CP4	-	1-CL	kp-K60	-	z5-CA	ta2-Re	ta-CP1	20-IR24
	t-K59	-	5-M	-	Kh-K3	7-M	-	zt-T	12-M	-	-
Code	1	1	1	1	1	1	1	1	1	1	1
	2	2	2	-	2	2	-	2	2	2	2
	4	-	4	-	4	4	-	4	4	-	-
Ex. Blast isolates virulence to all genes	S	S	S	S	S	S	S	S	S	S	S
	S	S	S	-	S	S	-	S	S	S	S
	S	-	S	-	S	S	-	S	S	-	-
	7	3	7	1	7	7	1	7	7	3	3

Example blast race No. of isolate that is virulence to all differential varieties (genes)

is composed IRBL19-A, IRBL20-IR24 in the ones place, IRBLta-K1, IRBLta-CP1 in the tens place, and IRBLta2-Pi, IRBLta2-Re, IRBL12-M in the hundreds place, which are multiallelic or closely linked.

Designation system

A race number is shown in the total of the code number of monogenic lines, which are compatible to a blast fungus. To indicate which of each part is *Pii*, *Pik*, *Piz* and *Pita* locus, the symbols i, k, z and ta, were marked in front of each multiallelic part, respectively. For example, when a blast isolate is pathogenic to all the differential varieties, the race number of the isolate is shown as "73-i7-k177-z17-ta773."

This system offers sufficient range for regularity, flexibility and expansion to take in new resistance identified in the future. Atkins et al. (1967) and Goto et al.(1967) developed eight differential varieties in 1967 and selected prior to genetic analysis of possible candidates for a set of international differential varieties. International races were determined by the susceptibility of the eight key varieties selected prior to genetic analysis. The race was numbered in each group of key variety. This system has sufficient regularity if the number of differential varieties does not change. When differential varieties are increased along with the introduction of the

new resistance gene, extensibility is poor.

Also the international differential system for rice blast race in this paper was proposed at the 4th International Rice Blast Conference in 2007 and at annual meetings of the Japanese society of Plant Pathology in 2006 and 2008. The system was developed after some degree of revision. Although the proposed system has not yet been completed, because of leaving verification of details, this system has the ability to contribute to studies of blast fungus and resistance in rice plants. We shall consider a better system as an international differential system is taken due to the accumulation of more perspectives, discussion and information.

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Protocols for the sampling of diseased specimens and evaluation of blast disease in rice

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Introduction

This protocol was written for scientists who were facing the problems of blast disease and want to start the study. When we decide a pathogenic race of Pyricularia oryzae using the new differential lines, the observation of infection type formed by leaf blade of seedling and the conversion to the binarization in resistance (R) or susceptible (S) are required. However, actually, we come up against the case that hesitates to judge R or S due to various conditions. So it is necessary to make criteria of the judgment, a point to keep in mind clear during the spread of new differential system, so that lead us determination not depend on a researcher. In this protocol, from the sampling of the pathogenic fungus to evaluation of the infection type concretely is mentioned. We want in particular you put panel at hand and refer on the occasion of evaluation of the infection type. Though blast resistance also included another type called a partial resistance, this manual is limited to the evaluation method for complete resistance to participate in determination of the pathogenic race.

This protocol includes as following:

I. Medium preparation
II. Sampling of diseased specimens
III. Monoconidial isolation
IV. Preparation of long-term storage (Stock culture)
V. Preparation of plant materials for inoculation
VI. Inoculum production
VII. Counting of spores using hemacytometer
VIII. Coordinated inoculation procedure (Spraying method)
IX. Disease assessment
X. Designation of new international differential system
XI. List of International Rice Blast Line (LTH monogenic lines)

I. Medium preparation

a) Water agar

[Purposes] for monoconidial isolation
[Materials]

Bacto Agar	30 g
Distilled water	1,000 ml
Streptomycin	40 mg

[Procedure]

1. Weigh 30 g of Bacto agar.
2. Put it in a 2-liter capacity flask.
3. Add 1000 ml *double distilled water* ($D^2H_2O^1$) or deionized water.
4. Plug with cotton and autoclave for 15 min at 15 psi.
5. Before plating 3% water agar add 40 mg streptomycin per liter.

When you promote growth of mycerium, add 5g sugar and 2g yeast extract.

b) Prune agar

[Purposes] for preparation of long-term storage (Stock culture)
[Materials]

Prune agar (PA)	3 pcs
Lactose	5 g
Yeast extract	1 g
Agar	20 g (If stick bar, 17 g if bacto)
D^2H_2O	1000 ml

[Procedure]

1. Boil the prunes in 500 ml distilled deionized water (D^2H_2O) for one hour.

2. Dissolve the agar by heating in 500 ml distilled deionized water (D^2H_2O).
3. Mash the prunes thoroughly and strain or filter the prune juice through cheese cloth or nylon mesh.
4. Pour the strained prune juice into the agar.
5. Adjust the volume to 1000 ml by adding distilled deionized water (D^2H_2O).
6. Add the lactose and yeast extract and stir well.
7. Adjust the pH to 6.5
8. Before it cools, dispense the melted agar into tubes (for slants – 10 ml) or flasks (for plates – 250 ml).
9. Plug with cotton and autoclave for 15 min at 15 psi.
10. Before plating prune agar, add 10 mg of streptomycin for every 250 ml media.

Note: Gulaman bar is locally available and much cheaper. Please use this for most experiments.

c) Rice flour agar

[Purposes] for preparation of long-term storage (Stock culture)

[Materials]

Complete rice flour	15 g
Yeast extract	4 g
Agar	15 g
Distilled water	1,000 ml

[Procedure]

Same as the Prune agar

d) Oatmeal agar

[Purposes] for inoculum production

[Materials]

Rolled oats	30-50 g
Sucrose	5 g
Agar	16 g
Distilled water	1,000 ml

[Procedure]

1. Boil the rolled oats in 500 ml D^2H_2O for 15 min. This may need constant stirring.
2. Add the agar to 500 ml D^2H_2O and heat to dissolve.
3. Strain or filter the "juice" through a strainer.
4. Pour the strained rolled oats (oatmeal "juice") into the agar. Adjust the volume to 1 liter with D^2H_2O.
5. Add the 16g sucrose and stir well.
6. Dispense into tubes (for slants – 10 ml) or flasks (for plates – 250 ml).
7. Plug with cotton and autoclave TWICE for 40 min at 15 psi.
8. Before plating oatmeal agar, add 10 mg of streptomycin for every 250 ml oatmeal agar.

II. Sampling of diseased specimens

Rice growth stage suitable for a sampling: Leaf blast season or panicle blast season.

Suitable place for collection: less windy place such as hill side where dew stays longer time. Leaf color of rice plant is dark among rice field.

Point 1: Parts to observe: Specimens of neck, grain, branch diseased blast and young aged lesion in leaf are easy to isolate the spore than fully elongated lesion in leaf.

Point 2: Rapid dryness management:

[Procedure]

1. Put collected sample inside between coffee filter.
2. Write down the information such as the date, place (name of place, longitude, latitude, altitude), paddy field/upland field/other, rice (cultivar name)/wild rice/weed.
3. Dry it quickly in the room temperature.
4. For the most dried samples, put silica gel and seal the container (coffee filter), and keep in 4℃ refrigeration.

The diseased leaves unattended in room temperature for one month make few sporogenesis, and fail to separate into single spore in the next step procedure.

When you put diseased leaves in the moist chamber,

Fig. 1. Oatmeal agar medium poured in Petri dishes

Fig. 2. The coffee filter put the information of the specimen

Fig. 3. The diseased plants stored with silica gel in the plastic box

the sporogenesis is good if the intensity of green leaf is high. If it is dull, keeping state is not good, and sporogenesis is bad.

III. Monoconidial isolation

Method 1) without a microscope

[Procedure]

1. Cut infected leaf or panicle samples in 3-5 cm sections.
2. Place cut sections on moist filter paper in a Petri dish.
3. Incubate plate for 24 hr at room temperature.
4. Examine lesions under a dissecting microscope.
5. Using very fine tip Pasteur pipettes (tips melted by heating with a gas burner, pulled and then cooled), pick conidial masses and spread on plated 3% water agar containing streptomycin (10 mg/250 ml agar).
6. Incubate plates for 24 h at room temperature.
7. Pick germinated conidia and transfer to prune agar slants.
8. Incubate at room temperature for four days. The prune agar cultures will be used to inoculate liquid cultures to prepare stock cultures (See the protocol for Medium preparation).
9. Be sure you pick a single conidium.

If any collected leaf samples remain after the monoconidial isolation, put them properly in a plastic bag and keep them in the cold room at about 4℃ for future use.

Method 2) for single spore isolation using a microscope

[Procedure]

Same as above until 4 in Method 1)

5. Prepare a microscope
6. Set an insect pin (No.1) or a glass needle in the rubber stopper and align an axis.
7. Put 3% agar plate on the stage inside out.
8. Focus a spore on the agar plate.
9. Move an insect needle to the upper portion using condenser adjustment knob and focus the tip of needle on a spore.
10. Pick a single conidia and down some needles once, and then transfer it to the other place of agar plate.
11. Incubate the plates at room temperature for 1 day.
12. Confirm the germination of spore and no contamination of other fungus or bacteria.
13. Incubate more 2 or 3 days to see the mycerium, transfer to rice flour agar plates.

The rice flour agar cultures will be used to prepare stock cultures (See the protocol for Medium preparation.

Fig. 4. Cultivation of blast fungus on field infected stem or leaf in wet paper

Fig. 5. Selection of single spore

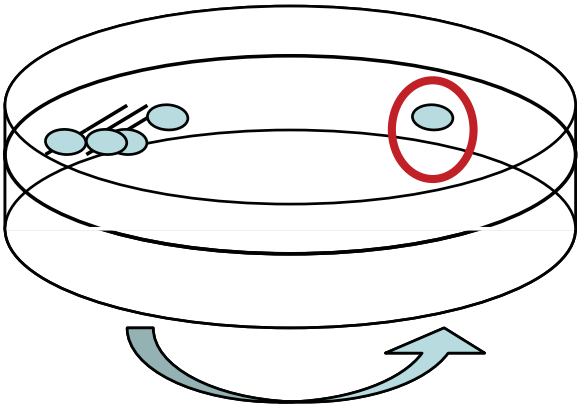


Fig. 6. Selection of single spore on water agar

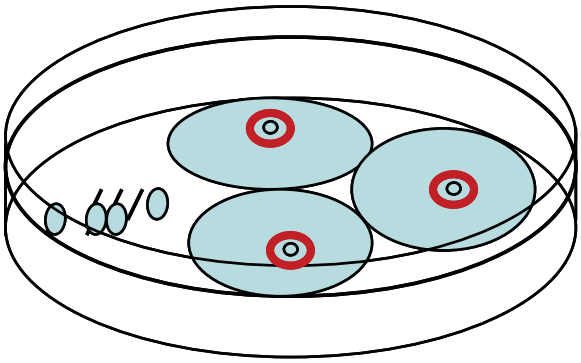


Fig. 7. Cultivation on water agar after 1 day at room temperature (ca. 25-28 ℃)

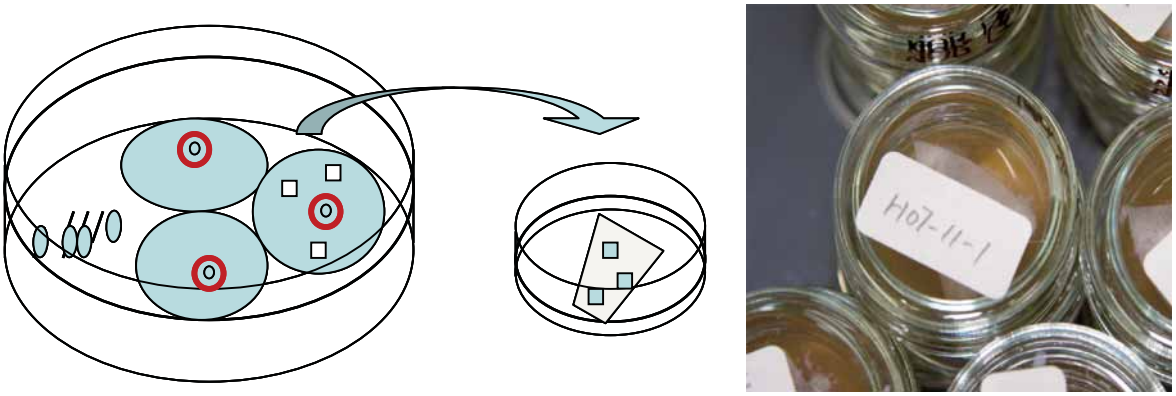


Fig. 8. Cultivation of blast fungus on rice flour agar medium for long-term storage of the blast isolate

IV. Preparation of long-term storage (Stock culture)

Method 1) IRRI method

The following steps describe the removal of paper disks from slants.

(If isolate is very important or will be used often, prepare more of this one.)

(If there appears to be contamination or no germination from the stock culture, report this to all concerned.)

[Procedure]

1. Cut the filter paper into small pieces about 3 mm disks.
2. Put about 750-1000 pieces of paper disks into a 50-ml flask.
3. Plug the flask containing paper disks with cotton, cover cotton plug with aluminum foil then sterilize 2x by autoclaving for 15 min at 15 psi twice.
4. Put sterile paper disks on the surface of 4-day-old prune agar slant growth of *P. oryzae* [see step 4 of previous protocol (Monoconidial Isolation).]
5. Incubate at room temperature (25-27 ℃) for 25 days.
6. After 25-day growth period, carefully remove colonized paper disks taking extra care not to include any agar, and place in 2x sterilized coin envelopes (#00 brown coin envelopes, Miracle Envelope & Specialty Papers, 4.3×7cm in size).
7. Keep the envelopes in a cool, safe, and dry place for 14 days.
8. Place the envelope in a plastic bag containing silica gel dessicant (indicating type, 12-28 mesh gr 408). The color of this gel is blue after dehydration in the oven – simultaneously done with the petri plates.
9. Seal the bag (use of 2 plastic bags is preferred) and store in cold storage -20℃ or -70 ℃. The silica gel should be changed whenever the color changes from blue to pink.

Method 2) NIAS method

[Procedure]

The following steps describe the removal of paper disks from plates and the preservation of collected samples.

1. Cut the filter paper (Advantec 101) into small pieces about 15 mm square.
2. Put them in a petri dish, cover with aluminum foil then sterilize by autoclaving for 15 min at 15 psi.
3. Put a sterile paper disk on the surface of rice flour agar plate, inoculate by a few fungal mycelium block of *P. oryzae* using transfer needle.

4. Incubate at room temperature (25-27 ℃) for 4-5 days until the filter paper is covered by fungal mycelium (Fig. 9).
5. Carefully remove colonized filter paper square, and place it in Petri dish laid a sterilized filter paper (Fig. 10).
6. Dry it in desiccator at room temperature for 3 days .
7. Cut the filter paper into small pieces about 3-5 mm square (Fig. 11).
8. Carefully remove cut filter papers, and place in sterilized coin envelopes.
9. Place the envelope in a plastic bag containing silica gel dessicant (indicating type, 12-28 mesh gr 408). The color of this gel is blue after dehydration in the oven – simultaneously done with the petri plates.
10. Seal the bag and store in cold storage -20 ℃ or -70 ℃. The silica gel should be changed whenever the color changes from blue to pink. Stocked isolates still alive over 10 years (Fig. 12).

Fig. 9. Mycerium grown on the filter paper in Petri dish with a diameter of 3cm

Fig.10. Dried filter paper with mycelium

Fig.11. Cut filter paper into small pieces before storing

Fig.12. Isolates stored in -20C freezer

V. Preparation of plant materials for inoculation

Method 1) IRRI protocol

[Procedure]

1. If it is necessary, break seed dormancy by placing seeds in incubator at 50° for 3 days.
2. Treat test materials with 2% Chlorox for 10 min, wash 4x with D^2H_2O.
3. To pre-germinate seeds, place the seeds in petri dishes on wet blotting paper for 4 days at about 30℃ (if it is necessary, rinse blotting paper and seeds with D^2H_2O).
4. Choose what kind of soil to use and the kind of container or pot that suits your experiment.
5. Transplant test materials in the greenhouse.
6. After 3 weeks, test plants will be ready for inoculation (5-6 leaf stage, including an imperfect leaf, 21 DAS).
7. In case of rare plant materials wherein seeds are very few (like *O. nivara*, *O. rufipogon*, *O. minuta* etc), dehull seeds after seed dormancy is broken, then proceed to the next step.

[The 4-5 leaf stages of wild species appear 34 DAS, while in *O. sativa*, it is about 21 DAS]

Chlorox: 5.25% solution of sodium hypochlorite

Method 2) NIAS method

[Procedure]

1. If necessary, break seed dormancy by placing seeds in incubator at 40° for 1 day and 50° for 4 days.
2. Treat test materials with seed disinfectant.
3. To pregerminate seeds, place the seeds in Petri dishes of water for 2-3 days at about 28 ℃. Change water every day to supply oxygen.
4. Choose what kind of soil to use and the kind of container or pot that suits your experiment.
5. Sow 5-10 seeds each line.
6. If it is possible, incubate 28℃ for 2day to emergence uniformly.
7. After growing 3 weeks in green house, plants will be ready for inoculation (5-6 leaf stage, including an imperfect leaf, actually changes by season from 14 to 21 days).

VI. Inoculum production

Method 1) IRRI protocol

1. To revive stored culture, place a colonized paper disk on a prune agar slant, and allow to grow at room temperature for about 7-10 days.
   1) Revive 3 disks (3 slants) per isolate. After 3-4 days, choose the best two of the three that were revived. Use these two best slants to multiply.
2. The number of slants needed depends on how many ml of inoculum you need. Two 10-day-old slants can be used for 10 ml of macerated mycelium and can be spread on 5-7 plates for sporulation, which can produce 100-150 ml of inoculum at 100,000 spores /ml depending on the isolate used.
   1) When the stock culture disks are few (less than 20-40 in the coin envelope), revive 1-2 disks, and add new sterile disks as specified below (See the Preparation of Stock Culture) to prepare more stock culture disks.
3. Using a transfer needle, slice off as much of the mycelium as possible from the slant, while taking the least amount of agar possible. Transfer the mycelium to a test tube containing 10 ml of sterile D^2H_2O. (This should be done in a clean transfer hood to avoid contamination.)
4. Macerate the mycelium by mashing with a transfer needle or spatula.
5. Pour the suspension of mycelial fragments and conidia onto the surface of prune agar plates and spread the inoculum (2 mls/plate that would be 5 mls per slant used).
6. Incubate the plates at about 27℃ for 7 days.
7. Scrape the fungal growth with a "rubber policeman" (a hard rubber spatula). Sterilized glass slides could be a substitute.
8. Leave the scraped plates OPEN under a fluorescent lights for 3-4 days to induce sporulation.
9. Before harvesting conidia, record the appearance of the scraped plates (of each isolate) and the number of plates to be used. When counting spores take note of the appearance of the conidia – Are they normal in color or shape? Is there a contaminant?
10. Pour 10-20 mls distilled water into the Petri dish and gently scrape the surface re-growth with a sterilized rubber policeman or glass slide.
11. Filter the conidial suspension through 4 layers of cheese cloth or through nylon mesh.
12. Determine the conidial concentration using a hemacytometer (see below).

13. Adjust the concentration to 10^5 (100,000) conidia per milliliter (dilute with distilled water if spore count is high). Scrape some more plates if count if low.
14. To aid in the adhesion of the inoculum to the leaves of the plants, add a tiny drop of Tween 20 (final concentration approximately 0.02%) and shake well before using. Alternatively, 0.5% gelatin can be added.

Method 2) NIAS method

1. To revive stored culture, place a colonized paper disk on a oatmeal agar plate, and allow to grow at about 25 ℃ for about 12-13 days. Use Petri dish 20mm in depth. The number of plates needed depends on how many ml of inoculum you need (Fig. 13).
2. One 12-day-old plate can produce 50-150 ml of inoculum at 100,000 spores /ml depending on the isolate used. Forty-five ml of inoculum is enough for spray inoculation to each 5 plants of 30 LTH monogenic lines.
3. Scrape the surface of the fungal growth with a toothbrush. The scraped plates opened in the tray, covered with wrapping film pitted several holes, and leave under a fluorescent lights for 3-4 days to induce sporulation (Fig. 14-19).
   1) Before scraping plates and harvesting conidia, record the appearance of the plates (of each isolate) and the number of plates to be used. When counting spores take note of the appearance of the conidia − Are they normal in color or shape? Is there a contaminant?
4. Pour 10-20 mls distilled water into the Petri dish and gently scrape the surface of sporulated plates with a paintbrush (Fig. 20).
5. Filter the conidial suspension through 4 layers of cheese cloth or through nylon mesh (Fig. 20).
6. Determine the conidial concentration using a hemacytometer (see below).
   1) Adjust the concentration to 10^5 (100,000) conidia per milliliter (dilute with distilled water if spore count is high). Scrape some more plates if count if low.
   2) To clear a infection type on differential variety, the concentration of spores needs to be changed to the susceptibility of rice plant through the season or by resistance gene harboring in each line. For example, in the line having *Pish* or *Pita*, inoculation of too high concentration of spores result in fused lesions, and difficult to determine whether the infection types of the line is susceptible or resistance reaction. In this case, the concentration from 2 to 5 $\times 10^4$ (20,000 to 50,000) conidia per milliliter is enough.
7. To aid in the adhesion of the inoculum to the leaves of the plants, add a tiny drop of Tween 20 (final concentration approximately 0.01%) and shake well before using. Alternatively, 0.5% gelatin can be added.

Fig.13. Multiplication of fungus with paper filter stock

Fig.14. Sporulation of blast fungus culture under fluorescent lamp

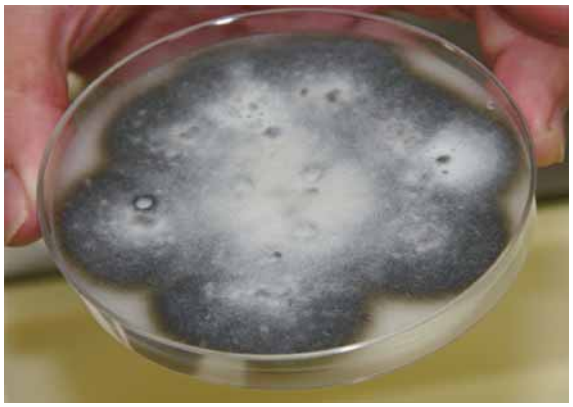


Fig.15. Cultivation of fungus after 12 days at 25 C

Fig.16. Clean the surface with toothbrush

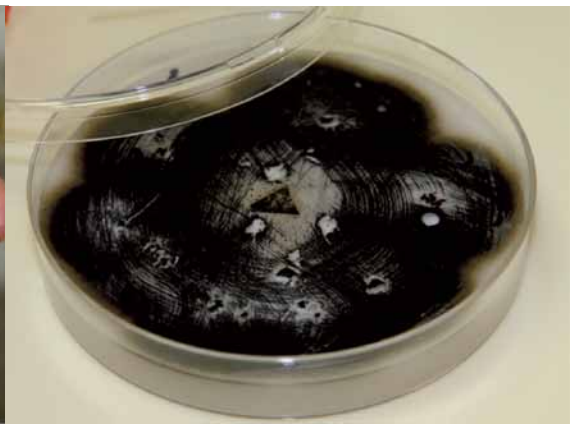


Fig.17. Enhance the spore appearance by the cleaning

Fig.18. Make the holes for the exchange the air in rapped box

Fig.19. Cultivation of balst spore under the fluorescent light
The spore covering the surface of the medium appeared after 3-4 days

Fig.20. Scraping plates and harvesting conidia

VII. Counting of spores using hemacytometer

1. There are 8-1 mm square squares on the hemacytometer
2. Place one drop of a spore suspension on the hemacytometer
3. Put a coverslip to expel some contents.
4. Count spores in ONE 1mm square surrounded in a red.
   (The volume in one square is approximately 10^{-4} ml.)
   So the number of cells per ml (cells/ml) equals:
   Average count per square x dilution factor x 10^4
   So: If there are 10 spores per mm square, then the spore count is 10 x 10^4 = 10^5
   Now: If there are 4 spores per square, then the spore count is 4 x 10^4 = 40,000
   Why? One square (16 little squares) = 1 mm^2
   WITH COVERSLIP (the volume becomes 10^{-4} ml = 1/10000 ml)
   If there are 4 spores in a square, 4 spores/0.00001 ml = 40,000 spores/ml

Fig.21. Counting area in hemacytometer

Count the spores on the line in two sides among four sides.

VIII. Coordinated inoculation procedure (Spraying method)

1. Prepare oatmeal agar or prune agar as described above.
2. Preparation of plant material as described above.
   1) Schedule the inoculum production to begin with step one on the same day seeds are sown for the plants which are going to be evaluated.
   2) Be sure to include susceptible and resistant checks in the planting.
3. Grow for three weeks (to 5-6 leaf stage) for inoculation.
4. Inoculum production

As described above

Measure the conidial concentration with a hemacytometer to obtain a final concentration of 10^5 (100,000) conidia per milliliter.

   1) If spore count is too high, dilute with sterile D^2H_2O
   2) If spore count is too low, scrape some more plates to obtain more spores, using the same D^2H_2O suspension until the desired concentration is obtained.

Add a few drops of Tween 20 to the suspension and shake well.

   1) Use approximately 10 µl per 100 ml of suspension (to 0.01%). Alternatively, use 0.5-1% gelatin instead of Tween 20.
   2) After inoculum preparation and spore counting are finished, proceed at once to inoculation. If two or more hours elapse before inoculation, spors may germinate in the suspension and this will adversely affect the pathogenicity test. If many isolates need to be used and the time to prepare the inoculum becomes longer, glass flasks containing the prepared suspensions may be put in a cool place (on ice) until ready to start spraying.

5. Inoculation

Method 1) IRRI procedure

Be sure that all equipment is sterile and properly labeled like D^2H^2O, tips, glassware, and sprayers. Dew chambers should be decontaminated.

1. Prepare about 10-20ml of inoculum per pot of five test plants.
2. Test plants are planted in 15-20cm diameter cups with 5 plants per cup. Plants are inoculated at approximately 21 days after sowing.
   1) Assemble sprayer and rinse with 95% EtOH, then sterile D^2H_2O.
3. To inoculate place pots on a rotating rack and spray all plants simultaneously while rotating the rack to ensure even coverage of the leaves.
4. Spray the conidial suspension onto the plants until runoff.
   1) When different plants are inoculated with different isolates, be sure that leaves of the plants do not touch or drip onto other inoculated plants inside the dew chamber.
5. Place the plants inside the dew chamber at 25 ℃ for 20h.
6. And then transfer them to a greenhouse high humidity room (mist room) at 25-30 ℃.
   1) Take care that the mist room spray nozzles do not drip into the pots or food containers, because excess moisture at this time can cause adverse physiological reactions leading to the death of otherwise healthy leaves and even whole plants.
   2) Observe time periods properly. Inoculated plants should be in the dew chamber for 24h and mist room for 6 days. Scoring should be no more than 8 days after inoculation.

Fig.22. Dew chamber at IRRI

Fig.23. Mist room at IRRI maintained at 26 ℃

Method 2) Using dew chamber by cooling the outer case (NIAS)

1. Prepare about 5-15 ml of inoculum per pot of thirty test plants.
   1) Test plants are planted in 20 x 5 x 10 height case with 30 plants per case. Plants are inoculated at approximately 21 days after sowing (leaf age ca.4.6).
   2) Assemble sprayer and rinse with 95% EtOH, then sterile D^2H_2O.
   3) To inoculate place pots on a rotating rack and spray all plants simultaneously while rotating the rack to ensure even coverage of the leaves.
2. Spray the conidial suspension onto the plants until visible.
   1) When different plants are inoculated with different isolates, be sure that leaves of the plants do not touch or drip onto other inoculated plants inside the dew chamber.
3. Place the plants inside the dew chamber at 25℃ for 20-24h, then transfer them to a green house (humidity approximately 60%) at 25-30 ℃.
4. Observe time periods properly. Scoring should be 7 to 8 days after inoculation.

Fig.24. Spray inoculation on turn table

Fig.25. Inoculated plant in dew chamber

IX. Disease assessment

1. Infection type on individual differential lines

Susceptibility of the test plants is assessed by examining the leaves for blast symptoms which appear on the latest expanding leaf. Five to seven days after inoculation, infection type is rated as the following (Goto and Yamanaka, 1968, Mackill and Bonman, 1992):

0 = No evidence of infection

1 = Brown specks smaller than 0.5mm in diameter, No sporulation.
Uniform or scattered brown specks, no sprulation

2 = Brown specks about 0.5-1.00mm in diameter, no sporulation.
Small lesions with distinct tan centers surrounded by a darker brown margin approximately 1mm in diameter, No sporulation.

3 = Roundish to elliptical lesion about 1-3 mm in diameter with gray center surrounded by brown margins, lesions capable of sporulation.
Small eyespot lesions less than one and a half times the interval between thin veins or less than 1.5mm in diameter surrounded by dark brown, lesions capable of sporulation.

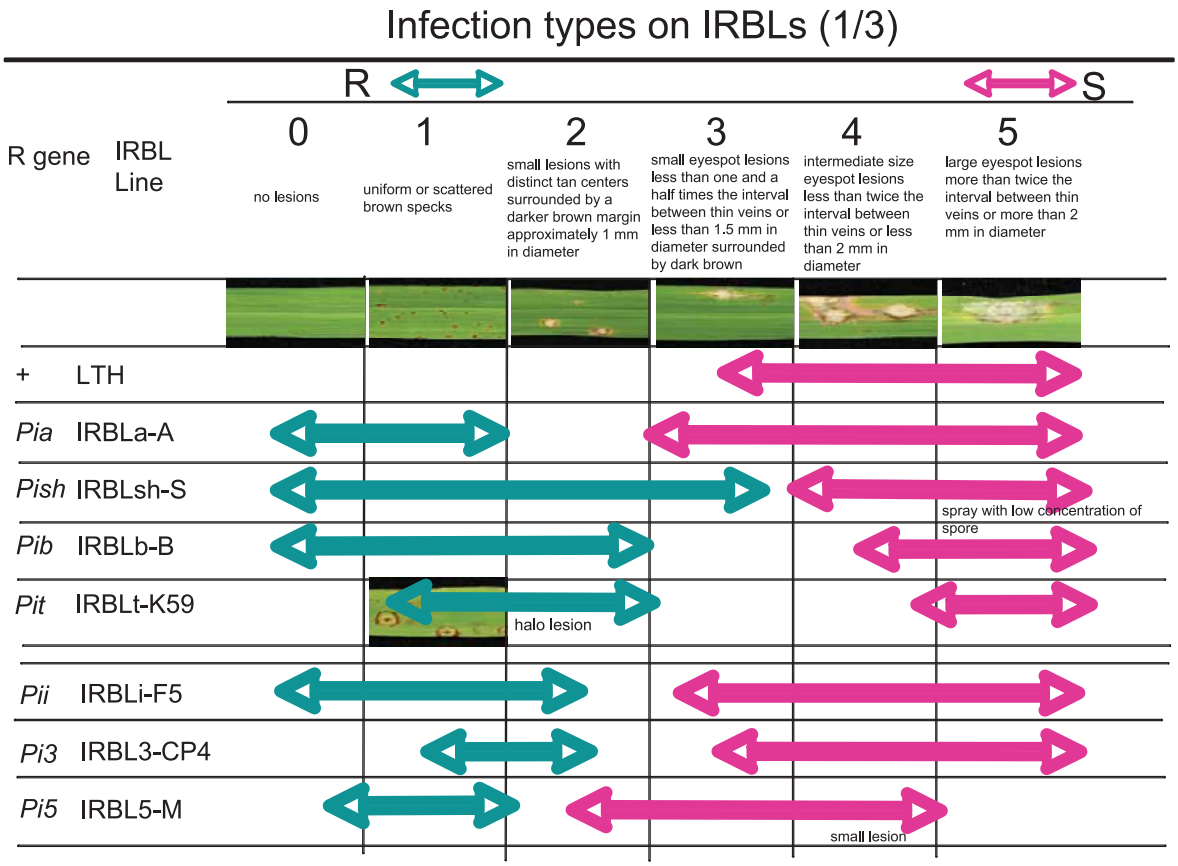
4 = Typical spindle shaped blast lesion capable of sporulation, 3 mm or longer with necrotic gray centers and water soaked brown margins little or no coalescence of lesion.
Intermediate size eyespot lesions less than twice the interval between thin veins or less than 2 mm in diameter.

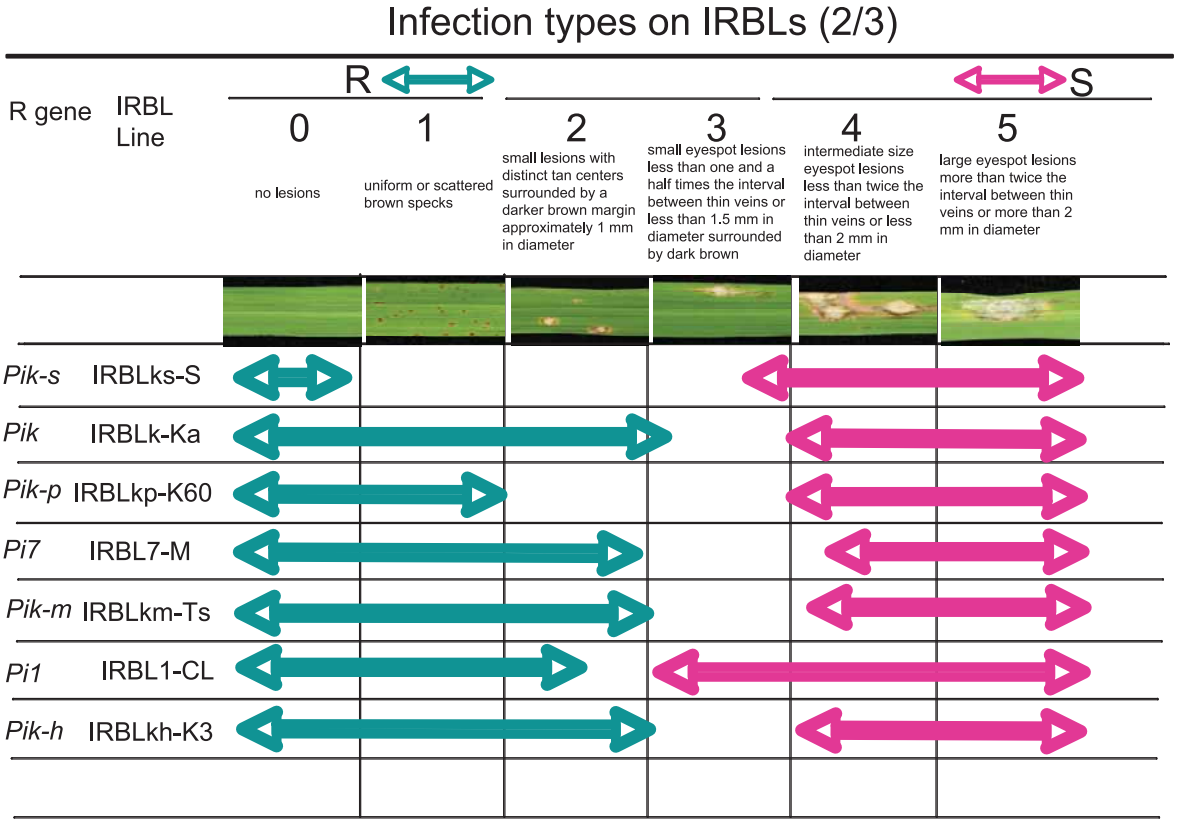
5 = Lesions as in 4 but about half of one or two leaf blade killed by coalescence of lesion.
Large eyespot lesions more than twice the interval between thin veins or more than 2 mm in diameter.

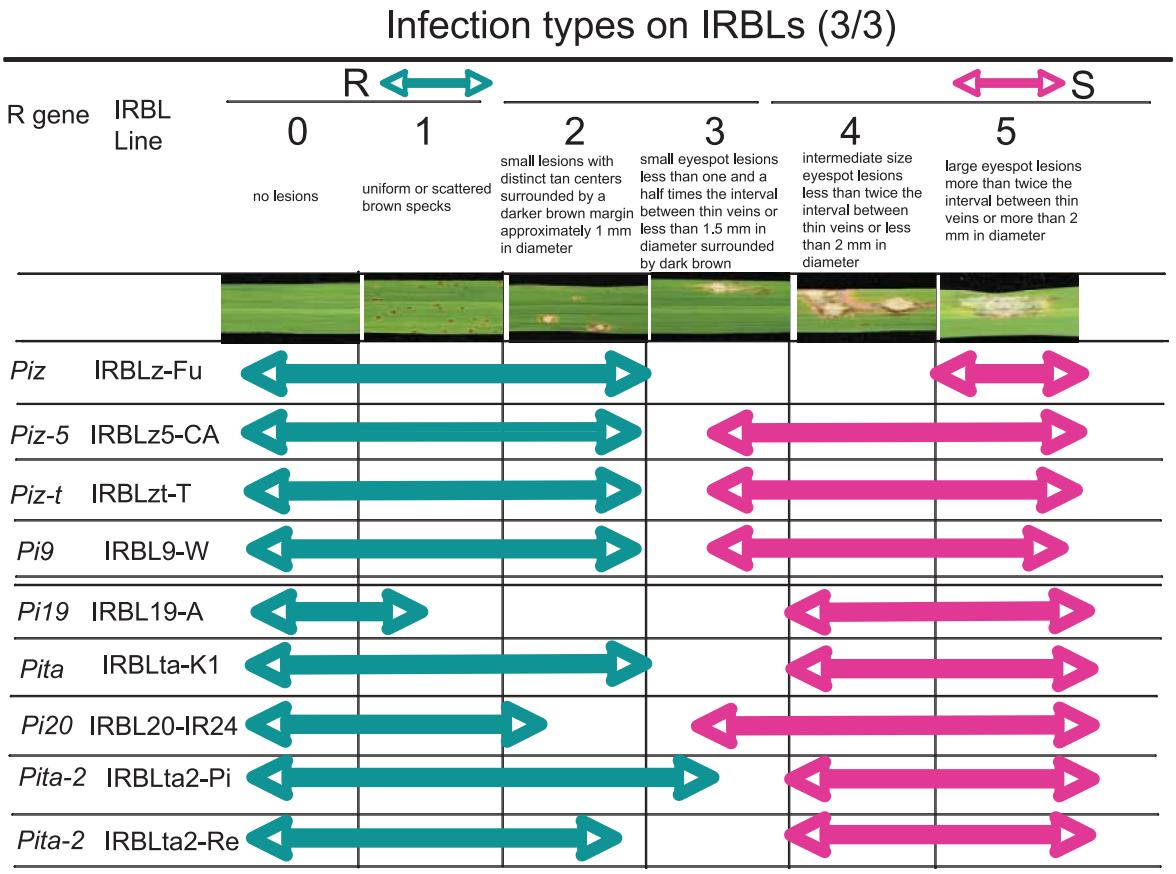
1) Infection type 0 to 2 is resistance reaction, and infection type 3 to 5 is susceptible reaction basically.
2) When we inoculated it into the rice plant which the most upper leaf extremely slightly emergence, the lesion developed on a tip of the leaf becomes sensitive, and there is a case more than infection type 3, but does not determine as the susceptible lesion.

Also record the presence or absence of sporulation on lesions after incubation for 24 h at 27 ℃.

Table 1. A standard decisions for susceptible or resistance reaction each LTH monogenic line







X. Designation of new international differential system

1. Each race code number has following five parts divided by hyphen (i.e. 1st-2nd-3rd-4th -5th).
2. The 1st part of the race code number has a two-digit number and is composed of LTH, and IRBLa-A in ones place, IRBLsh-B (or IRBLsh-S), IRBLb-B and IRBLt-K59 in tens place.
3. The 2nd part has one digit number and is composed IRBLi-F5, IRBL3-CP4, and IRBL5-M, which are multiallelic or closely linked.
4. The 3rd part has a three-digit number and is composed of IRBLk-Ka, IRBLkp-K60 and IRBL7-M in ones place, IRBLkm-Ts, IRBL1-CL and IRBLkh-K3 in tens place, IRBLks-S, in hundreds place, which are multiallelic or closely linked.
5. The 4th part has a two-digit number and is composed IRBLz-Fu, IRBLz5-CA, and IRBLzt-T in ones place, IRBL9-W in tens place, which are multiallelic or closely linked.
6. The 5th part has a three-digit number and is composed IRBL19-A, IRBL20-IR24 in ones place, IRBLta-K1, IRBLta-CP1 in tens place, IRBLta2-Pi, IRBLta2-Re, IRBL12-M in hundreds place, which are multiallelic or closely linked.
7. We put in front of 2nd to 5th part i, k, z, ta which showed locus of the multiple allele.
8. A race number is sum of a code number showing a susceptible reaction.
9. "U" mentioned before a number shows an international blast pathogenicity race.

Table 2. New designation system for blast races based on the reaction of monogenic line with LTH genetic background

Group	I		II	III			IV		V		
Locus	-		*Pii*	*Pik*			*Piz*		*Pita*		
Target resistance gene	*Pish*	+	*Pii*	*Pik-s*	*Pik*-m	*Pik*	*Pi9*(t)	*Piz*	*Pita-2*	*Pita*	*Pi19*
	Pib	*Pia*	*Pi3*	-	*Pi1*	*Pik-p*	-	*Piz-5*	*Pita-2*	*Pita*	*Pi20*(t)
	Pit	-	*Pi5*(t)	-	*Pik-h*	*Pi7*(t)	-	*Piz-t*	*Pi12*(t)	-	-
Monogenic line (IRBL)	sh-S	LTH	i-F5	ks-S	km-Ts	k-Ka	9-W	z-Fu	ta2-Pi	ta-K1	19-A
	b-B	a-A	3-CP4	-	1-CL	kp-K60	-	z5-CA	ta2-Re	ta-CP1	20-IR24
	t-K59	-	5-M	-	Kh-K3	7-M	-	zt-T	12-M	-	-
Code	1	1	1	1	1	1	1	1	1	1	1
	2	2	2	-	2	2	-	2	2	2	2
	4	-	4	-	4	4	-	4	4	-	-
Ex. Blast isolates virulence to all genes	S	S	S	S	S	S	S	S	S	S	S
	S	S	S	-	S	S	-	S	S	S	S
	S	-	S	-	S	S	-	S	S	-	-
	7	3	7	1	7	7	1	7	7	3	3

Example blast race No. of isolate that is virulence to all differential varieties (genes): U73-i7-k177-z17-ta733.

Table 3. Comparison of a race number of standard rice blast strains of Japan by the Japanese differential system and the international differential system

Blast strain	Differentiation system	
	Japanese race	International race
Mu-95	J001.2	U31-i0-k100-z00-ta401
Kyu89-246	J003.0	U13-i0-k100-z00-ta001
95Mu-29	J003.2	U33-i0-k100-z00-ta401
Shin83-34	J005.0	U11-i7-k100-z00-ta001
Ina86-137	J007.0	U13-i7-k100-z02-ta001
31-4-151-11-1	J007.2	U33-i7-k100-z00-ta001
Kyu92-22	J017.1	U13-i7-k107-z00-ta001
1804-4	J031.1	U11-i0-k177-z00-ta001
TH68-126	J033.1	U13-i0-k177-z00-ta001
TH68-140	J035.1	U11-i7-k177-z00-ta001
24-22-1-1	J037.1	U13-i7-k177-z00-ta001
Ai79-142	J037.3	U33-i7-k177-z00-ta001
Kyu9439013	J047.0	U13-i7-k100-z01-ta001
TH69-8	J071.1	U11-i0-k177-z01-ta001
Sasamori121	J077.1	U13-i7-k177-z01-ta001
Ina93-3	J301.0	U11-i0-k100-z00-ta331
GFOS8-1-1	J303.0	U13-i0-k100-z00-ta333
0528-2	J333.1	U13-i0-k107-z00-ta331
Ao92-6-2	J337.1	U13-i7-k177-z00-ta331
Mu-183	J337.3	U33-i7-k177-z00-ta331
IW81-04	J437.1	U13-i7-k177-z06-ta001
Ai74-134	J477.1	U13-i7-k177-z05-ta001

Table 4. List of monogenic lines as the differential varieties

Variety Name	Target resistance gene	Glume color
IRBLt-K59	*Pit*	Purple
IRBLsh-S	*Pish*	Brown
IRBLsh-B	*Pish*	Brown
IRBLb-B	*Pib*	Purple
IRBLz-Fu	*Piz*	Brown
IRBLz5-CA-1	*Piz-5* = *Pi2* (t)	Purple
IRBLz5-CA-2	*Piz-5* = *Pi2* (t)	Brown
IRBLzt-T	*Piz-t*	Brown
IRBL9-W	*Pi9*	Brown
IRBLi-F5	*Pii*	Brown
IRBL3-CP4	*Pi3*	Purple
IRBL5-M	*Pi3*	Brown
IRBLa-A	*Pia*	Purple
IRBLks-F5	*Pik-s*	Purple
IRBLk-K	*Pik*	Purple
IRBLkp-K60	*Pik-p*	Brown
IRBLkh-K3	*Pik-h*	Purple
IRBLkm-Ts	*Pik-m*	Brown
IRBL1-CL	*Pi1*	Brown
IRBL7-M	*Pi7* (t)	Purple
IRBL12-M	*Pi12* (t)	Purple
IRBL19-A	*Pi19*	Brown
IRBLta-K1	*Pita* = *Pi4* (t)	Purple
IRBLta-CT2	*Pita* = *Pi4* (t)	Purple
IRBLta-CP1	*Pita* = *Pi4* (t)	Purple
IRBLta2-Pi	*Pita-2*	Purple
IRBLta2-Re	*Pita-2*	Brown
IRBL20-IR24	*Pi20* (t)	Brown
LTH	Susceptible	Purple

This list was modified form the Tsunematsu et al., 2000, Fukuta et al. 2004, and Kobayashi et al., 2007, partially.

