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-

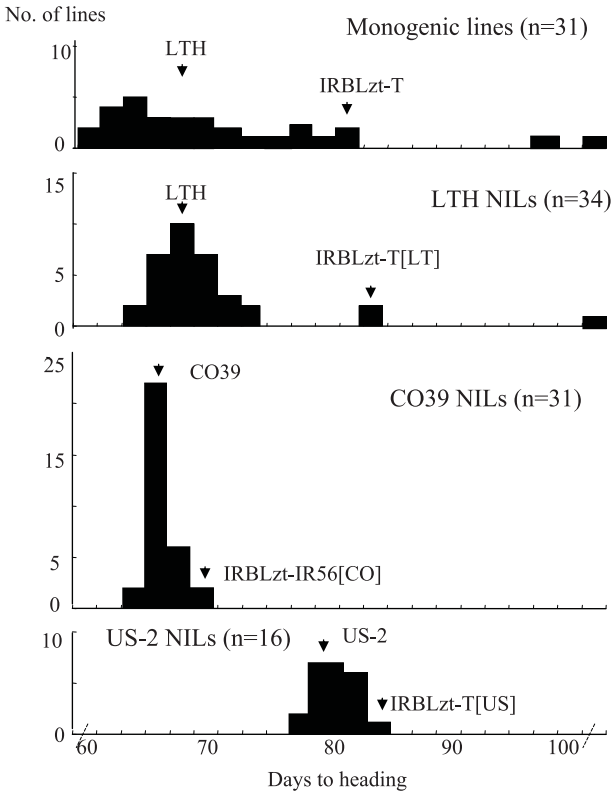

Fig. 1. Distributions of heading date in differential varieties at JIRCAS, Tsukuba, Japan, in 2006

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

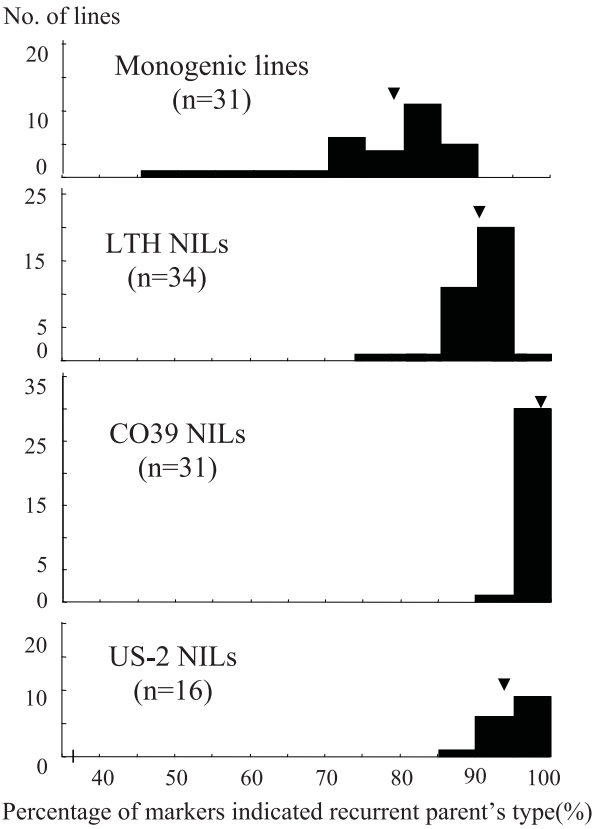

Fig. 2. Distribution of differential varieties for substitution ratio of recurrent parents' genetic backgrounds in each differential variety set