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Zeigler, D. Mackill, J. Bandong, M. Bernardo, E. Borromeo, A. Bordeos, R. Scott, C. Dahu, T. Inukai, T. Vergel de Dios-Mew, A.O. Mackill, P. Reimers, B. Consignado, G.L. Wang, E. Silab, and B. Consignado and P. Reimers compiled it in 1992.

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Genetic characterization of universal differential varieties for blast resistance developed under the IRRI-Japan Collaborative Research Project using DNA markers in rice (*Oryza sativa* L.)

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Abstract

Under the IRRI-Japan Collaborative Research Project, The International Rice Research Institute (IRRI) and the Japan International Research Center for Agricultural Sciences (JIRCAS) have developed four universal differential variety sets, which comprise monogenic lines (MLs) with the Japonica-type variety Lijian-xintuan-heigu (LTH) genetic background, and near isogenic lines (NILs) with LTH, Indica-type variety CO39, and universal susceptible line US-2 genetic backgrounds. These target 24 resistance genes: *Pia*, *Pib*, *Pii*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pish*, *Pita*(*Pi4*), *Pita-2*, *Pit*, *Piz*, *Piz-5*(*Pi-2*), *Piz-t*, *Pi1*, *Pi3*(t), *Pi5*(t), *Pi7*(t), *Pi9*, *Pi11*(t), *Pi12*(t), *Pi19*(t) and *Pi20*(t), and the MLs have been distributed to more than 15 countries. To characterize these chromosome components and confirm the introgression of chromosome segments harboring resistance genes, graphical genotypes were investigated using 162 simple sequence repeat (SSR) markers distributed across all 12 rice chromosomes. The chromosome components of the three sets of NILs were more uniform than those of MLs when compared to the corresponding recurrent parent. Several introgression segments, which corresponded to the locations of blast resistance genes, were also confirmed. Almost all the MLs were developed by backcrossing one or two times, while some lines were backcrossed three to five times with the recurrent parent, LTH. The restoration rate of genomic chromosomes of 31 MLs relative to LTH ranged from 50 to 90.0%, averaging 77.3%. All LTH, CO39, and US-2 NILs were developed by backcrossing six times with each recurrent parent. The genome restoration rate relative to the parent in 34 LTH NILs ranged from 75.6% to 96.9%, averaging 90.6%; lower than the theoretical value of 99%. The 31 CO39 NILs showed a genome restoration rate greater than 90% relative to the recurrent parent, averaging 97.3%. The 16 US-2 NILs were highly similar to those of the recurrent parent: these genome restoration rates ranged from 88.9% to 98.8%, averaging 94.6%. Genetic characterizations of four universal differential sets of varieties were carried out using DNA markers. This DNA marker data combined with resistance genes is potentially very useful for marker-assisted selection (MAS) in breeding programs since the differential varieties can be applied as gene sources.

Keywords: Resistance gene, differential variety, chromosome component, Blast (*Pyricularia oryzae* Cavara.), rice (*Oryza sativa* L.)

Introduction

Blast, caused by *Pyricularia oryzae* Cavara is one of the most destructive diseases of rice (*Oryza sativa* L.). The use of resistant varieties is an efficient method to control this disease. Differential varieties are important materials for improving blast resistance and for pathological studies. The relationship between the host plant (resistance genes) and pathogen (avirulence genes) can be explained by the gene-for-gene theory (Flor 1956). Therefore, virulence genotypes of pathotypes can be inferred when resistance genotypes are known for each differential variety and these can distinguish pathotypes (races) based on their reaction pattern or qualitative differences in reactions to different pathogen strains. In Japan, sets of 9 (Yamada et al. 1976) and 12 varieties (Kiyosawa et al. 1984) had been selected and used as blast differential varieties. However, these varieties are not readily available in other countries because several of these varieties contained not only the target resistance genes, but also harbored other gene(s) in their genetic backgrounds.

At the International Rice Research Institute (IRRI), near-isogenic lines (NILs) for blast resistance were developed for only four resistance genes, *Pi1*, *Pi3*(t), *Piz-5*, and *Pita*(*Pi4*), with the genetic background of the Indica-type rice CO39, and these were used as differential varieties (Mackill et al. 1985; Mackill and Bonman 1992). A set of NILs with a susceptible Japonica-type variety Lijiang-xintuan-heigu (LTH) genetic background targeting six resistance genes, *Pita*, *Pita-2*, *Pib*, *Pik*, *Pik-m*, and *Pik-p*, was developed by Ling et al. (1995). However, the number of resistance genes covered by these NILs is limited.

To provide more useful materials than have been previously developed, the IRRI-Japan Collaborative Research Project released the monogenic lines (MLs) for blast resistance as the first set of international standard differential varieties (Tsunematsu et al. 2000). These MLs were developed to target 24 resistance genes; *Pia*, *Pib*, *Pii*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pish*, *Pita*, *Pita-2*, *Pit*, *Piz*, *Piz-5*(*Pi-2*), *Piz-t*, *Pi1*, *Pi3*(t), *Pi5*(t), *Pi7*(t), *Pi9*, *Pi11*(t), *Pi12*(t), *Pi19*(t), and *Pi20*(t), and have been distributed to more than 15 countries by the IRRI-Japan Collaborative Research Project or International Network for Genetic Evaluation of Rice (INGER) in IRRI, and are being used as a source of differential varieties against blast disease (Fukuta et al., 2004a). The project has also been developing NILs with three different genetic backgrounds, LTH, the Indica-type variety CO39, and the universal susceptible line US-2, which was derived from a cross between an Indonesian landrace, Kencana, and Indica-type variety, Takanari, for targeting these resistance genes (Fukuta et al. 2004b; Kobayashi et al. 2007).

The reaction patterns of these differential varieties relative to the representative blast isolate from Japan and Philippine, as well as their morphological and agronomical characters, have been studied previously (Fukuta et al. 2004a, 2004b; Yanoria et al. 2004, Yanoria et al.; 2006a, 2006b, 2006c, 2006d), while genetic studies of these materials have not been completed to date. Genotyping of these materials using molecular markers is important to determine the genetic components within each of these differential varieties. Moreover, since differential varieties also offer great potential as genetic sources for breeding of blast resistance, availability of molecular markers linked with the resistance gene is necessary for marker-assisted breeding. The clarification of the genetic components of differential varieties and confirmation of introgressed blast resistance genes using molecular markers were carried out. The development of the set of differential varieties is reviewed and the perspective for further development under the IRRI-Japan Collaborative Research Project, and the utilization of differential varieties are discussed.

Materials and methods

1. Development of differential varieties

The blast resistance genes were introduced into the three different genetic backgrounds by recurrent backcross breeding. Three susceptible varieties, a Chinese Japonica-type, Lijiang-xintan-heigu (LTH), an Indica-type, CO39, and a hybrid progeny line, US-2, derived from a cross between an Indonesian landrace, Kencana (Yanoria et al. 2000), and an Indica-type, Takanari, were used as the recurrent parents. Furthermore, a total of 25 varieties were used as donor parents of blast resistance genes (Table 1, 2).

A total of 20 Philippines strains that were clarified by their pathogenicity and selected as the differential blast isolates (Yanoria et al. 2000), were used to estimate the resistance genes by the reaction pattern. The avirulent blast isolates were inoculated to confirm the presence and homozygosity of the corresponding genes in each developing progeny. Ten seedlings per line raised in plastic trays were inoculated at the fourth or fifth leaf stage by spraying with a spore suspension. Six or seven days after inoculation, each line was evaluated by their susceptibility based on the degree of infection of each seedling. The reaction was classified on a scale of 0 to 5, as described previously by Mackill and Bonman (1992), with slight modifications. In cases where the biggest lesion was elongated less than or more than 3 mm, the plant was rated as 3^- or 3^+, respectively. The reactions of differential varieties were categorized and summarized into four reaction classes wherein 0-2 were resistant (R), 3^- was moderately resistant (M), 3^+ was moderately susceptible (MS), and 4-5 were considered susceptible (S). LTH and CO39 were used as susceptible control varieties.

Table 1. Differential varieties developed by IRRI-Japan Collaborative Research Project

Target resistance gene	Chromosome[1)]	No. of donor variety used	Differential varieties			
			ML	NIL		
				LTH	CO39	US-2
Pit	1	1	1	-	-	-
Pish	1	4	2	-	5	-
Pib	2	3	1	1	2	-
Piz	6	1	1	-	-	-
Piz-5 (=*Pi2*)	6	3	2	3	1	-
Piz-t	6	2	1	1	1	1
Pi9	6	1	1	1	-	1
Pi11(t)	8	1	1	-	-	-
Pii	9	1	1	-	-	-
Pi3(t)	9	1	1	2	-	-
Pi5(t)	9	1	1	1	1	1
Pia	11	3	2	3	-	2
Pik	11	2	1	1	2	1
Pik-s	11	5	2	3	1	2
Pik-p	11	1	1	-	1	1
Pik-h	11	1	1	1	1	1
Pik-m	11	1	1	-	1	-
Pi1	11	1	1	1	1	1
Pi7(t)	11	1	1	1	1	1
Pita(=*Pi4*)	12	5	3	3	1	3
Pita-2	12	4	2	2	3	-
Pi12(t)	12	1	1	-	-	1
Pi19(t)	12	1	1	-	-	-
Pi20(t)	12	1	1	-	-	-
Unknown	-	16	-	10	9	-
Total	-	62	31	34	31	16

24 resistance genes were targeted for the development of differential varieties.
Several donor varieties were used for each differential variety.
ML: monogenic lines, LTH: a Chinese Japonica-type variety, Lijianxintuanheigu. CO39: an Indica-type variety. US-2: Universal susceptible variety developed Indica and Japonica-types varieties' cross by Andoh and Hayashi (unpublished)
[1)] Chromosome locating resistance gene

Table 2. Reaction patterns of monogenic lines and near isogenic lines with the LTH, CO39, and US-2 genetic backgrounds to blast isolates from the Philippines

Resistance gene	Designation	IRBL	Donor variety	Blast Isolates																		
				PO6-6	CA 89	43	CA41	M64-1-3-9-1	M39-1-3-8-1	M39-1-2-21-2	M36-1-3-10-1	JMB 8401	IK81-25	IK81-3	BN111	V850256	V850196	V86010	JMB840610	BN209	M101-1-2-9-1	B90002	C923-49
Pit	IRBLt-K59	15	K 59	MS-S	S	S	S	S	S	S	S	M-MS	MS-S	MS	S	S	S	S	S	S	MS-S	S	S
Pish	IRBLsh-S	16	Shin 2	R-M	M	M	R-M	M	M	R	R	M	R	R-M	R-M	R-M	R-M	M	S	M	R-M	R-M	M
	IRBLsh-S[CO]-1	-	Shin 2	M	M	M	MS	S	M	M	M	M	M	S	MS	M	M	M	M	M	M	R	R
	IRBLsh-S[CO]-2	-	Shin 2	M	M	M	MS	S	M	M	M	M	M	M	M	M	M	M	M	R-M	R-M	R	R
	IRBLsh-B	17	BL 1	R-M	R-M	M	R-M	M	M	R-M	R	R	R	R-M	R-M	M	R-M	M	R	R-M	R	M	M
	IRBLsh-B[CO]	-	BL 1	M	M	M	MS	S	M	M	M	M	M	MS	M	M	M	M	M	M	R	R	R
	IRBLsh-Ku[CO]	-	Kusabue	M	M	M	M	MS	M	M	M	M	M	S	M	M	M	M	M	M	R	R	R
Pib	IRBLb-B	14	BL 1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	M	R	R-M	R	R	R
	IRBLb-B[LT]	-	BL 1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	M	R	R-M	R	R	R-M
	IRBLb-B[US]	-	BL 1	S	MS	R-M	M	S	MS	M	M	S	M	S	MS	MS	MS	M	R	R	R	R-M	R-M
	IRBLb-IT13[CO]	-	IRAT13	S	S	S	S	S	S	S	M	S	S	M	S	S	S	M	R	R	R	R	R
	IRBLb-W[CO]	-	WHD-1S-75-1-127	S	S	S	S	S	S	S	S	S	S	S	S	S	S	M	R	R	R	R	R
Piz	IRBLz-Fu	9	Fukunishiki	R-M	M	R	M	R-M	R-M	R-M	R	R-M	R	R	S	M	R	R-M	M	R-M	R	M	R
	IRBLz-Fu[CO]	-	Fukunishiki	M	M	M	MS	MS	MS	M	M	M	M	S	M	M	M	M	MS	M	M	R	R
Piz-5 (*Pi2*(t))	IRBLz5-CA-1	10	C101A51	R	M	M	R	M	R	R-M	R	R	R	R	R	R	R-M	M	R	M	S	M	M
	IRBLz5-CA-2	31	C101A51	R	R	R	R	R-M	R-M	R	R	R	R-M	R	R	R	R	R	R	M	S	R-M	R
	IRBLz5-CA[LT]	-	C101A51	R-M	R-M	R-M	R-M	R-M	R-M	R-M	R	R	R	R-M	R	R-M	M	M	R-M	R-M	S	M	MS
	IRBLz5-CA[CO]	-	C101A51	R-M	R-M	R-M	R-M	R-M	R-M	R-M	R	R-M	R	R	R	R	R-M	R-M	M	R-M	S	R	R
	IRBLz5-TA[LT]	-	Tadukan	R-M	R-M	R-M	R	R	R	R	R	R	R	R	R	R	R-M	R	R	R-M	S	R	R
	IRBLz5-K60[LT]	-	K 60	R-M	R	R	R	R	R-M	R-M	R	R	R	R	R	R	R-M	R	R	R-M	S	R	R-M

Piz-t	IRBLzt-T	11	Toride 1	S	S	S	S	R	R	S	S	S	S	S	S	S	S	R	R-M	S	S	R	R
	IRBLzt-T[LT]	-	Toride 1	S	S	S	S	R	R	S	S	S	S	S	S	S	S	R-M	R	S	S	R	R-M
	IRBLzt-T[US]	-	Toride 1	S	S	S	S	R-M	R-M	S	MS	S	S	S	S	S	S	M	R-M	S	S	MS	R
	IRBLzt-IR56[CO]	-	IR 56	S	S	S	S	R-M	R	S	S	S	S	S	S	S	S	M	R-M	S	S	R	R
Pi9	IRBL9-W	22	WHD-1S-75-1-127	R	R	R	R	R-M	M	R-M	R	R	R	R	R	R	R-M	S	R	R	R	R	R
	IRBL9-W[LT]	-	WHD-1S-75-1-127	R	R	R	R	R-M	R	M	R	R	-	R	R-M	R	M	R	R	R	R	R	R
	IRBL9-W[US]	-	WHD-1S-75-1-127	R-M	M	R	R	M	M	R	R	R-M	M	R	R-M	R-M	S	R	R	M	R	R-M	R
Pi11(t)	IRBL11-Zh	30	Zhaiyeqing	S	MS-S	S	S	S	S	S	MS	MS	MS-S	M	S	S	MS-S	R	R	R-M	R	R	R
Pii	IRBLi-F5	3	Fujisaka 5	R	S	R	S	S	M	R-M	S	S	R	R-M	R	S	R	S	R	S	S	MS-S	S
Pi3(t)	IRBL3-CP4	19	C104PKT	R	S	R	S	S	R-M	R	S	R	R	M	R-M	S	R	S	S	S	S	S	S
	IRBL3-CP4[LT]-1	-	C104PKT	R-M	S	R-M	S	S	M	M	S	R-M	-	M	R-M	S	R-M	S	S	S	S	S	S
	IRBL3-CP4[LT]-2	-	C104PKT	R-M	S	R-M	S	S	M	M	S	R	-	MS	R-M	S	R	S	S	S	S	S	S
	IRBL3-TB[LT]	-		M	S	R-M	S	S	M	M	S	R-M	-	MS	R-M	S	R	S	S	S	S	S	S
Pi5(t)	IRBL5-M	20	RIL 249 (Moro.)	R	S	R	M	M	R	R	R-M	R	R	R-M	R-M	M-MS	R	S	M	MS	R	M	MS
	IRBL5-M[LT]	-	RIL 249 (Moro.)	R-M	S	R-M	S	S	M	M	S	R	-	MS	R-M	S	R	S	S	S	S	R	R
	IRBL5-M[CO]	-	RIL 249 (Moro.)	R-M	S	R-M	S	S	S	M	R-M	R-M	S	S	R-M	S	R	S	S	M	R	R	R
	IRBL5-M[US]	-	RIL 249 (Moro.)	M	S	R	S	S	MS	R	MS	R-M	R-M	S	R-M	S	R-M	S	S	S	S	S	S
Pia	IRBLa-A	1	Aichiasahi	S	S	S	S	S	S	S	S	S	S	MS	S	S	S	S	S	S	MS-S	R	R
	IRBLa-A[LT]	-	Aichiasahi	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R
	IRBLa-A[US]	-	Aichiasahi	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R-M	S	S	S	R	R
	IRBLa-C	2	CO39	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	MS-S	R	R
	IRBLa-C[LT]	-	CO39	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R
	IRBLa-C[US]	-	CO39	S	S	S	S	S	S	S	MS	S	S	S	S	S	S	S	S	S	S	R	R
	IRBLa-Z[LT]	-	Zenith	S	S	S	S	S	S	S	MS	S	S	S	S	S	S	S	S	S	S	R	R

Pik-s	IRBLks-F5	4	Fujisaka 5	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S
	IRBLks-F5*	-	Fujisaka 5	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S
	IRBLks-F5[US]	-	Fujisaka 5	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S
	IRBLks-S	5	Shin 2	M	M	M	R-M	M	M	MS	M	M	M	R-M	M	M	R	R	M	M	R-M	M-MS	M
	IRBLks-S[LT]	-	Shin 2	S	S	S	S	S	S	S	S	S	S	S	S	S	R	MS	S	S	S	S	S
	IRBLks-B40[LT]	-	B40	S	S	S	S	S	S	S	MS	S	S	MS	S	S	R	M	S	S	MS	S	S
	IRBLks-Zh[LT]	-	Zhaiyeqing 8	S	S	S	S	S	S	S	S	S	S	S	S	S	R	M	S	S	MS-S	S	S
	IRBLks-CO[CO]	-	Caloro	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R-M	S	S	M	R	R
Pik	IRBLk-K	6	Kanto 51	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk-K[LT]	-	Kanto 51	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk-K[CO]	-	Kanto 51	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk-K[US]	-	Kanto 51	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk-Ku[CO]	-	Kusabue	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
Pik-h	IRBLkh-K3	8	K 3	R	R	R	R	S	S	S	M-MS	S	M	R	R	R	R	R	R	R	R	R	R
	IRBLkh-K3[LT]		K 3	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLkh-K3[CO]		K 3	R	R	R	R	S	S	S	MS	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLkh-K3[US]		K 3	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
Pik-m	IRBLkm-Ts	25	Tsuyuake	R	R	R	R	S	S	S	M	S	M-MS	R	R	R	R	R	R	R	R	R	R
	IRBLkm-Ts[CO]		Tsuyuake	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
Pik-p	IRBLkp-K60	7	K 60	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLkp-K60[CO]		K 60	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLkp-K60[US]		K 60	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
Pi1	IRBL1-CL	18	C101LAC	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBL1-CL[LT]		C101LAC	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBL1-CL[CO]		C101LAC	R	R	R	R	S	S	S	S	S	S	R	R	R	M	R	R	R	R	R	R
	IRBL1-CL[US]		C101LAC	R	R	R	R	S	S	S	MS	S	S	R	R	R	R	R	R	R	R	R	R
Pi7(t)	IRBL7-M	21	RIL 29 (Moro.)	R	R	R	R	S	S	S	S	S	S	R	R	R	R	S	R	R	R	R	R
	IRBL7-M[LT]		RIL 29 (Moro.)	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBL7-M[CO]		RIL 29 (Moro.)	R	R	R	R	S	S	S	S	S	S	R	R	R	M	R	R	R	R	R	R
	IRBL7-M[US]		RIL 29 (Moro.)	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R-M	R

Unknown	IRBLk*-F14[LT]	-	F-14-3	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk*-Z[LT]	-	Zenith	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk*-NP[LT]	-	NP125	R-M	R-M	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R-M	R-M	-	-
	IRBLk*-Du[LT]	-	Dular	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk*-F25[LT]	-	F-25-3	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk*-F66[LT]	-	F-66-10	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk*-F21[CO]		F-21-6	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk*-F14[CO]		F-14-3	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk*-F25[CO]		F-25-3	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk*-F40[CO]		F-40-3	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk*-F59[CO]		F-59-1	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk*-F66[CO]		F-66-10	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk*-K86[CO]		KU86	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R
	IRBLk*-S[CO]		Shin2	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R-M	R	R	R
Pi12(t)	IRBL12-M	23	RIL 10	S	S	S	S	S	S	S	S	S	S	S	S	S	M	M	R	R	R	R	R
	IRBL12-M[US]		RIL 10	S	S	S	S	S	S	S	R-M	S	S	S	S	S	R	R-M	S	S	S	S	S
Pi19	IRBL19-A	24	Aichiasahi	S	S	S	MS-S	S	S	S	MS	MS	S	S	S	S	S	S	S	S	S	S	S
Pita=Pi4(t)	IRBLta-K1	12	K1	MS	S	S	R	R	R	S	M-MS	M	R	R	S	R	R	S	S	S	S	R	S
	IRBLta-K1[LT]		K1	S	S	R	R	R	R	S	S	S	R	R	S	R	S	S	S	S	S	MS	M
	IRBLta-K1[US]		K1	S	S	R	M	R	R-M	S	S	S	R	R	S	M	R	R	S	S	S	S	S
	IRBLta-CT2	13	C105TTP2L9	S	S	S	R	R	R	S	MS	MS	R	R	S	R	R	S	S	S	M-S	R	S
	IRBLta-CT2[LT]		C105TTP2L9	S	S	R	R-M	R-M	R-M	S	S	S	R	R	S	R-M	R-M	S	S	S	S	MS	M
	IRBLta-CT2[US]		C105TTP2L9	S	S	R	MS	MS	S	S	S	S	R	R	S	MS	R	S	S	S	S	S	S
	IRBLta-CP1	29	C101PKT	S	S	S	R-M	M	M	S	S	MS	R	R	S	R	R	S	S	S	S	M	S
	IRBLta-CP1[US]		C101PKT	S	S	R	M	MS	S	S	S	S	R	R	S	M	R	S	S	S	S	S	S
	IRBLta-Zh[LT]		Zhaiyeqing	S	S	R	R-M	R-M	M	S	S	S	R	R	S	R-M	S	S	S	S	S	MS	M
	IRBLta-Ya[CO]		Yashiromochi	S	S	S	R	R	R-M	S	MS	S	R	R	MS	R	R-M	S	MS	S	M	R	R

Unknown	IRBLta*-Me[CO]		Metica	S	S	S	R	R	M	S	S	S	R	R	S	R	R-M	S	S	S	S	R	R
Pita-2	IRBLta2-Pi	27	Pi No. 4	S	S	S	R	R-M	R	R	R	R	R	R	R	R	R	R	R-M	R	R	R	S
	IRBLta2-Pi[LT]		Pi No. 4	S	S	R	R	R-M	R	R	R	R	R	R	R	R	R	R	R-M	R-M	R-M	S	S
	IRBLta2-Pi[CO]		Pi No. 4	S	S	S	R	R	R-M	R	R	R-M	R	R	R	R	R	R	M	R-M	R	R	R
	IRBLta2-Re	28	Reiho	S	S	S	R	R-M	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S
	IRBLta2-Re[CO]		Reiho	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R-M	R-M	R	R	R	R
	IRBLta2-Fu[LT]		Fujisaka 5	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R-M	R	R	S	R-M
	IRBLta2-IR64[CO]		IR64	S	S	S	R-M	R	R-M	R	R	R-M	R	R	R	R	R	R	M	R	R	R	R
Pi20	IRBL20-IR24	26	ARL 24	S	S	S	S	S	S	R	R	S	MS-S	MS-S	R	S	S	R	S	S	S	R	R
Unknown	IRBL*-CR[LT]		Carreon	R-M	R-M	R-M	M	R-M	R-M	M	R	R	-	R	R	M	S	M	R	S	R-M	MS	MS
	IRBL*-TP[LT]		Tapochooz	R	R-M	R	R	R	R-M	R	R	R	-	R	R	R-M	R	R-M	R	R-M	R	R-M	R-M
	IRBL*-IT13a[LT]		IRAT13	M	MS	R-M	M	R-M	M	M	R-M	M	-	R-M	M	MS	M	R-M	R	R	R	R-M	R-M
	IRBL*-IT13b[LT]		IRAT13	MS	MS	M	M	R-M	M	M	M	M	-	R-M	M	MS	M	MS	R	M	M	M-MS	MS
Susceptible control	LTH		-	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	CO39		-	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R
	US-2		-	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

LTH, CO39, and US-2 are susceptible controls. CO39 harbors *Pia*.

2. Characterization of differential varieties

Agronomical traits including heading date and several morphological characteristics of the developed lines were evaluated to confirm the genetic uniformity and similarity to the corresponding recurrent parent.

To describe the genetic components of the differential varieties, the graphical genotype concept (Young and Tanksley, 1989) were clarified. In this concept, introgressed markers of the donor parents are plotted onto the genomic map of the recipient parent. This is similar to cytological karyotypes in describing an entire genome in a single graphic image, but different in that graphical genotypes are inferred from molecular data. In the previous study, approximately 160 simple sequence repeat (SSR) markers developed by Temnykh et al. (2001) were applied for genotyping of MLs and CO39 NILs (Yanoria et al. 2006a, 2006d). To complete this dataset, we used SSR markers developed by McCouch et al. (2002) to develop graphical genotypes of all differential varieties lines including MLs, LTH NILs, CO39 NILs and US-2 NILs.

A simple DNA extraction method described by Wang et al. (1993) for PCR analysis was used. Leaves from rice plants grown in a greenhouse were placed into a 2 ml micro tube, and combined with 100 µl NaOH (0.25 N). The leaf tissue was ground using a vibration mill machine (Retsch type MM300, Retsch GmbH & Co. KG, Germany) using the 25 [1/s] setting for 1 min. A total of 400 µl 100 mM Tris (pH 8.0) was added into the tube, and well mixed. The tube was centrifuged at 10,000 rpm for 10 min, and then the supernatant (~200µl), which contained the extracted DNA, was transferred to a new tube. The whole genome DNA was stored at -20℃.

To genotype differential varieties, a total of 162 SSR markers were selected from the core set developed and mapped by McCouch et al. (2002). PCR analysis was performed in a 15 µl PCR mixture containing 5.05 µl sterile H2O, 0.15 µl *Taq* polymerase (5 U/µl) (Takara Bio Inc, Japan), 1.5 µl 10x PCR Buffer (supplied with 15 mM $MgCl_2$), 0.3 µl dNTP (2.5 mM), 0.75 µl primer-F, 0.75 µl primer-R, 1.5 µl dye and 5 µl DNA at a concentration of 5 ng/µl. PCR was performed with the following profile: 35 cycles (1 min at 94 ℃ , 1 min at 55 ℃ , 2 min at 72 ℃) and extension of 5 min at 72 ℃. PCR products were visualized by gel electrophoresis on 4% (w/v) agarose. The polymorphic bands were recorded in each line and compared with the banding pattern of the recurrent parents LTH, CO39, and US-2.

Results

Monogenic lines (MLs) and three sets of near isogenic lines (NILs) were developed as the differential varieties of rice blast by recurrent backcross breeding. The first set consisted of 31 MLs targeting 24 blast resistance genes, *Pia*, *Pib*, *Pii*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pish*, *Pita*(*Pi4*), *Pita-2*, *Pit*, *Piz*, *Piz-5*(*Pi-2*), *Piz-t*, *Pi1*, *Pi3*(t), *Pi5*(t), *Pi7*(t), *Pi9*, *Pi11*(t), *Pi12*(t), *Pi19*(t), and *Pi20*(t) (Tsunematsu et al. 2000). The second set consisted of NILs with LTH genetic background targeting 15 rice blast resistance genes, *Pib*, *Piz-5*, *Piz-t*, *Pi3*(t), *Pi9*, *Pi5*(t), *Pia*, *Pik-s*, *Pik*, *Pik-h*, *Pi1*, *Pi7*(t), *Pik** (allele not yet identified), *Pita*, and *Pita-2*, and four unknown resistance genes, *Pita**, which showed the same reaction patterns of *Pita*. The third set of differential varieties consisted of NILs with the Indica-type variety CO39 genetic background targeting 14 major rice blast resistance genes, *Pish*, *Pib*, *Piz-5*, *Piz-t*, *Pi5*(t), *Pik-s*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pi1*, *Pi7*(t), *Pita*, and *Pita-2* and two unidentified alleles of *Pik* (*Pik**) and *Pita* (*Pita**). The last set consisted of NILs with the universal susceptible variety US-2 genetic background targeting 12 rice blast resistance genes, *Pia*, *Pik*, *Pik-h*, *Pik-p*, *Pik-s*, *Pi1*, *Pi7*(t), *Pita*, *Pi12*(t), *Piz-t*, *Pi9* and *Pi5*(t) (Table.1).

The MLs were designated as 'IRBL' lines (IRRI bred blast resistance lines) followed by the resistance gene, and then the abbreviation of the resistant donor variety. For example, IRBLa-A is a line with the resistance gene *Pia* originating from Aichi Asahi as a donor parent. These lines have been distributed to more than 30 institutes around the world through the International Network for Genetic Evaluation of Rice (INGER) and the IRRI-Japan Collaborative Research Project, IRRI. In the cases of the NILs, the abbreviation for the genetic backgrounds, LTH [LT], CO39 [CO], or US-2 [US], followed the donor name.

1. Characterization of agricultural traits

As a result of an evaluation of culm length, panicle length, days to heading, 100-grain weight, panicle number, spikelet fertility, leaf length, and leaf width of MLs and the three types of NILs over four cropping seasons, several significant differences were observed among these differential variety sets, although these lines are completely fixed in terms of their agronomical traits.

The period to heading of LTH was 68 days after transplanting at the paddy field, and those of MLs varied form 60 to 120 days in JIRCAS, Tsukuba city, Ibaraki, Japan. In the LTH, CO39, and US-2 NILs, it varied from 65 to 110, from 65 to 70, and 76 to 84 days, respectively. These results indicate that the variation in each differential variety set was changed according to the times of recurrent backcrosses with the parents. In addition, four differential varieties, IRBLzt-t, IRBLzt-T[LT], IRBLzt-IR56[CO], and IRBLzt-T[US], which contained Piz-t, showed late heading relative to the other lines in the same variety set (Fig. 1). Yokoo et al. (1980) reported that the major photoperiod sensitive gene *Se-1*, which is located on chromosome 6, is linked with the resistance gene *Piz-t*. These four lines may have introduced the target resistance gene with the photoperiod sensitive gene.

The same tendencies in variation among differential variety sets were observed in the other agronomical traits (data not shown).

2. Reaction pattern of differential varieties

All lines in the differential variety sets, MLs, LTH NILs, CO39 NILs, and US-2 NILs, were fixed with the resistance genes and showed clear reactions to 20 blast isolates from the Philippines. The data against the target resistance gene in each donor variety were compared among the differential varieties with the different genetic backgrounds, and confirmed the expected reactions to the standard differential blast isolates (Table 2).

The number of differential varieties that showed the same reaction pattern in each target resistance gene and confirmed the non-introgression of any additional resistance gene was 17, 23, 14, and 7 for MLs, LTH NILs, CO39 NILs, and US-2 NILs, respectively. In the other lines, 14 in the MLs, 11 in the LTH NILs, 17 in the CO39 NILs, and 9 in the US-2 NILs, showed additional resistance reaction(s), and it was predicted that some minor or other major resistance gene(s) may be harbored in these genetic backgrounds. The resistance genes *Pish* from Shin2 and BL1, *Pib* from BL1 and IRAT13, *Piz* from Fukunishiki, *Pi9* from WHD-1S-75-1-127, *Pi5* from Moroberican, *Pi12* from Moroberican, *Pia* from Aichi-

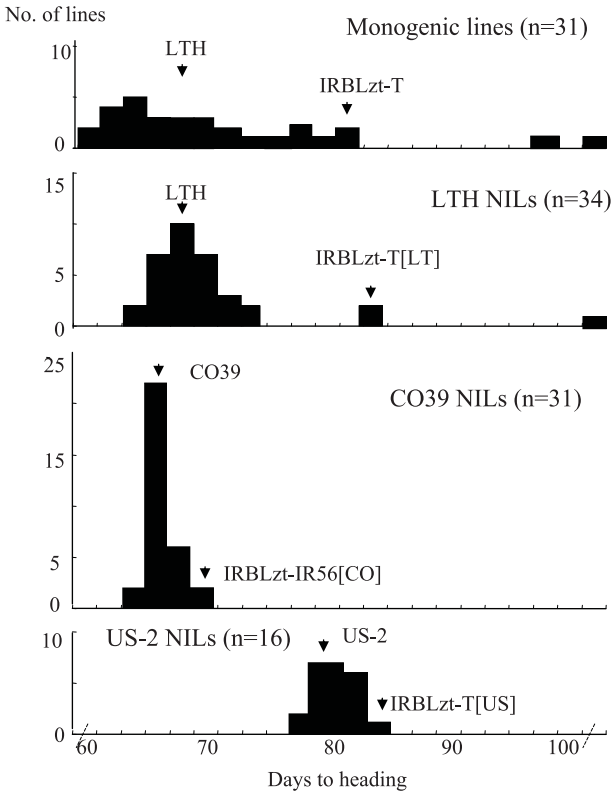

Fig. 1. Distributions of heading date in differential varieties at JIRCAS, Tsukuba, Japan, in 2006

All lines introduced with *Piz-t* showed late heading in relative to the other lines.

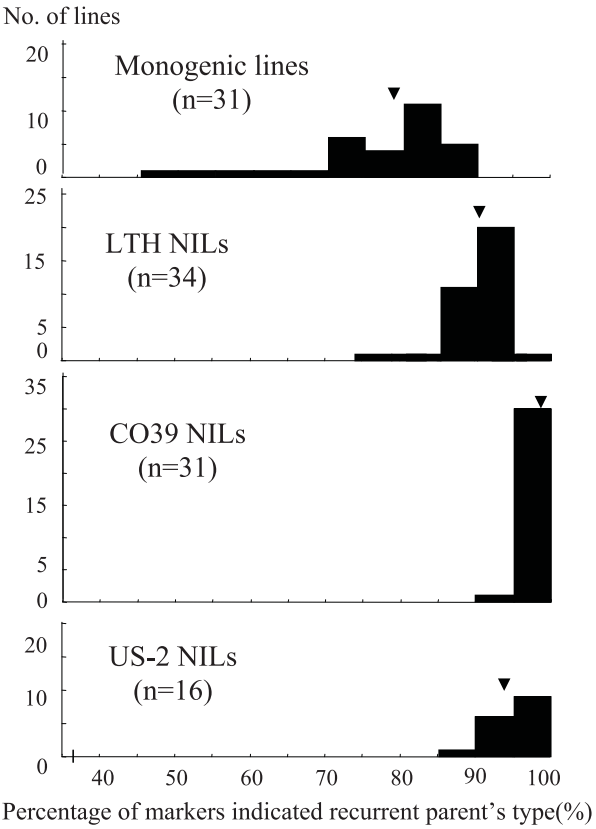

Fig. 2. Distribution of differential varieties for substitution ratio of recurrent parents' genetic backgrounds in each differential variety set

Triangles indicate the average among the differential varieties with the same genetic background.

Table 3. Summary of the development and characterization of differential varieties

Analysis for resistance gene	ML	NIL		
		LTH	CO39	US-2
Number of lines developing	31	34	31	16
Number of resistance genes	24	14	14	12
Pure reaction to blast isolates from the Philippines	17	23	14	7
Identification of the introgression	28	27	17	13

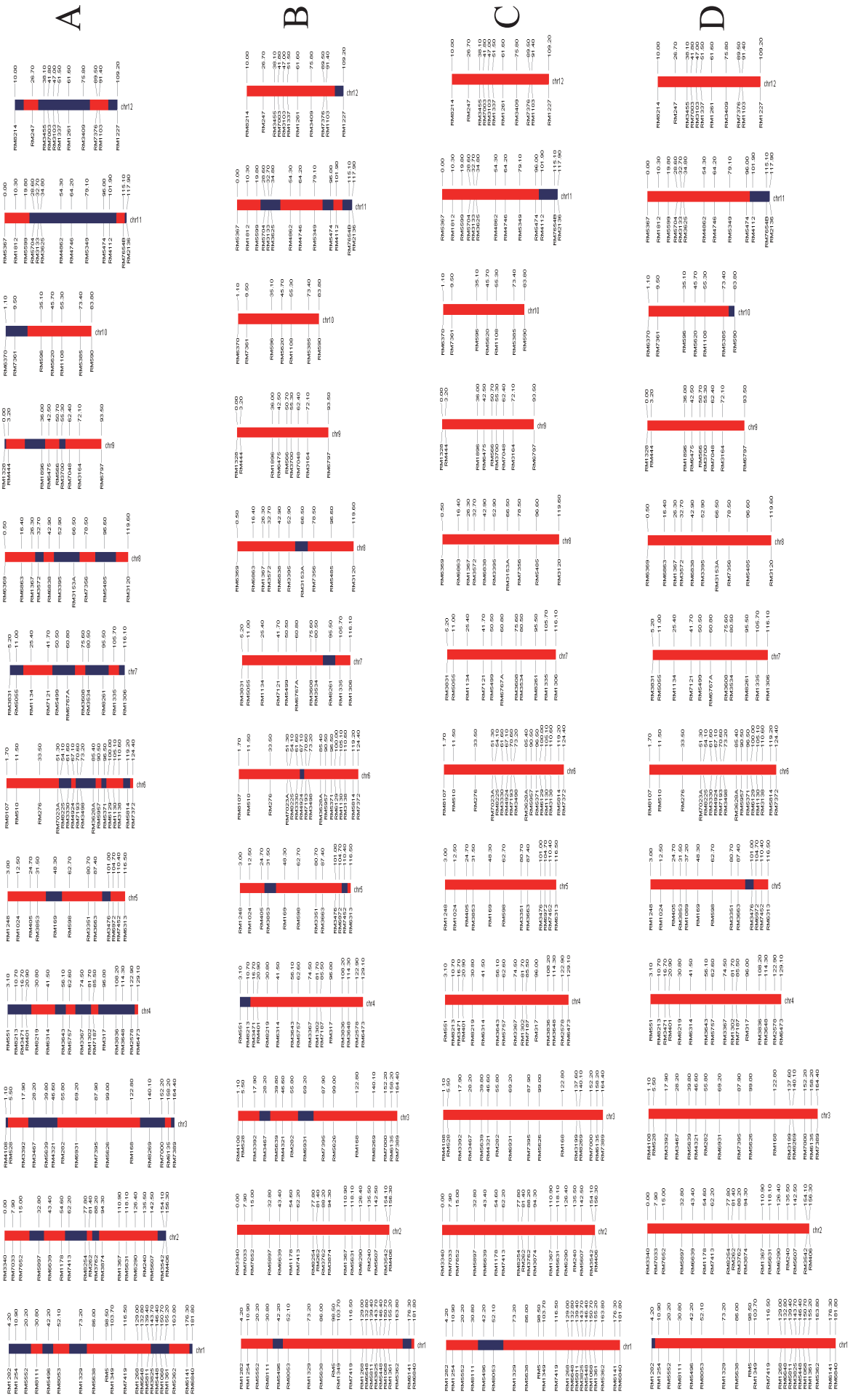

Fig. 3. Graphical genotypes of four differential varieties with introduced *Pik* from a donor, Kanto51

Red and blue colors in each chromosome indicate the genetic backgrounds of recurrent parents and introgressions from donor variety, Kanot51.
Pik was mapped in the terminal region of chromosome 11.
A: IRBLk-K, B: IRBLk-K[LT], C: IRBLk-K[CO], IRBLk-K[US]

ashahi, *Pik-h* from K3, *Pik-s* from Shin2 and Caloro, *Pita* from K1, C105TTP2L9, C101PKT, Zhaiyeqing, and Yashiromoch, and *Pita-2* from Pi No.4, Reiho, and Fujisaka 5 (Table 3) were introduced into the lines showing additional resistance reactions. These donor varieties are known to harbor several resistance genes in their genetic backgrounds.

3. Genetic characterization using DNA markers

Several introgression segments, which corresponded to the locations of blast resistance genes, were confirmed in almost all MLs and LTH, CO39, and US-2 NILs. These differential varieties were developed by backcrossing one to six times. The restoration rates of genomic chromosomes of 31 MLs of LTH ranged from 50% to 90.0% and averaged 77.3%, of 34 LTH NILs

Table 4. Genetic component of monogenic lines based on the polymorphism data of DNA markers

Designation	Resistance gene	Chr.	Donor variety	Generation (2006)	Number of SSR markers (%)				
					LTH type		No-LTH type		Total
IRBLt-K59	*Pit*	1	K59 (J)	BC_2F_{15}	130	(81.3)	30	(18.8)	160
IRBLsh-S	*Pish*	1	Shin2 (J)	BC_1F_{20}	126	(78.8)	34	(21.3)	160
IRBLsh-B	*Pish*	1	BL1 (J)	BC_1F_{18}	141	(88.1)	19	(11.9)	160
IRBLb-B	*Pib*	2	BL 1 (J)	BC_1F_{15}	117	(73.1)	43	(26.9)	160
IRBLz-Fu	*Piz*	6	Fukunishiki (J)	BC_1F_{20}	80	(50.0)	80	(50.0)	160
IRBLz5-CA-1	*Piz-5* (=*Pi2*(t))	6	C101A51 (I)	BC_3F_{18}	142	(88.8)	18	(11.3)	160
IRBLz5-CA-2	*Piz-5*	6	C101A51 (I)	BC_5F_{16}	143	(89.4)	17	(10.6)	160
IRBLzt-T	*Piz-t*	6	Toride 1 (J)	BC_1F_{20}	133	(83.1)	27	(16.9)	160
IRBL9-W	*Pi9*	6	WHD-1S-75-1-127 (I)	BC_3F_{18}	144	(90.0)	16	(10.0)	160
IRBL11-Zh	*Pi11*(t)	8	Zhaiyeqing (J)	BC_2F_{18}	133	(83.1)	27	(16.9)	160
IRBLi-F5	*Pii*	9	Fujisaka 5 (J)	BC_1F_{20}	113	(70.6)	47	(29.4)	160
IRBL3-CP4	*Pi3*(t)	9	C104PKT (I)	BC_2F_{18}	136	(85.0)	24	(15.0)	160
IRBL5-M	*Pi5*(t)	9	RIL249 (Moroberekan) (I)	BC_3F_{18}	114	(71.3)	46	(28.8)	160
IRBLa-A	*Pia*	11	Aichi Asahi (J)	BC_1F_{20}	117	(73.1)	43	(26.9)	160
IRBLa-C	*Pia*	11	CO39 (I)	BC_1F_{20}	93	(58.1)	67	(41.9)	160
IRBLk-Ka	*Pik*	11	Kanto 51 (J)	BC_1F_{19}	84	(52.5)	76	(47.5)	160
IRBLks-F5	*Pik-s*	11	Fujisaka 5 (J)	BC_1F_{20}	130	(81.3)	30	(18.8)	160
IRBLks-S	*Pik-s*	11	Shin 2 (J)	BC_1F_{20}	110	(68.8)	50	(31.3)	160
IRBLkp-K60	*Pik-p*	11	K 60 (J)	BC_1F_{18}	125	(78.1)	35	(21.9)	160
IRBLkh-K3	*Pik-h*	11	K 3 (J)	BC_1F_{15}	136	(85.0)	24	(15.0)	160
IRBLkm-Ts	*Pik-m*	11	Tsuyuake (J)	BC_1F_{16}	116	(72.5)	44	(27.5)	160
IRBL1-CL	*Pi1*	11	C101LAC (I)	BC_2F_{18}	136	(85.0)	24	(15.0)	160
IRBL7-M	*Pi7*(t)	11	RIL29 (Moroberekan) (I)	BC_3F_{18}	130	(81.3)	30	(18.8)	160
IRBLta-K1	*Pita* (=*Pi4*(t))	12	K 1 (J)	BC_2F_{18}	125	(78.1)	35	(21.9)	160
IRBLta-CT2	*Pita*	12	C105TTP2L9 (I)	BC_3F_{18}	134	(83.8)	26	(16.3)	160
IRBLta-CP1	*Pita*	12	C101PKT (I)	BC_5F_{16}	135	(84.4)	25	(15.6)	160
IRBLta2-Pi	*Pita-2*	12	Pi No.4 (J)	BC_1F_{14}	114	(71.3)	46	(28.8)	160
IRBLta2-Re	*Pita-2*	12	Reiho (J)	BC_1F_{16}	128	(80.0)	32	(20.0)	160
IRBL12-M	*Pi12*(t)	12	RIL10 (Moroberekan) (I)	BC_2F_{18}	137	(85.6)	23	(14.4)	160
IRBL19-A	*Pi19*(t)	12	Aichi Asahi (J)	BC_1F_{17}	133	(83.1)	27	(16.9)	160
IRBL20-IR24	*Pi20*(t)	12	ARL24 (I)	BC_1F_{16}	97	(60.6)	63	(39.4)	160
			Minimum		80	(50.0)	16	(10.0)	160
			Maxim		144	(90.0)	80	(50.0)	160
			Average		123.6	(77.3)	36.4	(22.7)	160

SSR markers distributed across 12 rice chromosomes, were used to clarify the component of each line.
I: Indica-type, J: Japonica-types.

ranged from 75.6% to 96.9% and averaged 90.6%, and the 31 CO39 NILs showed higher than 90% genome restoration rates relative to the recurrent parent, averaging 97.3%. The 16 US-2 NILs were highly similar to those of the recurrent parent: these genome restorations rates ranged from 88.9% to 98.8%, and averaged 94.6%. Thus, the ratio of restorations in each differential variety changed according to the number of backcrosses and recurrent parents (Fig. 2, 3).

Monogenic lines

The genotyping for 31 MLs was carried out using 160 SSR markers distributed throughout the rice genome. Most of the MLs were developed by backcrossing one to two times, but some lines were developed by backcrossing three to five times with the recurrent parent, LTH. The result demonstrated that the substitution to LTH ranged from 50% to 90.0% with an average of 77.3% among 31 MLs. Three MLs, IRBLz-Fu, IRBLa-C, and IRBLk-Ka, exhibited a frequency of recurrent type markers of 50.0%, 58.1%, 52.5%, respectively, which is substantially lower than the expected level for one time backcross breeding of 75% (Table 4, Fig. 2).

Pit was introduced from the Japonica-type variety K59 into the ML IRBLt-K59. The frequency of recurrent type, LTH, was 81.3%, and the other chromosome segments were distributed on all chromosomes, except chromosome 10. The gene *Pit* has been mapped on the short arm of chromosome 1, near the RFLP marker *R1613* (Kaji et al., 1997), and an introgression of donor segment was detected in this region by the SSR marker RM5552 (Fig. 4).

Pish originating from either the Shin2 or BL1 Japonica-type varieties was introduced into the MLs IRBLsh-S and IRBLsh-B, respectively. Based on polymorphism data of SSR markers, frequencies of LTH types of SSR markers were 78.8 and 88.1 % in IRBLsh-S and IRBLsh-B, respectively. *Pish* has been mapped on the long arm of chromosome 1 and is flanked by the SSR markers *RM212* and *OSR3* (*RM226*) (Araki et al., 2003). Both lines IRBLsh-S and IRBLsh-B showed the same polymorphism patterns in the SSR markers *RM3825* and

SSR marker	Distanc form terminal of short arm	Differential variety
		IRBLt-K59
RM1282	4.2	R
RM1254	10.9	R
RM5552*	20.2	I
RM8111	30.8	R
RM5496	42.2	R
RM8053	52.1	R
RM1329	73.2	I
RM5638	86.0	R
RM5	98.5	R
RM1349	103.7	R
RM7419	116.5	R
RM1268	129.0	R
RM6648	132.8	R
RM5811	139.4	R
RM3825	143.7	R
RM5448	146.4	R
RM1068	150.7	R
RM1361	155.2	R
RM5362	163.8	R
RM6141	176.3	R
RM6840	181.8	I

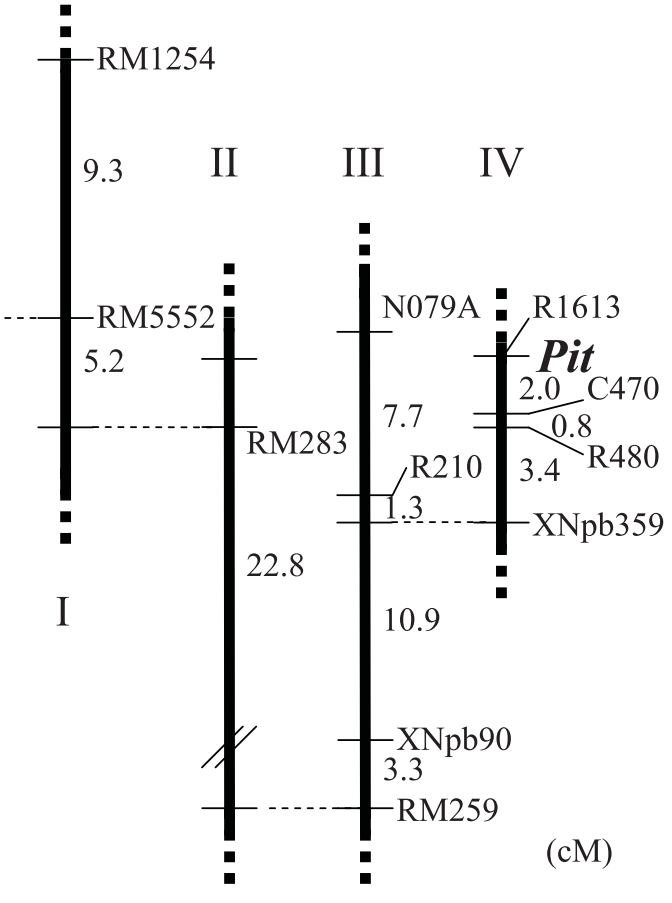

Fig. 4. Graphical genotypes of chromosome 1 of differential varieties containing *Pit*

R and I indicate the polymorphism patterns of the recurrent parent and the donor variety K59, respectively.
* Tightly linked DNA marker with targeted resistance gene.
I: McCouch et al. (2002), II: Temnysh et al. (2001), III: Fukuta et al. (2002), IV: Kaji et al. (1997)

RM1361 on the long arm of chromosome 1, where *Pish* was mapped, and the introgressions were confirmed (Fig. 5)

A monogenic line, IRBLb-B, which contained *Pib* from the Japonica-type line BL1, showed the same polymorphism patterns of LTH in 73.1% of SSR markers used. Several introgressions from the donor were detected in all chromosomes, except chromosome 9. *Pib* was mapped in the terminal region of chromosome 2 (Miyamoto et al. 1996; Monna et al. 1997; Wang et al., 1999). The SSR markers *RM3542* and *RM406* in the terminal region of chromosome 2 showed the donor polymorphism patterns (Fig. 6).

The blast resistance genes *Piz*, *Piz-5* (=*Pi2*), *Piz-t*, and *Pi9*(t) were clustered on chromosome 6 and had been mapped by Hittalmani et al. (2000), Liu et al. (2002) Conaway et al. (2003), Hayashi et al. (2004, 2006), Fjellstrom et al. (2006), Causse et al. (1994), Brar and Khush (1997), Liu et al. (2002), and Deng et al. (2006). A monogenic line, IRBLz-Fu, harbors *Piz* from the Japonica-type variety Fukunishiki. The genome restoration rate of this line relative to the recurrent parent, LTH, was 50%, which is quite a low frequency, while the donor segments were present on all chromosomes, except chromosome 9. Conaway et al. (2003) and Fjellstrom et al. (2006) reported that *Piz* linked closely with the SSR marker *RM6838*, which is located near the centromere of chromosome 6. The graphical genotype of IRBLz-Fu showed the intro- gression segment in the middle region of chromosome 6, thereby confirming the introgression of *Piz* in this line. Another *Piz* allele, *Piz-t*, from the Japonica-type variety Toride1, was introduced into IRBLzt-T. The frequency of LTH-type SSR markers was 83.1%. The presence of the donor segment in the *Piz* locus region was confirmed by several SSR markers. Two MLs from differing generations, IRBLz5-CA-1 and IRBLz5-CA-2, harbor *Piz-5* from the Indica-type variety C101A51. The genetic components of these lines were similar to the recurrent parent, with frequencies of the donor parent segment remaining in their genomes of 11.3% and 10.6%, respectively. *Piz-5* has been mapped in the same region with *Piz* near the centromere of chro-

SSR marker	Distanc from terminal of short arm	Differential variety						
		IRBLsh-S	IRBLsh-B	IRBLsh-Ku[CO]	IRBLsh-S[CO]-1	IRBLsh-S[CO]-2	IRBLsh-B[CO]	IRBLsh-Fu[CO]
RM1282	4.2	R	R	R	R	R	R	R
RM1254	10.9	I	R	R	R	R	R	R
RM5552	20.2	R	R	R	R	R	R	R
RM8111	30.8	R	R	R	R	R	R	R
RM5496	42.2	I	I	R	R	R	R	I
RM8053	52.1	R	R	R	R	R	R	I
RM1329	73.2	R	R	R	R	R	R	R
RM5638	86.0	R	R	R	R	R	R	R
RM5	98.5	R	R	R	R	R	R	R
RM1349	103.7	R	R	R	R	R	R	R
RM7419	116.5	R	R	R	I	R	R	R
RM1268	129.0	R	R	I	I	I	I	R
RM6648	132.8	R	R	I	I	I	I	R
RM5811	139.4	R	R	R	I	I	I	R
RM3825	143.7	I	I	R	R	R	R	R
RM5448	146.4	R	R	R	R	R	R	R
RM1068	150.7	R	R	R	R	R	R	R
RM1361	155.2	I	I	R	R	R	R	R
RM5362	163.8	R	R	R	R	R	R	R
RM6141	176.3	R	R	R	R	R	R	R
RM6840	181.8	I	R	R	R	R	R	R

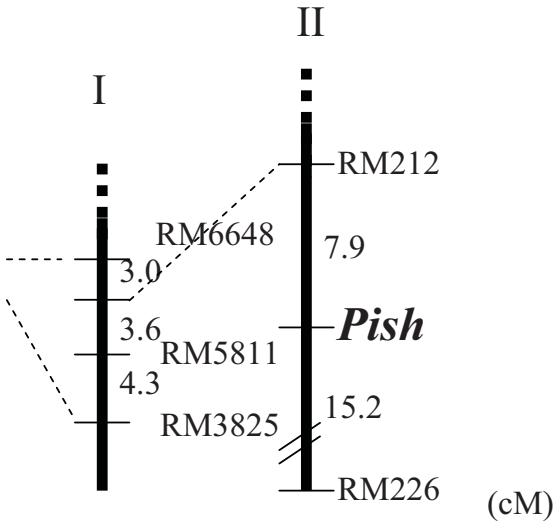

Fig. 5. Graphical genotypes of chromosome 1 of differential varieties containing *Pish*

R and I indicate the polymorphism patterns of recurrent parents and donor varieties, respectively.
* Tightly linked DNA marker with targeted resistance gene targeted.
I: McCouch et al. (2002), II: Fukuta et al. (2002)

mosome 6 (Mew et al., 1994; Hittalmani et al. 2000). This resistance gene was closely linked with the RFLP markers *RG64* and *R2123* (Liu et al. 2002). To estimate the location of *Piz-5* on the chromosomes of the MLs, we used linkage maps from Fukuta et al. (2000), which combined both SSR and RFLP markers. Graphical genotype analyses demonstrated that IRBLz5-CA-1 and IRBLz-5-CA-2 had donor segments in the middle region of chromosome 6, thereby confirming the introgression of *Piz-5*. The blast resistance gene *Pi9* originated from the wild rice *Oryza minuta* and transferred into the elite breeding line IR31917 (Brar and Khush, 1997). This gene was known to be tightly linked or may actually be allelic with the *Piz* locus (Causse et al., 1994, Liu et al., 2002). The ML IRBL9-W was developed for targeting the *Pi9* gene from the introgression line WHD-1S-75-127. The genetic structure of this line was similar to the LTH parent, with a genome restoration rate relative to the recurrent parent of 90.0%, despite several donor segments still remaining on chromosomes 1, 4, 6, 7, 8, 11, and 12. The resistance gene *Pi9* has been mapped to the same region as *Piz-5*, which is near the centromere of chromosome 6 and closely linked with the RFLP markers *RG64* and *R2123* (Liu et al. 2002). The presence of a donor segment in the middle of chromosome 6 in the graphical genotype of IRBL9-W indicated the introgression of the targeted segment (Fig. 7).

The ML IRBLi-F5 contains the *Pii* gene derived from the Japonica-type variety Fujisaka 5. Based on polymorphism data of 160 SSR markers, the frequency of the recurrent type on IRBLi-F5 genome was 70.6% with several introgression segments distributed across all 12 chromosomes. The resistance gene *Pii* has been previously mapped to chromosome 6 (Shinoda et al., 1971), but recent studies confirmed that this gene is located on chromosome 9 and tightly linked with *Pi3*(t) and *Pi5*(t) (Pan et al. 1998, 2003; Jeon et al. 2003; Yi et al. 2004). The presence of donor segments on the long arm of chromosome 9 suggested the introgression of *Pii* in this line. The gene *Pi3*(t) from the CO39 NIL, C104-PKT, which derived from the Japonica-type variety Pai-Kan-Tao (PKT) (Inukai et al. 1994), was introduced into the ML IRBL3-CP4. The genetic structure of this line is similar to the LTH parent with a genome restoration rate

SSR marker	Distanc from terminal of short arm	Differential variety			
		IRBLb-B	IRBLb-B[LT]	IRBLb-IT13[CO]	IRBLb-W[CO]
RM3340	0	R	R	R	R
RM7033	7.9	R	R	R	R
RM7652	15	R	R	R	R
RM5897	32.8	R	R	R	R
RM6639	43.4	R	R	R	R
RM1178	54.6	R	R	R	R
RM7413	62.2	I	R	R	R
RM8254	77.8	I	R	R	R
RM262	81.4	R	R	R	R
RM3762	88.2	I	R	R	R
RM3874	94.3	R	R	R	R
RM1367	110.9	R	R	R	R
RM5631	118.1	I	I	R	R
RM6290	126.4	R	R	R	R
RM240	135.5	R	R	R	R
RM5607	142.5	R	R	R	R
RM3542	154.1	I	I	R	R
RM406	156.3	I	R	R	R

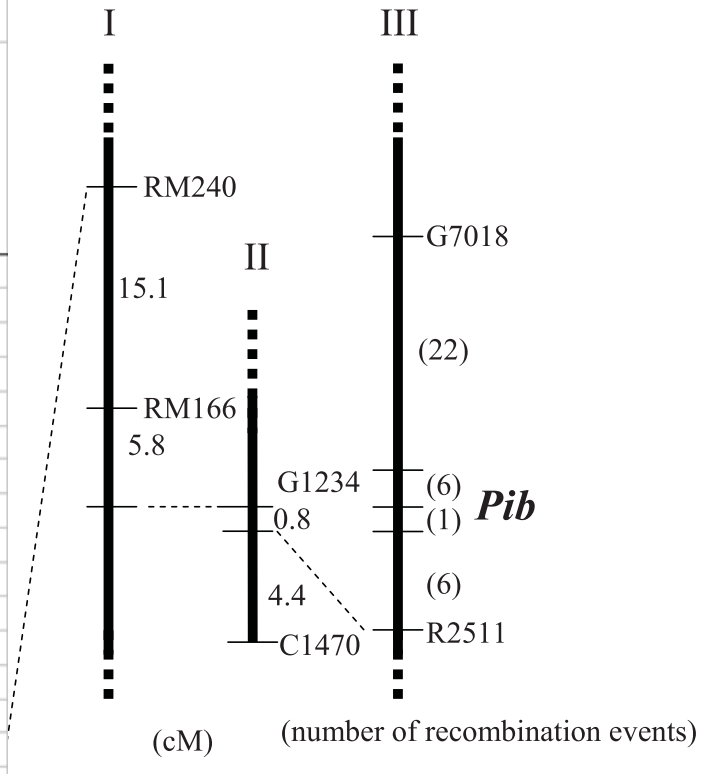

Fig. 6. Graphical genotypes of chromosome 2 of differential varieties containing *Pib*

R and I indicate the polymorphism patterns of recurrent parents and donor varieties, respectively.
* Tightly linked DNA marker with targeted resistance gene.
I: Fukuta et al. (2002), II: Harushima et al. (1998), III: Wang et al. (1999)

SSR marker	Distanc from terminal of short arm	Differential variety													
		IRBLz-Fu	IRBLz5-CA-1	IRBLz5-CA-2	IRBLzt-T	IRBL9-W	IRBLz5-CA[LT]	IRBLz5-TA[LT]	IRBLz5-K60[LT]	IRBLzt-T[LT]	IRBL9-W[LT]	IRBLz5-CA[CO]	IRBLzt-IR56[CO]	IRBLzt-T[US]	IRBL9-W[US]
RM8107	1.7	R	R	R	R	R	R	R	R	R	R	-	-	-	-
RM510	11.5	R	R	R	R	R	R	R	R	R	R	R	R	-	-
RM276	33.5	R	R	I	R	I	I	R	R	R	R	R	R	R	R
RM7023A	51.3	R	R	I	R	R	I	R	R	R	R	R	R	R	R
RM8225	54.1	R	I	I	I	I	I	I	I	R	I	R	R	R	I
RM3330	61.6	R	R	I	I	I	I	I	R	R	I	R	R	R	R
RM4924	67.1	I	I	I	I	I	I	R	I	R	I	I	R	R	R
RM7193	70.6	R	I	R	I	I	R	R	I	R	I	R	R	I	I
RM3498	73.2	R	I	R	I	R	R	R	I	R	I	R	R	R	R
RM3628A	85.4	R	I	R	I	R	R	R	R	R	I	R	R	R	I
RM5957	90.5	R	I	R	R	R	R	R	R	I	I	R	R	R	I
RM5371	96.5	I	I	R	I	R	R	R	R	R	R	R	R	R	R
RM6129	100.0	R	R	R	R	R	R	R	R	R	R	R	R	R	R
RM1130	105.1	R	I	R	R	R	R	R	R	R	R	R	R	R	R
RM3138	110.6	I	R	R	R	R	R	R	R	R	R	R	R	R	R
RM5814	119.2	R	R	R	R	R	R	R	R	R	R	R	R	R	R
RM7372	124.4	I	R	R	R	R	R	I	R	I	R	R	R	R	R

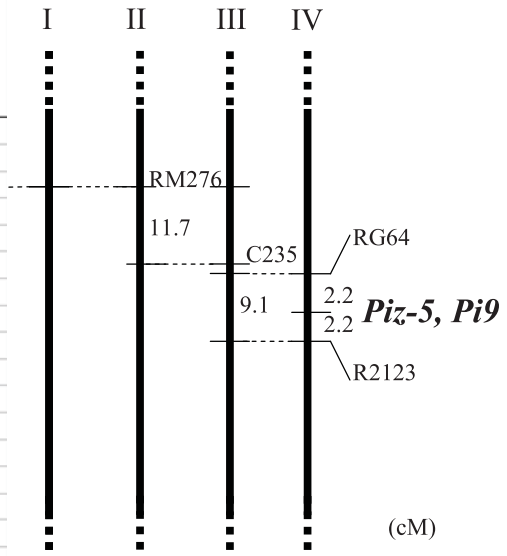

Fig. 7. Graphical genotypes of chromosome 6 of differential varieties containing either *Piz*, *Piz-t*, *Piz-5*, or *Pi9*

R and I indicate the polymorphism pattern of recurrent parents and donor varieties, respectively.
*Tight linked DNA marker with resistance gene targeted.
I: McCouch et al. (2002), II: Fukuta et al. (2002), III: Harushima et al. (1998), IV: Liu et al. (2002)

SSR marker	Distanc from terminal of short arm	Differential variety							
		IRBLi-F5	IRBL3-CP4	IRBL5-M	IRBL3-CP4[LT]-1	IRBL3-CP4[LT]-2	IRBL5-M[LT]	IRBL5-M[CO]	IRBL5-M[US]
RM1328	0.0	I	R	R	R	R	R	-	R
RM444	3.2	R	I	R	R	R	R	R	R
RM1896	36.0	I	I	I	I	I	I	R	I
RM6475	42.5	R	R	I	I	I	I	R	I
RM566	50.7	R	R	I	I	R	R	R	I
RM3700	55.3	I	I	I	I	R	R	R	I
RM7048	62.4	R	I	I	R	R	R	R	R
RM3164	72.1	R	I	I	R	R	R	R	R
RM6797	93.5	R	R	I	R	R	R	R	R

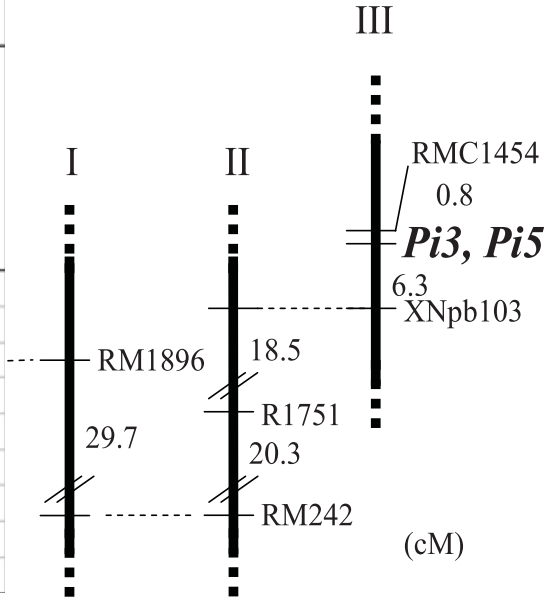

Fig. 8. Graphical genotypes of chromosome 9 of differential varieties introduced *Pii*, *Pi3*(t), or *Pi5*(t)

R and I indicate the polymorphism pattern of recurrent parents and donor varieties, respectively.
*Tight linked DNA marker with resistance gene targeted.
I: McCouch et al. (2002), II: Fukuta et al. (2002), III: Jeon et al. (2003)

SSR marker	Distanc form terminal of short arm	Differential variety						
		IRBLa-A	IRBLa-C	IRBLa-A[LT]	IRBLa-CO[LT]	IRBLa-Z[LT]	IRBLa-A[US]	IRBLa-C[US]
RM5367	0.0	R	R	R	R	R		
RM1812	10.3	R	I	I	R	R		
RM5599	19.8	R	I	I	R	R	R	R
RM5704	28.6	I	I	I	I	I	R	R
RM3133	32.7	I	I	I	I	I	I	R
RM3625*	34.8	I	R	R	R	R	I	I
RM4862*	54.3	I	I	I	I	I	I	I
RM4746	64.2	I	I	I	R	I	I	R
RM5349	79.1	R	I	R	R	I	R	R
RM5474	96.0	I	I	R	R	R	R	R
RM4112	101.9	R	R	R	R	R	I	R
RM7654B	115.1	R	R	R	I	R	R	R
RM2136	117.9	R	R	R	I	R	R	R

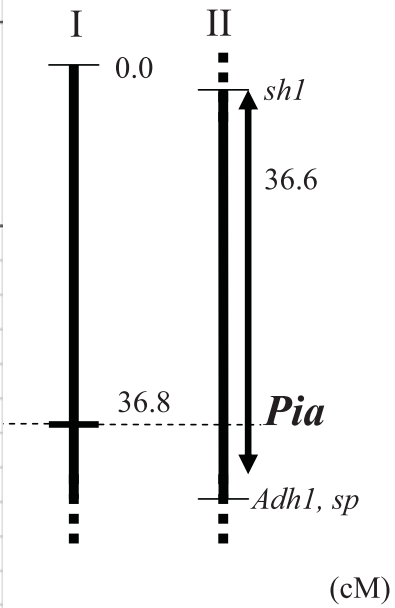

Fig. 9. Graphical genotypes of chromosome 11 of differential varieties containing *Pia*

R and I indicate the polymorphism patterns of recurrent parents and donor varieties, respectively.
* Tightly linked DNA marker with targeted resistance gene.
I: Nakamura et al. (2006), II: Goto et al. (1981)

relative to the recurrent parent of 85.0%. *Pi3*(t) has been mapped on chromosome 9, near the *Pi5*(t) gene, which is closely linked with the RFLP markers *XNpb103* and *C1454* (Jeon et al. 2003). Graphical analysis of IRBL3-CP4 revealed that the introgression segment from the donor parent occurs mainly on chromosome 9, therefore supporting the introgression of the targeted segment in this line. The blast resistance gene *Pi5*(t) was first introduced in the recombinant inbred line RIL249 from the traditional African cultivar Moroberekan and previously mapped to chromosome 4 (Wang et al. 1994). Recently, this gene was shown to be located on chromosome 9 and clustered with the *Pii* and *Pi3*(t) genes (Jeon et al. 2003; Yi et al. 2004). The *Pi5*(t) gene from RIL249 was introduced to IRBL5-M. Based on SSR data, 71.3% of the chromosomal components of IRBL5-M had been restored in the LTH parent. The graphical genotype of IRBL5-M showed that the donor segment on its genome was mainly distributed across chromosomes 1, 7, and 9. *Pi5*(t) has been mapped to chromosome 9 and is flanked by the RFLP markers *XNpb103* and *C1454* (Jeon et al. 2003). The abundance of introgression segments on chromosome 9 indicated the introgression of *Pi5*(t) in this line (Fig. 8).

Two MLs targeting the Pia gene, IRBLa-A and IRBLa-C, were developed using a Japonica-type variety Aichi Asahi and an Indica-type variety CO39 as donor parents, respectively. The genomic components of IRBLa-A were more similar to IRBLa-C, compared to the recurrent parent LTH. The genetic components of IRBLa-A showed a frequency of recurrent parent type chromosomes of 73.1%, with some introgression on chromosome 11 indicating that *Pia* had been incorporated in this line. Another ML, IRBLa-C, showed many introgression segments in which, until the BC_1F_{20} generation, the frequency of donor segments was 41.9% and the segments were mainly distributed on chromosomes 1, 2, 6, and 11 (Fig. 9).

The resistance genes *Pik*, *Pik-s*, *Pik-p*, *Pik-h*, and *Pik-m* are thought to be allelic and clustered on the long arm of chromosome 11, near the telomere (Inukai et al. 1994; Hayashi et al. 2006). The *Pik* gene from the Japonica-type variety Kanto51 was introduced to IRBLk-Ka. DNA analysis revealed that the genome restoration rate of this line relative to the recurrent parent was very low with a frequency of LTH segments in its genome in the BC_1F_{19} generation of 52.5%. Fjellstrom et al. (2004) reported that the *Pik* gene was located near the telomere of chromosome 11 and closely linked with the SSR markers *RM1233* and *RM224*. The introgression of the donor segment in the terminal region of chromosome 11 of IRBLk-Ka indicated the presence of the targeted seg-

ment in this line, despite the high frequency of the donor segment in other regions of chromosome 11. *Pik-s*, one of the *Pik* alleles, from the Japonica varieties Fujisaka5 and Shin2, were introduced into IRBLks-F5 and IRBLks-S, respectively. The genetic components of IRBLks-F5 were similar to the LTH parent with a frequency of the recurrent type segment in its genome of 81.3%. The introgression segment in the terminal region of chromosome 11 of this line indicated the introgression of *Pik-s*. Introgression of the targeted segment of *Pik-s* has also been confirmed in IRBLks-S, where the donor segment was present in the terminal region of chromosome 11. However, there are still many undesirable donor segments remaining in other regions of the IRBLks-S genome, in which the genome restoration rate of this line relative to the recurrent parent was 68.8%. *Pik-p* was introduced into the ML IRBLkp-K60 from the Japonica-type variety K60. 78.1% of the chromosome components of this line were similar to the recurrent parent. Introgression of the targeted segment in the IRBLkp-K60 genome was detected on the long arm of chromosome 11. *Pik-h* and *Pik-m* from the Japonica-type varieties K3 and Tsuyuake were introduced into the MLs IRBLkh-K3 and IRBLkm-Ts, respectively. Genome restoration rates relative to the recurrent parent of these two lines were 85.0% and 72.5%, respectively. The presence of donor segments in the terminal region of their chromosome 11 indicated the introgression of the targeted segments of *Pik-h* and *Pik-m*. *Pi1* and *Pi7*(t) have been mapped in the same region as the *Pik* alleles on the long arm of chromosome 11, near the telomere (Mew et al., 1994; Yu et al., 1996; Hittalmani et al., 2000; Wang et al., 1994; Campbell et al., 2004). *Pi1* and *Pi7*(t) were introduced into the MLs IRBL1-CL and IRBL7-M, respectively. The ML IRBL1-CL, which harbors *Pi1* from the NIL C101LAC, showed a genome restoration rate relative to the recurrent parent of 85.0%; whereas IRBL7-M, which harbors *Pi7*(t) from the Indica-type variety Moroberekan, showed a genome restoration rate relative to the recurrent parent of 81.3%. Both *Pi1* and *Pi7*(t) have been reported by Campbell et al. (2004) to be located between the RFLP markers *R251* and *S10003*. The presence of donor segments in the terminal region of IRBL1-CL and IRBL7-M indicated the introgression of the targeted segments. The location of the resistance genes was estimated based on a linkage map developed by Fukuta et al. (2000), which combined both RFLP and SSR markers (Fig. 10).

The MLs IRBLta-K1, IRBLta-CT2, and IRBLta-CP1, harbor the *Pita* gene from the Japonica-type variety K1 and the NILs C105TTP2L9 and C101PKT, respectively. These resistance genes have been intensively studied and mapped near the centromere of chromosome 12 (Mew et al. 1994; Yu et al. 1996; Hittalmani et al. 2000; Bryan et al. 2000; Jia et al. 2002; Jia et al. 2004; Hayashi et al. 2006). The genetic components were similar to the LTH parent with genome restoration rates relative to the recurrent parent of 78.1%, 83.8% and 84.4%, respectively. Jia et al. (2004) reported that the SSR markers *RM155* and *RM7102* were closely linked with the *Pita* gene on chromosome 12. Based on this information, we suggested that the donor segment, which was present in the centromere region of chromosome 12 of IRBLta-K1, IRBLta-CT2, and IRBLta-CP1, indicated the introgression of *Pita*. The resistance gene *Pita-2* from the Japonica-type varieties Pi No.4 and Reiho were introduced into IRBLta2-Pi and IRBLta2-Re, respectively. The frequency of the LTH-type with SSR markers in these two MLs was 71.3% and 80.0%, respectively. The resistance gene *Pita-2* is thought to be tightly linked with *Pita* and has been mapped in the same region near the centromere of chromosome 12 (Fjellstrom et al. 2004; Hayashi et al. 2006). Introgressed segments found near the centromere region of IRBLta2-Pi and IRBLta2-Re indicated that *Pita-2* has been incorporated in these lines; even though several undesirable segments were included in their genomes. The gene *Pi19* from the Japonica-type variety Aichi Asahi was introduced into ML IRBL19-A. This resistance gene was reported to be located on chromosome 12 and thought to be allelic or closely linked with *Pita-2* (Hayashi et al. 1998). Polymorphism data of SSR markers revealed that the genome restoration rate relative to the recurrent parent of this line was 83.1%. The graphical genotype of IRBL19-A showed that a donor segment was detected in the same region of *Pita* alleles on chromosome 12. Another resistance gene, which is also located on chromosome 12, is *Pi20*(t) (Imbe et al. 1997). The *Pi20*(t) gene from the recombinant inbred line ARL24 (Asominori/IR24) was introduced to IRBL20-IR24. DNA analysis showed that the genome restoration rate of this line relative to the recurrent parent was quite low at 60.6%. The graphical genotype analysis of IRBL20-IR24 confirmed that the introgressed segment was present near the centromere of chromosome 12, where *Pi20*(t) is located (Fig. 11).

The blast resistance gene *Pi11* (formerly *Pizh*) from the Japonica-type variety Zhaiyeqing was introduced into the ML IRBL11-Zh. DNA analysis using 160 SSR markers showed that the chromosome component of this line was 83.1% similar to the LTH parent. Several introgressed segments were found to be distributed across all chromosomes of IRBL11-Zh, except chromosome 8. Previously the resistance gene *Pi11* was mapped to chromosome 8 (Zhu et al. 1993; Causse et al. 1994). Evaluation of IRBL11-Zh against several blast isolates showed a similar reaction pattern with the line harboring the *Pib* gene. The graphical genotype of IRBL11-Zh supported this result in which the introgression segment was not detected on chromosome 8, but rather the introgression segment was found on the terminal region of chromosome 2, where *Pib* is located. The presence of the resistance gene *Pi11* may have been incorrectly identified by the introgression of *Pib* into the LTH genetic background (Fig. 12).

SSR marker	Distanc from terminal of short arm	Differential variett																																									
		IRBLk-Ka	IRBLks-F5	IRBLks-S	IRBLkp-K60	IRBLkh-K3	IRBLkm-Ts	IRBL1-CL	IRBL7-M	IRBLk-K[LT]	IRBLkh-K3[LT]	IRBLks-S[LT]	IRBLks-B40[LT]	IRBLks-Zh[LT]	IRBLk*-F14[LT]	IRBLk*-Z[LT]	IRBLk*-NP[LT]	IRBLk*-D[LT]	IRBLk*-F25[LT]	IRBLk*-F66/LT	IRBL1-CL[LT]	IRBL7-M[LT]	IRBLks-CO[CO]	IRBLk-Ku[CO]	IRBLk-Ka[CO]	IRBLkh-K3[CO]	IRBLkm-Ts[CO]	IRBLkp-K60[CO]	IRBLk*-F21[CO]	IRBLk*-F14[CO]	IRBLk*-F25[CO]	IRBLk*-F40[CO]	IRBLk*-F59[CO]	IRBLk*-F66[CO]	IRBLk*-K86[CO]	IRBLk*-S[CO]	IRBL1-CL[CO]	IRBL7-M[CO]	IRBLk-K[US]	IRBLkh-K3[US]	IRBLkp-K60[US]	IRBLks-F5[US]	IRBLks-S[US]	IRBL1-CL[US]	IRBL7-M[US]
RM5367	0.0	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R
RM1812	10.3	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
RM5599	19.8	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
RM5704	28.6	I	I	I	I	R	I	R	R	I	I	I	R	R	I	R	R	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
RM3133	32.7	I	I	I	I	R	R	R	R	I	R	R	R	R	R	R	R	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
RM3625	34.8	I	I	I	I	R	I	I	I	I	I	I	R	R	I	R	R	I	I	I	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
RM4862	54.3	I	R	R	I	R	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
RM4746	64.2	I	R	R	R	I	I	R	R	R	R	R	I	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
RM5349	79.1	I	I	I	R	R	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	I	R	R
RM5474	96.0	I	R	I	R	R	I	R	R	I	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
RM4112	101.9	I	I	I	I	R	R	R	I	R	R	R	R	R	R	I	I	R	I	R	R	I	I	I	I	R	R	R	R	R	I	R	I	R	R	I	R	R	I	I	I	I	I	R	I
RM7654B	115.1	R	R	R	I	I	I	R	I	I	I	R	R	I	I	R	R	I	I	I	R	I	I	I	I	R	R	R	R	R	I	R	R	I	I	I	I	R	R	R	R	R	R	R	R
RM2136	117.9	I	I	I	I	I	I	I	I	I	I	R	R	I	I	I	I	I	I	I	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

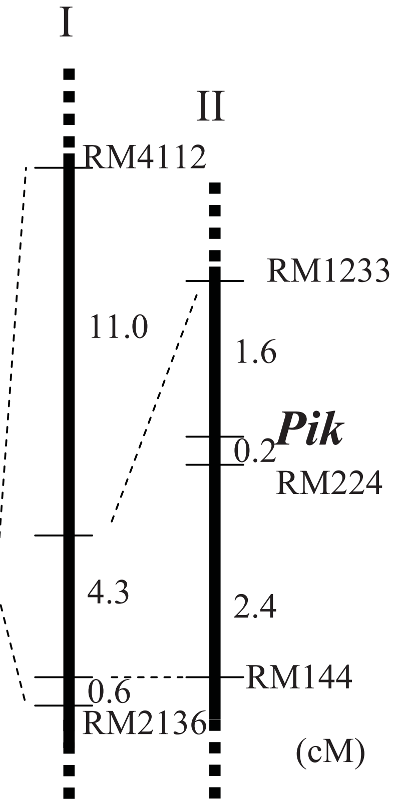

Fig. 10. Graphical genotypes of chromosome 11 of differential varieties containing *Pik* alleles and *Pi7*(t)

R and I indicate the polymorphism patterns of recurrent parents and donor varieties, respectively.
* Tightly linked DNA markers with targeted resistance gene.
I: Map by McCouch et al. (2002), II: Map by Fjellstrom et al. (2004)

SSR marker	Distanc from terminal of short arm	Differential variety																			
		IRBLta-K1	IRBLta-CT2	IRBLta-CP1	IRBLta2-Re	IRBLta-CT2[LT]	IRBLta-K1[LT]	IRBLta-Zh[LT]	IRBLta-Ya[CO]	IRBLta-K1[US]	IRBLta-CP1[US]	IRBLta-CT2[US]	IRBLta*-Me[CO]	IRBLta2-Pi	IRBLta2-Fu[LT]	IRBLta2-Pi[LT]	IRBLta2-Pi[CO]	IRBLta2-Re[CO]	IRBLta2-IR64[CO]	IRBL19-A	IRBL20-IR24
RM8214	10.0	I	I	I	I	R	R	R	R	R	R	I	R	I	I	R	R	R	R	R	I
RM247	26.7	R	I	R	R	R	R	I	R	R	R	I	R	R	R	R	R	R	R	R	I
RM3455	38.1	I	I	I	I	R	I	I	R	R	R	I	R	I	I	I	I	R	R	I	I
RM7003	41.8	I	I	I	I	R	I	I	I	I	R	I	I	I	I	I	I	R	I	R	R
RM3103	47.0	I	I	I	I	R	I	I	R	R	R	I	I	I	I	I	I	R	I	R	I
RM1337	51.5	I	I	I	I	I	I	I	R	I	I	I	I	I	I	I	I	I	I	I	I
RM1261	61.6	I	R	I	I	I	R	I	R	I	R	I	R	I	R	I	I	I	I	I	R
RM3409	75.8	I	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
RM7376	89.5	R	R	I	R	I	R	I	R	R	R	R	R	R	R	R	R	I	I	R	R
RM1103	91.4	R	R	I	R	I	R	I	R	R	R	R	R	R	R	R	R	I	I	R	R
RM1227	109.2	I	I	I	R	I	I	I	R	I	R	R	R	R	I	R	R	R	R	R	I

Fig.11. Graphical genotypes of chromosome 12 of differential varieties containing *Pita*, *Pita-2*, *Pi19*(t), and *Pi20*(t)

R and I indicate the polymorphism patterns of recurrent parents and donor varieties, respectively.
* Tightly linked DNA marker with targeted resistance gene.
I: McCouch et al. (2002), II: Jia et al. (2004)

SSR marker	Distanc from terminal of short arm	Diffrential variety: IRBL11-Zh
Chromosome 8		
RM6369	0.5	R
RM6863	16.4	R
RM1367	26.3	R
RM3572	32.7	R
RM6838	42.9	R
RM3395	52.9	R
RM3153A	66.5	R
RM7356	78.5	R
RM5485	96.6	R
RM3120	119.6	R
Chromosome 2		
RM3340	0	R
RM7033	7.9	R
RM7652	15	R
RM5897	32.8	R
RM6639	43.4	R
RM1178	54.6	R
RM7413	62.2	R
RM8254	77.8	R
RM262	81.4	I
RM3762	88.2	I
RM3874	94.3	R
RM1367	110.9	R
RM5631	118.1	R
RM6290	126.4	R
RM240	135.5	R
RM5607	142.5	I
RM3542	154.1	I
RM406	156.3	I

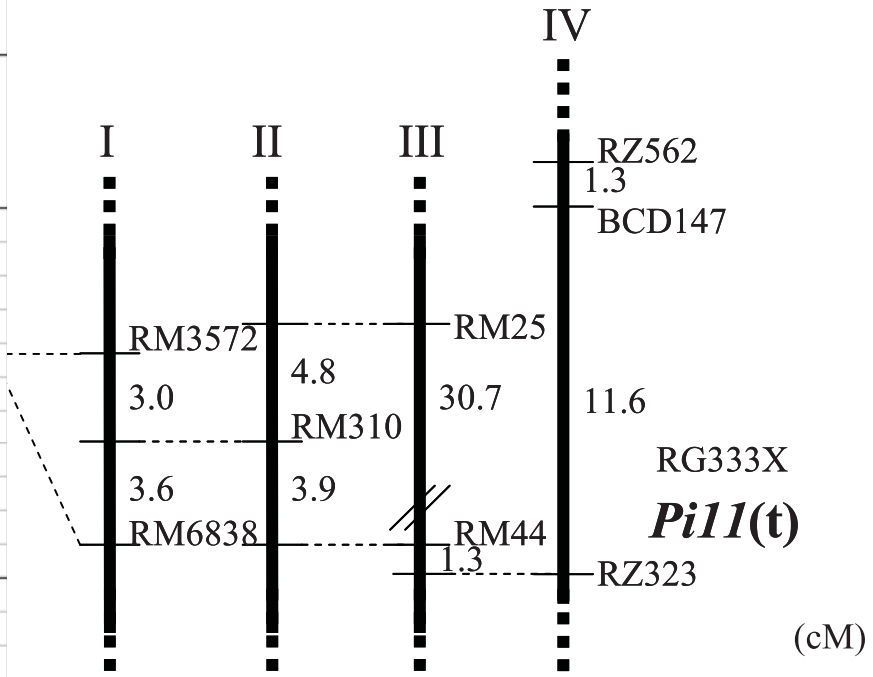

Fig. 12. Graphical genotypes of chromosomes 2 and 8 of differential varieties containing *Pi11*

R and I indicate the polymorphism patterns of recurrent parents and donor varieties, respectively. The region containing *Pib* on chromosome 2 was found to contain an introgression from the donor variety.
* Tightly linked DNA marker with targeted resistance gene.
I: McCouch et al. (2002), II: Temnykh et al., 2001, III: Cho et al. (1998), IV: Causse et al. (1994)

SSR marker	Distanc from terminal of short arm	Diffrential variety	
		IRBL12-M	IRBL12-M[US]
Chromosome 12			
RM8214	10	R	R
RM247	26.7	R	R
RM3455*	38.1	R	R
RM7003*	41.8	R	R
RM3103*	47	R	R
RM1337	51.5	R	R
RM1261	61.6	R	R
RM3409	75.8	R	R
RM7376	89.5	R	R
RM1103	91.4	R	R
RM1227	109.2	R	R
Chromosome 2			
RM3340	0	R	R
RM7033	7.9	R	R
RM7652	15	R	R
RM5897	32.8	I	R
RM6639	43.4	R	R
RM1178	54.6	R	R
RM7413	62.2	R	R
RM8254	77.8	I	R
RM262	81.4	R	R
RM3762	88.2	R	R
RM3874	94.3	R	R
RM1367	110.9	R	R
RM5631	118.1	R	R
RM6290	126.4	R	R
RM240	135.5	R	R
RM5607	142.5	R	R
RM3542	154.1	I	R
RM406	156.3	I	R

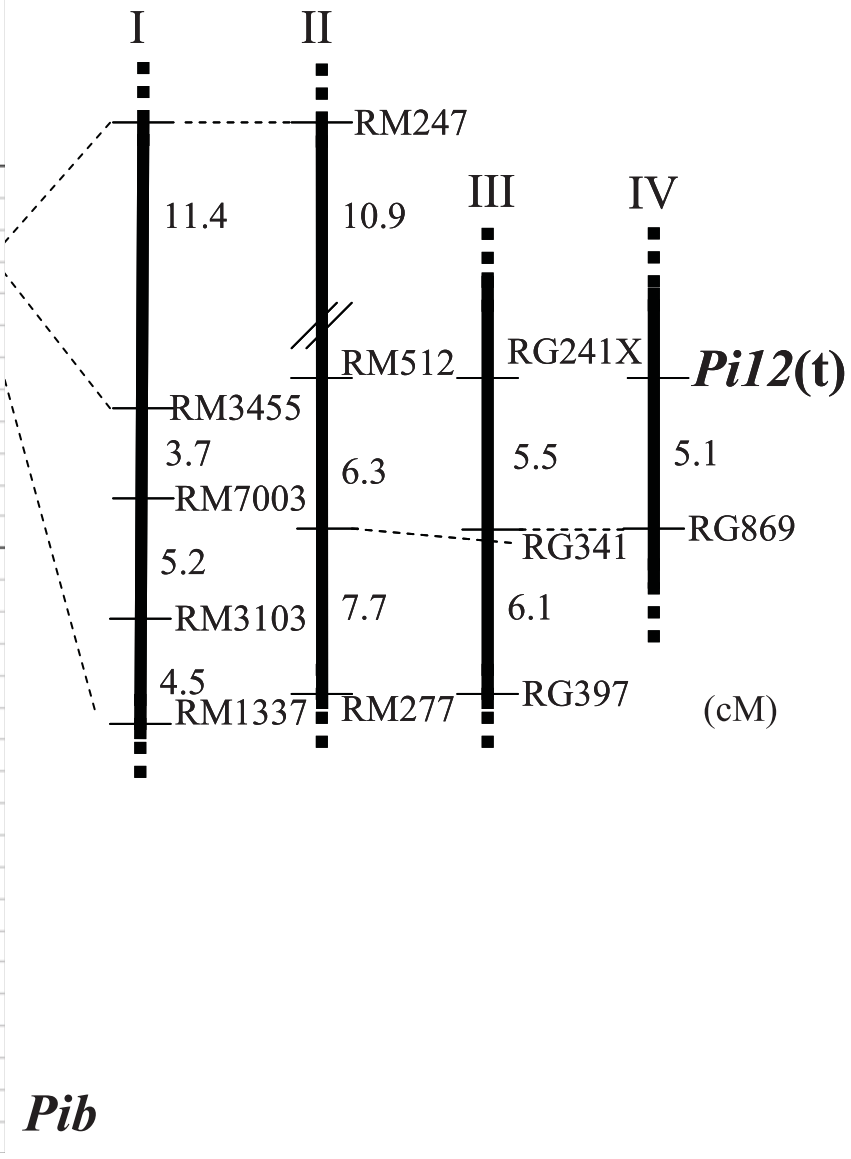

Fig. 13. Graphical genotypes of chromosomes 2 and 12 of differential varieties containing *Pi12*(t)

R and I indicate the polymorphism patterns of recurrent parents and donor varieties, respectively. The region containing *Pib* on chromosome 2 was found to contain an introgression from the donor variety.