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

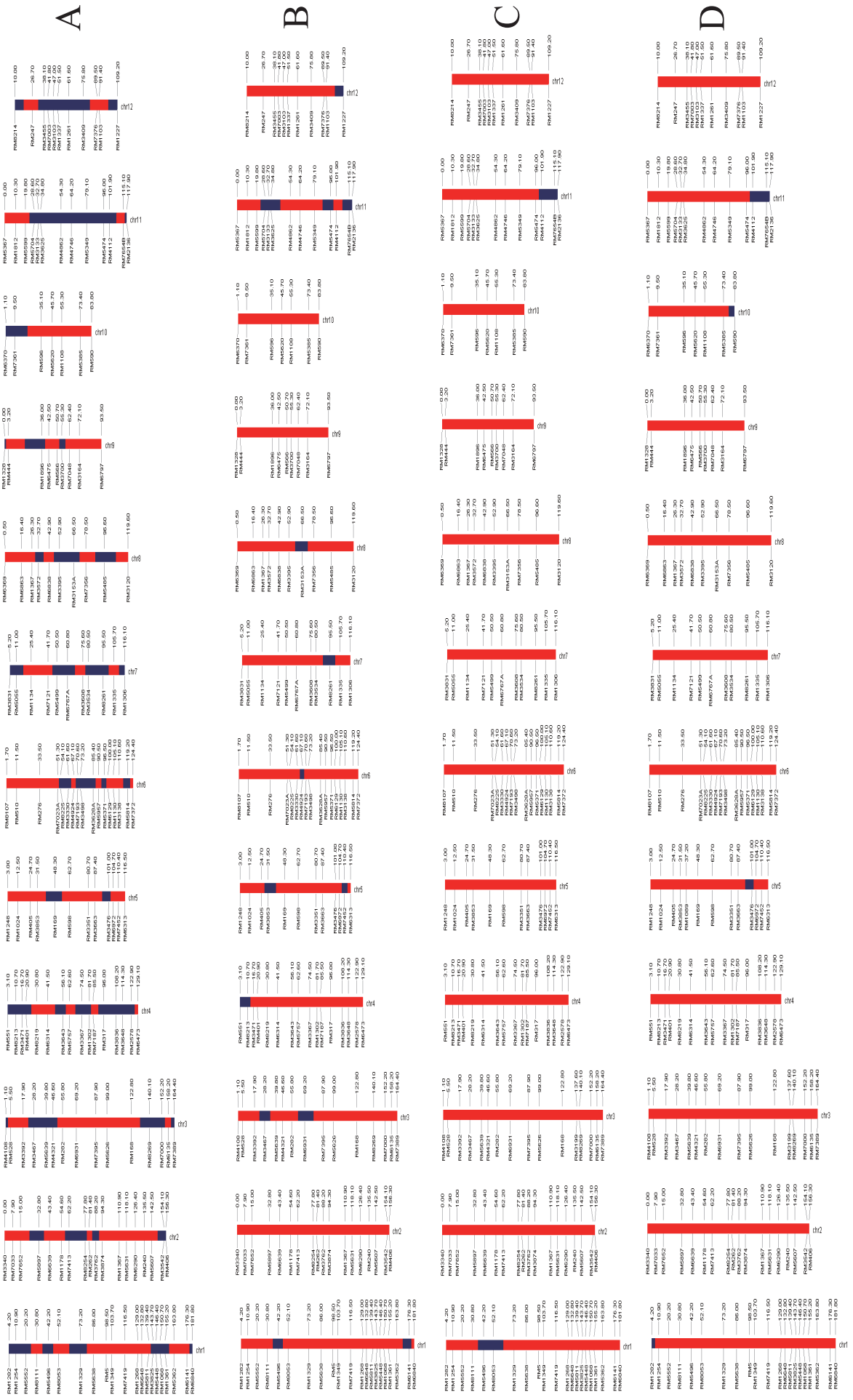

Fig. 3. Graphical genotypes of four differential varieties with introduced *Pik* from a donor, Kanto51

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

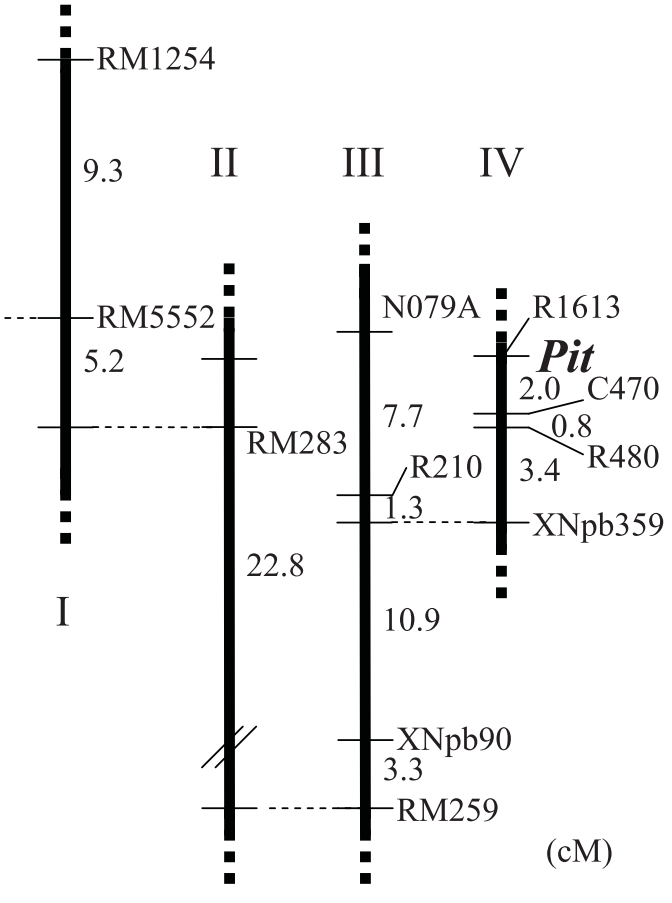

Fig. 4. Graphical genotypes of chromosome 1 of differential varieties containing *Pit*