* Tightly linked DNA marker with targeted resistance gene.

I: McCouch et al. (2002), II: Temnykh et al., 2001, III: Causse et al. (1998), IV: Zheng et al., (1995)

The resistance gene *Pi12*(t), which was derived from RIL10 (Moroberekan), was introduced into IRBL12-M. 85.6% of chromosome components of this line were similar to the recurrent parent LTH. The remaining 14.4% of the genome that was non-recurrent was distributed across chromosomes 1, 2, 3, 5, 6, 7, 9, and 11. The resistance gene *Pi12*(t) was mapped near the centromere of chromosome 12 (Zheng et al. 1996). Recent studies showed that the reaction pattern of IRBL12-M compared to several blast isolates corresponded to a line that carried the *Pib* gene. The graphical genotype of IRBL12-M demonstrated that the introgressed segment was present in the terminal region of chromosome 2 and not on chromosome 12. In IRBL12-M, the introgression of *Pi12*(t) may have been incorrectly identified and rather the *Pib* gene was introduced in the breeding process (Fig. 13).

LTH near isogenic lines

The MLs have been further backcrossed with LTH to generate NILs. A total of 23 lines for 14 resistance genes, *Pia*, *Pib*, *Pik*, *Pik-h*, *Pik-s*, *Pita*, *Pita-2*, *Piz-5*, *Piz-t*, *Pi1*, *Pi3*(t), *Pi5*(t), *Pi7*(t), and *Pi9*, have been developed. All NILs were developed by backcrossing six times with the LTH as a recurrent parent. Polymorphism data of 160 markers revealed that the rate of genome restoration relative to the LTH parent ranged from 75.6% to 96.9% with an average of 90.6%. These genome restoration rates were lower than the expected levels (Table 5, Fig. 2).

Pib from the Japonica-type variety BL1 was introduced into the LTH NIL IRBLb-B[LT]. DNA analysis demonstrated that the genomic components of this NIL were 91.9% similar to the recurrent parent. 8.1% of the donor segment was present in the IRBLb-B/LT genome, and this was distributed across all chromosomes, except chromosomes 1, 5, and 6. The resistance gene *Pib* has been mapped to the long arm of chromosome 2, near the telomere region (Miyamoto et al. 1996, Monna et al. 1997; Wang et al. 1999; Fjellstrom et al. 2004). The introgression of the donor segment in the terminal region of chromosome 2 of IRBLb-B[LT] indicated that *Pib* was incorporated in this line (Fig. 6).

The resistance gene *Piz-5* from three different donor parents, the isogenic line C101A51, the Indica-type variety Tadukan, and the Japonica-type variety K60, was introgressed into the NILs IRBLz5-CA[LT], IRBLz5-TA[LT], and IRBLz5-K60[LT], respectively. 90% of SSR markers in the three NILs were similar to the recurrent parent. The resistance gene *Piz-5*, a *Piz* allele, was mapped to a region near the centromere of chromosome 6 (Mew et al. 1994; Hittalmani et al. 2000). Liu et al. (2002) had previously reported that the RFLP markers *RG64* and *R2123* were closely linked to this resistance gene. The presence of the donor segment near the centromere of chromosome 6 in IRBLz5-CA[LT] indicated that *Piz-5* was introgressed in this line. Similar results were demonstrated in the NILs IRBLz5-TA[LT] and IRBLz5-K60[LT] and the presences of donor segments in the targeted region indicated the introgression of *Piz-5*. Another *Piz* allele, *Piz-t*, from the Japonica-type variety Toride 1 was introduced into NIL IRBLzt-T[LT]. SSR marker analysis showed that the genome restoration rate relative to the recurrent parent was 86.3%, which was lower than expected for a six-time backcross. The donor segment that remained in this line was distributed across all chromosomes, except chromosomes 8, 9, and 10. *Piz-t* was mapped in the same region as *Piz* and *Piz-5*, near the centromere of chromosome 6 (Hayashi et al. 2004, 2006). Despite the presence of donor chromosome segments in chromosome 6 of IRBLzt-T[LT], the introgression of *Piz-t* in the predicted region could not be confirmed in this study. A NIL IRBL9-W[LT] harboring *Pi9* from WHD-1S-75-127, showed that the frequency of recurrent parent markers was 88.8%, and the donor segment was present on chromosomes 1, 2, 4, 6, 11, and 12. The resistance gene *Pi9* was mapped to chromosome 6, which is closely linked to the *Piz* locus (Causse et al. 1994; Liu et al. 2002). The RFLP markers *RG64* and *R2123* have been mapped near this resistance gene (Liu et al., 2002). The presence of the donor segment in the middle region of chromosome 6 indicated the introgression of the targeted segment in NIL IRBL9-W[LT] (Fig. 7).

The NILs IRBL3-CP4[LT]-1 and IRBL3-CP4[LT]-2 harbor the *Pi3*(t) gene from the CO39 NIL C104PKT. The resistance gene originally came from the Japonica-type variety Pai-Kan-Tao (PKT) (Inukai et al. 1994). The genetic components of IRBL3-CP4[LT]-1 and IRBL3-CP4[LT]-2 were similar to those of LTH, with genome restoration rates relative to the recurrent parent of 91.9% and 91.3%, respectively. *Pi3*(t) has been mapped to the long arm of chromosome 9 and thought to be closely linked with *Pii* and *Pi5*(t) (Pan et al. 1998, 2003; Jeon et al. 2003; Yi et al. 2004). Jeon et al. (2003) reported the RFLP markers *XNpb103* and *C1454* were closely linked to the *Pi3*(t) and *Pi5*(t) genes. The introgression of donor segments on chromosome 9 of IRBL3-CP4[LT]-1 and IRBL3-CP4[LT]-2 indicated the presence of *Pi3*(t). The resistance gene *Pi5*(t) from African cultivar Moroberekan was introduced to the NIL IRBL5-M[LT]. The chromosome component of this line was 90% similar to the recurrent parent LTH. Donor segments were detected on chromosomes 9, 11, and 12, and these covered 10% of the IRBL5-M[LT] genome. The resistance gene *Pi5*(t) was mapped to chromosome 9 and is tightly linked with the RFLP markers *XNpb103* and *C1454* (Jeon et al. 2003). Graphical genotype analysis of IRBL5-M[LT] showed the presence of the donor segment in the same region as these markers, indicating the introgression of *Pi5*(t) (Fig. 8).

Table 5. Genetic components of near isogenic lines with LTH genetic background based on the polymorphism data of DNA markers

Designation	Target resistance gene	Chromosome	Donor variety	Generation (2006)	Number of SSR marker (%)				
					Recurrent type (LTH)		Non recurrent type		Total
IRBLb-B[LT]	*Pib*	2	BL1 (J)	BC_6F_{15}	147	(91.9)	13	(8.1)	160
IRBLz5-CA[LT]	*Piz-5* (=*Pi2*(t))	6	C101A51 (I)	BC_6F_{17}	145	(90.6)	15	(9.4)	160
IRBLz5-TA[LT]	*Piz-5* (=*Pi2*(t))	6	Tadukan (I)	BC_6F_{14}	150	(93.8)	10	(6.3)	160
IRBLz5-K60[LT]	*Piz-5* (=*Pi2*(t))	6	K60 (J)	BC_6F_{14}	146	(91.3)	14	(8.8)	160
IRBLzt-T[LT]	*Piz-t*	6	Toride (J)	BC_6F_{16}	138	(86.3)	22	(13.8)	160
IRBL9-W[LT]	*Pi9*	6	WHD-1S-75-1-127(I)	BC_6F_{17}	142	(88.8)	18	(11.3)	160
IRBL3-CP4[LT]-1	*Pi3*(t)	9	C104PKT (I)	BC_6F_{17}	147	(91.9)	13	(8.1)	160
IRBL3-CP4[LT]-2	*Pi3*(t)	9	C104PKT (I)	BC_6F_{17}	146	(91.3)	14	(8.8)	160
IRBL5-M[LT]	*Pi5*(t)	9	RIL249 (I)	BC_6F_{17}	144	(90.0)	16	(10.0)	160
IRBLa-A[LT]	*Pia*	11	Aichi Asahi (J)	BC_6F_{16}	121	(75.6)	39	(24.4)	160
IRBLa-CO[LT]	*Pia*	11	CO39 (I)	BC_6F_{16}	137	(85.6)	23	(14.4)	160
IRBLa-Z[LT]	*Pia*	11	Zenith	BC_6F_{15}	147	(91.9)	13	(8.1)	160
IRBLk-K[LT]	*Pik*	11	Kanto51 (J)	BC_6F_{15}	142	(88.8)	18	(11.3)	160
IRBLkh-K3[LT]	*Pik-h*	11	K3 (J)	BC_6F_{15}	148	(92.5)	12	(7.5)	160
IRBLks-S[LT]	*Pik-s*	11	Shin2 (J)	BC_6F_{15}	143	(89.4)	17	(10.6)	160
IRBLks-B40[LT]	*Pik-s*	11	B40	BC_6F_{15}	148	(92.5)	12	(7.5)	160
IRBLks-Zh[LT]	*Pik-s*	11	Zhaiyeqing8 (J)	BC_6F_{15}	149	(93.1)	11	(6.9)	160
IRBL1-CL[LT]	*Pi1*	11	C101LAC (I)	BC_6F_{17}	133	(83.1)	27	(16.9)	160
IRBL7-M[LT]	*Pi7*	11	RIL29 (I)	BC_6F_{17}	155	(96.9)	5	(3.1)	160
IRBLta-CT2[LT]	*Pita* (=*Pi4*(t))	12	C105TTP2L9	BC_6F_{17}	148	(92.5)	12	(7.5)	160
IRBLta-K1[LT]	*Pita* (=*Pi4*(t))	12	K1	BC_6F_{15}	144	(90.0)	16	(10.0)	160
IRBLta-Zh[LT]	*Pita* (=*Pi4*(t))	12	Zhaiyeqing8 (J)	BC_6F_{15}	141	(88.1)	19	(11.9)	160
IRBLta2-Fu[LT]	*Pita-2*	12	Fukunishiki (J)	BC_6F_{16}	147	(91.9)	13	(8.1)	160
IRBLta2-Pi[LT]	*Pita-2*	12	Pi No.4	BC_6F_{12}	151	(94.4)	9	(5.6)	160
IRBLk*-F14[LT]	Unknown	Unknown	F-14-3	BC_6F_{16}	152	(95.0)	8	(5.0)	160
IRBLk*-Z[LT]	Unknown	Unknown	Zenith	BC_6F_{15}	149	(93.1)	11	(6.9)	160
IRBLk*-NP[LT]	Unknown	Unknown	NP125	BC_6F_{15}	147	(91.9)	13	(8.1)	160
IRBLk*-D[LT]	Unknown	Unknown	Dular	BC_6F_{15}	145	(90.6)	15	(9.4)	160
IRBLk*-F25[LT]	Unknown	Unknown	F-25-3	BC_6F_{15}	143	(89.4)	17	(10.6)	160
IRBLk*-F66[LT]	Unknown	Unknown	F-66-10	BC_6F_{15}	143	(89.4)	17	(10.6)	160
IRBLx*-CR[LT]	Unknown	Unknown	Carreon	BC_6F_{15}	143	(89.4)	17	(10.6)	160
IRBLx*-TP[LT]	Unknown	Unknown	Tapochooz	BC_6F_{15}	152	(95.0)	8	(5.0)	160
IRBLx*-IT[LT]-1	Unknown	Unknown	IRAT13	BC_6F_{12}	148	(92.5)	12	(7.5)	160
IRBLx*-IT[LT]-2	Unknown	Unknown	IRAT13	BC_6F_{12}	148	(92.5)	12	(7.5)	160
		Minimum			121	(75.6)	5	(3.1)	160
		Maxim			155	(96.9)	39	(24.4)	160
		Average			145.0	(90.6)	15.0	(9.4)	160

k*: The allele of *Pik* was expected to introgress into the LTH genetic background based on the reaction patterns to blast isolates from the Philippines.
x*: Unknown resistance gene was introgressed into the LTH genetic background.
SSR markers distributed across 12 rice chromosomes were used to determine the component of each line.
I: Indica-type, J: Japonica-types

The resistance gene *Pia* derived from three different cultivars, namely Aichiasahi, CO39 and Zenith, was introduced to the LTH NILs IRBLa-A[LT], IRBLa-CO[LT], and IRBLa-Z[LT], respectively. Polymorphism analysis revealed that the genome restoration rate of IRBLa-A[LT] relative to the recurrent parent after backcrossing six times was very poor at 75.6%. The other LTH NILs with the *Pia* gene, IRBLa-CO[LT] and IRBLa-Z[LT], showed a higher frequency of the recurrent type segment than IRBLa-A[LT], with genome restoration rates relative to the LTH parent of 85.6% and 91.9%, respectively. However, the presence of the donor segment on chromosome 11 in these three lines indicated the introgression of *Pia* (Fig. 9).

Several blast resistance genes are clustered in the terminal region of chromosome 11, which are thought to be tightly linked or even allelic, and include *Pik*, *Pik-h*, *Pik-s*, *Pi1* and *Pi7*(t) (Inukai et al. 1994; Fjellstrom et al. 2004; Hayashi et al. 2006). The resistance gene *Pik* from the Japonica-type variety Kanto51 was introduced into the LTH NIL IRBLk-K[LT]. In the BC_6F_{15} generation, the genome restoration rate relative to the LTH parent IRBLk-K[LT] was 88.8% with a few donor segments still remaining on the chromosome. The resistance gene *Pik* was previously reported to be flanked by the SSR markers *RM1233* and *RM224* (Fjellstrom et al. 2004). Using this information, we predicted the location of the introgression segment that corresponded to the *Pik* alleles. The graphical genotype of IRBLk-K[LT] revealed the presence of introgressed segments in that region, indicating the introgression of *Pik*. The chromosome component of IRBLkh-K3[LT], which harbors the *Pik-h* gene derived from the Japonica-type variety K3, was similar to the LTH parent. The genome restoration rate of this line relative to the recurrent parent was 92.5%, with a few introgression segments on chromosomes 1, 4, 6, 7, 11, and 12. The presence of a donor segment in the terminal region of chromosome 11 indicated the introgression of *Pik-h* in IRBLkh-K3[LT]. Three LTH NILs, IRBLks-S[LT], IRBLks-B40[LT], and IRBLks-Zh[LT], were developed for targeting the *Pik-s* gene from the donor parents, Shin2, B40, and Zhaiyeqing8, respectively. Relative to the recurrent parent, 90% of the genetic components of these three LTH NILs were restored. Introgression was only confirmed in IRBLks-Zh[LT], where *Pik-s* was located close to the telomere of chromosome 11, whereas in IRBLks-S[LT] and IRBLks-B40[LT], this region could not be differentiated from the recurrent type. Several new blast resistance genes designated as *Pik**, which are thought to be allelic with *Pik*, have been introgressed to LTH NILs, including IRBLk*-F14[LT], IRBLk*-Z[LT], IRBLk*-NP[LT], IRBLk*-D[LT], IRBLk*-F25[LT] and IRBLk*-F66[LT], from the donor varieties F-14-3, Zenith, NP125, Dular, F-25-3, F-66-10, C101LAC, and RIL29 (Moroberekan), respectively. Genetic components of these NILs were similar to the recurrent parent. The introgression of the donor segment in the terminal region of chromosome 11 in these six NILs indicated the presence of the targeted segment. The resistance gene *Pi1*, which maps to chromosome 11 and is thought to be linked with the *Pik* alleles (Mew et al. 1994; Yu et al. 1996; Hittalmani et al. 2000), was introduced into NIL IRBL1-CL[LT]. Analysis of 160 SSR markers revealed that 83.1% of chromosome components of this line were similar to the LTH parent, with introgression segments mainly present on chromosomes 3 and 6. Campbell et al. (2004) reported that *Pi1* is located in the same region as the *Pik* locus between the RFLP markers *R251* and *S10003*. The presence of the donor segment on chromosome 11, indicated that *Pi1* had introgressed in IRBL1-CL[LT]. The LTH NIL IRBL7-M[LT] harbors the *Pi7*(t) gene from Moroberekan, which is located in the same region as *Pi1* (Wang et al. 1994; Campbell et al. 2004). DNA analysis showed that 96.9% of the genomic components of IRBL7-M[LT] were similar to the recurrent parent with some introgression near the telomere of chromosome 11, indicating that the targeted segment had been incorporated in this line (Fig. 10).

The LTH NILs IRBLta-CT2[LT], IRBLta-K1[LT], and IRBLta-Zh[LT] contained the *Pita* gene derived from C105TTP2L9, K1, and Zhaiyeqing8, respectively. Polymorphism analysis using SSR markers showed that the genome restoration rates of these three NILs were 90.0%, 88.1% and 91.9%, respectively. *Pita* has been mapped near the centromere of chromosome 12 (Mew et al. 1994; Yu et al. 1996; Hittalmani et al. 2000; Bryan et al. 2000; Jia et al. 2002; Jia et al. 2004; Hayashi et al. 2006). The SSR markers *RM155* and *RM7102* are reported to be tightly linked with this resistance gene (Jia et al. 2004). The presence of the introgressed segment in IRBLta-CT2[LT], IRBLta-K1[LT] and IRBLta-Zh[LT], which correspond to the region of these two markers, indicates the incorporation of the targeted segment in these NILs. The LTH NILs IRBLta2-Fu[LT] and IRBLta2-Pi[LT] that harbor the resistance gene *Pita-2*, which is thought to be allelic to *Pita* (Fjellstrom et al. 2004; Hayashi et al. 2006), also showed introgression in the centromere region of chromosome 12. The *Pita-2* in these NILs originated from the Japonica-type varieties Fukunishiki and Pi No.4, respectively. Approximately 90% of the genomic components of these lines resembled the recurrent parent (Fig. 11).

The LTH NILs IRBLx-CR[LT] and IRBLx-TP[LT] harbored unknown blast resistance genes derived from Carreon and Tapochooz, respectively. These genes are thought to be allelic with *Piz*, which is located near the centromere of chromosome 6. The genetic components of IRBLx-CR[LT] and IRBLx-TP[LT] were similar to those of the recurrent parents, and the graphical genotypes revealed the introgression of some donor segments in their chromosomes including an introgressed segment in chromosome 6. The LTH NILs IRBLx-IT[LT]-1 and IRBLx-IT[LT]-2 contain an unknown blast resistance

gene from IRAT13, which is thought to be allelic with *Piz* on chromosome 6(Data were not shown). The frequency of recurrent parent type markers in these two sister lines are similar, but their graphical genotype showed a different pattern. IRBLx-IT[LT]-1 exhibited introgression of the donor segment near the centromere, whereas IRBLx-IT[LT]-1 showed introgression of the donor segment in the terminal region of chromosome 6. However, further studies are needed to identify these resistance genes.

CO39 near isogenic lines

An Indica-type rice, CO39, was used to develop NILs for blast resistance. CO39 is susceptible to blast fungus, but is reported to harbor the resistance gene *Pia*. A total of 31 NILs have been developed to more than the BC_6F_{10} generation for 14 resistance genes, *Pib*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pish*, *Pita*, *Pita-2*, *Piz-5*, *Piz-t*, *Pi1*, *Pi5*(t), and *Pi7*(t). This is the first differential variety set with Indica-type genetic background for a large number of resistance genes. These NILs were analyzed using 161 SSR markers, which are distributed across all 12 rice chromosome. DNA analysis revealed that the genetic components of the 31 NILs and each of the CO39 NILs showed genome restoration rates relative to the recurrent parent of more than 90%, with an average of 97.3% (Table 6, Fig.2).

DNA analysis showed that the genetic components of five NILs, IRBLsh-Ku[CO], RBLsh-S[CO]-1, IRBLsh-S[CO]-2 IRBLsh-B[CO] and IRBLsh-Fu[CO], were similar to those of the recurrent parent. Genetic components of IRBLsh-Ku[CO] containing *Pish* from the Japonica-type variety Kusabue were almost identical to the recurrent parent with genome restoration rates of 98.8%, and only two SSR markers corresponded to the donor segment on chromosome 1 (Fig.5). Araki et al. (2003) reported that the *Pish* gene was located on chromosome 1 and flanked by the SSR markers *RM212* and *OSR3* (*RM226*). Therefore, this study validated the introgression of *Pish* in IRBLsh-Ku[CO]. The NILs IRBLsh-S[CO]-1 and IRBLsh-S[CO]-2 are sister lines that contain the *Pish* gene from the Japonica-type variety Shin 2. The genome restoration rate relative to the recurrent parent IRBLsh-S[CO]-1 was 96.3%, which was higher than that of IRBLsh-S[CO]-2 at 91.3%. Some donor segments were present on chromosomes 1 and 9 of IRBLsh-S[CO]-1, whereas in IRBLsh-S[CO]-2 donor segments were present on chromosomes 1, 3, 4, 6, 7, 9, and 10. The introgressed segments on chromosome 1 of these two NILs indicated the presence of *Pish*. *Pish* was introduced into IRBLsh-B[CO] from the Japonica-type variety BL1 and the genetic components were similar to those of CO39 with a frequency of recurrent type segments in its genome of 96.3%. Introgression segments were found in chromosomes 1, 4, and 12. The donor segment was present on the long arm of chromosome 1 of this line, which is the same region where *Pish* is located (Araki et al., 2003). *Pish* from the Japonica-type variety Fukunishiki was introduced into IRBLsh-Fu[CO]. 96.9% of the genetic components in this line were restored relative to the recurrent parent CO39, but no introgressions were detected on the long arm of chromosome 1, but rather the donor segment was found on the terminal region of chromosome 11.

The *Pib* genes from the Indica-type varieties IRAT13 and WHD-1S-175-1-127 were introduced into the NILs IRBLb-IT13[CO] and IRBLb-W[CO], respectively. Their genetic components were almost identical to those of the recurrent parent with genome restoration rates of 96.9% and 99.4%, respectively. The introgression of *Pib*, which was mapped on the terminal region of chromosome 2 (Miyamoto et al. 1996; Monna et al. 1997; Wang et al. 1999; Fjellstrom et al. 2004), could not be identified in either line (Fig. 6). Donor segments were detected on chromosomes 6, 7, 9, and 12 of IRBLb-IT23[CO], and in the short arm of chromosome 7 in IRBLb-W[CO].

The resistant genes *Piz-5* and *Piz-t* were introduced into IRBLz5-CA[CO] and IRBLzt-IR56[CO], respectively. The genetic components of these two NILs were almost identical relative to the recurrent parent with genome restoration rates of 98.8% and 99.4%, respectively. Introgressed segments in IRBLz5-CA[CO] were present in the middle region of chromosome 6 and on the short arm of chromosome 9, while in IRBLzt-IR56[CO], the donor segment was only present on the short arm of chromosome 1. *Piz-5* has been mapped in the same region as the *Piz* locus near the centromere of chromosome 6 (Mew et al. 1994; Hittalmani et al. 2000), and the RFLP markers *RG64* and *R2123* were closely linked with this resistance gene (Liu et al. 2002). A genetic map by Fukuta et al. (2000) was then used to determine the location of *Piz-5* on chromosome 11 of the NILs. Graphical genotype analysis showed that IRBLz5-CA[CO] harbored donor segments in the region of *Piz-5*, thus indicating the introgression of the targeted segment. In contrast, the introgression of *Piz-t* in the NIL IRBLzt-IR56[CO] could not be determined (Fig. 7). It will need the more detail analysis in the region of *Piz-5* on chromosome 6 to detecte the segment introgressed in IRBLzt-IR56[CO].

The blast resistance gene *Pi5*(t) in IRBL5-M[CO] is derived from the recombinant inbred line RIL249, which was derived from a cross between Moroberekan and CO39. Polymorphism data showed that 99.4% of the chromosome components of IRBL5-M[CO] were similar to the recurrent parent. From the data, only one of the markers used showed a differential pattern between IRBL5-M[CO] and CO39, which was located on the long arm of chromosome 11. Recent studies indicated that the *Pi5*(t) gene was located on chromosome 9 and linked with the *Pii* and *Pi3*(t) genes (Jeon et al. 2003; Yi et al. 2004). Therefore, the introgression of *Pi5*(t) in IRBL5-M[CO] could not be confirmed (Fig. 8).

Table 6. Genetic components of near isogenic lines with CO39 genetic background based on the polymorphism data of DNA markers

Designation	Resistance gene	Chromosome	Donor variety	Generation (2006)	Number of SSR marker and frequency (%)				
					CO39		Non-CO39 type		Total
IRBLsh-Ku[CO]	*Pish*	1	Kusabue (J)	BC_6F_{15}	159	(98.8)	2	(1.2)	161
IRBLsh-S[CO]-1	*Pish*	1	Shin2 (J)	BC_6F_{15}	155	(96.3)	6	(3.7)	161
IRBLsh-S[CO]-2	*Pish*	1	Shin2 (J)	BC_6F_{15}	147	(91.3)	14	(8.7)	161
IRBLsh-B[CO]	*Pish*	1	BL1 (J)	BC_6F_{15}	155	(96.3)	6	(3.7)	161
IRBLsh-Fu[CO]	*Pish*	1	Fukunishiki	BC_6F_{15}	156	(96.9)	5	(3.1)	161
IRBLb-IT13[CO]	*Pib*	2	IRAT13 (I)	BC_6F_{15}	156	(96.9)	5	(3.1)	161
IRBLb-W[CO]	*Pib*	2	WHD-1S-175-1-127 (I)	BC_6F_{15}	160	(99.4)	1	(0.6)	161
IRBLz5-CA[CO]	*Piz-5* (=*Pi2*(t))	6	C101A51 (I)	BC_6F_{15}	159	(98.8)	2	(1.2)	161
IRBLzt-IR56[CO]	*Piz-t*	6	IR56 (I)	BC_6F_{15}	160	(99.4)	1	(0.6)	161
IRBL5-M[CO]	*Pi5*	9	RIL249 (I)	BC_6F_{15}	160	(99.4)	1	(0.6)	161
IRBLks-CO[CO]	*Pik-s*	11	Caloro	BC_6F_{15}	157	(97.5)	4	(2.5)	161
IRBLk-Ku[CO]	*Pik*	11	Kusabue (J)	BC_6F_{15}	156	(96.9)	5	(3.1)	161
IRBLk-Ka[CO]	*Pik*	11	Kanto51 (J)	BC_6F_{15}	156	(96.9)	5	(3.1)	161
IRBLkh-K3[CO]	*Pik-h*	11	K3 (J)	BC_6F_{15}	158	(98.1)	3	(1.9)	161
IRBLkm-Ts[CO]	*Pik-m*	11	Tsuyuake (J)	BC_6F_{15}	160	(99.4)	1	(0.6)	161
IRBLkp-K60[CO]	*Pik-p*	11	K60 (J)	BC_6F_{15}	161	(100.0)	0	(0.0)	161
IRBL1-CL[CO]	*Pi1*	11	C101LAC (I)	BC_6F_{15}	159	(98.8)	2	(1.2)	161
IRBL7-M[CO]	*Pi7*	11	RIL29	BC_6F_{15}	156	(96.9)	5	(3.1)	161
IRBLta-Ya[CO]	*Pita* (=*Pi4*(t))	12	Yashiromochi (J)	BC_6F_{15}	158	(98.1)	3	(1.9)	161
IRBLta2-Pi[CO]	*Pita-2*	12	Pi No.4	BC_6F_{15}	156	(96.9)	5	(3.1)	161
IRBLta2-Re[CO]	*Pita-2*	12	Reiho (J)	BC_6F_{15}	155	(96.3)	6	(3.7)	161
IRBLta2-IR64[CO]	*Pita-2*	12	IR64 (I)	BC_6F_{15}	153	(95.0)	8	(5.0)	161
IRBLk*-F21[CO]	Unknown	Unknown	F-21-6	BC_6F_{15}	160	(99.4)	1	(0.6)	161
IRBLk*-F14[CO]	Unknown	Unknown	F-14-3	BC_6F_{15}	155	(96.3)	6	(3.7)	161
IRBLk*-F25[CO]	Unknown	Unknown	F-25-3	BC_6F_{15}	154	(95.7)	7	(4.3)	161
IRBLk*-F40[CO]	Unknown	Unknown	F-40-3	BC_6F_{15}	161	(100.0)	0	(0.0)	161
IRBLk*-F59[CO]	Unknown	Unknown	F-59-1	BC_6F_{15}	157	(97.5)	4	(2.5)	161
IRBLk*-F66[CO]	Unknown	Unknown	F-66-10	BC_6F_{15}	157	(97.5)	4	(2.5)	161
IRBLk*-K86[CO]	Unknown	Unknown	KU86	BC_6F_{15}	157	(97.5)	4	(2.5)	161
IRBLk*-S[CO]	Unknown	Unknown	Shin2 (J)	BC_6F_{15}	158	(98.1)	3	(1.9)	161
IRBLta*-Me[CO]	Unknown	Unknown	Metica (J)	BC_6F_{13}	156	(96.9)	5	(3.1)	161
		Minimum			147	91.3	0	0	161
		Maxim			161	100.0	14	8.7	161
		Average			156.91	97.5	4.0909	2.5	161

k*: The allele of *Pik* was expected to introgress into the CO 39 genetic background based on the reaction patterns to blast isolates from the Philippines.
ta*: The allele of *Pita* was expected to introgress into the CO 39 genetic background based on the reaction patterns to blast isolates from the Philippines.
SSR markers distributed across 12 rice chromosomes were used to determine the component of each line.
I: Indica-type, J: Japonica-types.

Several blast resistance genes occur near the telomere of chromosome 11, including *Pik-s*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p* (Fjellstrom et al. 2004; Hayashi et al. 2006), *Pi1* (Inukai et al. 1994; Mew et al. 1994; Yu et al. 1996; Hittalmani et al. 2000) and *Pi7*(t) (Wang et al. 1994; Campbell et al. 2004), and were introduced into the CO39 genetic background to develop NILs (Fig. 10). *Pik-s* from the USA Indica-type variety Caloro was introduced into IRBLks-CO[CO]. The genetic compo- nents of this line were almost identical to the recurrent parent with a genome restoration rate of 97.5%, and the donor segments were present on chromosomes 1 and 11. The introgression segment on the terminal region of chromosome 11 of IRBLks-CO[CO], which is the same region as the *Pik* locus that was mapped between the SSR markers *RM1233* and *RM224* (Fjellstrom et al. 2004), thus validated the introgression of *Pik-s* in this line. The Japonica-type varieties Kusabue and Kanto51 were the donors of the *Pik* gene for NILs IRBLk-Ku[CO] and IRBLk-Ka[CO], respectively. DNA analysis showed genome restoration rates relative to the recurrent parent of both lines were 96.9%. The donor segments were present on chromosomes 5 and 11 of IRBLk-Ku[CO], whereas the introgression segments were found on chromosomes 1 and 11 in IRLk-Ka[CO]. Since the *Pik* gene was reported to be located in the terminal region of chromosome 11 (Fjellstrom et al. 2004; Hayashi et al. 2006), the presence of the donor segments in this region both in IRBLk-Ku[CO] and IRBLk-Ka[CO] indicated the introgressions of *Pik* in these lines. These graphical genotypes of IRBLkh-K3[CO], IRBLkm-Ts[CO], and IRBLkp-K60[CO] showed the non-polymorphic patterns in comparison with CO39. Therefore, the introgression of the three genes, *Pik-h*, *Pik-m*, and *Pik-p* could not be confirmed in this study. The blast resistance genes *Pi1* and *Pi7*(t), which are located near the telomere of chromosome 11, were introduced into IRBL1-CL[CO] and IRBL7-M[CO], respectively. Analysis of SSR markers showed that 98.8% of the genetic components of IRBL1-CL[CO], which harbors *Pi1* from the isogenic line C101LAC, were similar to those of the recurrent parent. The resistance gene *Pi1* was mapped between the RFLP markers *R251* and *S10003*, in the same region as *Pi7*(t) (Campbell et al. 2004). The introgression segment, which was only present in the terminal region of chromosome 11 of IRBL1-CL[CO], strongly indicated the introgression of *Pi1* in this line. IRBL7-M[CO], which contained the *Pi7*(t) gene from the Indica-type variety Moroberekan, showed a genome restoration rate relative to the recurrent parent of 96.9%. Donor segments were distributed across chromosomes 1, 2, 3, and 12 of the IRBL7-M[CO] genome. In this study, we could not confirm the introgression of *Pi7*(t) in the terminal region of chromosome 11, where the gene had been mapped by Campbell et al. (2004).

Eight NILs harboring unknown genes, which were expected to contain one allele of *Pik* based on the reaction patterns to standard differential blast isolates, were designated as *Pik**, and were developed using eight donor varieties, F-21-6, F-14-3, F-25-3, F-40-3, F-59-1, F-66-10, KU86, and Shin2. Introgression of donor segments was confirmed in the terminal region of chromosome 11 in four NILs, IRBLk*-F25[CO], IRBLk*-F66[CO], IRBLk*-K86[CO] and IRBLk* S[CO], but not in the other four, IRBLk*-F21[CO], IRBLk*-F14[CO], IRBLk*-F40[CO], or in IRBLk*-F59[CO]. The genomic components of the NILs showing introgression were almost identical to the recurrent parent, and for NIL IRBLk*-F40[CO] we were unable to find any differences with the recurrent parent (Fig. 10).

The blast resistance gene *Pita* on chromosome 12 near the centromere (Mew et al. 1994; Yu et al. 1996; Hittalmani et al. 2000; Bryan et al. 2000; Jia et al. 2002; Jia et al. 2004; Hayashi et al. 2006) has been introduced into CO39 NIL IRBLta-Ya[CO] from the Japonica-type variety Yashiromochi. Polymorphism data inferred from 161 SSR markers revealed that 98.1% of the genetic components of IRBLta-Ya[CO] were similar to the recurrent parent, with a few introgression segments on chromosomes 11 and 12. The SSR markers *RM155* and *RM7102* were reported to be closely linked with the *Pita* gene on chromosome 12 (Jia et al. 2004). The graphical genotype of IRBLta-Ya[CO] revealed the introgression segment on the short arm of chromosome 12, near the region in which *Pita* has been mapped, supporting the introgression of *Pita* in this line. Three NILs, IRBLta2-Pi[CO], IRBLta2-Re[CO], and IRBLta2-IR64[CO], which harbor the *Pita-2* gene from the donor varieties Pi No.4, Reiho, and IR64, respectively, showed that their chromosome components were almost identical to those of CO39, with frequencies of recurrent parent segments in each line of 96.9%, 96.3%, and 95.0%, respectively. The resistance gene *Pita-2* has been mapped in the same region as *Pita* near the centromere of chromosome 12 (Fjellstrom et al. 2004; Hayashi et al. 2006). Graphical genotype analyses of IRBLta2-Pi[CO], IRBLta2-Re[CO], and IRBLta2-IR64[CO] showed the introgression segments on chromosome 12, indicating the introgressions of *Pita-2* in these lines (Fig. 11).

An unknown resistance gene, which was thought to be allelic with *Pita* and designated as *Pita**, was introduced into CO39 NIL IRBLta*-Me[CO] from the Japonica-type variety Metica. DNA analysis revealed that the chromosome components of this line were similar to the recurrent parent with a genome restoration rate of 96.9%, while introgression segments were present on chromosomes 3, 9, and 12. According to the genetic map of the *Pita* gene by Jia et al. (2004), the introgression segment that was present in the middle region of chromosome 12 suggested the introgression of *Pita* in IRBLta*-Me[CO].

US-2 near isogenic lines

LTH and CO39 are good blast-susceptibility controls and have the same genetic backgrounds as the differen-

tial varieties. However, the seeds of these two lines are difficult to obtain in sufficient amounts because they lodge easily after heading under tropical conditions. US-2 was developed as a 'universal susceptible' rice line, which was produced from a cross between an Indonesian landrace, Kencana, and an Indica-type, Takanari and was not known to have any blast resistance genes in the genetic background (Fukuta et al., 2004b). NILs with universal susceptible variety US-2 genetic background have been developed for 12 rice blast resistant genes, *Pia*, *Pik*, *Pik-h*, *Pik-p*, *Pik-s*, *Pita*, *Piz-t*, *Pi1*, *Pi5*(t), *Pi7*(t), *Pi9*(t), and *Pi12*(t). These materials are expected to be universal differential varieties since they can be easily adapted to tropical and temperate conditions. A total of 16 US-2 NILs targeting 12 resistance genes and the recurrent parent US-2 line were used in this study. These 16 NILs with US-2 genetic background were subjected to DNA analysis to examine their chromosomal components. DNA analyses using 162 SSR markers revealed that chromosome components of all US-2 NILs were almost identical to those of the US-2 parent. All US-2 NILs were developed by six times backcrossing, except IRBLks-F5[US]. Genome restoration rates relative to US-2 in NILs ranged from 88.9% to 98.8% with an average of 94.6%. (Table 7, Fig. 2).

The NILs IRBLzt-T[US] and IRBL9-W[US] harbor *Piz-t* and *Pi9*(t), respectively, which are located on chromosome 6 and are closely linked to each other (Hayashi et al. 2004, 2005; Causse et al. 1994; Brar and Khush 1997; Liu et al. 2002; Deng et al. 2006). The genetic components of these two NILs are almost identical to those of the recurrent parent with genome restoration rates of 96.9% and 93.2%, respectively. In IRBLzt-T[US], the donor segments were present on chromosomes 1, 4, 6, and 11, while in IRBL9-W[US] the donor segments were present on chromosomes 1, 5, 6, 8, and 11. *Pi9* has been previously mapped near the centromere of chromosome 6 and is flanked by the RFLP markers *RG64* and *R2123* (Liu et al. 2002). Since the location of *Piz-t* is thought to be closely linked with the *Pi9* gene, the presence of the donor segment on chromosome 6, both in IRBLzt-T[US] and IRBL9-W[US], confirmed the introgression of *Piz-t* and *Pi9* (Fig. 7).

The *Pi5*(t) gene from the Indica-type variety Morobberekan was introduced into the US-2 NIL IRBL5-M[US]. Polymorphism data based on SSR markers showed that genome restoration rates relative to the recurrent parent of this line was 95.1%, while donor segments were present on chromosomes 2, 4, and 9. *Pi5*(t) was reported to be linked to the RFLP markers *XNpb103*

Table 7. Genetic components of near isogenic lines with US-2 genetic background based on the polymorphism data of DNA markers

Designation	Resistance gene	Chromosome	Donor variety	Generation (2006)	Number of SSR marker and frequency (%)				
					US-2 type		Non US-2 type		Total
IRBLa-A[US]	*Pia*	11	Aichi Asahi	BC_6F_8	146	(90.1)	16	(9.9)	162
IRBLa-C[US]	*Pia*	11	CO39	BC_6F_8	157	(96.9)	5	(3.1)	162
IRBLk-K[US]	*Pik*	11	Kanto51	BC_6F_9	156	(96.3)	6	(3.7)	162
IRBLkh-K3[US]	*Pik-h*	11	K3	BC_6F_8	155	(95.7)	7	(4.3)	162
IRBLkp-K60[US]	*Pik-p*	11	K60	BC_6F_8	160	(98.8)	2	(1.2)	162
IRBLks-F5[US]	*Pik-s*	11	Fujisaka5	BC_6F_8	160	(98.8)	2	(1.2)	162
IRBLks-S[US]	*Pik-s*	11	Shin2	BC_6F_8	153	(94.4)	9	(5.6)	162
IRBL1-CL[US]	*Pi1*	11	C101LAC	BC_6F_8	150	(92.6)	12	(7.4)	162
IRBL7-M[US]	*Pi7*	11	RIL29(Moro)	BC_6F_8	155	(95.7)	7	(4.3)	162
IRBLta-K1[US]	*Pita* (=*Pi4*(t))	12	K1	BC_6F_9	148	(91.4)	14	(8.6)	162
IRBLta-CP1[US]	*Pita* (=*Pi4*(t))	12	C101PKT	BC_6F_9	154	(95.1)	8	(4.9)	162
IRBLta-CT2[US]	*Pita* (=*Pi4*(t))	12	C105TTP2L9	BC_6F_9	144	(88.9)	18	(11.1)	162
IRBL12-M[US]	*Pi12*	12	RIL10	BC_6F_9	152	(93.8)	10	(6.2)	162
IRBLzt-T[US]	*Piz-t*	6	Toride1	BC_6F_9	157	(96.9)	5	(3.1)	162
IRBL9-W[US]	*Pi9*	6	WHD-IS-75-127	BC_6F_8	151	(93.2)	11	(6.8)	162
IRBL5-M[US]	*Pi5*	9	RIL249(Moro)	BC_6F_9	154	(95.1)	8	(4.9)	162
	Minimum				144	(88.9)	2	(1.2)	162
	Maxim				160	(98.8)	18	(11.1)	162
	Average				153.3	(94.6)	8.8	(5.4)	162

SSR markers distributed across 12 rice chromosomes were used to determine the component of each line.
I: Indica-type, J: aponica-types.

and *C1454* on the short arm of chromosome 9 (Jeon et al. 2003). Based on this information and using a genetic map by Fukuta et al. (2000) and Harushima et al. (1998), we predicted that the donor segment present on chromosome 9 of IRBL5-M[US] corresponded to *Pi5*(t) (Fig.8).

The blast resistance gene *Pia* from the Japonica-type variety Aichiasahi and the Indica-type variety CO39 were introduced into the US-2 NILs IRBLa-A[US] and IRBLa-C[US], respectively. SSR analysis showed that the genetic components of IRBLa-A[US] and IRBLa-C[US] were almost identical to those of US-2 with frequencies of recurrent segment types on their chromosomes of 90.1% and 96.9%, respectively. Donor segments were found on chromosomes 1, 11, and 12 in IRBLa-A[US], and on chromosomes 3, 4, and 11 in IRBLa-C[US]. The presences of donor segments on the short arm of chromosome 11 suggested the introgressions of *Pia* in IRBLa-A[US] and IRBLa-C[US] (Fig.9).

The *Pik* gene from the Japonica-type variety Kanto51 was introduced into the US-2 NIL IRBLk-K[US]. 96.3% of the genetic components of this line were similar to those of the recurrent parent with introgression segments present on chromosomes 1, 5, 10, and 11. *Pik* has been mapped in the terminal region, near the telomere of chromosome 11, flanked by the SSR markers *RM1233* and *RM224* (Fjellstrom et al. 2004). The graphical genotype of IRBLk-K[US] clearly showed the donor segment near the telomere of chromosome 11, thus indicating the introgression of *Pik* in this line. The blast resistance genes *Pik-h*, *Pik-p*, and *Pik-s* were thought to be allelic with *Pik* and clustered in the terminal region of chromosome 11 (Fjellstrom et al. 2004; Hayashi et al. 2006; Inukai et al. 1994; Mew et al. 1994; Yu et al. 1996; Hittalmani et al. 2000). The chromosome components of IRBLkh-K3[US], which contained *Pik-h* from the Japonica-type variety K3, showed a 95.7% genome restoration rate relative to the recurrent parent. A few donor segments, located on chromosomes 1, 3, 4, and 11, remained in the genome of IRBLkh-K3[US]. The presence of an introgression segment in the terminal region of chromosome 11 validated the introgression in this line. 98.8% of the genetic components of IRBLkp-K60[US], which harbors the *Pik-p* gene from the Japonica-type variety K60, were restored to the recurrent parent. Introgression segments were present in the long arm of chromosomes 10 and 11 of this line. This also supported the introgression of *Pik-p*, which was located in the same region as the *Pik* locus in the terminal region of chromosome 11. *Pik-s* from Japonica-type varieties, Fujisaka5 and Shin2 were introduced into the NILs IRBLks-F5[US] and IRBLks-S[US], respectively. The frequencies of recurrent types in the IRBLks-F5[US] and IRBLks-S[US] genomes were 98.8% and 94.4%, respectively. Both NILs showed introgressed segments near the telomere of chromosome 11. *Pi1* from the isogenic line C101LAC and *Pi7*(t) from recombinant inbred line RIL249 was introduced into IRBL1-CL[US] and IRBL7-M/US, respectively. DNA analysis revealed that chromosome components of IRBL1-CL[US] and IRBL7-M[US] were almost identical to those of the recurrent parent, with mean genomic restoration rates of 92.6% and 95.7%, respectively. The blast resistance genes *Pi1* and *Pi7*(t) were reported to be located in the terminal region of chromosome 11, the same region as the *Pik* locus, and mapped between the RFLP markers *R251* and *S10003* (Campbell et al. 2004). Based on this information, we predicted the location of *Pi1* and *Pi7*(t) in IRBL1-CL[US] and IRBL7-M[US], respectively. The presence of the donor segment in the terminal region of IRBL1-CL[US] validated the introgression of *Pi1*, while in IRBL7-M[US] the introgressed segment was present some distance from the telomere of chromosome 11 (Fig. 10).

Three NILs, IRBLta-K1[US], IRBLta-CP1[US], and IRBLta-CT2[US], have been developed to target the *Pita* gene from different donor varieties.. This resistance gene has been mapped near the centromere region of chromosome 12 (Mew et al. 1994; Yu et al. 1996; Hittalmani et al. 2000; Bryan et al. 2000; Jia et al. 2002; Jia et al. 2004; Hayashi et al. 2006). Polymorphism data showed that IRBLta-K1[US], which harbors the *Pita* gene from the Japonica-type variety K1, showed genome restoration rates relative to the recurrent parent of 91.4% and had several donor segments remaining on chromosomes 3, 4, 6, 11, and 12. The SSR markers *RM155* and *RM7102* were reported to be closely linked to the *Pita* gene on chromosome 12 (Jia et al. 2004). An introgression segment near the centromere of chromosome 12 was detected in the same region as *Pita* as reported by Jia et al. (2004). The NILs IRBLta-CP1[US] and IRBLta-CT2[US], which each contained *Pita* from the isogenic lines C101PKT and C105TTP2L9, respectively, also revealed the introgression in the aforementioned regions suggesting that the targeted segment had been introduced in their genome. However, the genome restoration rate of IRBLta-CP1[US] was more similar to the recurrent parent than that of IRBLta-CT2[US]. Frequencies of the recurrent type in the genome of these two lines were 95.1% and 88.9%, respectively (Fig. 11).

The NIL IRBL12-M[US] harbors *Pi12*(t) from RIL10 (Moroberekan). Based on DNA analysis, the genome restoration rate relative to the recurrent parent of this line was 93.8%, with some introgression segments present on chromosomes 1, 5, 6, and 11. This resistance gene had been previously mapped near the centromere of chromosome 12 (Zheng et al. 1996). From graphical genotype analysis of IRBL12-M[US], we were unable to find any introgression segments on chromosome 12. IRBL12-M[US] may not have acquired *Pi12*(t) through the breeding process, and therefore further analysis should be performed to verify the introgression of the *Pi12*(t) gene in this line (Fig. 13).

Discussion

Monogenic lines were used in the first set of international differential varieties to target a large number of resistance genes, while harboring only a single resistance gene in each genetic background (Tsunematsu et al. 2000; Kobayashi et al. 2007). It means that MLs are the first standard differential varieties set which were minimized the influence(s) of genetic background using the susceptible variety, LTH. These differential varieties are useful tools to clarify the pathogenesis of blast races and to identify the resistance genes in rice varieties based on the gene-for-gene theory (Flor 1971). These varieties can provide useful information for breeding programs to improve blast resistance. These lines are also offered as useful materials for genetic and molecular biological studies of blast resistance and as important breeding materials to improve blast resistant rice varieties. IRRI and JIRCAS have been continuously developing blast resistance NILs, even after releasing the MLs. A set of NILs is the most suitable material for race differentiation. The LTH NILs were made the uniform in the morphological traits and maked easier to evaluation of inoculation test. Co 39 NILs adapte in tropical condition in compare with the materials with LTH genetic background. These NILs with LTH and CO39 backgrounds will not only the differential varieties, but also can be used as gene sources in breeding programs for Japonica and Indica-type rice varieties, respectively. Although NILs with the US-2 genetic background will be more efficient tools, they are still under development and will take time to be established.

These differential varieties are useful for rice breeding, but the breeding process to develop such materials is laborious and time-consuming. These varieties were developed by a backcross breeding method and investigated for the existence of the resistance gene by inoculation with suitable blast isolates in every generation. This process is very difficult when the resistance gene introduced from a donor parent harbors more than one resistance gene. Several differential varieties, 14 in MLs, 11 in LTH NILs, 17 in CO39 NILs and 9 in US-2 NILs, were expected to contain some additional minor or other major resistance gene(s) in their genetic backgrounds. These donor varieties, Shin2, BL1, IRAT13, Fukunishiki, WHD-1S-75-1-127, Moroberican, Aichiashahi, K3, Caloro, K1, Zhaiyeqing, and Yashiromoch, Pi No.4, Reiho, and Fujisaka 5, were used as sources of different resistance genes. These results highlight the difficulties involved in the introduction of resistance genes from the varieties that harbor multiple resistance genes in their genetic background, based on inoculation tests of the blast fungus. Moreover, we demonstrated the linkage between *Piz-t* in two donor varieties, Toride 1 and IR56, and the photosensitive gene, *Se-I*, in the middle of chromosome 6 in four differential varieties, ML, LTH NILs, CO39 NILs and US-2 NILs. These results suggested that the tight linkage between *Piz-t* and *Se-1* would be problem for rice breeding and the limitations of the selection based on the inoculation test with avirulent blast isolates or to remove additional traits that link tightly with resistance gene. However, this information will be useful to understand the relationship between the resistance gene and other agronomic traits.

Confirmation of the existence of a single resistance gene can be effectively helped by analysis at the molecular level. The availability of various DNA markers in the public domain as well as information of location of resistance genes in the chromosomes of rice is very useful in determining the introgression of a resistance gene in an isogenic line. Using around 160 selected SSR markers from Cornell University (McCouch et al. 2002), which are distributed across all 12 rice chromosomes, we have analyzed DNA from four sets of differential varieties and verified the chromosome components of each line through graphical genotype analysis. The results from our study revealed that the chromosome components of differential varieties that comprised NILs were more uniform than those of MLs relative to the respective recurrent parent. For instance, the genome restoration rate relative to the recurrent parent of 31 MLs with LTH genetic background varied from 50% to 90% with an average of 77.3%, while 34 NILs that were developed by six times backcrossing using the same genetic background, showed genome restoration rates ranging from 75.6% to 96.9% with an average of 90.6%, despite these frequencies still being lower than the expected level of 99%. Polymorphism data from SSR markers used in this study showed that the average genome restoration rates of CO39 NILs with Indica-type variety genetic background and US-2 NILs with the hybrid Indica-Japonica genetic background were 97.5% and 94.6%, respectively, which are higher than the average rate of LTH NILs of 90.6%. The degree of genome restoration varied according to the time of backcross or type of recurrent parents among universal differential variety sets and these corresponded with the phenotypic variations (data not shown). We have identified several introgression segments on the genome of differential varieties corresponding to the location of blast resistance genes, which were previously reported in other studies. Therefore, further study should address those lines to clarify the introgression of resistance genes by segregation analysis between DNA markers and resistance genes. The information of DNA markers in each differential variety will be useful in blast resistance breeding for targeting these genes and removing the additional chromosome segments in these genetic backgrounds. Furthermore, the association between undesirable introgression segments that were detected in some differential varieties with the resistance genes should be clarified.

In addition to the 24 targeted genes, lines for other resistance genes including QTL, field resistance and pan-

icle blast resistance will be developed with this genetic background, to determine the effect of each gene and QTL or for analysis of these abilities in more advanced studies, such as gene pyramiding.

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Identification of blast resistance genes in IRRI-bred rice varieties by segregation analysis based on a differential system

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Abstract

A differential system for rice blast disease consists of standard blast isolates (*Pyricularia oryzae* Cavara..) and differential rice varieties (*Oryza sativa* L.) with known resistance genes. Monogenic lines for blast resistance have been newly developed at the International Rice Research Institute (IRRI) as international differential varieties and differential blast isolates from the Philippines were selected. Using a differential system based on the gene-for-gene theory, blast resistance genes were estimated in the IRRI-bred elite rice varieties. At least seven kinds of resistance genes -- *Pi20*(t), *Pita*, *Pik** (one of the *Pik* allele genes except *Pik-s*), *Pik-s*, *Pib*, *Piz-t*, and *Pii* or *Pi3* -- were estimated in 42 rice varieties on the basis of reaction patterns to 14 standard blast isolates. The reaction patterns of the IRRI varieties were compared with those of the blast monogenic lines. To confirm this gene estimation, genetic analysis was done using segregating populations derived from crosses between the IRRI varieties and a susceptible Indica-type variety, CO 39. The BC_1F_2 populations (with CO 39 as recurrent parent) were segregated for reaction to the specific standard isolates. Furthermore, the estimated genes were confirmed by allelism test against the blast resistance monogenic lines. As a result of the segregation analysis of 10 of 42 IRRI-bred varieties, seven genes -- *Pi20*(t), *Pita*, *Pik**, *Pia*, *Pib*, *Pik-s* -- and *Piz-t*, were identified. Genes *Pia*, *Pib*, *Pik-s*, and *Piz-t* were not estimated by reaction patterns to the blast isolates but identified by genetic analysis in some varieties. The effectiveness of the differential system, which is based on conventional methods and which does not require advanced facilities, is discussed as a fundamental tool to provide essential information to develop breeding programs for blast resistance.

Keywords: rice (*Oryza sativa* L.), blast (*Pyricularia oryzae* Cavara), differential system, genetic analysis, resistance, IRRI variety

Introduction

Blast disease, caused by *Pyricularia oryzae* Cavara is a serious disease of rice (*Oryza sativa* L.) worldwide. The use of host resistance is one of the most economical and effective means of controlling blast disease. Resistance to blast disease is governed by a gene-for-gene relationship between resistance gene in the host and avirulence gene in the blast pathogen (Kiyosawa, 1972; Silue et. al., 1992). Genetic studies performed in this context led to the identification of many blast resistance genes. Genetic studies in Indica-type varieties have been limited because the extremely changeable virulence of the blast fungus (Ou, 1985; Bonman et al., 1986) and the presence of several resistance genes in Indica-type varieties' genetic background (Mackill et al.; 1985; Yu et al., 1987) made these studies complicated.

The limitation in genetic studies on blast resistance could also be attributed to a lack of suitable differential system for blast resistance genes in the tropics. The 12 Japonica-type differential varieties (DVs) for blast resistance were selected by Yamada et al. (1976) and Kiyosawa (1981), but those DVs are known to carry additional gene(s) showing resistance to tropical blast isolates, which masked the targeted gene's reaction (Noda et al., 1999). To address this limitation, Yanoria et al. (2000) tried to clarify the pathogenicities of blast isolates from the Philippines using DVs and lines. Several blast isolates with distinct pathogenicities were selected and studied in detail by Tsunematsu et al. (2000) using a set of monogenic lines that contains 23 kinds of single resistance genes with the genetic background of a Chi-

nese variety Lijiang-xin-tuan-heigu (LTH).

In South and Southeast Asia, rice varieties bred in IRRI are widely grown and account for more than 80% of total rice production in this region (Khush, 1990). And these IRRI-bred varieties have been used for breeding materials in many countries of Tropical region. Using the differential system consisting of newly developed monogenic lines and differential blast isolates from the Philippines, kinds of blast resistance genes in IRRI-bred varieties were identified. In this paper, methodologies of gene identification are explained following 3 steps with special emphasis on a case of IR64, which were distributed and cultivated the most widely and was still one of leading varieties in tropical region At first, kind of blast resistance gene was estimated by reaction patterns to differential blast isolates (I). To confirm the gene estimation, segregation analysis using backcross progenies with a susceptible variety (II) and allelism test with DVs were conducted (III).

I. Gene estimation based on reaction pattern to differential blast isolates

Materials and methods

1. Rice plant materials

A total of 42 IRRI-bred rice varieties were used for blast resistance gene estimation (Table 1). The widely grown varieties, IR8, IR24, IR36, IR64 and IR72 were included. Eight varieties, designated as PSBRc by the Philippine Seed Board, which are IRRI developed varieties, were also included.

Nine monogenic lines, IRBL20-IR24 (*Pi20*(t)), IRBLta-CP1 (*Pita*), IRBLk-K (*Pik*), IRBLks-F5 (*Piks*), IRBLa-A (*Pia*), IRBLb-B (*Pib*), IRBLzt-T (*Pizt*), IRBLi-F5 (*Pii*), and IRBL3-CP4 (*Pi3*) were tested for comparison.

2. Inoculation and evaluation of resistance

Pregerminated seeds of each variety or population were sown on soil in a 26 × 35 cm plastic tray. Ten grams of ammonium sulfate was applied to each tray as basal fertilizer and 1 g was added 1 wk before inoculation. In all cases, susceptible cultivars CO39 and LTH were included in each tray to check the success of inoculation and the virulence of blast isolates used.

Fourteen blast isolates with known avirulence (Yanoria et al., 2000, Telebanco-Yanoria et al. 2007) from the Philippines were used for inoculation. Seedlings at the four-leaf stage were sprayed with 40-50 ml spore suspension per tray adjusted to 10^5 spores ml^{-1}. Trays were placed inside the wet jute sacks for 18-24 h, and transferred to an air-conditioned glasshouse room at 23±3℃/30±5℃ night and day temperature. Blast incidence on each seedling was examined 6-7 d after inoculation. The reaction was classified into 0-5 scales with slight modifications as described previously by Mackill and Bonman (1992). Seedlings rated 0-2 were considered resistant (R), 3 was moderate resistant (M), and those rated 4-5, susceptible (S).

3. Resistance gene estimation

Gene(s) were estimated by comparing reaction patterns to the differential blast isolates of IRRI varieties with those of blast monogenic lines (Table 2). Using the Philippine blast isolates, 4 alleles of *Pik* loci (*Pik*, *Pik-h*, *Pik-m*, and *Pik-p*) can not be differentiated because all the monogenic lines with these genes showed the same reaction pattern (Tsunematsu et al. 2000). Therefore, those alleles were designated tentatively as *Pik** in this paper.

Although total 14 differential isolates were involved in this study, reactions to 3 distinct isolates, M36-1-3-10-1, IK81-25, and PO6-6, were considered for grouping of varieties and primary gene estimation as a first step. As a second step, reaction patterns to the other 11 isolates were also taken into consideration for gene estimation (Table 2).

Results and discussion

1. Gene estimation in IR64

IR64 was resistant to 12 (M36-1-3-10-1, IK81-25, IK81-3, BN111, V850256, M39-1-3-8-1, M64-1-3-9-1, M39-1-2-21-2, BN209, V850196, B90002, and C923-49) out of 14 standard blast isolates tested (Table 3). Among the 9 monogenic lines tested, 6 lines (IRBL20-IR24, IRBLta-K1, IRBLb-B, IRBLa-A, IRBLks-F5, and IRBLzt-T) were resistant to the blast isolates which were avirulent to IR64. These results suggested that 6 blast resistant genes (*Pi20*(t), *Pita*, *Pib*, *Pia*, *Pik-s*, and *Piz-t*) which were harbored in the respective monogenic lines were estimated to exist in the genetic background of IR64. Three genes, *Pi20*(t), *Pita*, and *Pib* showed relatively wide spectrum of resistance and covered the resistance of the other 3 genes, *Pia*, *Pik-s*, and *Piz-t*. Therefore, the latter 3 were not estimated directly but possible to be harbored in the genetic background.

The other 3 monogenic lines, IRBLk-Ka, IRBLi-F5, and IRBL3-CP4 were resistant to a blast isolate PO6-6 which was virulent to IR64 (Table 3). This suggested that 3 resistant genes, *Pik*, *Pii*, and *Pi3* were not harbored in the genetic background of IR64.

2. Gene estimation in IRRI-bred varieties

IRRI-bred varieties showed various patterns to the differential blast isolates. The reaction patterns of these varieties were compared with those of DVs (Table 4). These varieties were classified into 8 variety groups (VG) based on reaction patterns to three distinct blast isolates, M36-1-3-10-1, IK81-25, and PO6-6 (Table 4). These 3 isolates showed incompatibility with 3 resistance genes, *Pi20*(t) resistant to M36-1-3-10-1, *Pita* resistant to IK81-25, and *Pik* resistant to PO6-6. From these specific reactions, presence or absence of these 3 genes was estimated in the varieties for each VG.

Table 1. List of IRRI-bred rice varieties used for identifing the blast resistance genes

Variety	Year of release	Ecosystem suited	Line designation	Cross combination
IR 5	1967	Irrigated	IR5-47-2	Peta/ tangkai Rotan
IR 8	1966	Irrigated	IR8-288-2	Pata/Dee-geo-woo-gen
IR20	1969	Irrigated	IR523-E576	IR262-24-3/TKM6
IR22	1969	Irrigated	IR579-160-2	IR8/Tadukan
IR24	1971	Irrigated	IR661-1-140-3	IR8/IR127-2-2
IR26	1973	Irrigated	IR1541-102-7	IR24/TKM6
IR 28	1974	Irrigated	IR2061-214-3-8-2	IR833///IR1561-149-1//IR24*4/O. nivara
IR 29	1974	Irrigated	IR2061-464-4-14-1	IR833///IR1561-149-1//IR24*4/O. nivara
IR 30	1974	Irrigated	IR2153-159-1-4	IR1541-102-6-3//IR24*4/O. nivara
IR32	1975	Irrigated	IR2070-747-6-3-2	IR20*2/O.nivara//CR94-13
IR34	1975	Irrigated	IR2061-213-2-17	IR833-6-2-1-1//IR1561-149-1//IR24*4/O.nivara
IR36	1976	Irrigated	IR2071-625-1-252	IR1561-228-1-2/IR737//CR94-13
IR38	1976	Irrigated	IR2070-423-2-5-6	IR20*2/O.nivara//CR94-13
IR 40	1977	Irrigated	IR2070-414-3-9	IR20*2/O.nivara//CR94-13
IR42	1977	Irrigated	IR2071-586-5-6-3	IR1561-228-1-2/IR737//CR94-13
IR 43	1978	Irrigated	IR1529-430-3	IR305-3-17-1-3/IR661-1-140-3
IR 44	1978	Irrigated	IR2863-38-1-2	IR1529-680-3/CR94-13//IR480-5-9-3
IR 45	1978	Irrigated	IR2035-242-1	IR1416-128-5/IR1364-37-3-1//IR1824-1
IR 46	1978	Irrigated	IR2058-78-1-3-2	IR1416-128-5/IR1364-37-3-1//IR1366-120-3-1/IR1539-111
IR 48	1979	Irrigated	IR4570-83-3-3	IR1702-74-3-2/IR1721-11-6-8-3//IR2055-481-2
IR 50	1979	Irrigated	IR9224-117-2-3-3-2	IR2153-14-1-6-2/IR28//IR36
IR 52	1980	Irrigated	IR5853-118-5	Nam Sa-gui 19/IR2071-88//IR2061-214-3-6-20
IR 54	1980	Irrigated	IR5853-162-1-2-3	Nam Sa-gui 19/IR2071-88//IR2061-214-3-6-20
IR 56	1982	Irrigated	IR13429-109-2-2-1	IR4432-53-33/PTB33//IR36
IR 58	1983	Irrigated	IR9752-71-3-2	IR28/Kwang-chang-ai//IR36
IR 60	1983	Irrigated	IR13429-299-2-1-3	IR4432-53-33/PTB33//IR36
IR 62	1984	Irrigated	IR13525-43-2-3-1-3-2	PTB33/IR30//IR36
IR 64	1985	Irrigated	IR18348-36-3-3	IR5657-33-2-1/IR2061-465-1-5-5
IR 65	1985	Irrigated	IR21015-196-3-1-3	Batatais/IR36//IR52
IR 66	1987	Irrigated	IR32307-107-3-2-2	IR13240-108-2-2-3/IR9129-209-2-2-2-1
IR 68	1988	Irrigated	IR28224-3-2-3-2	IR19660-73-4/IR2415-90-4-3-2//IR54
IR 70	1988	Irrigated	IR28228-12-3-1-1-2	IR19660-73-4/IR54//IR9828-36-3
IR 72	1988	Irrigated	IR35366-90-3-2-1-2	IR19661-9-2-3/IR1576-199-3-3//IR9129-209-2-2-2-1
IR74	1988	Irrigated	IR32453-20-3-2-2	IR19661-131-1-2/IR15795-199-3-3
PSBRc 1	1990	Upland	IR10147-113-5-1-1-5	KN-1B-361-1-8-6/IR1750-F5B-3//BPI76*9/Dawn
PSBRc 2	1991	Irrigated	IR32809-26-3-3	IR4215-301-2-2-6/BG90-2//IR19661-131-1-2
PSBRc 4	1991	Irrigated	IR41985-111-3-2-2	IR4547-4-1-2/IR1905-81-3-1//IR25621-94-3-2
PSBRc 10	1992	Irrigated	IR50404-57-2-2-3	IR33021-39-2-2/IR32429-47-3-2-2
PSBRc 18	1994	Irrigated	IR51672-62-2-1-1-2-3	IR24594-204-1-3-2-6-2/IR28222-9-2-2-2-2
PSBRc 20	1994	Irrigated	IR57301-195-3-3	IR35293-125-3-2-3/IR32429-47-3-2-2/PSBRc4
PSBRc 28	1995	Irrigated	IR56381-139-2-2	IR28239-94-2-3-6-2/IR64
PSBRc 30	1995	Irrigated	IR58099-41-2-3	IR72/IR24632-34-2

IRRI (1995) and Peng and Khush (2003) were modified.

Table 2. Reaction pattern of monoginic lines to standard blast isolates from the Philippines

Monogenic lines as differential varieties (resistance gene)	Standsrd differential blast isolates from the Philippines													
	M36-1-3-10-1	IK81-25	PO6-6	IK81-3	BN111	Ca89	V850256	M39-1-3-8-1	M64-1-3-9-1	M39-1-2-21-2	BN209	V850196	B90002	C923-49
IRBL20-IR24 (*Pi20*(t))	R	S	S	S	R	S	S	S	S	R	S	S	R	R
IRBLta-CP1 (*Pita*)	S	R	S	R	S	S	R	R	R-M	S	S	R	R	S
IRBLk-Ka (*Pik*)	S	S	R	R	R	R	R	S	S	S	R	R	R	R
IRBLks-F5 (*Pik-s*)	S	S	S	S	S	S	S	S	S	S	S	R	S	S
IRBLa-A (*Pia*)	S	S	S	S	S	S	S	S	S	S	S	S	R	R
IRBLb-B *(Pib*)	S	S	S	S	S	S	S	S	S	S	R-M	S	R	R
IRBLzt-T (*Piz-t*)	S	S	S	S	S	S	S	R	R	S	S	S	R	R
IRBLi-F5 (*Pii*)	S	R-M	R	R-M	R	S	S	M	S	R-M	S	R	S	S
IRBL3-CP4 (*Pi3*(t))	S	R	R	M	R	S	S	R-M	S	R	S	R	S	S

Monogenic lines were developed as a set of diffrential varieties by Tsunematsu et al. 2000
Standard diffential blast isolates from the Philippines were selected by Telebanco-Yanoria et al. 2008.

Table 3. Blast resistance gene estimation in IR64 based on the differential system

Rice genotype (Resistance gene harbored in the genetic background)		Standard differential blast isolates from the Philippines													
		M36-1-3-10-1	IK81-25	PO6-6	IK81-3	BN111	Ca89	V850256	M39-1-3-8-1	M64-1-3-9-1	M39-1-2-21-2	BN209	V850196	B90002	C923-49
IR64	(*Pi20*(t), *Pita*, *Pib*)* (*Pia*, *Pik-s*, *Piz-t*)**	R	R	S	R	R	S	R	R	R	R	R	R	R	R
IRBL20-IR24	(*Pi20*(t))	R	S	S	S	R	S	S	S	S	R	S	S	R	R
IRBLta-K1	(*Pita*)	S	R	S	R	S	S	R	R	R-M	S	S	R	R	S
IRBLb-B	(*Pib*)	S	S	S	S	S	S	S	S	S	S	R-M	S	R	R
IRBLa-A	(*Pia*)	S	S	S	S	S	S	S	S	S	S	S	S	R	R
IRBLks-F5	(*Pik-s*)	S	S	S	S	S	S	S	S	S	S	S	R	S	S
IRBLzt-T	(*Piz-t*)	S	S	S	S	S	S	S	R	R	S	S	S	R	R
IRBLk-Ka	(*Pik*)	S	S	R	R	R	R	R	S	S	S	R	R	R	R
IRBLi-F5	(*Pii*)	S	R-M	R	R-M	R	S	S	M	S	R-M	S	R	S	S
IRBL3-CP4	(*Pi3*(t))	S	R	R	M	R	S	S	R-M	S	R	S	R	S	S

S; susceptible, M; moderate resistant, and R; resistant.
*Estmated resistatce genes harboried in the IR64 genetic background, based on the reaction patterns to standard differential blast isolates from the Philippines
** Possible resistance gene harbored in the genetic background. These reaction of resistance genes are masked by the estimated genes which show the wide spectrums to the blast isolates
Three genes, *Pik*, *Pii*, and *Pi3*(t)are not harbored in the genetic background of IR64. Reaction patterns of the monogenic lines do not agree with that of IR64.

Table 4. Variety classification based on reaction pattern to 3 selected blast isolates

Variety Group and differential variety	Reaction to standard blast isolate[1)]			Estimated gene(s) harboring in the genetic background		
	M36-1-3-10-1	IK81-25	PO6-6			
IRBL20-IR24	R	S	S	*Pi20*(t)	-	-
IRBLta-CP1	S	R	S	-	*Pita*	-
IRBLk-K	S	S	R	-	-	*Pik*
VG1	S	S	S	-	-	-
VG2	R	S	S	*Pi20*(t)	-	-
VG3	S	R	S	-	*Pita*	-
VG4	M	S	R	-	-	*Pik*
VG5	R	S	R	*Pi20*(t)	-	*Pik-*
VG6	S	R	R	-	*Pita*	*Pik*
VG7	R	R	S	*Pi20*(t)	*Pita*	-
VG8	R	R	R	*Pi20(*t)	*Pita*	*Pik*

S; susceptible, M; moderate resistant, and R; resistant.

1) Blast isolates were selcted from the Philippines by Telebanco-Yanoria et al. 2008

Five IRRI varieties, IR20, IR28, IR30, IR45, and IR66, were susceptible to all 3 selected isolates and classified as VG1a (Table 4). None of the *Pi20*(t), Pita, or *Pik** were estimated in these varieties. These five varieties showed resistance to 4 isolates, BN209, V850196, B90002, and C923-49 (Table 5). These results can be explained by presence of *Pib* and *Pik-s* in VG1 (Table 2 and 5). Two varieties, IR29 and IR34 in VG1b showed resistance to 4 more isolates, IK81-25, IK81-3, M39-1-3-8-1, and M64-1-3-9-1. The resistant reactions to M39-1-3-8-1, and M64-1-3-9-1 were attributed to the presence of *Piz-t* in these 2 varieties, while those to the other 2 isolates, IK81-25 and IK81-3 were to unknown gene(s). From these reaction patterns, VG1 was further classified into 2 subgroups; i.e. VG1a (IR20, IR28, IR30, IR45, and IR66 estimated with *Pib* and *Pik-s*) and VG1b (IR29 and IR34 with *Pib*, *Pik-s*, *Piz-t* and unknown gene).

Seven varieties, IR8, IR22, IR24, IR26, IR43, PSBRc2, and PSBRc30, showed resistance to a blast isolate M36-1-3-10-1 and were classified as VG2 (Table 4). VG2 was estimated to harbor *Pi20*(t), because this gene is incompatible with the isolate M36-1-3-10. Resistance genes, *Pita* or *Pik** were thought to be absent in VG2, because these varieties was susceptible to both IK81-25 and PO6-6 which are avirulent to these genes. All 7 varieties in this group also showed resistance to 3 isolates, BN209, B90002, and C923-49, which were avirulent to *Pib* (Table 2 and 5). These results suggested that varieties in VG2 were likely to harbor *Pi20*(t) and *Pib*.

Furthermore, VG2 was divided into 3 subgroups, VG2a, VG2b, and VG2c based on differences in reaction to 3 isolates, M39-1-3-8-1, M64-1-3-9-1, and V850196 (Table 5). The varieties in subgroups, VG2a and VG2c showed resistance to V850196 whereas VG2b showed moderate resistance. Resistance to this isolated was estimated to be controlled by a resistance gene *Pik-s*, because *Pita* nor *Pik** was not estimated in VG2. The moderate resistance to this isolate of VG2b containing only one variety IR43 could be attributed to the presence of an unknown gene. VG2b was resistant to M39-1-3-8-1 and moderately resistant to M64-1-3-9-1. This reaction pattern could not be explained by resistance gene in the DVs used in this study. VG2c which included only one variety, PSBRc2, was resistant to M39-1-3-8-1 and M64-1-3-9-1 likely because of the presence of *Piz-t*.

Among the IRRI varieties tested, many of them were resistant to IK81-25 and susceptible to both M36-1-3-10 and PO6-6 (Table 4). From this reaction pattern, 17 varieties, IR5, IR32, IR36, IR38, IR40, IR42, IR44, IR50, IR52, IR54, IR58, IR60, IR62, IR65, IR68, IR72, and PSBRc4, were classified as VG3 and considered to harbor *Pita* and not to harbor *Pi20*(t) or *Pik**. Resistance to IK81-3, V850256, M39-1-3-8-1, M64-1-3-9-1, V850196, and B90002 were considered to be attribution of *Pita* (Table 2 and 5). VG3 varieties were also resistant to blast isolates BN209 and C923-49 which were avirulent to *Pib*. Therefore, VG3 were estimated to harbor *Pita* and *Pib*. The presence of *Pita* and *Pib* did not contradicted to susceptible reaction to PO6-6, M36-1-3-10-1, BN111, Ca89, and M39-1-2-21-2.

Table 5. Summary of estimated resistance gene in IRRI-bred ricevarieties using standard differential blast isolates from the Philippines

Variety Group	Monogenic lines and IRRI-bred varieties	Standard differential blast isolate														Resistance genes estimated and harboring in the genetic background						
		M36-1-3-10-1	IK81-25	PO6-6	IK81-3	BN111	Ca89	V850256	M39-1-3-8-1	M64-1-3-9-1	M39-1-2-21-2	BN209	V850196	B90002	C923-49							
	IRBL20-IR24	R	S	S	S	R	S	S	S	S	R	S	S	R	R	*Pi20*(t)	-	-	-	-	-	-
	IRBLta-CP1	S	R	S	R	S	S	R	R	R	S	S	R	R-M	M	-	*Pita*	-	-	-	-	-
	IRBLk-K	S	S	R	S	S	S	S	S	S	S	R-M	S	R	R	-	-	*Pik*	-	-	-	-
VG1a	IR20, IR28, IR30, IR45, IR66	S	S	S	S	S	S	S	S	S	S	R	R	R	R	-	-	-	*Pik-s*	*Pib*	-	-
VG1b	IR29, IR34	S	R-M	S	R-M	S	S	S	R	R	S	R	R	R	R	-	-	-	*Pik-s*	*Pib*	*Piz-t*	-
VG2a	IR8, IR22, IR24, IR26, PSBRc30	R	S	S	S	R	S	S	S	S	R	R	R	R	R	*Pi20*(t)	-	-		*Pib*	-	-
VG2b	IR43	R	S	S	S	R	S	S	R	M	R	R	M	R	R	*Pi20*(t)	-	-		*Pib*		*Pix*
VG2c	PSBRc2	R	S	S	S	R	S	S	R	R	R	R	R	R	R	*Pi20*(t)	-	-		*Pib*	*Piz-t*	
VG3	IR5, IR32, IR36, IR38, IR40, IR42, IR44, IR50, IR52, IR54, IR58, IR60, IR62, IR65, IR68, IR72, PSBRc4	S	R	S	R	S	S	M	R	R	S	R	R	R	R	-	*Pita*			*Pib*	-	-
VG4	PSBRc1	M	S	R	M	R	R	R	S	S	S	R	R	R	R		-	*Pik**		-	-	*Pix*
VG5	IR74	R	S	R	R	R	R	R	S	S	R	R	R	R	R	*Pi20*(t)	-	*Pik**		-	-	-
VG6	IR56, IR70	S	R	R	R	R	R	R	R	R	S	R	R	R	R		*Pita*	*Pik**		-	-	-
VG7a	IR46, IR48, IR64, PSBRc28	R	R	S	R	R	S	R	R	R	R	R	R	R	R	*Pi20*(t)	*Pita*	-		*Pib*	-	-
VG7b	PSBRc10, PSBRc18, PSBRc20	R	R	R	R	R	S	R	R	R	R	R	R	R	R	*Pi20*(t)	*Pita*	-		*Pib*	-	*Pii* or *Pi3*(t)

S; susceptible, M; moderate resistant, and R; resistant.
*Pik**: One of the allele of *Pik* gene expect for *Pik-s*.
These standard differential blast isolates from the Philippines can not classify the multiple alleles of *Pik*; *Pik*, *Pik-h*, *Pik-m*, and *Pik-p*.
Pix: Unknow gene

Only one variety, PSBRc1 was resistant to PO6-6, susceptible to IK81-25, and moderately resistant to M36-1-3-10. This variety was classified as VG4 and estimated to harbor *Pik** but not *Pita* (Table 4). *Pi20*(t) was thought to be absent in PSBRc1, because this variety was susceptible to M39-1-2-21-2 which is incompatible with the gene (Table 2 and 5). The moderate resistance of this variety to M36-1-3-10 was probably conferred by unknown gene(s) other than genes in DVs tested.

IR74 was susceptible to only 1 isolate IK81-25 among the 3 isolate for primarily estimation of 3 genes. This variety was classified as VG5 and estimated to harbor *Pi20*(t) and *Pik** among the 3 genes (Table 4). The presence of these genes did not contradict to the susceptible reaction to M39-1-8-1 and M64-1-3-9-1 of this variety (Table 5).

Two varieties, IR56 and IR70 were resistant to PO6-6 and IK81-25 and susceptible to M36-1-3-10-1 (Table 4). These varieties were classified as VG6 and estimated to harbor *Pita* and *Pik** among the 3 selected genes for primary estimation. The reaction pattern of VG6 was corresponded with that of additional effect pattern of IRBLta-CP1 with *Pita* and IRBLk-Ka with *Pik* (Table 2 and 5).

Four varieties, IR46, IR48, IR64, and PSBRc28, were resistant to 2 isolates, M36-1-3-10-1 and IK81-25, and estimated to have *Pi20*(t) and *Pita* by primary estimation and classified as VG7 (Table 4). Three varieties, PSBRc10, PSBRc18, and PSBRc20 were resistant to PO6-6 in addition to those 2 isolates. These 3 varieties were estimated to have *Pi20*(t) and *Pita* but not *Pik**, because these varieties were susceptible to Ca89 which was avirulent to *Pik** (Table 5). These 3 varieties were classified as VG7b, while the former 4 were classified as VG7a. These 7 varieties were resistant to BN209 which was avirulent to *Pik** and *Pib* (Table 2 and 5). *Pik** was not considered to be harbored in these varieties as mentioned earlier. Therefore, *Pib* was estimated in these varieties in addition to *Pi20*(t) and *Pita*. Resistance to the blast isolate, PO6-6 in varieties classified as VG7b, was estimated to be due to the presence of *Pii* or *Pi3*.

II. Segregation analyses using backcross populations

Materials and methods

1. Rice plant materials

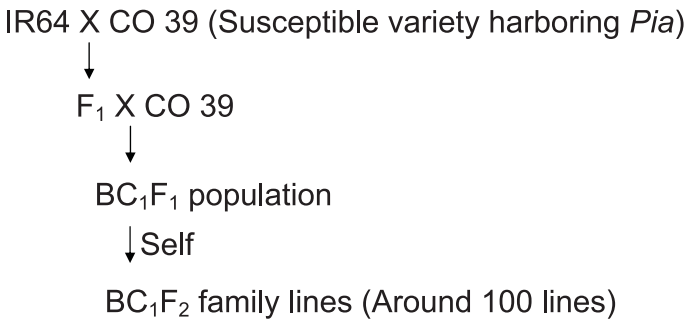
As shown in table 3, resistance to a certain blast isolate is governed by one or more genes in a variety. Segregation analysis using the BC_1F_2 family lines was conducted to determine the number of genes conferring resistance to the specific blast isolate. Six IRRI varieties representing several VGs, IR34 (VG1b), IR36 and IR60 (VG3), IR74 (VG5), and IR46 and IR64 (VG7a), were crossed with a susceptible check variety CO39 (a case of IR64 is shown in Fig. 1). The F_1 plants were backcrossed with CO39 as the recurrent parent to generate BC_1F_1. The BC_1F_2 family lines with population sizes ranging from 53 to 104 were generated by selfing and used for the segregation analysis.

2. Inoculation and evaluation of resistance

The same procedure described above was employed for blast inoculation test. Differential blast isolates from the Philippines with known pathogenecity were used for the inoculation test to identify resistance genes. Each line consisting of 20 seedlings was inoculated with selected blast isolates avirulent to the expected genes.

3. Estimation of number of resistance genes

Following the procedure proposed by Toriyama et al. (1986), the number of genes conferring resistance in a variety was estimated from the segregation of BC_1F_2 lines to each blast isolate. Three ratios, 1:1, 3:1, and 7:1, for segregating for resistance (heterozygote) against susceptible (homozygote) lines were expected for one, two, and three genes controlling resistance, respectively (Fig. 2, Fig. 3, and Fig. 4, respectively). For example as shown in Fig. 2, where a single dominant gene in target rice



Appendix 1. Hybrid population for segregation analysis of resistance genes in the IRRI bred variety IR64

The hybrid population was developed by back crossing between IR64 and the susceptible variety CO 39 as the recurrent parent.

variety confers the resistance, ratio of number of resistant against susceptible BC_1F_1 plants and that of segregating against susceptible BC_1F_2 family lines are 1:1.

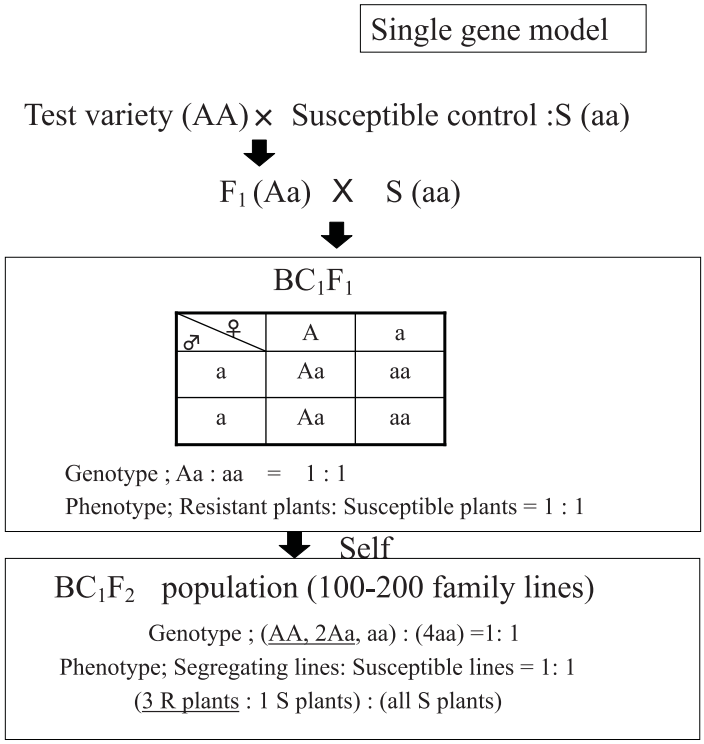
Segregation analysis using F_2 or F_3 family lines are also possible for estimation of number of resistance genes, although it was not employed in this study. The expected ratios for all resistant plants : segregation : all susceptible plants are 1:2:1 and 7:8:1 in F_3 family lines for a single and two dominant gene(s) control, respectively (Fig. 5 and 6, respectively).

Results and discussion

1. Estimation of number of resistance genes in IR64

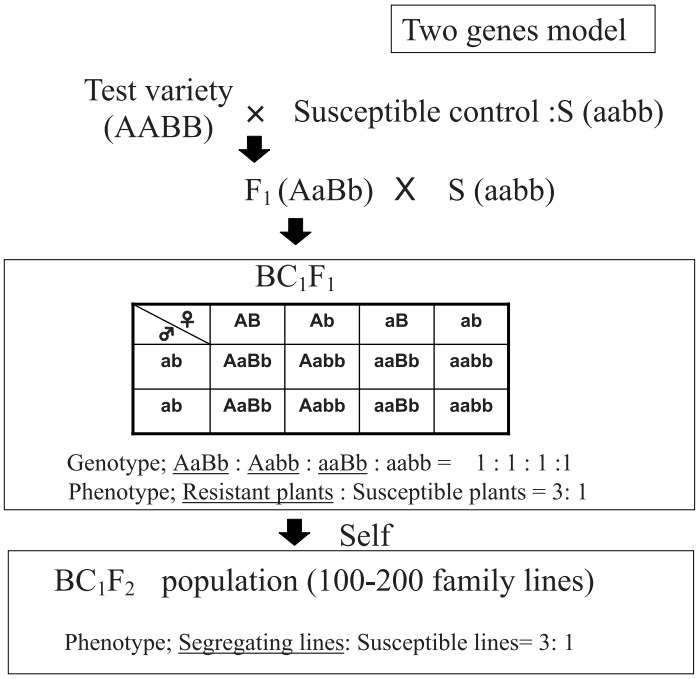
In IR64 of VG7a, 72 BC_1F_2 lines were generated by backcross with CO39 (Fig. 1). These lines were tested against four isolates, BN111, IK81-25, M64-1-3-9-1, and BN209 (Table 6). The ratios of number of all resistant lines (resistant homozygote) : segregating lines (heterozygote) : all susceptible lines (susceptible homozygote) in response to two isolates, BN111 and IK81-25 were well fit to 0:1:1 (χ^2=0.06, p=0.81, and χ^2=1.39, p=0.24, respectively). These results suggested that the resistance to each of these 2 isolates was controlled by a single dominant gene. The ratios in number of lines of all resistant : segregating : all susceptible to the remaining two isolates, M64-1-3-9-1 and BN209 were well fit to 0:3:1 (χ^2=0.67, p=0.41 and χ^2=0.08, p=0.78, respectively). From these results, it was estimated that the resistance to each of these 2 isolates was controlled by 2 dominant genes.

Isolate BN111 is avirulent to *Pi20*(t) and *Pik** (Table 3). A single dominant gene controlling resistance of IR64 to this isolate was estimated to be *Pi20*(t) but not *Pik**, because the gene *Pik** was not estimated in IR64 in the previous estimation by differential system (Table 5 and 6). Similarly, a single dominant gene for resistance to IK81-25 was estimated to be *Pita* (Table 3 and 6). The remaining 2 blast isolates, M64-1-3-9-1 and BN209 which were estimated to be controlled by 2 genes, were avilurent to *Pita* and *Piz-t*, and to *Pib*, respectively (Table 3). From these results, four genes (*Pi20*(t), *Pita*, *Piz-t*, and *Pib*) were detected in IR64 to control the segregation for resistance to the isolates used. However, one of the two genes could not be identified in the segregation analysis using BN209.



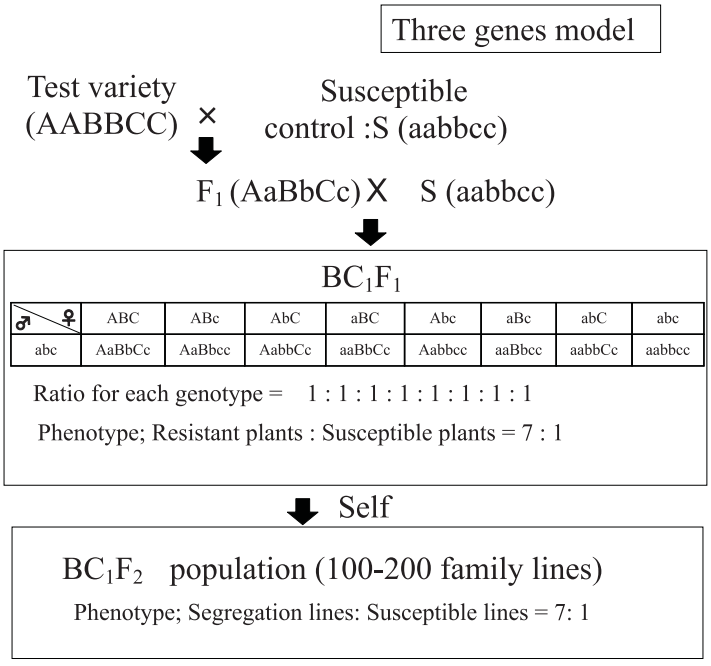
Appendix 2. Segregation analysis for blast resistance gene using BC_1F_2 plants or BC_1F_2 family lines – single gene model

A; resistance gene harbored in the genetic background. Where a single dominant gene in a test rice variety confers resistance, the ratio of number of resistant to susceptible BC_1F_1 plants and that of segregating to susceptible BC_1F_2 family lines is 1 : 1.
Avirulence blast pathogen for the resistance gene, A, is used in segregation analysis. Avirulence pathogen is selected by prior inoculation test on the test variety. When the virulent blast isolate is used, all progeny will be susceptible.



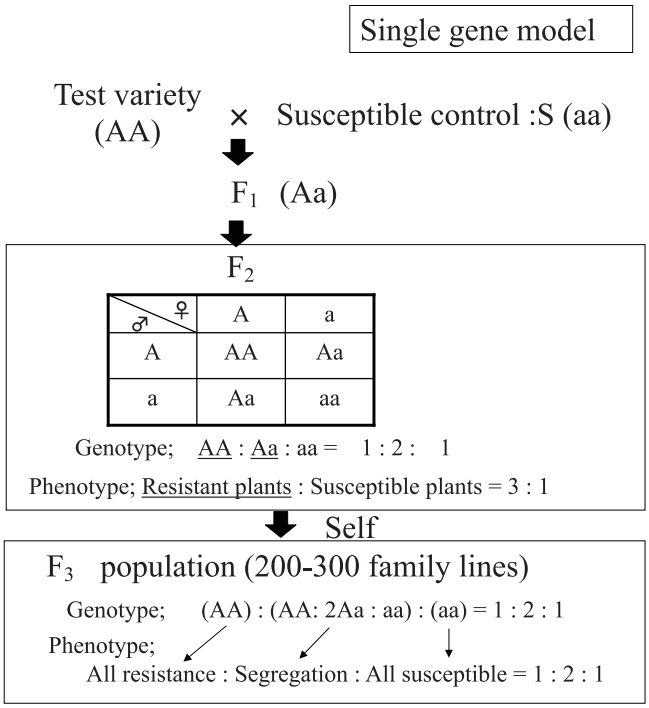
Appendix 3. Segregation analysis for blast resistance gene using BC_1F_2 plants or BC_1F_2 family lines – two gene model

A, B; Resistance genes harbored in the genetic background.
Where two dominant genes of test rice variety confer resistance independently, the segregation ratio in the BC_1F_1 plants for resistant (heterozygote) and susceptible (homozygote) plants is 3:1. Accordingly, the ratio of number of BC_1F_2 family lines segregating for resistance to all susceptible lines is 3:1.
A blast isolate avirulent to the differential variety with a known resistance gene is used for the inoculation test.



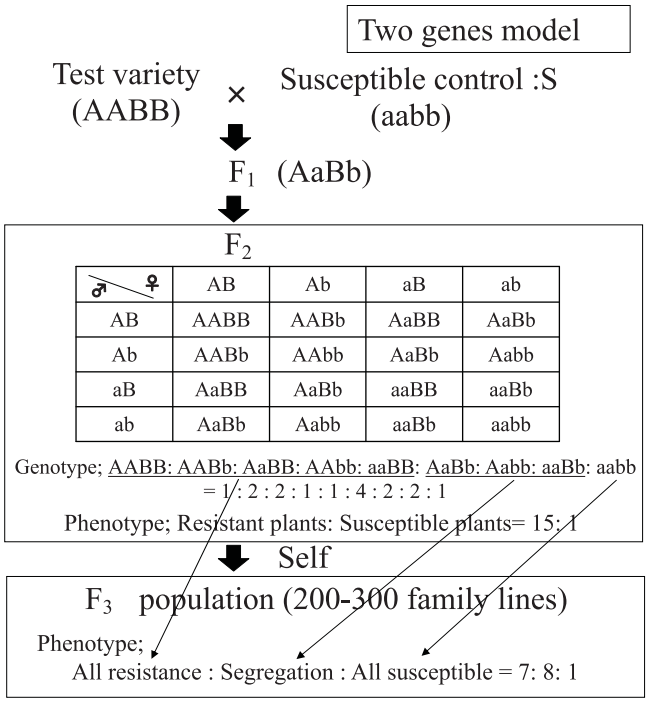
Appendix 4. Segregation analysis for blast resistance gene using BC_1F_2 plants or BC_1F_2 family lines – three gene model

A, B, C; Resistance genes harbored in the background.
Where three dominant genes in the genetic background of test rice variety confer resistance independently, the segregation ratio in the BC_1F_1 plants for resistant (heterozygote) and susceptible (homozygote) plants is 7:1. Accordingly, the ratio of number of BC_1F_2 family lines with segregating for resistance to all susceptible lines is 7:1.
A blast isolate avirulent to the differential variety with known resistance gene is used for the inoculation test.



Appendix 5. Segregation analysis for blast resistance genes using F_2 plants or F_3 family lines – single gene model

A; Resistance gene harbored in the genetic background. Where a single dominant gene in the genetic background of a test rice variety confers resistance, the ratio for the number of resistant homozygote : heterozygote : susceptible homozygote F_2 plants is 1:2:1. Accordingly, the ratio for number of F_3 family lines is 1(all R): 2 (segregation): 1 (all S). A blast isolate avirulent to the differential variety with the known resistance gene is used for the inoculation test.



Appendix 6. Segregation analysis for a blast resistance gene using F_2 plants or F_3 family lines – two gene model

A, B; Resistance genes harbored in the genetic background. Where two dominant genes in the genetic background of a test rice variety confer resistance independently, the ratio for the number of resistant homozygotes with at least one gene: heterozygote : susceptible homozygote F_2 plants is 7:8:1. Accordingly, the ratio for the number of F_3 family lines is 7 (all R): 8 (segregation) : 1 (all S). A blast isolate avirulent to the differential variety with the known resistance gene is used for the inoculation test.

2. Estimation of number of resistance genes in IRRI-bred varieties

Table 7 shows the results of the segregation analyses in six BC_1F_2 populations. In IR34 (VG1b), three genes, *Pik-s*, *Pib*, and *Piz-t*, were previously estimated (Table 5), hence, genetic analysis was performed to confirm these genes. A total of 104 BC_1F_2 family lines were examined for segregation to five isolates, M39-1-3-8-1, M64-1-3-9-1, V850196, BN209, and B90002. Against M39-1-3-8-1 and M64-1-3-9-1, 48 lines were segregating resistant and 56 lines were susceptible homozygote, and a co-segregation for resistance was recognized between these isolates. The segregation fit a 1:1 ratio expected of a single dominant gene control. Since both isolates were avirulent to *Piz-t* and *Pita*, but IR34 in VG1 was previously estimated not to have *Pita*, the segregation for resistance was therefore governed by *Piz-t*. To isolates V850196 and BN209, the segregation rates between segregating resistant and susceptible homozygote lines fit a 3:1 ratio, suggesting that two dominant genes controlled the resistance to each isolate. One of the genes was identified as *Pik-s* to V850196 based on the estimation by reaction patterns, but the other one could not be identified by the differential system. Similarly, *Pib* was identified as one of the resistance genes to BN209 but the other one was not identified. The same lines were also examined for *Pia*, a resistance gene that was not estimated by reaction patterns because *Pia* was masked by *Pi20*(t), *Pita*, or *Pik**. All the lines showed a resistant reaction to isolate B90002, which was avirulent to *Pia*. Therefore, *Pia* was identified in IR34 since it was known to be included in the recurrent parent, CO39 (Tsunematsu et al., 2000). On the basis of these results, the presence of *Pib*, *Pik-s*, and *Piz-t* was confirmed in IR34, while another gene *Pia* and at least an unknown one were also detected.

Two varieties from VG 3, IR36 and IR60, previously estimated to harbor *Pita* and *Pib*, were analyzed (Table 7). Results showed that *Pita* could be identified in IR36 using isolates IK81-3, and V850196 which are avirulent to *Pita*. The 61 BC_1F_2 lines derived from a cross between IR36 and CO39 exhibited single gene segregation to IK81-3. The segregation for resistance was due to *Pita*. To isolates V850196, the segregation rates between segregating resistant and susceptible homozygote lines fit a 3:1 ratio, suggesting that two dominant genes controlled the resistance. Therefore, *Pib*, which was previously estimated and incompatible to this isolate, was identified in IR36 as one of the gene conferring the resistant to this isolate. *Pik-s* was also identified in IR36 as another resistance gene to V850196 with considering the reaction pattern (Table 5). Similarly, the resistance to BN209 that has avirulence to *Pib* and *Pik** among the gene tested, was considered to be controlled by 2 dominant genes. Previous analysis showed that *Pik** was not included in VG 3; hence, segregation for resistance to BN209 was related to *Pib* and another unknown gene. As a result of segregation analysis, 3 genes, *Pib*, *Pita* and *Pik-s* as well as one unknown gene were confirmed to be harbored in IR36.

The presence of two genes, *Pita* and *Pib*, was analyzed in IR60 of VG3. Using 53 BC_1F_2 lines, a digenic segregation to each of the three isolates, IK81-3, M64-1-3-9-1, and V850196 was obtained, and three genes controlled were estimated for resistance to BN209 (Table 7). IK81-3 is avirulent to *Pita* and *Pik**, while the latter was not present in VG3 by the previous estimation. M64-1-3-9-1 was avirulent to *Pita* and *Piz-t*, V850196 to *Pita* and *Pik-s*, and BN209 to *Pib* and *Pik** in the differential system. Thus, 3 genes, *Pita*, *Pik-s*, and *Pib*, were considered to be related in the segregation. However, the other one gene for resistance to IK81-3 and two genes for resistance to BN209 could not be identified by reaction patterns of the differential system.

IR74 in VG5 was estimated to harbor 2 genes, *Pi20*(t) and *Pik** by reaction patterns (Table 5). This variety was

Table 6. Segregation for resistance in BC_1F_2 populations derived from a cross between IR64 and a susceptible variety CO 39 as the recurrent parent

Differntial blast isolate	Reaction of monogenic line				No. of BC_1F_2 lines				χ^2 value		*P* (df =1)	Estimated no. of resistance gene	Estimated gene
	IRBL20-IR24 (*Pi20*)	IRBLta-K1(*Pita*)	IRBLb-B (*Pib*)	IRBLzt-T (*Piz-t*)	All resistant plants	Segregating	All susceptible	Total	1:1	3:1			
BN111	R	S	S	S	0	37	35	72	0.06	-	0.81	1	*Pi20*(t)
IK81-25	S	R	S	S	0	41	31	72	1.39	-	0.24	1	*Pita*
M64-1-3-9-1	S	R	S	R	0	51	21	72	-	0.67	0.41	2	*Pita*, *Piz-t*
BN209	S	S	R-M	S	0	52	17	69	-	0.08	0.78	2	*Pib*, one unknown

analyzed using 76 BC_1F_2 lines for segregation to six isolates: PO6-6 and Ca89 for identification of *Pik**, M36-1-3-10-1 for *Pi20*(t), BN111 for *Pi20*(t) and *Pik**, BN209 for *Pib* and *Pik**, and B90002 for *Pia*, *Pik**, *Pi20*(t), *Pib*, and *Piz-t* (Table 7). In the segregation analysis on BC_1F_2 lines, resistance was controlled by a single dominant gene judging from the segregation ratio of 1:1 involving three isolates, PO6-6, Ca89, and M36-1-3-10-1. A co-segregation was also observed between isolates PO6-6 and Ca89. *Pik** was identified to control the segregation for PO6-6 and Ca89, and *Pi20*(t) was for isolate M36-1-3-10-1. A digenic segregation to isolates BN111 and BN209 were observed. One of the genes, *Pik**, was identified to be common in the segregation to both isolates.

Table 7. Segregation for resistance in BC_1F_2 populations derived from the crosses between IRRI-bred varieties and a susceptible variety CO 39 as the recurrent parent

Variety (VG)	Isolate	No. of lines				χ^2 value[1)]			*P* (df=1)	Estimated no. of resistance gene	Estimated gene[2)]
		All resistant	Segregating resistant	All susceptible	Total	1:1	3:1	7:1			
IR34 (VG1b)	M39-1-3-8-1	-	48	56	104	0.62[a]	-	-	0.43	1	***Piz-t***
	M64-1-3-9-1	-	48	56	104	0.62[a]	-	-	0.43	1	***Piz-t***
	V850196	-	77	27	104	-	0.02	-	0.89	2	***Pik-s***, one unknown
	BN209	-	79	25	104	-	0.02	-	0.89	2	***Pib***, one unknown
	B90002	104	0	0	104	-	-	-	-		*Pia*
IR36 (VG3)	IK81-3	-	35	26	61	1.32	-	-	0.25	1	***Pita***
	V850196	-	47	14	61	-	0.13	-	0.72	2	***Pita***, *Pik-s*
	BN209	-	45	16	61	-	0.05	-	0.82	2	***Pib***, one unknown
IR60 (VG3)	IK81-3	-	36	17	53	-	1.41	-	0.24	2	***Pita***, one uknown
	M64-1-3-9-1	-	40	13	53	-	0.02	-	0.89	2	***Pita***, *Piz-t*
	V850196	-	37	16	53	-	0.76	-	0.38	2	***Pita***, *Pik-s*
	BN209	-	48	5	53	-	-	0.46	0.5	3	***Pib***, two unknown
IR74 (VG5)	M36-1-3-10-1	-	36	40	76	0.22	-	-	0.64	1	***Pi20*(t)**
	BN111	-	51	25	76	-	2.52	-	0.11	2	***Pi20*(t), *Pik****[3)]
	PO6-6	-	34	42	76	0.84[b]	-	-	0.36	1	***Pik****
	Ca89	-	34	42	76	0.84[b]	-	-	0.36	1	***Pik****
	BN209	-	54	22	76	-	0.63	-	0.43	2	***Pik****, *Pib*
	B90002	76	0	0	76	-	-	-	-		*Pia*
IR46 (VG7a)	M36-1-3-10-1	-	58	42	100	2.56[d]	-	-	0.11	1	***Pi20*(t)**
	BN111	-	58	42	100	2.56[d]	-	-	0.11	1	***Pi20*(t)**
	IK81-25	-	59	41	100	3.24[c]	-	-	0.07	1	***Pita***
	M64-1-3-9-1	-	59	41	100	3.24[c]	-	-	0.07	1	***Pita***
	V850196	-	79	21	100	-	0.85	-	0.36	2	***Pita***, *Pik-s*
	BN209	-	81	19	100	-	1.92	-	0.17	2	***Pib***, one unknown
IR64 (VG7a)	BN111	-	37	35	72	0.06	-	-	0.81	1	***Pi20*(t)**
	IK81-25	-	41	31	72	1.39	-	-	0.24	1	***Pita***
	M64-1-39-1	-	51	21	72	-	0.67	-	0.41	2	***Pita***, *Piz-t*
	BN209	-	52	17	69	-	0.08	-	0.78	2	***Pib***, one unknown

1) Common letters (a, b, c, and d) indicate co-segregation among isolates.

2) Resistance genes described by bold letters were those estimated by the differential system (see Table 5).

3) *Pik**: One of the alleles *Pik*, *Pik-h*, *Pik-m*, or *Pik-p*.

The other genes segregating to BN111 and BN209 corresponded to *Pi20*(t) and *Pib*, respectively. In the analysis of segregation to B90002, all the lines were resistant, suggesting that IR74 carries the same *Pia* gene found in CO39. These results revealed that besides *Pita* and *Pik** confirmed in IR74, two other genes, *Pia* and *Pib* were identified in this variety.

Two varieties in VG 7a, IR46 and IR64, were analyzed to confirm the presence of *Pi20*(t), *Pita*, and *Pib*. In the analysis of IR46 involving 100 BC_1F_2 lines and four isolates, M36-1-3-10-1, BN111, IK81-25, and M64-1-3-9-1, a 1:1 ratio suggesting the monogenic segregation, and the co-segregation for resistance to BN111 and M36-1-3-10-1, and to IK81-25 and M64-1-3-9-1 were observed (Table 7). Since BN111 and M36-1-3-10-1 are avirulent to *Pi20*(t), and IK81-25 and M64-1-3-9-1 to *Pita*, the presence of *Pi20*(t)and *Pita* in IR46 was confirmed. A 3:1 ratio observed between segregating resistant and susceptible homozygote lines suggested that the segregation for resistance to isolates V850196 and BN209 was controlled by two dominant genes. In the reaction pattern of differential system, V850196 was avirulent to *Pita*, *Pik** and *Pik-s*, and BN209 to *Pib* and *Pik**. The gene *Pik** was estimated not to be harbored in VG 7a by reaction pattens. The other genes, *Pita* and *Pik-s*, were related to the segregation for resistance to V850196, and *Pib* and an unknown gene to BN209. Thus, *Pi20*(t), *Pita*, *Pik-s*, and *Pib*, and one unknown gene were identified in IR46.

III. Allelism tests of the resistance genes using differential varieties

Materials and methods

1. Rice plant materials

Nine IRRI rice varieties from 7 different VGs, IR34 (VG1b), IR24 (VG2a), IR36 and IR60 (VG3), PSBRc1 (VG4), IR74 (VG5), IR56 and IR70 (VG6), and IR64 (VG7b), were crossed with DVs with known blast resistance genes. The DVs were Toride 1 for *Piz-t*; BL 1 for *Pib*; Yashiromochi, C105TTP2L9, and C101PKT for *Pita*; Tsuyuake with *Pik-m* and Kanto 51 with *Pik* for *Pik**; and IR24 for three genes, *Pib*, *Pik-s*, and *Pi20*(t) (Imbe et al.1997). The F_2 populations derived from each cross were tested.

2. Inoculation and evaluation of resistance

The same procedure described above was employed for blast inoculation test. The F_2 populations were inoculated with selected blast isolates, which were avirulent to particular genes.

3. Identification of resistance gene

The allelic relationship of the genes was determined based on the segregation for resistance to a particular blast isolate in the F_2 populations. If the segregation did not include susceptible plants, it was assumed that both parents had the same or an allelic gene (Fig. 7). Otherwise, it was considered that the resistance was governed by different allele genes (Fig. 8).

Same allele case

Test variety (A^TA^T) × Differential variety Y (A^DA^D)

F_1 (A^TA^D)

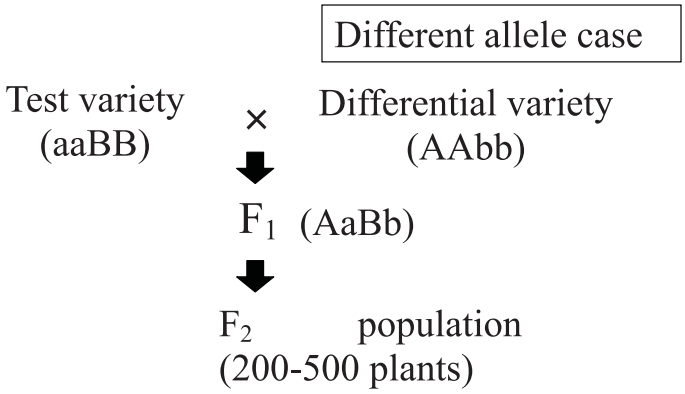
F_2 population (200-500 plants)

♂ \ ♀	A^T	A^D
A^T	A^TA^T	A^DA^T
A^D	A^TA^D	A^DA^D

Phenotype; All resistant plants

Appendix 7. Allelism test using an F_2 population derived from a cross between a test rice variety and differential varieties – a case of the same allele

AT, AD; resistance gene in the rice test variety and differential variety, respectively.
Inoculation test is carried out with a blast isolate avirulent to the differential variety.



♂ \ ♀	AB	Ab	aB	ab
AB	**AABB**	**AABb**	**AaBB**	**AaBb**
Ab	**AABb**	**AAbb**	**AaBb**	**Aabb**
aB	**AaBB**	**AaBb**	aaBB	aaBb
ab	**AaBb**	**Aabb**	aaBb	aabb

•Segregation ratio in case of inoculation with a blast isolate avirulent only to the differential variety.
→ Resistance plants: Susceptible plants = 3 : 1
•Segregation ratio in case of inoculation with a blast isolate avirulent to both the differential and the test variety.
→ Resistance plants : Susceptible plants = 15 : 1

Appendix 8. Allelism test using F_2 population derived from the cross between rice test variety and differential variety – a case of different allele

A, B; resistance gene in the differential and the test variety, respectively.
A blast isolate avirulent to the differential variety carrying the known resistance gene is used for the inoculation test.

Results and discussion

1. Gene identification in IR64

The F_2 populations were generated from crosses between IR64 of VG7a and 5 DVs, IR24 for *Pi20*(t), *Pik-s* and *Pib*, C105TTP2L9 and C101PKT for *Pita*, Toride 1 for *Piz-t*, and IR56 for *Pita* and *Pik**. When the F_2 populations derived from cross between IR64 and IR24 were tested against 3 blast isolates, BN111 avirulent to *Pi20*(t) and *Pik*, V850196 avirulent to *Pita*, *Pik* and *Pik-s*, and BN209 avirulent to *Pik* and *Pib*, no population shows any susceptible plants to these isolates (Table 8). These results indicated that IR64 was revealed to harbor the same alleles of *Pi20*(t), *Pik-s* and *Pib* in IR24 by inoculation test against BN111, V850196 and BN209, respectively. The F_2 plants derived from crosses between IR64 and 2 DVs for *Pita*, C105TTP2L9 and C101PKT were all resistant to a blast isolate, IK81-25 avirulent to *Pita*. This suggested that IR64 also harbored the same allele of *Pita* in those DVs. Similarly, *Piz-t* was identified in IR64, when the F_2 plants derived from cross between IR64 and Toride 1 which is a DV for *Piz-t* were all resistant to both M39-1-3-8-1 and M64-1-3-9-1 which are avirulent to the gene. In the F_2 population derived from a cross between IR64 and IR56, all tested plants were resistant to IK81-25 avirulent to *Pita*. On the other hand, a 3:1 segregation ratio (χ^2= 0.22, p=0.64) was observed in the same population for resistance to PO6-6, which is avirulent to *Pik**. This resistance was suggested to be governed by a single gene, *Pik** in IR56. These results confirmed that IR64 harbored 6 resistance genes, *Pi20*(t), *Pita*, *Pib*, *Pik-s*, *Piz-t*, and one unknown.

2. Gene identification in IRRI-bred varieties

To confirm the presence of *Piz-t* and *Pib* in IR34 in VG1b, F_2 populations derived from crosses with 2 DVs, Toride 1 for *Piz-t* and BL 1 for *Pib* were tested against the selected blast isolates. The F_2 population derived from cross with Toride 1 was inoculated with 2 blast isolates, M64-1-3-9-1 and M39-1-3-8-1 both avirulent to Piz-t (Table 9). As a result, all plant of the population was resistant to these isolate. Thus, the resistance gene in IR34 to these isolate was the same as that in Toride 1, *Piz-t*. In the same manner, F_2 population derived from cross with BL1 did not show any susceptible plants to blast isolate BN209 avirulent to *Pib*. These results suggested the presence of *Piz-t* and *Pib* in IR34.

All F_2 plants derived from the cross between IR24 in VG2a and a DV, BL 1 for *Pib* was resistant to BN209

avirulent to *Pik** and *Pib* (Table 9). These results suggested the presence of *Pib* in IR24 and supported the previous report by Imbe et al. (1997).

Four kind of F_2 population were generated from crosses between IR36 in VG3 and 4 DVs; C105TTP2L9 and C101PKT for *Pita*, Toride 1 for *Piz-t*, and BL 1 for *Pib*. When these populations were tested against 4 isolates, IK81-25 (avirulent to *Pita*), M391-3-8-1 and M64-4-1-3-9-1 (avirulent to *Pita* and *Piz-t*), and BN209 (avirulent to *Pib*), all F_2 plants showed resistance. These results confirmed the presence of *Pita*, *Piz-t*, and *Pib* in IR36. The presence of *Piz-t* was also confirmed by the allelism test between IR34 and IR36 using M39-1-3-8-1 and M64-1-3-9-1. Using F_2 population derived from a cross between IR36 and IR24, the allelism of *Pib* and *Pik-s* was confirmed by inoculation test against blast isolate BN209 (avirulent to *Pib*) and V850196 (avirulent to *Pik-s*), respectively. The reaction of *Piz-t* was masked by *Pita* and could not be estimated in the previous analysis based on reaction pattern. However, *Piz-t* was identified in IR36 through allelism tests. Thus, BC_1F_2 analysis and allelism tests revealed that IR36 carries at least four genes— *Pita*, *Pik-s*, *Pib*, and *Piz-t*.

The F_2 populations derived from crosses of IR60 in VG3 with IR34 and IR24 did not segregate any susceptible plants to 4 isolates, BN209 (avirulent to *Pib* and *Pik**), V850196 (avirulent to *Pita*, *Pik-s* and *Pik**), M39-1-3-8-1 and M64-1-3-9-1 (avilurent to *Pita* and *Piz-t*) (Table 9). IR34 was confirmed to harbor *Pia*, *Pib*, *Piz-t*, and *Pik-s* in this study. IR24 was also confirmed to harbor *Pia*, *Pib*, *Pik-s*, and *Pi20*(t), previously. IR60 was estimated not to harbor *Pik** by reaction pattern, while the resistance to M39-1-3-8-1 and M64-1-3-9-1 was governed by *Piz-t* which was estimated also in IR34. From these results, IR60 was revealed to have 3 genes, *Pib*, *Piz-t*, and *Pik-s*.

PSBRc1 of VG4 was crossed with IR56, which was estimated to be carrying *Pita* and *Pik** to generate F_2 population. All of the F_2 plants was resistant to isolates PO6-6 avirulent to *Pik** and BN111 avirulent to both *Pik** and *Pi20*(t) (Table 9). Since *Pi20* was not estimated in VG4 by reaction patterns in the previous analysis, the resistance of F_2 plants to these isolates suggested the allelism of *Pik** in PSBRc1 and IR56. The presence of *Pita* was also verified in this population using IK81-25 which is avirulent to *Pita*. Results showed that the F_2 plants segregated into 406 resistant and 126 susceptible, which fit the 3:1 ratio (χ^2=0.51, p=0.43). This indicated that a single dominant gene, *Pita* previously estimated in IR56 governed the segregation, because PSBRc1 was estimated not to have *Pita* by reaction pattern.

Table 8. Allelism test for resistance using F_2 populations derived from the crosses between IR64 and differential varieties

Cross combination (Resistance gene harbored in parent)		Differntial blast isolate used for the seregation analysis of resistance	Reaction of monogenic line						No. of plants[1)]			χ^2 value (3:1)	*P* (df =1)	Identified gene
			IRBL20-IR24 (*Pi20*)	IRBLta-K1 (*Pita*)	IRBLk-Ka (*Pik*)	IRBLb-B (*Pib*)	IRBLzt-T (*Piz-t*)	IRBLks-F5 (*Pik-s*)	R	S	Total			
IR24 (*Pib*, *Pi20*(t), *Pik-s*)	/ IR64 (*Pi20*(t), *Pita*, *Pib*)	BN111	R	S	R	S	S	S	302	0	302	-	-	*Pi20(t)*
		V850196	S	R	R	S	S	R	227	0	227	-	-	*Pik-s*
		BN209	S	S	R	R-M	S	S	222	0	222	-	-	*Pib*
IR64	/ C105TTP2L9 (*Pita*)	IK81-25	S	R	S	S	S	S	274	0	274	-	-	*Pita*
IR64	/ C101PKT (*Pita*)	IK81-25	S	R	S	S	S	S	132	0	132	-	-	*Pita*
IR56 (*Pita*, *Pik**)	/ IR64	IK81-25	S	R	S	S	S	S	296	0	296	-	-	*Pita*
		PO6-6	S	S	R	S	S	S	193	60	253	0.22	0.64	*Pik** in IR56
Toride1 (*Piz-t*)	/ IR64	M39-1-3-8-1	S	R	S	S	R	S	124	0	124	-	-	*Piz-t*
		M64-1-3-9-1	S	R	S	S	R	S	133	0	133	-	-	*Piz-t*

1) R; resistant, S; susceptible.

2) *Pik**: One of the alleles *Pik*, *Pik-h*, *Pik-m*, or *Pik-p*.

IR24 was expected not to habor Pita based on the reaction pattern to differential blast isolates in previous reasearch.

IR24 and IR64 were expected not to harbor Pik based on the reaction patterns to differential blast isolates in previous reasearch.

Toride 1 dose not harbor *Pita*

Table 9. Allelism test for resistance using F_2 populations derived from the crosses between IRRI-bred and differential varieties

Variety (VG)	Cross combination (Resistance gene(s) harbored in parent)		Isolate	No. of plants[1)]			χ^2 value (3:1)	*P* (df = 1)	Identified gene
				R	S	Total			
IR34	Toride1 (*Piz-t*)	/ IR34 (*Pia*, *Pik-s*, *Pib*, *Piz-t*)	M39-1-3-8-1	94	0	94	-	-	*Piz-t*
(VG1b)			M64-1-3-9-1	84	0	84	-	-	*Piz-t*
	BL1 (*Pib*)	/ IR34	BN209	437	0	437	-	-	*Pib*
IR24 (VG2b)	BL1	/ IR24	BN209	161	0	161	-	-	*Pib*
IR36	IR36 (P*ita*, *Pib*, *Pik-s*)	/ C105TTP2L9 (*Pita*)	IK81-25	468	0	468	-	-	*Pita*
(VG3)	IR36	/ C101PKT (*Pita*)	IK81-25	167	0	167	-	-	*Pita*
	BL1	/ IR36	BN209	420	0	420	-	-	*Pib*
	IR24	/ IR36	V850196	295	0	295	-	-	*Pik-s*
			BN209	287	0	287	-	-	*Pib*
	Toride 1	/ IR36	M39-1-3-8-1	184	0	184	-	-	*Piz-t*
			M64-1-3-9-1	168	0	168	-	-	*Piz-t*
	IR34	/ IR36	M39-1-3-8-1	467	0	467	-	-	*Piz-t*
			M64-1-3-9-1	516	0	516	-	-	*Piz-t*
IR60	IR34	/ IR60 (*Pita*, *Pib*, *Pik-s*, *Piz-t*)	M39-1-3-8-1	302	0	302	-	-	*Piz-t*
(VG3)			M64-1-3-9-1	303	0	303	-	-	*Piz-t*
	IR24	/ IR60	V850196	235	0	235	-	-	*Pik-s*
			BN209	360	0	360	-	-	*Pib*
PSBRc1	IR56 (*Pita*, *Pik**)	/ PSBRc1 (*Pik**[2)])	PO6-6	531	0	531	-	-	*Pik**
(VG4)			BN111	526	0	526	-	-	*Pik**
			IK81-25	406	126	532	0.51	0.43	*Pita* in IR56
IR74	IR56	/ IR74 (*Pi20*(t), *Pik**)	PO6-6	560	0	560	-	-	*Pik**
(VG5)			BN111	585	0	585	-	-	*Pik**
			IK81-25	442	135	577	0.82	0.37	*Pita* in IR56
IR56	C101PKT	/ IR56	IK81-25	469	0	469	-	-	*Pita*
(VG6)			PO6-6	358	85	443	7.98	0.005	*Pik** in IR56
	Yashiromochi (*Pita*)	/ IR56	IK81-25	463	0	463	-	-	*Pita*
			PO6-6	370	127	497	0.08	0.78	*Pik** in IR56
	IR56	/ Kanto 51 (*Pik*)	PO6-6	440	0	440	-	-	*Pik**
			BN111	407	0	407	-	-	*Pik**
	IR56	/ Tsuyuake (*Pik-m*)	PO6-6	498	0	498	-	-	*Pik**
			BN111	496	0	496	-	-	*Pik**
IR70	IR56	/ IR70 (*Pita*, *Pik**)	IK81-25	567	0	567	-	-	*Pita*
(VG6)			PO6-6	577	0	577	-	-	*Pik**
			BN111	558	0	558	-	-	*Pik**

1) R; resistant, S; susceptible.
2) *Pik**; One of the alleles *Pik*, *Pik-h*, *Pik-m*, or *Pik-p*.

*Pik** was identified in IR74 by an allelism test with IR56 (Table 9). All the F_2 plants derived from a cross of IR74 with IR56 were resistant to PO6-6, which is avirulent to *Pik**, and BN111 avirulent to both *Pik** and *Pi20*(t), suggesting that IR74 had the same allele of *Pik** in IR56. The segregation ratio for resistance to IK81-25, which was avirulent to *Pita* fitted to a 3:1 ratio (χ^2= 0.82, p=0.37) expected for a single dominant gene control. This was thought to be due to *Pita* in IR56 but not in IR74.

In VG6, two genes, *Pita* and *Pik**, were previously estimated by reaction patterns in IR56 and IR70. To confirm their presence in these varieties, IR56 was crossed with 4 DVs, C101PKT and Yashiromochi for *Pita*, Kanto 51 and Tsuyuake for *Pik**, and with IR70 for *Pita* and *Pik**. On these 5 kinds of F_2 populations, 11 combinations of allelism test were carried out using 3 isolates: IK81-25 avirulent to *Pita*, PO6-6 to *Pik**, and BN111 to *Pik** and *Pi20*(t). Of these, two F_2 populations derived from crosses of IR56 with C101PKT and Yashiromochi showed a single gene segregation to PO6-6, suggesting that the segregation was due to *Pik** in IR56 (Table 9). The other 9 combinations did not segregate any susceptible plants, suggesting that the same gene in IR56 or IR70 and DVs controlled the resistance. Since blast isolates from the Philippines could not differentiate the *Pik* alleles, except *Pik-s*, no susceptible plants were obtained from the populations derived from the combinations among DVs, Kanto 51, Tsuyuake, and IR56 for *Pik**. These results confirmed that IR56 and IR70 harbored Pita and *Pik**.

Conclusions

A total of 42 IRRI-bred rice varieties were classified into seven variety groups and estimated at least seven blast resistance genes, *Pi20*(t), *Pita*, *Pik**, *Pib*, *Pik-s*, *Piz-t*, and *Pii* or *Pi3*(t), following a differential system using Philippine isolates of *P. grisea* (Ebron et al., 2004, Fukuta et al. 2007). In the present study, the presence of seven blast resistance genes, *Pi20*(t), *Pita*, *Pik**, *Pia*, *Pib*, *Pik-s*, and *Piz-t*, was confirmed through genetic analyses in 10 varieties: IR34 (VG1b), IR24 (VG2a), IR36 and IR60 (VG3), PSBRc1 (VG4), IR74 (VG5), IR56 and IR70 (VG6), and IR46 and IR64 (VG7a) (Table 5, 7, and 9). Four of these genes, *Pia*, *Pib*, *Pik-s*, and *Piz-t*, were identified also in some varieties. Two genes *Pib* and *Pik-s* or *Pik** were previously estimated in nearly all the varieties and their presence confirmed in the present study. The genes *Pita*, *Pik* alleles (*Pik** and *Pik-s*), *Piz-t*, and *Pib* were identified frequently in Indica-type varieties (Kiyosawa, 1966; Kiyosawa and Murty, 1969; Yokoo and Kiyosawa, 1970; Yokoo et al., 1978). Our results agreed with the findings of these previous studies.

The present study reports the genotypes of blast resistance in IRRI-bred rice varieties determined by segregation analyses using backcrossed progenies and allelism tests with DVs to confirm the estimated resistance genes and to postulate the masked ones in the IRRI-bred varieties' genetic backgrounds. The utility of genetic analyses based on the differential system was demonstrated in this study, and this information will be very useful whenever IRRI-bred varieties are used in any rice breeding program. Genetic analyses based on the differential system are useful tools for the identification of resistance genes, although these are complicated and relatively tedious. Over the past several years, two genes *Pita* and *Pib* have already been isolated and their detailed sequences described by Wang et al. (1999) and Jia et al. (2002), respectively. Based on this information, molecular markers specific to both genes were developed and can be used to identify the genotypes of *Pib* and *Pita* in Indica-type varieties. Therefore, Indica-type, IRRI-bred varieties, which harbor several resistance genes and show complex reactions, can be determined of these genotypes using molecular markers that can complement detection of the genes by standard genetic analysis.

The differential system, which is based on conventional methods and which does not require advanced facilities, was revealed to be useful as a fundamental tool to provide essential information to develop breeding programs for blast resistance.

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Comparison of DNA marker analysis of the blast resistance genes *Pib* and *Pita* in IRRI-bred rice varieties with gene estimation by conventional genetic analysis

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Abstract

Rice blast resistance genes are important for rice (*Oryza sativa* L.) improvement programs. DNA markers linked to resistance genes are a powerful tool to detect the presence of genes and are widely used in the selection of breeding materials through marker-assisted selection. This study was conducted to evaluate the detection ability of DNA markers for the rice blast resistance genes *Pib* and *Pita* in IRRI-bred rice varieties. Forty-two Indica-type varieties, which have been previously analyzed for the presence of *Pib* and *Pita* by conventional genetic analysis using a differential system involving standard blast isolates (*Pyricularia oryzae* Cavara) from the Philippines, were tested. To detect for the presence of *Pib* and *Pita*, previously reported PCR-based dominant markers were used. DNA fragments of *Pib* using the DNA marker Sub3-5 were amplified in 40 out of the 42 varieties examined. Additionally, DNA fragments of *Pita* using three gene specific markers were amplified in 27 or 28 varieties out of the 42 examined, depending on the marker used. The results of DNA marker analysis of 42 IRRI-bred rice varieties were largely consistent with those of previous gene estimations of *Pib* and *Pita* by a differential system. These results suggest that the efficiency of detecting blast resistance genes through use of DNA markers is dependent on the rice variety and the DNA markers. The proper markers for the *Pita* gene provide a basis for stacking other blast resistance genes into high-yielding and good-quality advanced breeding rice lines.

Keywords: DNA marker, IRRI-bred rice variety (*Oryza sativa* L.), *Pib*, *Pita*, resistance gene

Introduction

Rice blast resistance genes are important in rice (*Oryza sativa* L.) improvement programs. The resistance to blast disease is governed by a gene-for-gene relationship between the resistance gene in the host and the avirulence gene in the blast pathogen *Pyricularia oryzae* Cavara. (Silue et al. 1992). Around 40 blast resistance genes have been identified by genetic studies and five resistance genes, *Pib*, *Pita*, *Piz-5*(*Pi2*), *Piz-t*, and *Pi9*, have been isolated (Wang et al. 1999; Qu et al. 2006; Zhou et al. 2006). *Pib* and *Pita*, two major resistance genes, introgressed from Indica cultivars, encode predicted nucleotide binding proteins (Wang et al. 1999; Bryan et al. 2000). Specific gene markers are a powerful tool to detect the presence of genes and are widely used to select breeding materials through marker-assisted selection. Several gene specific markers of *Pib* and *Pita* have been developed using the genomic sequences of these genes in resistant and susceptible varieties (Wang et al. 1999; Jia et al. 2002; Fjellstrom et al. 2004).

Genetic studies of blast resistance were limited by the lack of a suitable differential system for blast resistance genes. The 12 Japonica-type differential varieties for blast resistance were selected by Yamada et al. (1976) and Kiyosawa (1981), but they carried additional gene(s) to tropical blast isolates, and these masked the reaction of the targeted gene (Noda et al. 1999). To bypass this limitation, the pathogenicities of blast isolates from the Philippines were determined using differential varieties and

lines (Yanoria et al. 2000). Several blast isolates with distinct pathogenicities were selected and studied in detail using a set of monogenic lines that contain 23 types of single resistance genes with the genetic background of a Chinese variety Lijiang-xin-tuan-heigu (Tsunematsu et al. 2000). Using this system, a total of seven resistance genes, *Pi20*, *Pita*, *Pik*† (one of *Pik* alleles, *Pik*, *Pik-h*, *Pik-m*, or *Pik-p*), *Pib*, *Pik-s*, *Piz-t*, and *Pii* or *Pi3*(t), in 42 IRRI (International Rice Research Institute) -bred rice varieties were targeted and 42 IRRI-bred varieties were classified into seven groups based mainly on the presence of *Pi20*, *Pita*, and one of *Pik*† allele gene (Ebron et al. 2004). To confirm the presence of resistance genes in IRRI-bred varieties, conventional genetic analysis, which comprise segregation analyses and allelism tests, were performed using BC_1F_2 and F_2 populations, respectively (Ebron et al. 2005).

During the 1970s and early 1980s, many IRRI-bred varieties were released and distributed worldwide, and cultivated directly in farmar's filed or used as important parental varieties in breeding programs. IR8, which was released in 1966, triggered the Green revolution in tropical countries of Asia (Hossain 1995). In the 1970s, IR36 was utilized for rice production in several Asian countries. IR64, which was released in 1985, has been widely accepted as a high-quality rice variety in many countries (Khush 1987). A more recently developed variety, IR72, displays a high yield potential, shorter growth duration, and enhanced resistance to several diseases and insect pests. IRRI-bred varieties and their progenies are now planted in rice crops worldwide. Therefore, it is important to understand blast resistance genes in IRRI-bred varieties for rice breeding.

In this study, the detection ability of gene specific markers for *Pib* and *Pita* was evaluated. Forty-two IRRI-bred varieties were analyzed using four PCR-based

Table 1. Gene identification by conventional genetic analysis for two resistance genes *Pib* and *Pita* in 42 IRRI-bred varieties

Variety group	IRRI-bred variety	*Pib*			*Pita*		
		Reaction pattern[1)]	BC_1F_2 analysis[2)]	Allelism test[2)]	Reaction pattern[1)]	BC_1F_2 analysis[2)]	Allelism test[2)]
VG 1a	IR20, IR28, IR30, IR45, IR66	+	-	-	-	-	-
VG 1b	IR29, IR34	+	+ (IR34)	+ (IR34)	-	-	-
VG 2a	IR8, IR22, IR24, IR26, PSBRc30	+	+ (IR24)	+ (IR24)	-	-	-
VG 2b	IR43	+	-	-	-	-	-
VG 2c	PSBRc2	+	-	-	-	-	-
VG 3	IR5, IR32, IR36, IR38, IR40, IR42, IR44, IR50, IR52, IR54, IR58, IR60, IR62, IR65, IR68, IR72, PSBRc4	+	+ (IR36, IR60)	+ (IR36, IR60)	+	+ (IR36, IR60)	+ (IR36)
VG 4	PSBRc1	unknown	-	-	-	-	-
VG 5	IR74	unknown	+	-	-	-	-
VG 6	IR56, IR70	unknown	-	-	+	-	+
VG 7a	IR46, IR48, IR64, PSBRc28	+	+ (IR46, IR64)	+ (IR64)	+	+ (IR46, IR64)	+ (IR64)
VG 7b	PSBRc10, PSBRc18, PSBRc20	+	-	-	+		

1) Classification of IRRI-bred varieties and genes *Pib* and *Pita* determined using a differential system involving Philippine blast isolates of *P. grisea* obtained and modified from Ebron et al. (2004).

2) Classification of IRRI-bred varieties and genes *Pib* and *Pita* determined using genetic analysis and allelism test referred from the data of Ebron et al. (2005).

+ or - indicates presence or absence of genes *Pib* and *Pita*, respectively.

dominant markers that were designed from the sequences of the blast resistance genes *Pib* and *Pita*. The results for detection of DNA fragments by PCR were compared with those of gene estimation for *Pib* and *Pita* by conventional genetic analysis.

Materials and methods

IRRI-bred rice varieties

A total of 42 Indica-type varieties at IRRI, which have been previously analyzed by conventional genetic methods, were used. Thirty-four out of the 42 Indica-type varieties are IR varieties, including IR8, IR24, IR36, IR64, and IR72, all of which are widely distributed and used in many countries. Eight out of the 42 Indica-type varieties, PSBRc1, PSBRc2, PSBRc4, PSBRc10, PSBRc18, PSBRc20, PSBRc28, and PSBRc30, were developed by IRRI and designated by the Philippine Seed Board. Six other varieties, Nipponbare, Yashiromochi, BL1, IRBLb-B (monogenic line for *Pib* derived from BL1), K1, and IRBLta-K1 (monogenic line for *Pita* derived from K1), were used as controls. The 42 IRRI-bred varieties were classified into seven groups, VG 1 to VG 7, based on a differential system (Table 1). In some cases, subgroups within a group were also identified because the variety reacted differentially to a particular isolate. VG 1, VG2, and VG 7 were further divided into two, three, and two subgroups, respectively.

DNA markers and genotyping

Whole genomic DNA was extracted from fresh leaves using the CTAB method (Murray and Thompson 1980). Primer set for *Pib*, Sub3-5 (5′- AGGGAAAAA TGGAAATGTGC -3′ and 5′-AGTAACCTTCTGCTGC CCAA-3′), was used to detect *Pib*, and primer sets for *Pita*, $Pita_{440}$ (YL153: 5′-CAACAATTTAATCATACA CG-3′ and YL154: 5′-ATGACACCCTGC-GATGCAA-3′), $Pita_{1042}$ (YL155: 5′-AGCAGGTTATAAGCTAGG CC-3′ and YL87: 5′-CTACCAACAAGTTCATCAAA-3′), and $Pita_{403}$ (YL100: 5′-CAATGCCGAGTGTGCAA AGG-3′ and YL102: 5′-TCAGGTTGAAGATGCATA GC-3′) were used for *Pita* in a previous study (Wang et al. 1999; Jia et al. 2002). The genotypes of *Pib* and *Pita* using these primers were then determined by PCR amplification in a DNA engine dyad Peltier thermal cycler (Bio-Rad). The 15 µl PCR reaction mixtures contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 200 µM dNTP, 0.2 µM primer, and 5-10 µg/ml of genomic DNA as template. The thermal cycler was programmed for a first denaturation step of 5 min at 95 ℃ , followed by 35 cycles, each of 30 s at 95 ℃, 30 s at 55 ℃ for three markers, Sub 3-5, $Pita_{440}$ (YL153/YL154), and $Pita_{1042}$ (YL155/YL87), and at 64.5 ℃ for Pita403 (YL100/YL102), and 30 s at 72 ℃ . The SSR products were resolved in 1.0% agarose gel by electrophoresis at 200 V for 1 h in 0.5 X TBE buffer. The gels were stained with ethidium bromide and photographed under ultraviolet light.

Results

Genotyping of Pib and Pita using DNA markers

BL1 and IRBLb-B carrying *Pib* were used as positive controls, while Yashiromochi and Nipponbare were used as negative controls. The presence and absence of the *Pib* genes in 42 IRRI-bred rice varieties and control varieties were confirmed using the DNA marker Sub3-5 (Fig. 1, Table 2). The DNA fragments of positive control varieties, BL1 and IRBLb-B carrying *Pib*, were amplified, while those of the negative control, Yashiromochi and Nipponbare, were not amplified. DNA fragments using Sub3-5 were amplified in all but two (PSBRc18 and IR54) of the 42 IRRI-bred rice varieties. Similarly, K1, IRBLta-K1, and Yashiromochi carrying *Pita* were used as positive controls, while Nipponbare was used as a negative control. The presence or absence of the *Pita* genes in 42 IRRI-bred rice varieties and control varieties was confirmed using three DNA markers, $Pita_{440}$ (YL153/YL154), $Pita_{1042}$ (YL155/YL87), and $Pita_{403}$ (YL100/YL102) (Fig.1, Table 2). The DNA fragments of positive control varieties, K1, IRBLta-K1, and Yashiromochi carrying *Pita* were amplified, while those of negative control, Nipponbare, were not amplified. Of the 42 IRRI-bred rice varieties examined, DNA fragments using either the $Pita_{440}$ or $Pita_{1042}$ markers were detected in 28 varieties. Using the $Pita_{403}$ marker, DNA fragments were amplified in 27 of the 42 IRRI-bred rice varieties examined.

Comparison between conventional genetic analysis and DNA marker analysis

The detection of DNA fragments for *Pib* in positive and negative control varieties was compared with the presence or absence of *Pib*. The DNA fragments of Sub3-5 in positive control varieties, BL1 and IRBLb-B, carrying *Pib* were detected while those of negative control varieties, Yashiromochi and Nipponbare, were not detected. For control varieties, the results of DNA fragments were consistent with the presence or absence of *Pib*. The detection of DNA fragments for *Pib* in 37 IRRI-bred rice varieties was consistent with the results of gene estimation for *Pib* using conventional genetic analysis, while those in two varieties were different from the results of gene estimation for *Pib* (Table 2). Although the two varieties, IR54 and PSBRc18, were found carrying *Pib* using conventional genetic analysis, DNA fragments for *Pib* were not detected. Three varieties, IR56, IR70, and PSBRc1, had not been previously examined for *Pib* using conventional genetic analysis. In summary, the results of DNA fragment detection for *Pib* in 42 IRRI-bred varieties were generally consistent with those of gene estimation by conventional genetic analysis, expected for IR54 and PSBRc18.

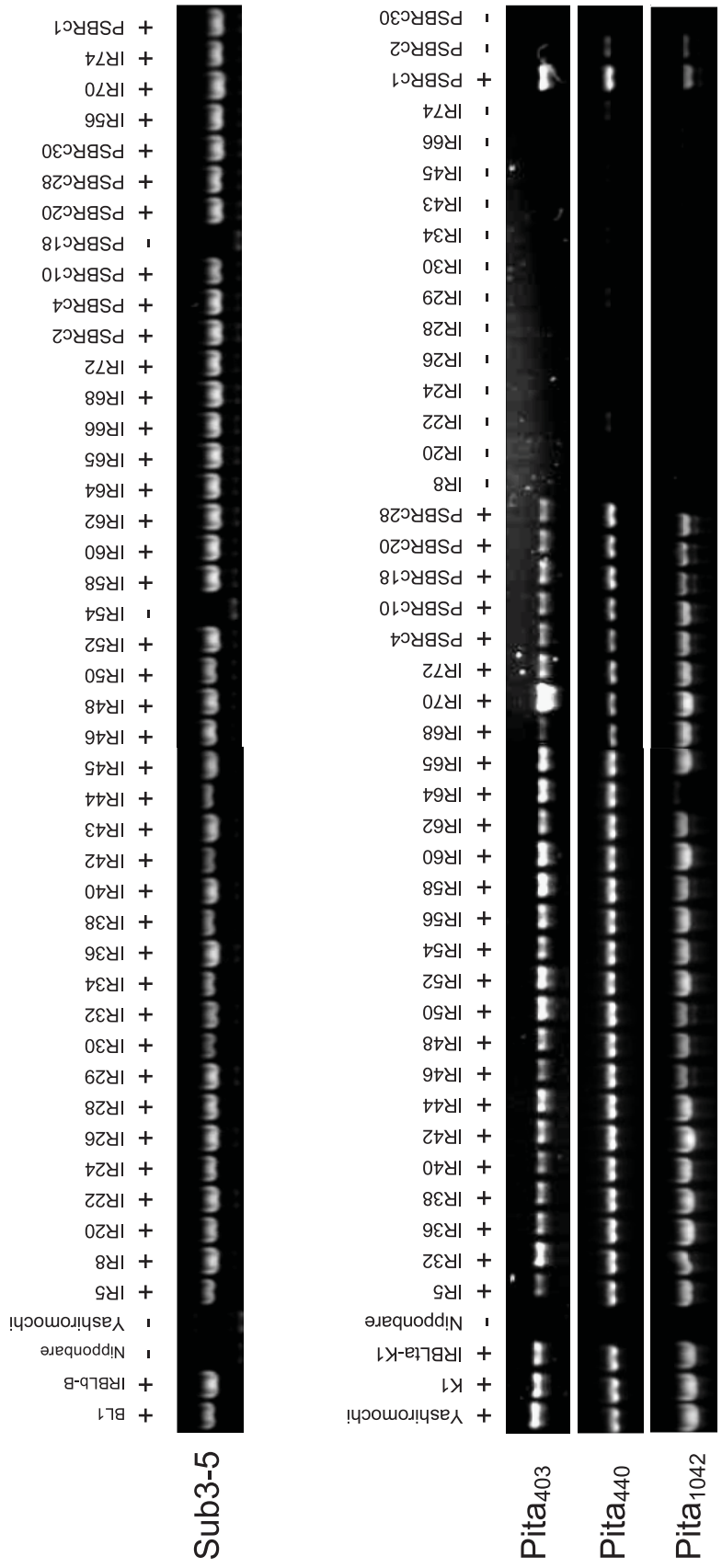

Fig. 1. Electorophoretic profiles of DNA markers, Sub3-5, $Pita_{440}$, $Pita_{1042}$, and $Pita_{403}$, used for detection of *Pib* and *Pita* in control varieties and 42 IRRI-bred varieties

Table 2. Gene identification by conventional genetic analysis and DNA marker for two resistance genes for *Pib* and *Pita* in 42 IRRI-bred varieties

IRRI-bred variety		Target resistance gene					
		Pib		*Pita*			
		Method of gene identification					
		Conventional genetic analysis	DNA marker Sub3-5	Conventional genetic analysis	DNA marker $Pita_{440}$	DNA marker $Pita_{1042}$	DNA marker $Pita_{403}$
1a[1)]	IR20	+	+	-	-	-	-
	IR28	+	+	-	-	-	-
	IR30	+	+	-	-	-	-
	IR45	+	+	-	-	-	-
	IR66	+	+	-	-	-	-
1b	IR29	+	+	-	-	-	-
	IR34	+	+	-	-	-	-
2a	IR8	+	+	-	-	-	-
	IR22	+	+	-	-	-	-
	IR24	+	+	-	-	-	-
	IR26	+	+	-	-	-	-
	PSBRc30	+	+	-	-	-	-
2b	IR43	+	+	-	-	-	-
2c	PSBRc2	+	+	-	+	+	-
3	IR5	+	+	+	+	+	+
	IR32	+	+	+	+	+	+
	IR36	+	+	+	+	+	+
	IR38	+	+	+	+	+	+
	IR40	+	+	+	+	+	+
	IR42	+	+	+	+	+	+
	IR44	+	+	+	+	+	+
	IR50	+	+	+	+	+	+
	IR52	+	+	+	+	+	+
	IR58	+	+	+	+	+	+
	IR60	+	+	+	+	+	+
	IR62	+	+	+	+	+	+
	IR65	+	+	+	+	+	+
	IR68	+	+	+	+	+	+
	IR72	+	+	+	+	+	+
	PSBRc4	+	+	+	+	+	+
	IR54	+	-	+	+	+	+
4	PSBRc1	/	+	-	+	+	+
5	IR74	+	+	-	-	-	-
6	IR56	/	+	+	+	+	+
	IR70	/	+	+	+	+	+
7a	IR46	+	+	+	+	+	+
	IR48	+	+	+	+	+	+
	IR64	+	+	+	+	+	+
	PSBRc28	+	+	+	+	+	+
7b	PSBRc10	+	+	+	+	+	+
	PSBRc20	+	+	+	+	+	+
	PSBRc18	+	-	+	+	+	+
Control	BL1	+	+	+	+	+	+
	IRBLb-B	+	+	+	+	+	+
	Yashiromochi	-	-	+	+	+	+
	Nipponbare	-	-	-	-	-	-

1) Variety group based on differential system.

'+ or - indicates presence or absence of *Pib* and *Pita*, respectively.

The DNA fragments of positive and negative control varieties for *Pita* were compared with the presence and absence of *Pita*. The DNA fragments of $Pita_{440}$, $Pita_{1042}$, and $Pita_{403}$ in positive control varieties, K1, IRBLta-K1, and Yashiromochi, carrying *Pita* were detected, while those of the negative control variety, Nipponbare, were not detected. For control varieties, the results of DNA fragment detection were consistent with the presence or absence of Pita. The detection of DNA fragments for Pita using two DNA markers, $Pita_{440}$ and $Pita_{1042}$, was consistent with the results of gene estimation for Pita using conventional genetic analysis in 40 out of the 42 IRRI-bred rice varieties (Table 2). Although PSBRc1 and PSBRc2 were not found carrying *Pita* by conventional genetic analysis, DNA fragments for *Pita* in these varieties were detected using $Pita_{440}$ and $Pita_{1042}$. The detection of DNA fragments for *Pita* using $Pita_{403}$ was consistent with the results of gene estimation for *Pita* using conventional genetic analysis in 41 out of the 42 IRRI-bred rice varieties. Although PSBRc1 was found not to carry *Pita* by conventional genetic analysis, DNA fragments for Pita were detected in PSBRc1. In summary, the results of DNA fragment detection for Pita in 42 IRRI-bred varieties were consistent with the results of gene estimation using conventional genetic analysis, expect for PSBRc1.

Discussion

The use of gene specific markers enabled the detection of the *Pib* and *Pita* genes in most IRRI-bred rice varieties that have been previously identified to contain these genes by an independent system. The *Pib* gene was previously found in IR54 and PSBRc18 by conventional genetic analysis (Ebron et al. 2004), but could not be detected using a DNA marker in this study (Table 2). For these varieties, further DNA analysis using other gene specific markers for *Pib*, such as Pibdom, which was developed by Fjellstrom et al. (2004), will be needed. Although three varieties, IR56, IR70, and PSBRc1, were not found to contain *Pib* by conventional genetic analysis, DNA fragments using gene specific marker were detected in three varieties. In previous analysis, IR56, IR70, and PSBRc1 were not found to carry *Pib* using conventional genetic analysis due to the narrow spectra of resistance in *Pib*. *Pib* showed a narrow spectra of resistance to blast isolates from the Philippines and this reaction pattern might be completely masked by the presence of $Pik^{\dagger}$. Therefore, the results of DNA analysis suggest that IR56, IR70, and PSBRc1 may contain the *Pib* gene.

Similarly, the gene specific markers, $Pita_{440}$, $Pita_{1042}$, and $Pita_{403}$, were useful and effective for *Pita* gene identification because results of DNA fragments in all IRRI-bred varieties, with the exception of PSBRc1 and PSBRc2, were the same as those obtained by conventional genetic analysis. Although the DNA fragments of *Pita* in PSBRc1 and PSBRc2 were detected, the results of the reaction pattern using conventional genetic analysis showed that PSBRc1 and PSBRc2 did not have the *Pita* gene. Additionally, allelism testing between IR56 carrying *Pita* and $Pik^{\dagger}$ and PSBRc1 carrying $Pik^{\dagger}$ showed that PSBRc1 did not contain the *Pita* gene. Thus, the sequence of the primer sites of $Pita_{440}$, $Pita_{1042}$ and $Pita_{403}$, (markers made from the Yashiromochi sequence) in PSBRc1 and PSBRc2 are almost identical to those in the Yashiromochi sequence. Parts of the *Pita* gene sequence, outside of these primer sites may differ between the Yashiromochi and the PSBRc1 and PSBRc2 varieties, because they were bred using different donors in Japan and Philippines. The new DNA marker for *Pita*, which can identify and differentiate *Pita* between Yashiromochi and the two IRRI-bred varieties, PSBRc1 and PSBRc2 will need based on the detail analysis in sequence level.

The utility of gene specific markers was demonstrated in this study, and gene detection using DNA analysis was in agreement with that of conventional genetic analysis. IR74 was not found with *Pib* using conventional genetic analysis because *Pib* was completely masked by the presence of $Pik^{\dagger}$, but IR74 was identified carrying *Pib* using segregation analysis (Table 1). The DNA fragments of gene specific markers for *Pib* in IR74 were also detected. Although *Pib* in IR74 was not detected using conventional genetic analysis, the amplification of DNA using *Pib* specific DNA marker were observed. IR74 did not harbor the functional *Pib* gene, but might remain the base sequence of the resistance gene with some modifications. This result suggests that precise gene detection was achieved using DNA analysis and conventional genetic analysis in the case of narrow resistance spectra. Three resistance genes, *Pi2*, *Piz-t*, and *Pi9*, have been cloned and sequenced (Qu et al. 2006; Zhou et al. 2006). Based on this information, gene specific markers for these genes will be developed to identify the genotypes of *Pi2*, *Piz-t*, and *Pi9*. Therefore, various varieties, which carry multiple resistance genes and show complex reactions, will be able to be identified using gene specific markers.

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Appendix 1. Protocol for DNA extraction using CTAB

CTAB extraction in small scale

1. Cut freeze-dried leaf (0.1 g: 1 cm x 5 cm) using scissors. Put small leaf pieces in 2.0 ml tube with three beads.
2. Break into pieces by bead shocker (1200 rpm, 2 m, 1 or 2 times). Remove the beads and add 750 µl of 1.5x CTAB extraction buffer and mix well.
3. Incubate in a water bath at 56℃ for 20-60 min
4. Add 600 µl of chloroform/isoamyl alcohol (24:1).
5. Gently agitate the tube in a shaker (40 rpm, 20 min)
6. Centrifuge the tube at 10000 rpm for 12 min.
7. Transfer the supernatant solution from the top phase to a new clean tube (1.5ml) using a 1 ml pipette.
8. Add 800 µl of CTAB precipitation buffer and slowly mix well.
9. Maintain for one day to two weeks (a longer period is better).
10. Centrifuge the tube at 13000 rpm for 10 min.
11. Gently pour off the supernatant and add 400 µl of high-salt TE-RNase. Mix well and remove pellet.
12. Maintain at 56 ℃ for 60 min.
13. After confirming pellet has resuspended completely, add 400 µl of 2-propanol and gently mix well.
14. Centrifuge the tube at 13000 rpm for 10 min and pour off the supernatant gently. Wash the precipitate by soaking with 400 µl of 70% ethanol.
15. Centrifuge the tube at 13000 rpm for 10 min.
16. Pour off the supernatant and invert the tubes. Completely dry the tubes.
17. Re-hydrate in 200 µl 1x TE buffer and check the scale of DNA concentration and electrophoresis.

Solutions for DNA extraction using the CTAB method

1.5 X CTAB EXTRACTION BUFFER

Components:		m.w./stock
1.5% CTAB		15 g
75 mM Tris- HCl	(1M, pH8.0)	75 ml
15 mM EDTA	(0.5M, pH8.0)	30 ml
1.05 M NaCl	(58.4)	61.4 g
Distilled water		to 1 liter

CTAB= Cetyl Trimethy Ammonium Bromide, $CH3(CH2)15(CH3)3NBr$ MW = 364.4

CTAB PRECIPITATION BUFFER

Components:		m.w./stock
1.0% CTAB		10 g
50 mM Tris-HCl	(1M, pH 8.0)	50 ml
10mM EDTA	(0.5M, pH 8.0)	20 ml
Distilled water		to 1 liter

Appendix 2. Protocol for PCR using SSR markers

A. Polymerase Chain Reaction to amplify the SSR marker

Note: Use miniscale extracted DNA (1:40 dilution).

Prepare the PCR reaction in a 96-well PCR plate. The components are in the table below.

Table. PCR cocktail components for SSR markers

Components of cocktail	Stock conc.	Final conc.	1 reaction 10µl, for SSR
Distilled water (dH_2O)	-	-	1.75 µl
PCR buffer (10XTB)	10X	1X	1 µl
dNTP	1 mM	0.1 mM	1 µl
Primer Forward	50 ng	2.5 ng	0.5 µl
Primer Reverse	50 ng	2.5 ng	0.5 µl
Taq polymerase	4 U/µl	1 U/10 µl	0.25 µl
Template DNA	5 ng/µl	25 ng/10µl reaction	5.0 µl
Total	-	-	10.0 µl

PCR profile for SSR markers

Turn on the thermocycler, place the 96-well PCR plate inside the block, close the heating lid and set the following program conditions:

1. 95 ℃ for 5 min (initial denaturation)
2. 35 cycles of the following steps
   95 ℃ for 30 s (denaturation)
   55 ℃ for 30 s (primer annealing, maybe adjusted)
   72 ℃ for 30 s (extension)
3. 20 ℃ untimed (storage)

B. Electrophoresis using 4.0% agarose and TBE buffer to check PCR product

1. Add 2-3 µl 10x loading buffers to the remaining PCR product prior to loading.
2. Load about 8 µl of sample in each well.

DNA size marker, e.g. 100 bp DNA ladder (50ng/µl), may be used for size determination.

PCR COMPONENTS

10x loading buffer

50% glycerol	25 ml
0.4% bromophenol blue	0.2 g
0.4% xylene cyanole	0.2 g

10x PCR buffer (1 liter)

dH_20	200 ml
1M Tris HCl (pH8.4)	500 ml
5M KCl	200 ml
150mM MgCl2	100 ml
Gelatin	0.1 g

1mM dNTP (4X 25 uM)

250 µl each dNTPs (100 mM)
24 ml dH_2O

Primers

100 mM stock,
5 mM working concentration:
Dilute 100 mM stock by adding 99 µl dH_2O for every 1 µl of 100 mM stock

Blast resistance genes and their selection markers in rice (*Oryza sativa* L.)

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Abstract

Blast is a serious disease caused by a fungal pathogen *Pyricularia oryzae* Cavara of rice (*Oryza sativa* L.). The use of resistant varieties is considered one of the most efficient ways of crop protection from the disease. In addition to a large amount of information accumulated during the long history of genetic studies on resistance to rice blast, recent progress in rice genomics has enabled us to use DNA markers for breeding the resistant varieties by marker assisted selection (MAS). In this report, we summarize the reported rice blast resistance genes and their selection markers to encourage further utilization for breeding. First, we assemble the information about the reported genes with regard to their number, chromosomal locations, patterns of resistance, donor strains, and molecular characterization of the cloned genes by reviewing the literature. In addition, we present some remaining issues about the nomenclature system and identification of the resistance genes. Then, we provide the first assembled list of the reported DNA markers for blast resistance genes, including the sequences of the primer pairs, genetic distances from the resistance genes, and cross combinations of the parental strains used to detect the polymorphisms. This information will help rice breeders to improve the resistance to rice blast by MAS.

Keywords: resistance gene, DNA marker, marker assisted selection (MAS), blast (*Pyricularia oryzae* Cavara), rice (*Oryza sativa* L.)

Introduction

Blast is a serious disease caused by a fungal pathogen *Pyricularia oryzae* Cavara of rice (*Oryza sativa* L.). It causes considerable damage to rice and crop loss in rice growing regions worldwide (Ou 1985; Latterell and Rossi 1986; Bonman and Mackill 1988). Although fungicides can be used to control rice blast, they generate additional costs in rice production and chemical contamination of environment and foods. Therefore, the use of resistant varieties is thought to be one of the most economically and environmentally efficient ways of crop protection from the disease.

A large amount of information has been accumulated during the long history of genetic studies on resistance to rice blast. In addition, recent progress in rice genomics will facilitate using the resistance genes in breeding by DNA marker assisted selection (MAS). However it is not easy for breeders to handle a large amount of information for DNA markers and there are no reports or databases that assemble reported marker information for rice blast resistance genes. In this report, we summarize the reported resistance gene information for rice blast and their selection markers. Such information will help rice breeders improve the resistance to rice blast through using MAS.

Overview of blast resistant genes

Since the first publication of the inheritance of host resistance to rice blast (Sasaki 1923), many reports of the resistance genes for rice blast have been published. To date, more than 70 genes and 347 quantitative trait loci (QTLs) have been detected (Bellini et al. 2008). To

encourage further utilization of these resistance genes for marker-assisted rice breeding, we first summarize this large amount of information. Although there have been many QTL mapping studies for rice blast resistance, it is still unclear whether many QTLs with minor effects can be used in marker-assisted breeding. Thus we will focus on only genes or QTLs with major effects in this review.

Number of rice blast resistance genes

The genes and major QTLs responsible for the rice blast resistance are listed in Table 1. To date, 96 genes or major QTLs have been reported.

Among the reported resistance genes, several gene symbols are synonymously used for the following two reasons.

1. The gene symbols were revised in accordance with the international committee on gene symbolization in 1995 (e.g., *Pib* and *Pis*, *Pita* and *Pi4*, *Piz* and *Pi2*, and, *Pi11* and *Pizh*).
2. The genes are suggested to be identical to each other based on their reaction pattern to blast isolates and/or linkage analysis (e.g. *Pi3*(t) and *Pi5*(t): Inukai et al. 1993, and *Pi1* and *Pi7*(t); Jeon et al. 2003).

In addition, several genes are suggested to be allelic (e.g., *Pi2*/*Piz*, *Piz-t*, and *Piz-5* on chromosome 6, *Pik*, *Pik-s*, *Pik-p*, *Pik-m*, *Pik-h*, and *Pik-g* on chromosome 11, and *Pita* and *Pita-2* on chromosome 12).

Characterization of the cloned resistance genes

As of now, eight resistance genes, *Pib* (Wang et al. 1999), *Pita* (Bryan et al. 2000), *Pik-h* (Sharma et al. 2005), *Pi9* (Qu et al. 2006), *Pi2* (Zhou et al. 2006), *Piz-t* (Zhou et al. 2006), *Pid2* (Chen et al. 2006), *Pi36* (Liu et al. 2007), and *Pi37* (Lin et al. 2007) have been isolated and cloned using map-based cloning strategies.

Seven of the cloned genes (*Pib*, *Pita*, *Pik-h*, *Pi9*, *Pi2*, *Piz-t*, *Pi36*, and *Pi37*) have the sequences including both nucleotide binding site (NBS) and leucine-rich repeat (LRR) domains, which are contained by the commonest class of plant resistance gene (Bent 1996; Hammond-Kosack and Jones 1997; Hulbert et al. 2001). The products of the NBS-LRR domain containing resistance genes seem to interact with the avirulence (Avr) gene of the pathogen and follow a gene-for-gene type resistance. Jia et al. (2000) showed that the product of the Avr gene for *Pita*, *Avr-Pita*, binds specifically to the LRR domain of the *Pita* protein by the yeast two hybrid system and an in vitro binding assay. This suggests that the product of the resistance gene binds directly with the effecter gene product of the pathogen to initiate the resistance gene mediated defense response.

Another cloned resistance gene, *Pid2*, encodes a receptor-like kinase protein with a predicted extracellular domain of a bulb-type mannose specific binding lectin (B-lectin) (Chen et al. 2006). Because of its novel extracellular domain, *Pid2* represents a new class of plant resistance genes.

Location of the resistance genes

To summarize the locations of the rice blast resistance genes in the genome, a genetic map with the positions of the reported genes was constructed (Figure 1). The map position was based on the high-density genetic map constructed by the Rice Genome Program (Chen et al. 2002; Harushima et al. 1998). The approximate genetic positions of the resistance genes were determined by identifying BAC or PAC clones that contained the sequences of the cloned gene or the flanking marker.

Many reports mention that the genes affecting blast resistance are colocalized on chromosome 6, 11, and 12 (Bryan et al. 2000; Wu et al. 2005). On chromosome 6, at least 14 genes and/or alleles (*Pi2*, *Piz*, *Piz-t*, *Piz-5*, *Pi8*(t), *Pi9*, *Pi13*, *Pi13*(t), *Pi25*(t), *Pi26*(t), *Pi27*(t) *Pid2*, *Pigm*(t), and *Pi40*(t)) have been mapped in the region near the centromere. Among them, *Pi2*, *Piz-t*, and *Pi9* are cloned and confirmed to be in the same genomic region. They are embedded in a gene cluster containing tandemly repeated NBS-LRR genes (Qu et al. 2006; Zhou et al. 2006). Zhou et al. (2006) revealed that *Pi2* and *Piz-t* are allelic and eight amino acid changes differentiate between them.

On the long arm of chromosome 11, at least nine genes (*Pi1*, *Pi7*, *Pi18*, *Pif*, *Pi34*, *Pi38*, *Pi44*(t), *PBR*, and *Pilm2*) and six alleles at the *Pik* locus (*Pik*, *Pik-s*, *Pik-p*, *Pik-m*, *Pik-h*, and *Pik-g*) have been mapped. Hayashi et al. (2006) revealed that three alleles of the *Pik* locus, *Pik*, *Pik-p*, and *Pik-m* are mapped on the same chromosomal region by linkage analysis using 300 to 2100 F_2 segregation populations. Although this result is consistent with the notion that these three genes are multiple alleles at the one locus, more detailed analysis is necessary to confirm that they are allelic. Sharma et al. (2005) identified and cloned the *Pik-h* gene from an Indica-type variety, Tetep. However its location is apart from the *Pik* cluster. The question whether the cloned *Pik-h* gene from Tetep is the same gene as the *Pik-h* gene first reported by Kiyosawa and Murty (1969) is under debate (Xu et al. 2008).

On chromosome 12, at least 17 resistance genes and/or alleles (*Pita*, *Pita-2*, *Pitq6*, *Pi6*(t), *Pi12*(t), *Pi12*(t), *Pi19*(t), *Pi20*(t), *Pi21*(t), *Pi24*(t), *Pi31*(t), *Pi32*(t), *Pi39*(t), *Pi62*(t), *Pi157*(t) *IPi*, and *IPi3*) have been mapped in the region near the centromere (the gene symbol, *Pi12*(t) is used for the different two genes as mentioned below).

Based on the data of genome-wide mapping of the NBS-LRR domain containing genes reported by Monosi et al. (2004), all three clusters of the rice resistance genes are closely associated with the clusters of NBS-LRR domain containing genes. Ballini et al. (2008) also reported that 80% of the complete resistance genes for rice blast colocalize with NBS-LRR candidates. These data suggest that non-random distribution of the resistance genes is partly due to localization of the NBS-LRR domain containing genes in the genome.

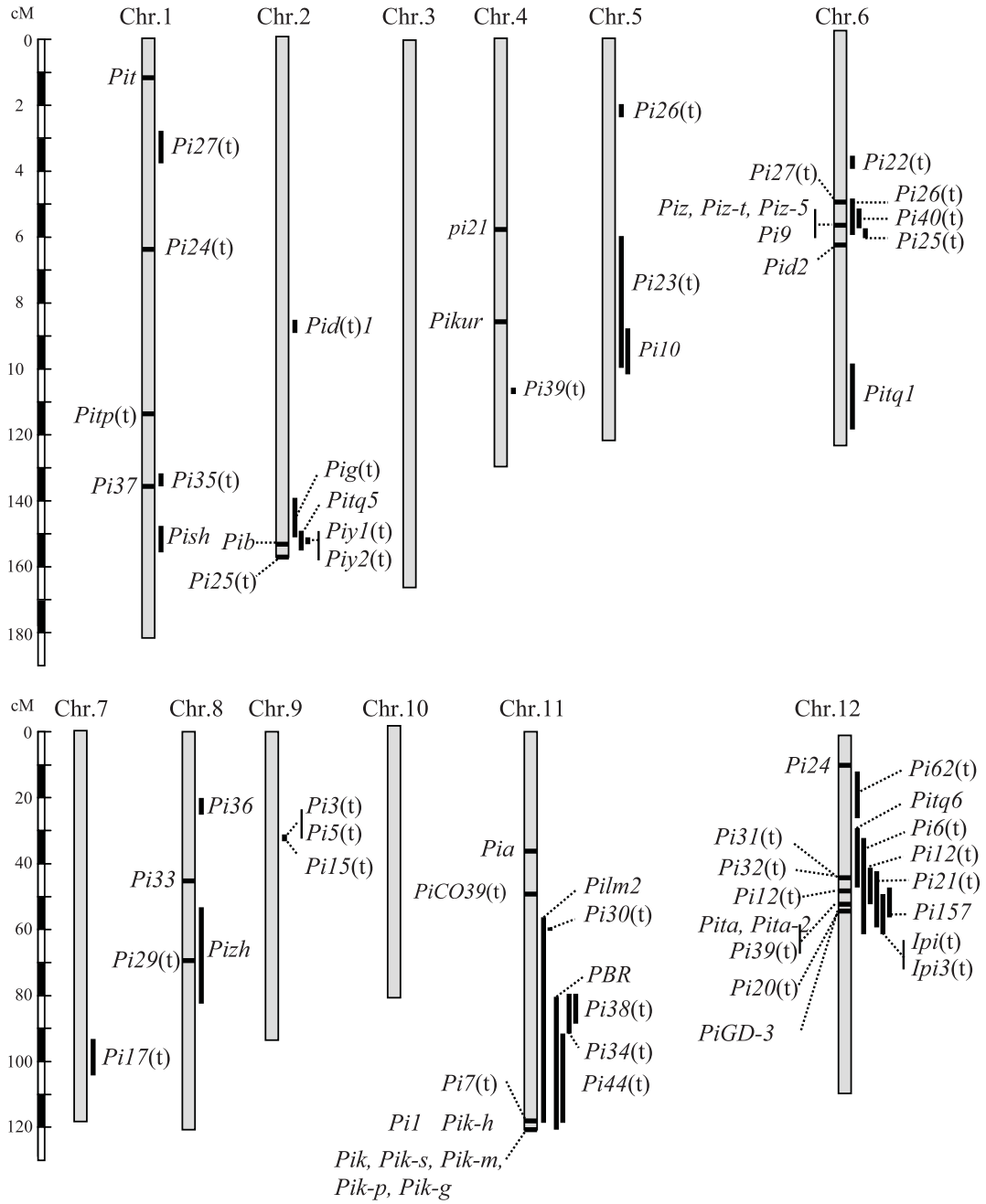

Fig. 1. Putative location of the blast resistance genes reported by 2008

The genetic location of the each genes are based on the public database (Oryzabase and Gramene) and each references (see Table 1).

Table 1. Summary of rice blast resistance genes

Gene	Map position (cM)[1)]	Donor		Type of resistance[2)]	Current status	Harboring varieties	Remarks	Reference
		Strain (original doner)	Type					
Chromosome 1								
Pit	12.2	Tjahaja	Japonica	C	Mapped within 770 kb	BL10, K59, Tongil	-	Hayashi et al. 2006
Pi27(t)	28.4-38.8	Q14	-	C	Mapped within 21.6cM	-	-	Zhu et al. 2004
Pi24(t)	64.4	Azucena	Japonica	-	QTL mapping	-	-	Sallaud et al. 2003
Pitp(t)	114.1	Tetep	Indica	-	Co-segregation marker was identified	-	-	Barman et al. 2004
Pi35(t)	132.0-136.6	Hokkai 188	Japonica	P	QTL mapping	-	-	Nguyen et al. 2006
Pi37	136.1	St. No. 1	Japonica	-	Cloned	-	-	Lin et al. 2007
Pish	148.7-154.8	Shin 2	Japonica	C	Mapped within 6.1cM	Nipponbare, Pi No. 4, Fukunishiki, Norin 22, Kusabue, BL1, Akihikari	-	Imbe et al. 1985, Fukuta et al. 2004
Chromosome 2								
Pid(t)*1*	87.5-89.9	Digu	Indica	-	Mapped within 11.8cM	-	-	Chen et al. 2004
Pig(t)	142.0-154.1	Guangchangzhan	Indica	-	Mapped within 10.3cM	-	-	Zhou et al. 2004
Pitq5	150.5-157.9	Teqing	Indica	C	QTL mapping	-	-	Tabien et al. 2000
Piy1(t)	153.2-154.1	Yanxian No.1	Indica	-	Mapped within 1.6 cM	-	-	Lei et al. 2005
Piy2(t)	153.2-154.1	Yanxian No.1	Indica	-	Mapped within 3.0 cM	-	-	Lei et al. 2005
Pib	154.1	Tohoku IL9	Japonica	C	Cloned	Tjina, BL1, IRT 13, WHD-1S-175-1-127, Teqing, Engkatek, Milek Kuning	synonimous to *Pis*	Hayasaka et al. 1995, Wang et al. 1999, Fjellstrom et al. 2004
Pi25(t)	157.9	IR64	Indica	-	QTL mapping	-	-	Sallaud et al. 2003
Pi14(t)	-	Maowang	-	C	Linkage analysis using Isozyme marker	-	linked to isozyme marker *Amp1*	Pan et al. 1996
Pi16(t)	-	AUS373	-	C	Linkage analysis using Isozyme marker	-	linked to isozyme marker *Amp1*	Pan et al. 1997
Chromosome 4								
pi21	58.6	Owarihatamochi	Japonica	P	QTL mapping	-	resessive	Fukuoka and Okuno 2001

Pikur1	86	Kuroka	Japonica	-	Linkage analysis using phenotypic marker	-	-	Goto 1988
Pi39(t)	107.4-108.2	Chubu 111 (Haonaihuan)	Japonica	-	Mapped within 0.3cM	-	-	Terashima et al. 2008
Pi(t)	-	-	-	-	-	-	-	Causse et al. 1994
Chromosome 5								
Pi26(t)	22.5-24.7	Azucena	Japonica	-	QTL mapping	-	-	Sallaud et al. 2003
Pi23(t)	59.3-99.5	Sweon 365	Japonica	-		-	-	Ahn et al. 1997
Pi10	88.5-102.8	Tongil	Indica	C	Mapped within 6.7cM	-	-	Naqbi et al. 1995, Naqbi and Chatto 1996
Chromosome 6								
Pi22(t)	38.7-41.9	Sweon 365	Japonica	-	-	-	-	Ahn et al. 1997
Pi26(t)	51.0-63.2	Gumei 2	Indica	-	QTL mapping	-	-	Wu et al. 2005
Pi27(t)	51.9	IR64	Indica	-	QTL mapping	-	-	Sallaud et al. 2003
Pi40(t)	54.1-61.6	IR65482-4-136-2-2 (Acc100882)	*Oryza australiensis*	-	Mapped within 1.8cM	-	-	Jeung et al. 2007
Piz-5	58.7	Tadukan	Indica	C	Cloned	K60, C101A51 (5173)	synonimous to *Pi2*(t), allelic to *Piz-t*, *Piz*, tightly linked with *Pi9*(t)	Zhou et al. 2006
Piz	58.7	Zenith	-	C	Mapped within 0.43cM	Fukei 67 Fukunishiki	allelic to *Piz-5*, *Piz-t*	Goto 1976, Goto et al. 1981, Hashimoto et al. 1998, Hayashi et al. 2006
Piz-t	58.7	Toride 1	Japonica	C	Cloned	IR56	allelic to *Piz-5*(*Pi2*)	Zhou et al. 2006
Pi9	58.7	75-1-127 (101141)	*Oryza minuta*	C	Cloned	-	tightly linked with *Piz-5* (*Pi2*)	Qu et al. 2006
Pi25(t)	63.2-64.6	Gumei 2	Indica	-	QTL mapping	-	-	Zhuang et al. 2001, Wu et al. 2005
Pid2	65.8	Digu	Indica	C	Cloned	-	-	Chen et al. 2006
Pigm(t)	65.8	Gumei 4	Indica	-	Mapped within 70 kb	-	-	Deng et al. 2005
Pitq1	103.0-124.4	Teqing	Indica	C	QTL mapping	-	-	Tabien et al. 2000

Pi8	-	Kasalath	Indica	C	Mapped using Isozyme marker	-	linked to isozyme markers, *Amp3*, *Pgi2* and *Piz-t*	Pan et al. 1995, Pan et al. 1996
Pi13(t)	-	Maowang	-	C	Mapped using Isozyme marker	-	linked to marker genes *Amp3* and C	Pan et al. 1996
Pi13	-	Kasalath	Indica	-	-	-	-	Hayasaka et al. 1995, Ballini et al. 2008
Chromosome 7								
Pi17(t)	94.0-104.0	DJ 123	-	C	-	-	linked to isozyme marker Est9	Pan et al. 1995, Iwata 1996
Chromosome 8								
Pi36	21.6-25.2	Q61	Indica	-	Cloned	-	-	Liu et al. 2007
Pi33	45.4	IR64, Bala	Indica	C	Mapped within 1.6cM	-	-	Berruyer et al. 2003
Pizh	53.2-84.8	Zhai-Ye-Quing	Indica	C	QTL mapping	-	synonimous to *Pi11*	Causse et al. 1994
Pi29(t)	69	IR64	Indica	-	QTL mapping	-	possibly identical to *Pi11*	Sallaud et al. 2003
PiGD-1(t)	-	Sanhuangzhan 2	-	-	QTL mapping	-	-	Liu et al. 2004
Chromosome 9								
Pii2(t)	-	Ishikari shiroke	Japonica	-	Linkage analysis using phenotypic marker	-	-	Kinoshita and Kiyosawa 1997
Pi5(t)	31.3-33.0	RIL125, RIL249, RIL260 (Moroberekan)	Japonica	C	Mapped within 170kb	-	tightly linked with *Pi3*(t)	Jeon et al. 2003
Pi3(t)	31.3-33.0	Pai-Kan-Tao	Japonica	C	Linkage analysis to other resistance genes	Taebaeg, C104PKT	tightly linked with *Pi5*(t)	Inukai et al. 1996
Pi15	31.3-34.9	GA25	-	C	Mapped within 0.7cM	-	linked with *Pi5*(t), *Pi3*(t), and *Pii*	Pan et al. 1996; 2003
Pii	-	Ishikari shiroke	Japonica	C	Linkage analysis using phenotypic marker	Fujisaka 5	-	Ise 1991
Chromosome 10								
Pi28(t)	114.7	Azucena	Japonica	-	QTL mapping	-	-	Sallaud et al. 2003
PiGD-2(t)	-	Sanhuangzhan 2		-	QTL mapping	-	-	Liu et al. 2004

Chromosome 11								
Pia	36	Aichi Asahi	Japonica	C		CO39, Zenith	-	Goto et al. 1981
PiCO39(t)	49.1	CO39	Indica	C	Mapped within 1.2cM	-	-	Chauhan et al. 2002
Pilm2	56.2-117.9	Lemont	Japonica	C	QTL mapping	-	synonimous to *Pib2*	Tabien et al. 2000; 2002
Pi30(t)	59.4-60.4	IR64	Indica	-	QTL mapping	-	-	Sallaud et al. 2003
Pi7(t)	71.4-84.3	RIL29 (Moroberekan)	Japonica	C	QTL mapping	-	identical to *Pi1*	Wang et al. 1994
Pi34	79.1-91.4	Chubu 32	Japonica	P	QTL mapping	-	-	Zenbayashi et al. 2002
Pi38	79.1-88.7	Tadukan	Indica	-	Mapped within 20cM	-	-	Gowda et al. 2006
PBR	80.5-120.3	St No.1	Indica	-	Mapped within 22.9cM	-	-	Fujii et al. 1995
Pb1	85.7-91.4	Modan	Indica	P	Mapped within 12.4cM	-	synonimous to *Pbst*	Fujii et al. 2000
Pi44(t)	91.4-117.9	RIL29 (Moroberekan)	-	C	-	-	-	Chen et al. 1999
Pik-h	101.9	Tetep	Indica	C	Mapped within 1.2cM	K3, Kaybonnet, Lemont, Lebonnet	allelic to *Pik*	Sharma et al. 2005, Fjellstrom et al. 2004
Pi1	112.1-117.9	C101LAC (Lac23)	-	C	Mapped within 11.4cM	-	identical to *Pi7*(t)	Hittalmani et al. 2000
Pik-m	115.1-117.0	Tsuyuake	Japonica	C	Mapped within 0.3cM	Tohoku IL4	allelic to *Pik*	Kaji and Ogawa 1996, Li et al. 2007
Pi18(t)	117.9	Sweon 365	Japonica	C	Mapped using RFLP marker	-	-	Ahn et al. 1996
Pik	119.9-120.3	Kusabue	Japonica	C	Mapped within 1.4cM	Kanto 51, Sasanishiki BL1	-	Hayasaka et al. 1996, Hayashi et al. 2006
Pik-p	119.9-120.3	HR22	-	C	Mapped within 2.8cM	K60	allelic to *Pik*	Hayashi et al. 2006
Pik-s	115.1-117.3	Shin 2	Japonica	C	Mapped within 2.7cM	Fujisaka 5, Caloro, B40, Zhaiyeqing 8, Bengal, M-201	allelic to *Pik*	Fjellstrom et al. 2004
Pik-g	-	GA20	-	C	Linkage analisis to other resistance genes	-	allelic to *Pik*	Pan et al. 1996
Pise1	-	Sensho	Japonica	-	Linkage analysis using phenotypic marker	-	synonimous to *Rb1*	Goto 1970

Pi f	-	Chugoku 31-1 (St. No.1)	Japonica	P	Linkage analysis using phenotypic markers	-	linked to *Pik*	Shinoda et al. 1971
Mpiz	-	Zenith	-	-	Linkage analysis using phenotypic markers	-	linked to *la*	Goto 1976
Pikur2	-	Kuroka	Japonica	-	Linkage analysis using phenotypic markers	-	linked to *la*	Goto 1988
Piisi	-	Imochi shirazu	Japonica	-	Linkage analysis using phenotypic markers	-	linked to *la*	Goto 1970
Chromosome 12								
Pi24(t)	10.3	Zhong 156	Indica	C	QTL mapping	Gumei 2, Yunxi 2, Q14, IR64	-	Zhuang et al. 1997, Koizumi 2007
Pi62(t)	12.2-26.0	Yashiromochi	Japonica	-	Mapped within 1.9cM	-	-	Wu et al. 1996
Pitq6	29.2-47.5	Teqing	Indica	C	QTL mapping	-	-	Tabien et al. 2000
Pi6(t)	32.6-63.2	Apura	-	C	-	-	-	McCouch et al. 1994
Pi12(t)	42.8-53	RIL10 (Moroberekan)	Japonica	C	-	-	-	Inukai et al. 1994
Pi21(t)	43.4-59.6	Sweon 365	Japonica	-	-	-	-	Ahn et al. 1997
Pi31(t)	44.3	IR64	Indica	-	QTL mapping	-	-	Sallaud et al. 2003
Pi32(t)	47.5	IR64	Indica	-	QTL mapping	-	-	Sallaud et al. 2003
Pi12(t)	47.6-48.2	K80 (Hong-jiao-zhan)	Indica	-	Linkage analysis using RFLP markers	-	-	Zheng et al. 1996
Ipi(t)	47.6-58.3	-	-	-	Linkage analysis using RFLP markers	-	-	Causse et al. 1994
IPi3(t)	47.6-58.3	-	-	-	Linkage analysis using RFLP markers	-	-	Causse et al. 1994
Pi157	49.5-62.2	Moroberecan	Japonica	-	Mapped within 9.5cM	-	-	Naqvi and Chattoo 1996
Pita	50.4	Taducan	Indica	C	Cloned	K1, C101PKT, Zhaiyeqing 8, Yashiromochi, C105TTP2L9	synonimous to *Pi4*, allelic to *Pita-2*	Bryan et al. 2000
Pita-2	50.4	Shimokita	Japonica	C	Mapped within 4.0cM	Pi No. 4, Reiho, IR64, Fukunishiki, Katy, Kaybonnet	allelic to *Pita*	Nakamura et al. 1997, Hayashi et al. 2006

Pi19(t)	-	Aichi Asahi	Japonica	C	Linkage analysis to other resistance genes	Aichi Asahi, Shin 2, Ishikari Shiroke, Fujisaka 5, Kusabue, Tsuyuake, Yashiro-mochi, K1, Pi No. 4, Toride 1, BL1, K59, Kanto 51, Fukunishiki, K60	linked to *Pita-2*	Hayashi et al. 1996, 1998, Iwata 1997
Pi39(t)	50.4	Q15	-	-	Mapped within 37kb	-	-	Liu et al. 2007
Pi20(t)	51.5-51.8	IR24	Indica	C	Mapped within 0.6cM	ARL 24	-	Imbe et al. 1997, Li et al. 2008
PiGD-3(t)	55.8	Sanhuangzhan 2	-	-	QTL mapping	-	-	Liu et al. 2005
Map position unidentified								
Pi67(t)	-	Tsuyuake	Japonica	-	-	-	-	Wu et al. 1996
Piis2	-	Imochi shirazu	Japonica	-	-	-	-	Goto 1970
Pise2	-	Sensho	Japonica	-	-	-	-	Goto 1970
Pise3	-	Sensho	Japonica	-	-	-	-	Goto 1970

1) The map position was based on the high-density genetic map constructed by the RGP. The approximate genetic positions of the resistance genes were determined by identifying BAC or PAC clones that contained the sequences of the cloned gene or the flanking marker.
2) C and P indicate complete resistance and partial resistance, respectively. The classifications of resistance pattern of each gene were followed according to Koizumi (2007) and each references (C: compete resistance, P: partial resistance).

Partial resistance

Blast resistance in rice is generally classified into complete and partial resistance. Complete resistance, caused by incompatible combinations between the host and pathogen strains, prevents reproduction of the pathogen, and the resistance is usually controlled by a major gene. Another form of resistance, partial resistance, is characterized by a decrease in the extent of pathogen reproduction in the compatible interaction (Parlevliet 1979). Although the partial resistance has been thought to be under polygenic control and show non pathogen race specific pattern of resistance, several recent studies suggest that not all the partial resistance have such characteristics. To date, four major genes (*Pif*, *pi21*, *Pb1*, and *Pi34*(t)) that control partial resistance are reported (Table 1; Yunoki et al. 1970; Fukuoka and Okuno 1997; Fujii et al. 1995, 2000; Zenbayashi-Sawata et al. 2002, 2005). Moreover, Zenbayashi-Sawata et al. (2005) reported that the interaction between the partial resistance gene *Pi34*(t) and a corresponding avirulence gene follows the gene-for-gene model, suggesting that the partial resistance gene does not always show non pathogen race specific pattern of resistance.

The molecular mechanism of the partial resistance genes is one of the topics for studying resistance genes. Fukuoka et al. (2005, 2007) revealed that one of the partial resistance genes, *pi21*, has sequences different from those of the previously reported complete resistance genes. Recently, Ballini et al. (2008) revealed that the reported QTLs for partial resistance are different from the mapped complete resistance genes with regard to colocalization with resistance gene analogs by meta-QTL analysis, which statistically estimates the position of one single QTL by combining the QTL obtained from different studies. These results were consistent with the notion that partial and complete resistance is governed by different types of genes.

Donor strains

Apart from two resistance genes (*Pi9* and *Pi40*(t)) that have been found in wild relatives, most rice blast resistance genes have been found in rice blast resistant cultivars. Tsunematsu et al. (2000) revealed that an Indica-type cultivar, CO 39, which has been used as a susceptible check strain also carried the rice blast resistance gene, *Pia*, and made a monogenic line harboring it. In addition, Chauhan et al. (2002) showed that CO 39 also carried another blast resistant gene, *PiCO39*(t). These observations suggest that even a variety previously considered to be susceptible might have the resistance gene which specially interacts with the unidentified isolates and is used as a donor parent of the resistance genes.

Telebanco-Yanoria et al. (2008a) surveyed genetic diversity of blast resistance in 922 rice varieties by using the standard differential blast isolates selected by Telebanco-Yanoria et al. (2008b). They revealed the relationships among the variations of the pattern of resistance for 20 standard blast isolates, geographical distribution, and the genetic variations characterized by the Isozyme types (Glaszman 1987) of the rice varieties. Such a study will help to find a novel resistance gene and enhance the diversity of the resistance genes used in rice breeding.

Problems in studying blast resistant genes

Nomenclature system

As shown in Table 1, 96 rice blast resistance genes have been reported. Information about these genes is available in databases such as Oryzabase (http://www.shigen.nig.ac.jp/rice/oryzabase/top/top.jsp) and Gramene (http://www.gramene.org/). However, the following problematic points remain to be solved.

1. The same gene symbol was given to different genes as Ballini et al. (2008) pointed out. There are at least nine redundant gene symbols, *Pi12*, *Pi13*, *Pi14*, *Pi21*, *Pi24*, *Pi25*, *Pi26*, *Pi27* and *Pi39*, that have been used to date.
2. Several studies do not follow the rules for naming and symbolization of blast resistance genes. If a blast resistance gene is identified, it should be designated with *Pi* followed by a numeral according to the Committee on Gene Symbolization (Kinoshita et al. 1994).
3. Different studies use different writing systems for the same resistance gene (e.g., *Pik-h*, *Pikh*, *Pi-kh*, Pik^h and $Pi\text{-}k^h$).

The rice blast research community should have responsibility for symbolizing the new gene to avoid a confusing situation.

Identification of the genes

As mentioned above, several gene symbols are synonymously used because they are suggested to be identical with each other based on their reaction pattern to blast isolates and/or linkage analysis (e.g., *Pi3*(t) and *Pi5*(t): Inukai et al. 1993, and *Pi1* and *Pi7*(t),: Jeon et al. 2003). However, it is difficult to confirm the identification of the two genes, because if both the two genes are dominant and tightly linked to each other, it is impossible to confirm that these genes are identical to each other by the simple allelism test. Similarly, although many of the resistance genes have been mapped on the same chromosomal region and are thought to be in a gene cluster, it is still unclear whether these genes are at tightly linked different loci or whether these genes are the alleles of one locus. Furthermore, several genes are suggested to be allelic without detailed confirmation of the allelic relationships in some cases. Information about whether the two genes are allelic is important for breeding because the alleles of one locus cannot be integrated into and fixed in one plant, while two genes at different loci can. More detailed analysis, such as high resolution mapping and positional cloning of the genes, is neces-

sary to confirm the allelic relationship of the genes.

Except for the tightly linked genes, genetic analysis based on gene segregation is a powerful tool for clarifying the identity of the genes. Toriyama et al. (1986) demonstrated that the estimation of genes with resistance to blast in rice varieties by using segregation analysis with the population derived from a cross between resistant and susceptible varieties was effective. Monogenic lines for blast resistance were developed as the first set of international standard differential variety (Tsunematsu et al. 2000, Fukuta et al. 2004b, Kobayashi et al. 2007). Such a differential system is very useful to estimate the identity of the genes by conventional segregation analysis.

Using MAS for blast resistance genes

Advantages of molecular markers for selecting resistance genes

Marker assisted selection (MAS) is a process whereby a DNA marker is used for indirect selection of the genes underlying target traits. With the fast development of molecular biotechnologies, MAS has been receiving more attention in recent years because it has advantages for the efficiency and effectiveness as compared to conventional phenotypic selection (reviewed by Collard et al. 2008; Xu and Crouch 2008). There are several advantages of using MAS for breeding instead of using conventional phenotypic selection. For example, MAS has the potential to save time and reduce the cost of breeding in cases where conventional phenotypic selection is particularly time-consuming or expensive to measure. Furthermore, selection based on DNA markers may be more reliable due to the influence of environmental factors on field trials.

MAS has been shown to be especially valuable in backcross breeding. Over 90% of the recurrent parental genotype can be recovered within two generations when a suitable number of markers (e.g., one marker every 10cM) and an adequate number of progeny are used for background selection (Tanksley et al. 1989; Xu and Crouch 2008). Since the complete resistance to rice blast is often controlled by a major gene, MAS seems useful for improving the complete resistance to rice blast by backcross breeding. In addition, MAS is a powerful tool for pyramiding two or more genes affecting blast resistance. In some cases, the phenotypic effect of the resistance gene is masked by that of another resistance gene, which is brought together into one plant, because blast resistance genes sometimes confer resistance to overlapping spectra of blast pathotypes. In this case, it is difficult to monitor the presence of multiple resistance genes without using MAS.

Markers suitable for MAS

Many types of DNA markers including restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), and cleaved amplified polymorphic sequences (CAPS), have been developed (Mohan et al. 1997). Among them, PCR markers, such as CAPS and SSRs require only small quantities of DNA from small tissue samples for genotyping. Therefore selection can be carried out at the seedlings stage. Thus these markers are cost-effective and advantageous for applied breeding.

Recently, Hayashi et al. (2006) developed a PCR-based marker system for nine rice blast resistance genes based on the information of single-nucleotide polymorphisms (SNPs) and small insertions/deletions (InDels). In this system, by using allele-specific PCR primers, the genotypes of SNP can be easily assessed according to the presence or absence of PCR-amplified products. Because SNPs and small InDels are highly abundant and widely dispersed throughout the genome in rice (Nasu et al. 2002; Yu et al. 2002, 2005; Feltus et al. 2004), such types of DNA markers can help generate a sufficient number of markers within target genomic regions.

Conventional markers developed for detecting rice blast resistance genes

To date, 8 of the rice blast resistance genes have been isolated by map based cloning and more than 14 genes have been finely mapped (Table 1). During the procedure of fine mapping of the resistance genes, DNA markers which are tightly linked to or co-segregated with the target genes can be obtained. These markers have the potential for use in MAS. In addition, several markers have been developed based on the information of the cloned resistance gene position (Jia et al. 2002; Fjellstrom et al. 2004; Hayashi et al. 2006; Kwon et al. 2008). These markers were designed according to DNA polymorphisms between resistant and susceptible varieties within or around the genes.

The PCR based markers reported to be tightly linked or co-segregated with the rice blast resistance genes are listed in Table 2. Although there are many reports of genetic linkage analysis using RFLP or AFLP markers, we excluded these types of markers from the list because RFLP or AFLP are laborious to use in a breeding program. Information for these conventional markers will encourage further utilization of the resistance genes for marker-assisted rice breeding.

Future perspectives of MAS for blast resistance genes

Because MAS uses DNA markers to indirectly select the phenotype, its efficiency is highly dependent on the strength of association between using DNA markers and genes responsible for the phenotypes. If there is a DNA marker that can distinguish the polymorphism underlying the phenotypic effect of the gene (i.e., gene specific marker or functional marker), such a marker has strong

association with the phenotype (McCouch et al. 2007). Although there have been many reports of conventional markers for rice blast resistance genes (Table 2), almost all reported markers are linked to the resistance genes (i.e., linkage marker) and there are few gene specific markers reported (Jia et al. 2002; Fjellstrom et al. 2004; Hayashi et al. 2006; Kwon et al. 2008). Since such linkage markers have weaker association with phenotype than gene specific markers, there are a few limitations on applying them to MAS as discuss below. Thus breeders should use them as a support tool for conventional phenotypic selection.

Recombination between markers and genes

Association between the linkage markers and the genes is mainly dependent on the genetic distance between them. In general, the association becomes stronger when more tightly linked markers are used. However, even though the marker is tightly linked to the gene, recombination between them will possibly occur during the breeding procedure using MAS (as discussed by Fjellstorm et al. (2004) about the relationship between the marker, Pibdom, and the gene, *Pib*). If recombination occurs, there will be no association between the marker and the phenotype and, thus, it will be impossible to select the phenotype by that marker. As a result, the plant that does not harbor the target gene may be selected as a false positive. To reduce the possibility a false positive selection, it is necessary to confirm the introgression of the genetic region where the target gene is by using the gene specific marker or two linkage markers which are on both sides of the flanking region of the gene.

Linkage drag

Even if one can confirm the introgression of the target genetic region by two linkage markers, another problem of the linkage drag still remains (Collard et al. 2008; Hospital 2001). When the linkage marker is used to introduce the resistance gene from the donor strain, not only the resistance gene, but also the chromosomal fragment between the resistance gene and linkage markers is inevitably introduced. Thus there is a probability that the undesirable genes on the chromosome of the donor strain are introduced together with the resistance genes.

Limitation for the universality of the markers

One of the big issues is knowing whether one marker set designed for specific cross or population can be applied to other crosses or populations. Because the polymorphisms detected in linkage marker systems do not affect the phenotype of the target gene, such polymorphisms do not always exist between resistance and susceptible strains. Therefore, one marker set which is useful in a specific cross combination does not always work well in other cross combinations. Although several reports showed that linkage markers can be successfully used to screen for cultivars with resistance to rice blast (Fjellstrom et al. 2004; Yi et al. 2004; Wang et al. 2007), almost all markers have never been evaluated as to whether they can be applied to other cross combinations. Information about the markers and their applicability to the combinations of the strains will be valuable for the rice breeding using MAS.

Conclusion

Recent progress in rice genomics has facilitated finding new resistance genes in blast disease. The volume of publications identifying the new resistance genes will increase in this era of genomics. To avoid confusion, it will be necessary to characterize the resistance genes and organize information about them in an easily understandable format.

For gene characterization, a differential system for rice blast is essential. In the IRRI-Japan Collaborative Research Project, we released the monogenic lines and have been developing NILs together with markers for MAS for blast resistance genes. These lines will be useful not only as gene sources for breeding blast resistance but also as sets of international standard differential varieties used for characterizing the resistance genes. As the number of resistance genes increases, the number of their selection markers applicable to MAS will increase in the future. To enhance the utilization of the selection markers in MAS, it is also necessary to integrate marker information into an easily utilizable database. In this report, we assembled the reported markers for the rice blast resistance genes. This information will encourage the application of MAS in rice breeding programs.

Table 2. Summary of conventional DNA markers for the rice blast resistance genes

Target gene	Type of marker	Marker name	Distance	Primer		Population used (Resistant /Susceptible varieties)	Restriction enzyme	Reference
				Forward	Reverse			
Chromosome 1								
Pit	SNP	t311	0.44	CGTGAACCCAAGGCACCAGTATTC (Koshihikari-specific primer)	CATGTAGTTCTGGATGTTGTAGCTACTC	K59/Koshihikari		Hayashi et al. 2006
				CGTGAACCCAAGGCACCAGTATTA (K59-specific primer)	CATGTAGTTCTGGATGTTGTAGCTACTC	K59/Koshihikari		Hayashi et al. 2006
	SNP	t256	0	GGATAGCAGAAGAACTTGAGACTG (Koshihikari-specific primer)	CATGTCTTTCAACATAAGAAGTTCTC	K59/Koshihikari		Hayashi et al. 2006
				GGATAGCAGAAGAACTTGAGACTA (K59-specific primer)	CATGTCTTTCAACATAAGAAGTTCTC	K59/Koshihikari		Hayashi et al. 2006
	SNP	t8042	0.28	CTCAAGATTGTATCGTCGACGACTA (Koshihikari-specific primer)	GAGAGGTTTGCAGCCAGACCAGG	K59/Koshihikari		Hayashi et al. 2006
				CTCAAGATTGTATCGTCGACGACTC (K59-specific primer)	GAGAGGTTTGCAGCCAGACCAGG	K59/Koshihikari		Hayashi et al. 2006
Pi27(t)	SSR	RM151	11.9	GGCTGCTCATCAGCTGCATGCG	TCGGCAGTGGTAGAGTTTGATCTGC	Q14/Q61		Zhu et al. 2004
	SSR	RN259	9.7	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT	Q14/Q61		Zhu et al. 2004
Pitp(t)	SSR	RM246	0	GAGCTCCATCAGCCATTCAG	CTGAGTGCTGCTGCGACT	Tetep/CO39		Barman et al. 2004
Pi35(t)	SSR	RM1216	<3.5	TTCCCCAATGGAACAGTGAC	AGGGTCTACCACCCGATCTC	Hokkai188 /Danghang-Shali		Nguyen et al. 2006
	SSR	RM1003	<3.5	GATTCTTCCTCCCCTTCGTG	TTCCTGTCAGAACAGGGAGC	St. No. 1/C101PKT, CO39, or AS20-1		Nguyen et al. 2006
Pi37	SSR	RM302	0	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC	St. No. 1/C101PKT, CO39, or AS20-1		Chen et al. 2005
	SSR	RM212	0	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG	St. No. 1/C101PKT, CO39, or AS20-1		Chen et al. 2005
	SSR	FPSM1	0.07	TTGAACATGATCCACCCCAC	ATTCCCGTAGCCGTAGAGTC	St. No. 1/C101PKT, CO39, or AS20-1		Chen et al. 2005

	SSR	FPSM2	0.14	GAAGGTCCATCAAACGCTGC	CTCGCGGACAAGACGATACG	St. No. 1/C101PKT, CO39, or AS20-1	Chen et al. 2005
	SSR	FPSM4	0	CCTTCCAGTCCTCGTTATCG	CCACGCGACCCTGTTTGAGA	St. No. 1/C101PKT, CO39, or AS20-1	Chen et al. 2005
	STS	S15628	0	GGATGAGCTCACCGAGCAAC	AGGCTATAACACTGCAGCGG	St. No. 1/C101PKT, CO39, or AS20-1	Chen et al. 2005
	STS	FSTS1	0	CGCTGCATGGCACTAACCCT	CAAGAGGCTGGAACAGACAC	St. No. 1/C101PKT, CO39, or AS20-1	Chen et al. 2005
	STS	FSTS2	0.14	GGAACTGCGGCGAAAGGAAT	TCAGGAAGCCGTACATTAGG	St. No. 1/C101PKT, CO39, or AS20-1	Chen et al. 2005
	STS	FSTS3	0	GCCGCTGTGGCCTCGTCAATCTACATCAAG	AAGGAAGAGGAGATCGCTATCGGAGGGGCA	St. No. 1/C101PKT, CO39, or AS20-1	Chen et al. 2005
	STS	FSTS4	0	CAGGCTCAGGAACGACACG	GCTACGACGCGCTGTGGAAT	St. No. 1/C101PKT, CO39, or AS20-1	Chen et al. 2005
Chromosome 2							
Pid1(t)	SSR	RM262	14.5	CATTCCGTCTCGGCTCAACT	CAGAGCAAGGTGGCTTGC	Digu/LTH	Chen et al. 2004
Pig(t)	SSR	RM166	2.4 -4.0	GGTCCTGGGTCAATAATTGGGTTACC	TTGCTGCATGATCCTAAACCGG	Guangchangzhan /LTH	Zhou et al. 2004
		RM208	1.2 -6.3	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC	Guangchangzhan /LTH	Zhou et al. 2004
Piy1	SSR	RM3248	1.3	AGAAGGTTGCTTTCTTGGCC	CTTGCAAGGTCTGTTGCATC	Yanxian No. 1/LTH	Lei et al. 2005
		RM20	1.7	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG	Yanxian No. 1/LTH	Lei et al. 2005
Piy2	SSR	RM3248	1.3	AGAAGGTTGCTTTCTTGGCC	CTTGCAAGGTCTGTTGCATC	Yanxian No. 1/LTH	Lei et al. 2005
		RM20	1.7	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG	Yanxian No. 1/LTH	Lei et al. 2005
Pib	SNP	b213**	-	GCATTAGATAGTGATGAAAGCCGA (Koshihikari-specific primer)	TGTTCATCCAGGCAATTGGC	BL1/Koshihikari	Hayashi et al. 2006
				GCATTAGATAGTGATGAAAGCCGG (BL1-specific primer)	TGTTCATCCAGGCAATTGGC	BL1/Koshihikari	
	SNP	b28	1.2	GACTCGGTCGACCAATTCGCA (Koshihikari-specific primer)	ATCAGGCCAGGCCAGATTTG	BL1/Koshihikari	Hayashi et al. 2006

				GACTCGGTCGACCAATTCGCC (BL1-specific primer)	ATCAGGCCAGGCCAGATTTG	BL1/Koshihikari	
	SNP	b2**	0	GCATTAGATAGTGATGAAAGCATA (Koshihikari-specific primer)	AATGGACTGGTGTTCATCCAGGC	BL1/Koshihikari	Hayashi et al. 2006
				GCATTAGATAGTGATGAAAGCCGG (BL1-specific primer)	AATGGACTGGTGTTCATCCAGGC	BL1/Koshihikari	
	SNP	b3989	1.2	TGTAAGCGCGGGATATCCGA (Koshihikari-specific primer)	TTGTGAGCTTTGCCACTCCAC	BL1/Koshihikari	Hayashi et al. 2006
				TGTAAGCGCGGGATATCCGG (BL1-specific primer)	TTGTGAGCTTTGCCACTCCAC	BL1/Koshihikari	
	SSR	RM138	3.1	AGCGCAACAACCAATCCATCCG	AAGAAGCTGCCTTTGACGCTATGG	Gilfmont/Te-Qing	Fjellstrom et al. 2004
	SSR	RM166	2.3	GGTCCTGGGTCAATAATTGGGTTACC	TTGCTGCATGATCCTAAACCGG	Gilfmont/Te-Qing	Fjellstrom et al. 2004
	SSR	RM208	0	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC	Gilfmont/Te-Qing	Fjellstrom et al. 2004
	SSR	RM266	2.2	TAGTTTAACCAAGACTCTC	GGTTGAACCCAAATCTGCA	Gilfmont/Te-Qing	Fjellstrom et al. 2004
	SNP	Pibdom***	0	GAACAATGCCCAAACTTGAGA	GGGTCCACATGTCAGTGAGC	Gilfmont/Te-Qing	Fjellstrom et al. 2004
	SSR	RM138	1.5	AGCGCAACAACCAATCCATCCG	AAGAAGCTGCCTTTGACGCTATGG	Maybelle/Te-Qing	Fjellstrom et al. 2004
	SSR	RM166	1.5	GGTCCTGGGTCAATAATTGGGTTACC	TTGCTGCATGATCCTAAACCGG	Maybelle/Te-Qing	Fjellstrom et al. 2004
	SSR	RM208	0	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC	Maybelle/Te-Qing	Fjellstrom et al. 2004
	SSR	RM266	1.5	TAGTTTAACCAAGACTCTC	GGTTGAACCCAAATCTGCA	Maybelle/Te-Qing	Fjellstrom et al. 2004
	-	NSb*	-	ATCAACTCTGCCACAAAATCC	CCCATATCACCACTTGTTCCCC	-	Kwon et al. 2008
Chromosome 4							
Pi21	STS	P702D03_#79	0	AGAAGGTGGAGTACGACGTGAAGA	AGTTTAGTGAGCCTCTCCACGATTA	Nipponbare, Aichi Asahi /Owarihatamochi	Fukuoka et al. 2007

Pi39	SSR	RM3843	0	ACCCTACTCCCAACAGTCCC	GGGGTCGTACGCTCATGTC	Chubu 111 /Mineasahi	Terashima et al. 2007
	SSR	RM5473	0	ACCACAAACGATCGCGTC	GAGATTAACGTCGTCCTCCG	Chubu 111 /Mineasahi	Terashima et al. 2007
Chromosome 5							
Pi10	InDel	OPF62700	<7	GGGAATTCGGTTTTACAACCACCG	GGGAATTCGGATCTCCGGGGGTAG	Tongil/CO39	Naqvi and Chattoo 1996
	InDel	OPF62700	<7	TTTTACAACCACCGGTTTTATGAC	ATCTCCGGGGGTAGAGCACTGTTT	Tongil/CO39	Naqvi and Chattoo 1996
Chromosome 6							
Pi40(t)	SSR	RM3330	2.4	ATTATTCCCCTCTTCCGCTC	AAGAAACCCTCGGATTCCTG	IR65482-4-136-2-2 /Jinbubeyo	Jeung et al. 2007
	SSR	RM527	1.1	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG	IR65482-4-136-2-2 /Jinbubeyo	Jeung et al. 2007
	CAPS	S2539	3.8	GGACTGAGATGGAATGTGCT	GTAGAGTGATGACAAAATGACAA	IR65482-4-136-2-2 /Jinbubeyo -	Jeung et al. 2007
Piz	InDel	z4794	0.32	CACGCCACCCTTCAATGGAGACT	TGAATGTGAGAGGTTGACTGTGG	Fukunishiki /Koshihikari	Hayashi et al. 2006
	SNP	z60510	0.11	GGAGTTGGTGCGACGGTGCCGTTAT (Koshihikari-specific primer)	GCGCGGACCGGCCAGCTAGGTGAC	Fukunishiki /Koshihikari	Hayashi et al. 2006
				GGAGTTGGTGCGACGGTGCCGTTAC (Fukunishiki-specific primer)	GCGCGGACCGGCCAGCTAGGTGAC	Fukunishiki /Koshihikari	Hayashi et al. 2006
	SNP	z5765	0.13	AATGTGAAATTGGATGAGCCGGATA (Koshihikari-specific primer)	TTACCGATGTTCGTCGCTCTCAGG	Fukunishiki /Koshihikari	Hayashi et al. 2006
				AATGTGAAATTGGATGAGCCGGATG (Fukunishiki-specific primer)	TTACCGATGTTCGTCGCTCTCAGG	Fukunishiki /Koshihikari	Hayashi et al. 2006
	SNP	z56592	0	GGACCCGCGTTTTCCACGTGTAC (Koshihikari-specific primer)	AGGAATCTATTGCTAAGCATGAC	Fukunishiki /Koshihikari	Hayashi et al. 2006
				GGACCCGCGTTTTCCACGTGTAA (Fukunishiki-specific primer)	AGGAATCTATTGCTAAGCATGAC	Fukunishiki /Koshihikari	Hayashi et al. 2006
	SNP	z565962	-	AAGAAATAATATTTTTGAAACATGGCAAAT (Koshihikari-specific primer)	CCATGGTGGTAACTGGTATGTG	Fukunishiki/ Koshihikari	Hayashi et al. 2006
				AAGAAATAATATTTTTGAAACATGGCAAAG (Fukunishiki-specific primer)	CCATGGTGGTAACTGGTATGTG	Fukunishiki /Koshihikari	Hayashi et al. 2006

Piz-t	InDel	z4794	0.41	CACGCCACCCTTCAATGGAGACT	TGAATGTGAGAGGTTGACTGTGG	Toride /Koshihikari	Hayashi et al. 2006
	SNP	z60510	0.17	GGAGTTGGTGCGACGGTGCCGTTAT (Koshihikari-specific primer)	GCGCGGACCGGCCAGCTAGGTGAC	Toride /Koshihikari	Hayashi et al. 2006
				GGAGTTGGTGCGACGGTGCCGTTAC (Toride 1-specific primer)	GCGCGGACCGGCCAGCTAGGTGAC	Toride /Koshihikari	Hayashi et al. 2006
	SNP	z5765	0.17	AATGTGAAATTGGATGAGCCGGATA (Koshihikari-specific primer)	TTACCGATGTTCGTCGCTCTCAGG	Toride /Koshihikari	Hayashi et al. 2006
				AATGTGAAATTGGATGAGCCGGATG (Toride 1-specific primer)	TTACCGATGTTCGTCGCTCTCAGG	Toride /Koshihikari	Hayashi et al. 2006
	SNP	zt56591	0	TTGCTGAGCCATTGTTAAACG (Koshihikari-specific primer)	ATCTCTTCATATATATGAAGGCCAC	Toride /Koshihikari	Hayashi et al. 2006
				TTGCTGAGCCATTGTTAAACA (Toride 1-specific primer)	ATCTCTTCATATATATGAAGGCCAC	Toride /Koshihikari	Hayashi et al. 2006
	SNP	zt5659	0	GGACCCGCGTTTTCCACGTGTAC (Koshihikari-specific primer)	CATCCACGGGCTCTCGGACATC	Toride /Koshihikari	Hayashi et al. 2006
				GGACCCGCGTTTTCCACGTGTAA (Toride 1-specific primer)	CATCCACGGGCTCTCGGACATC	Toride /Koshihikari	Hayashi et al. 2006
Pigm(t)	CAPS	C26348	2	GGGGAGTATCTGCCTATCTG	CGTCACCACCTTATCGTTC	Gumei 4/Maratelli *XbaI*	Deng et al. 2006
	InDel	S47656	2.3	CGGGCTTCTTCTCCTCCTT	TCCGCAATCTATCTGTTATCCTC	Gumei 4/Maratelli	Deng et al. 2006
Chromosome 8							
Pi36	SSR	RM5647	0.4	ACTCCGACTGCAGTTTTTGC	AACTTGGTCGTGGACAGTGC	Q61/Aichi Asahi, or LTH	Liu et al. 2005
	CAPS	CRG2	0.2	GGCCTCCTTCCCTTCTCTCT	TGTGGAGGACAACGGGAGAG	Q61/Aichi Asahi, or LTH *HinfI*	Liu et al. 2005
	CAPS	CRG3	0	GCTAGCAAGCATGGAGTTCGT	AGCGGGTAAGGTAGCATAGGT	Q61/Aichi Asahi, or LTH *HinfI*	Liu et al. 2005
	CAPS	CRG4	0	TAGCTACAAGACCGTCGTGCC	GGCATAGAGCACCCTCAGTTC	Q61/Aichi Asahi, or LTH *HaeIII*	Liu et al. 2005
Pi33	SSR	RM72	<11.5	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG	IR64/Azucena	Berruyer et al. 2003
	SSR	RM44	<11.5	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC	IR64/Azucena	Berruyer et al. 2003

Chromosome 9								
Pi5(t)	CAPS	94A20r	5.2	AATTCCATTCGCCACCGAGTGCTC	TCTCAGTATAGAACACTAACTCTA	RIL125, RIL249, orRIL260/CO39	*AvaI*	Jeon et al. 2003
	CAPS	76B14f	0	GTCTTGGACTTAAAGCACTACC	TGAGAAACTGGTTCAAATTGGC	RIL260/M202	*DraI*	Jeon et al. 2003
	CAPS	40N23r	0	TGTGAGGCAACAATGCCTATTGCG	CTATGAGTTCACTATGTGGAGGCT	RIL260/M202	*EcoRI*	Jeon et al. 2003
	SNP	JJ817*	-	GATATGGTTGAAAAGCTAATCTCA	ATCATTGTCCTTCATATTCAGAGT	RIL260/M202		Kwon et al. 2008
Chromosome 11								
Pia	CAPS	yca72	-	AGGAGAAGAAGCCACCAAGG	GAGCTGCCACATCTTCCTT	-	*HinfI*	Kwon et al. 2008
PiCO39(t)	CAPS	RGA8	0	AATAATCACAACACGGAAGAATCATGTG	AGGTAGCTTGAGTGAGACAAAACTGAGG	CO39/51583	*Sau3AI*	Chauhan et al. 2002
	CAPS	RZ141	10.5	GCCAAAATTGGATGTATAGCG	CGTGTTAAGACAATTCCACGTC	CO39/51583	*Sau3AI*	Chauhan et al. 2002
	CAPS	RGACO39	0	CTTTCCATTGAGTCTTGAAGTCTTTGT	GGTAACTAACTTGAGGGAACTTCCAGA	CO39/51583	*HindIII*	Chauhan et al. 2002
Pi38	SSR	RM206	4	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	Tadukan/CO39		Gowda et al. 2006
		RM21	16	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG	Tadukan/CO39		Gowda et al. 2006
Pik	InDel	k6816	1.4	TCGCCGATGCGGTTGATTTACTC	CGTATTTTGTGTTGTTAGGAGATAAGG	Kanto51/OSIL 235, or Koshihikari		Hayashi et al. 2006
		k2167	<1.4	CGTGCTGTCGCCTGAATCTG	CACGAACAAGAGTGTGTCGG	Kanto51/OSIL 235, or Koshihikari		Hayashi et al. 2006
	SNP	k6438	1.4	GCGACCCTGTCTTTGGACTGC (Kanto 51-specific primer)	GAATGATGAGGAGAGAAGGCTGTCG	Kanto51/OSIL 235, or Koshihikari		Hayashi et al. 2006
				GCGACCCTGTCTTTGGACTGG (OSIL235-specific primer)	GAATGATGAGGAGAGAAGGCTGTCG	Kanto51/OSIL 235, or Koshihikari		Hayashi et al. 2006
	SNP	k6415	-	CTAATGGAATTAAACGGTTGAGCTG (Kanto 51-specific primer)	ATCCCGATGTCATCGATCAC	Kanto51/OSIL 235, or Koshihikari		Hayashi et al. 2006
				CTAATGGAATTAAACGGTTGAGCTA (Koshihikari-specific primer)	ATCCCGATGTCATCGATCAC	Kanto51/OSIL 235, or Koshihikari		Hayashi et al. 2006

	SNP	k8823	0	GTTGTGGGTTCCTCTATACAACT (Kanto 51-specific primer)	GCATGACAGATGGAAGTGTAGATGG	Kanto51/OSIL 235, or Koshihikari	Hayashi et al. 2006
				GTTGTGGGTTCCTCTATACAACA (OSIL235-specific primer)	GCATGACAGATGGAAGTGTAGATGG	Kanto51/OSIL 235, or Koshihikari	Hayashi et al. 2006
	SNP	k8824	0	CCACGCTCCTAGCTACCCCG (Kanto 51-specific primer)	ACAAGGGAACCCAGAAACTC, ATCGCAGCGACTGTATGTGC	Kanto51/OSIL 235, or Koshihikari	Hayashi et al. 2006
				GTTGTGGGTTCCTCTATACAACA (Koshihikari-specific primer)	ACAAGGGAACCCAGAAACTC, ATCGCAGCGACTGTATGTGC	Kanto51/OSIL 235, or Koshihikari	Hayashi et al. 2006
	SNP	k3951	0	AAGTAACAACATGGTCAATAGTAC (Kanto 51-specific primer)	CCAGAATTTACAGGCTCTGG	Kanto51/OSIL 235, or Koshihikari	Hayashi et al. 2006
				AAGTAACAACATGGTCAATAGTAA (Koshihikari-specific primer)	CCAGAATTTACAGGCTCTGG	Kanto51/OSIL 235, or Koshihikari	Hayashi et al. 2006
	SNP	k39512	0	GCCACATCAATGGCTACAACGTC (OSIL235-specific primer)	CCAGAATTTACAGGCTCTGG	Kanto51/OSIL 235, or Koshihikari	Hayashi et al. 2006
				GCCACATCAATGGCTACAACGTT (Koshihikari-specific primer)	CCAGAATTTACAGGCTCTGG	Kanto51/OSIL 235, or Koshihikari	Hayashi et al. 2006
Pik-m	InDel	k6816	1.3	TCGCCGATGCGGTTGATTTACTC	CGTATTTTGTGTTGTTAGGAGATAAGG	Tsuyuake /99SL-44, or Koshihikari	Hayashi et al. 2006
		k2167	0	CGTGCTGTCGCCTGAATCTG	CACGAACAAGAGTGTGTCGG	Tsuyuake /99SL-44, or Koshihikari	Hayashi et al. 2006
	SNP	k641	1.3	GGCTGGAACACCAACATCCATGG (Tsuyuake-specific primer)	GCGCTGGACTTGGAACTAGTGC	Tsuyuake /99SL-44, or Koshihikari	Hayashi et al. 2006
				GCTGGGACACCAACATCCATGC (99SL-44-specific primer)	GCGCTGGACTTGGAACTAGTGC	Tsuyuake /99SL-44, or Koshihikari	Hayashi et al. 2006
	SNP	k6441	0	TGTAAAATACTTTCTATGCGCAGGT (Tsuyuake-specific primer)	GTTTATGGAGAGAGTAGTCGCTG	Tsuyuake /99SL-44, or Koshihikari	Hayashi et al. 2006
				TGTAAAATACTTTCTATGCGCAGGC (99SL-44-specific primer)	GTTTATGGAGAGAGTAGTCGCTG	Tsuyuake /99SL-44, or Koshihikari	Hayashi et al. 2006
	SNP	k4731	<3.5	GCAGATGCATCAGCCAGTGAGTG (Tsuyuake-specific primer)	GTGCAGGACCGGCACGCAG	Tsuyuake /99SL-44, or Koshihikari	Hayashi et al. 2006
				GCAGATGCATCAGCCAGTGAGTT (Koshihikari-specific primer)	GTGCAGGACCGGCACGCAG	Tsuyuake /99SL-44, or Koshihikari	Hayashi et al. 2006
	SNP	k7237	3.5	AGTGTGCCTCGTTGCCCTGTTCTG (Tsuyuake-specific primer)	TATAGCTTGCATTAGATCCTCTCTGTTGA	Tsuyuake /99SL-44, or Koshihikari	Hayashi et al. 2006

				AGTGTGCCTCGTTGCCCTGTTCTA (99SL-44-specific primer)	TATAGCTTGCATTAGATCCTCTCTGTTGA	Tsuyuake /99SL-44, or Koshihikari	Hayashi et al. 2006
Pik-p	SNP	k641	1.9	GGCTGGAACACCAACATCCATGG (K60-specific primer)	GCGCTGGACTTGGAACTAGTGC	K60/Koshihikari	Hayashi et al. 2006
				GCTGGGACACCAACATCCATGC (Koshihikari-specific primer)	GCGCTGGACTTGGAACTAGTGC	K60/Koshihikari	Hayashi et al. 2006
	SNP	k39575		GGTGTTTGGGAACCTGAACCCTG (60-specific primer)	TTTCTGTTCGTCGGATGCTC	K60/Koshihikari	Hayashi et al. 2006
				GGTGTTTGGGAACCTGAACCCTA (Koshihikari-specific primer)	TTTCTGTTCGTCGGATGCTC	K60/Koshihikari	Hayashi et al. 2006
	SNP	k403	0.97	CATCTTGACGACAACGACACCATTAGTTA (k60-specific primer)	CCAAAATGAACAAACCGATTCGAC	K60/Koshihikari	Hayashi et al. 2006
				CTTGACGACGACGACACCATTAGTTG (Koshihikari-specific primer)	CCAAAATGAACAAACCGATTCGAC	K60/Koshihikari	Hayashi et al. 2006
	SNP	k3957	0	ATAGTTGAATGAATGGAATGGAAC (K60-specific primer)	CTGCGCCAAGCAATAAAGTC	K60/Koshihikari	Hayashi et al. 2006
				ATAGTTGAATGAATGGAATGGAAT (Koshihikari-specific primer)	CTGCGCCAAGCAATAAAGTC	K60/Koshihikari	Hayashi et al. 2006
Pik-h	SSR	RM206	0.7	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	Tetep/HP2216	Sharma et al. 2002
	SSR	TRS26	0.7	GGAGAGCCAATCTGATAAGCA	CAACAAGAGAGGCAAATTCTCA	Tetep/HP2216	Sharma et al. 2002
	SSR	TRS33	0.6	AAGAAGAAGCGTACGCATGAAT	GTCCTGGAGGGGAGGAGA	Tetep/HP2216	Sharma et al. 2002
	SSR	RM144	4	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	Maybelle/ Kaybonnet	Fjellstrom et al. 2004
	SSR	RM224	0	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	Maybelle/ Kaybonnet	Fjellstrom et al. 2004
	SSR	RM1233	3.3	TTCGTTTTCCTTGGTTAGTG	ATTGGCTCCTGAAGAAGG	Maybelle/ Kaybonnet	Fjellstrom et al. 2004
	SSR	RM144	0.9	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	Maybelle/Lemont	Fjellstrom et al. 2004
	SSR	RM224	0	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	Maybelle/Lemont	Fjellstrom et al. 2004

	SSR	RM1233	0.8	TTCGTTTTCCTTGGTTAGTG	ATTGGCTCCTGAAGAAGG	Maybelle/Lemont	Fjellstrom et al. 2004
	SSR	RM144	1.7	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	(Vista/Lebonnet //Rosemont) /Katy	Fjellstrom et al. 2004
	SSR	RM224	1.1	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	(Vista/Lebonnet //Rosemont) /Katy	Fjellstrom et al. 2004
Pik-s	SSR	RM144	2.7	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	Maybelle/Bengal	Fjellstrom et al. 2004
	SSR	RM224	0	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	Maybelle/Bengal	Fjellstrom et al. 2004
	SSR	RM1233	0	TTCGTTTTCCTTGGTTAGTG	ATTGGCTCCTGAAGAAGG	Maybelle/Bengal	Fjellstrom et al. 2004
	SSR	RM144	5.4	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	Maybelle/M-201	Fjellstrom et al. 2004
	SSR	RM224	0	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	Maybelle/M-201	Fjellstrom et al. 2004
	SSR	RM1233	2.7	TTCGTTTTCCTTGGTTAGTG	ATTGGCTCCTGAAGAAGG	Maybelle/M-201	Fjellstrom et al. 2004
Chromosome 12							
Pita	SNP	ta642	1.2	GGTCAAACATGAAGTGAGATGGG (Yashiromochi-specific primer)	CTGCATCACACTTCTGATGAAC	Yashiromochi /Nipponbare	Hayashi et al. 2006
				GGTCAAACATGAAGTGAGATGGA (Nipponbare-specific primer)	CTGCATCACACTTCTGATGAAC	Yashiromochi /Nipponbare	Hayashi et al. 2006
	SNP	ta801	0	CAAGCCAAATCTGAATCTTACCAA (Yashiromochi-specific primer)	TATGGAAATGTTGCCCCAATCTG	Yashiromochi /Nipponbare	Hayashi et al. 2006
				CAAGCCAAATCTGAATCTTACCAT (Nipponbare-specific primer)	TATGGAAATGTTGCCCCAATCTG	Yashiromochi /Nipponbare	Hayashi et al. 2006
	SNP	ta3	0	GGAGTACGTGTCTTTTTCCATGTATA (Yashiromochi-specific primer)	CTTGGTCCTACCTGTCATACACAC	Yashiromochi /Nipponbare	Hayashi et al. 2006
				GGAGTACGTGTCTTTTTCCATGCATT (Nipponbare-specific primer)	CTTGGTCCTACCTGTCATACACAC	Yashiromochi /Nipponbare	Hayashi et al. 2006
	SNP	ta577	0	ATGAACACCACAGCCTAAACG (Yashiromochi-specific primer)	CAGACCCGAAACAACACTAGG	Yashiromochi /Nipponbare	Hayashi et al. 2006
				ATGAACACCACAGCCTAAACC (Nipponbare-specific primer)	CAGACCCGAAACAACACTAGG	Yashiromochi /Nipponbare	Hayashi et al. 2006

	SNP	ta5		CAGCGAACTCCTTCGCATACGCG (Yashiromochi-specific primer)	CGAAAGGTGTATGCACTATAGTATCC	Yashiromochi /Nipponbare	Hayashi et al. 2006
				CAGCGAACTCCTTCGCATACGCA (Nipponbare-specific primer)	CGAAAGGTGTATGCACTATAGTATCC	Yashiromochi /Nipponbare	Hayashi et al. 2006
	SNP	Pi-ta 440*	-	CAACAATTTAATCATACACG	ATGACACCCTGCGATGCAA	Katy, Drew, or Kaybonnet /Nipponbare or M-202	Jia et al. 2002
	SNP	Pi-ta 1042*	-	AGCAGGTTATAAGCTAGGCC	CTACCAACAAGTTCATCAAA	Katy, Drew, or Kaybonnet /Nipponbare or M-202	Jia et al. 2002
	SNP	Pi-ta 403*	-	CAATGCCGAGTGTGCAAAGG	TCAGGTTGAAGATGCATAGC	Katy, Drew, or Kaybonnet /Nipponbare or M-202	Jia et al. 2002
Pita-2	SNP	ta642	0	GGTCAAACATGAAGTGAGATGGG (Pi No. 4 -specific primer)	CTGCATCACACTTCTGATGAAC	Pi No. 4/Koshihikari	Hayashi et al. 2006
				GGTCAAACATGAAGTGAGATGGA (Koshihikari-specific primer)	CTGCATCACACTTCTGATGAAC	Pi No. 4/Koshihikari	
	SNP	ta801	0	CAAGCCAAATCTGAATCTTACCAA (Pi No. 4 -specific primer)	TATGGAAATGTTGCCCCAATCTG	Pi No. 4/Koshihikari	Hayashi et al. 2006
				CAAGCCAAATCTGAATCTTACCAT (Koshihikari-specific primer)	TATGGAAATGTTGCCCCAATCTG	Pi No. 4/Koshihikari	
	SNP	ta3	0	GGAGTACGTGTCTTTTTCCATGTATA (Pi No. 4 -specific primer)	CTTGGTCCTACCTGTCATACACAC	Pi No. 4/Koshihikari	Hayashi et al. 2006
				GGAGTACGTGTCTTTTTCCATGCATT (Koshihikari-specific primer)	CTTGGTCCTACCTGTCATACACAC	Pi No. 4/Koshihikari	
	SNP	ta577	4	ATGAACACCACAGCCTAAACG (Pi No. 4 -specific primer)	CAGACCCGAAACAACACTAGG	Pi No. 4/Koshihikari	Hayashi et al. 2006
				ATGAACACCACAGCCTAAACC (Koshihikari-specific primer)	CAGACCCGAAACAACACTAGG	Pi No. 4/Koshihikari	
	SNP	ta5		CAGCGAACTCCTTCGCATACGCG (Pi No. 4 -specific primer)	CGAAAGGTGTATGCACTATAGTATCC	Pi No. 4/Koshihikari	Hayashi et al. 2006
				CAGCGAACTCCTTCGCATACGCA (Koshihikari-specific primer)	CGAAAGGTGTATGCACTATAGTATCC	Pi No. 4/Koshihikari	

	SSR	OSM89	4.6	TTGGTCAAAGTTAGCATGGGAGGG	TTTGAACCGGGTGGCCCACATG	(Vista/Lebonnet //Rosemont) /Katy		Fjellstrom et al. 2004
	SSR	RM155	3.5	GAGATGGCCCCCTCCGTGATGG	TGCCCTCAATCGGCCACACCTC	(Vista/Lebonnet //Rosemont) /Katy		Fjellstrom et al. 2004
	SSR	OSM89	2.4	TTGGTCAAAGTTAGCATGGGAGGG	TTTGAACCGGGTGGCCCACATG	Maybelle/ Kaybonnet		Fjellstrom et al. 2004
	SSR	RM155	0.8	GAGATGGCCCCCTCCGTGATGG	TGCCCTCAATCGGCCACACCTC	Maybelle /Kaybonnet		Fjellstrom et al. 2004
	SSR	RM7102	1.1	TTGAGAGCGTTTTTAGGATG	TCGGTTTACTTGGTTACTCG	Maybelle /Kaybonnet		Fjellstrom et al. 2004
	SSR	OSM89	2.4	TTGGTCAAAGTTAGCATGGGAGGG	TTTGAACCGGGTGGCCCACATG	Kaybonnet/M-204		Fjellstrom et al. 2004
	SSR	RM7102	1.3	GAGATGGCCCCCTCCGTGATGG	TGCCCTCAATCGGCCACACCTC	Kaybonnet/M-204		Fjellstrom et al. 2004
Pi20(t)	SSR	RM1337	0	GCTGAGGAGTATCCTTTCTC	ACCATAGGAAGATCATCACA	IR24/Asominori		Li et al. 2008
	SSR	RM5364	0	GTATTACGCTCGATAGCGGC	GTATCCTTTCTCGCAATCGC	IR24/Asominori		Li et al. 2008
	SSR	RM7102	0	CGGCTTGAGAGCGTTTTTAG	TACTTGGTTACTCGGGTCGG	IR24/Asominori		Li et al. 2008
Pi39(t)	CAPS	39M6	0	GGTTCGGGTCTCTCAAGTA	CAACGGAGGAAGTAAGGAGA	IR24/Asominori	*HinfI*	Liu et al. 2007
	CAPS	39M7	0.09	GGTAGAGGCAGCAGGGTAAT	GTCGAGACACTGTCGGATTC	IR24/Asominori	*BstYI*	Liu et al. 2007

* The markers are designed to detect the polymorphisms within the resistance genes.
** The markers are designed to detect the polymorphisms in 5' UTR of the resistance genes.
*** The markers are designed to detect the polymorphisms in 3' UTR of the resistance genes.

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