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

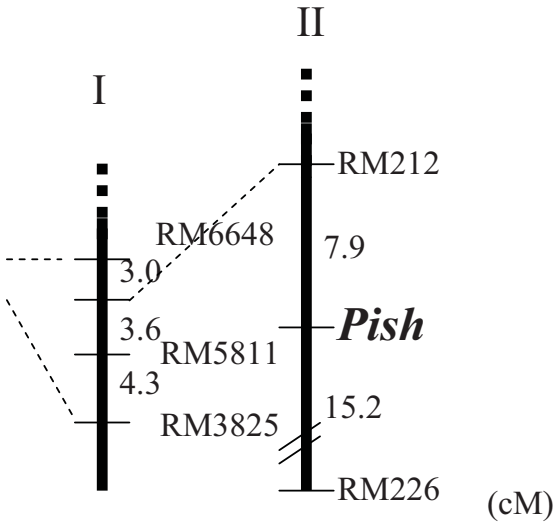

Fig. 5. Graphical genotypes of chromosome 1 of differential varieties containing *Pish*

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

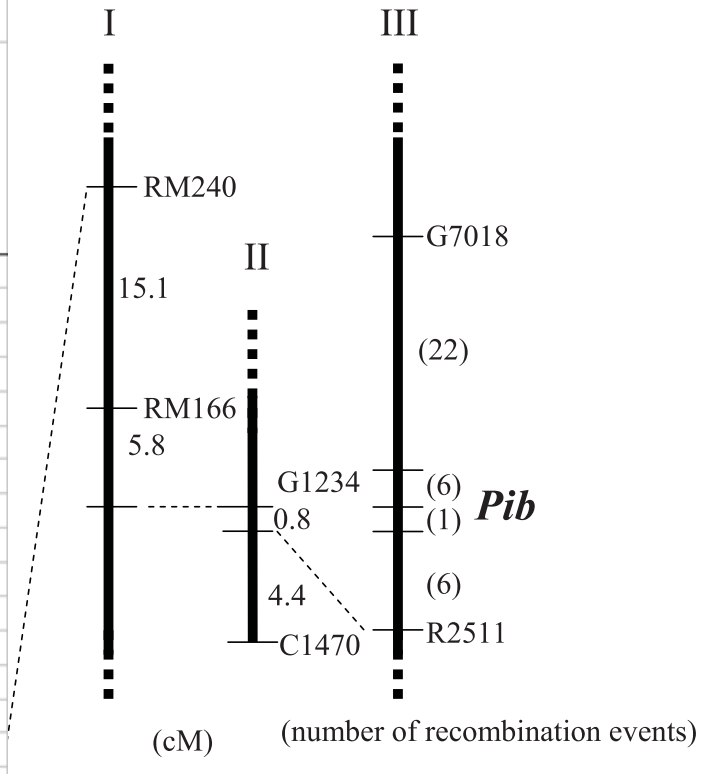

Fig. 6. Graphical genotypes of chromosome 2 of differential varieties containing *Pib*

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

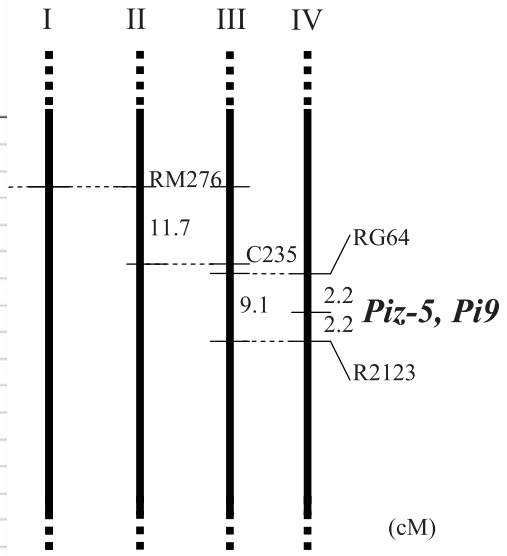

Fig. 7. Graphical genotypes of chromosome 6 of differential varieties containing either *Piz*, *Piz-t*, *Piz-5*, or *Pi9*

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

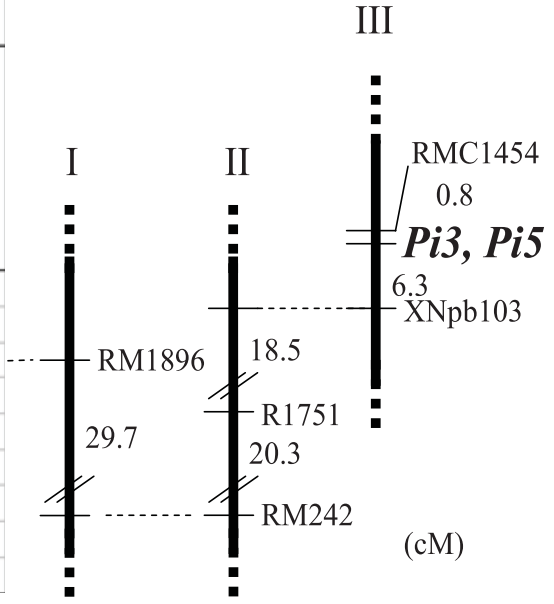

Fig. 8. Graphical genotypes of chromosome 9 of differential varieties introduced *Pii*, *Pi3*(t), or *Pi5*(t)

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

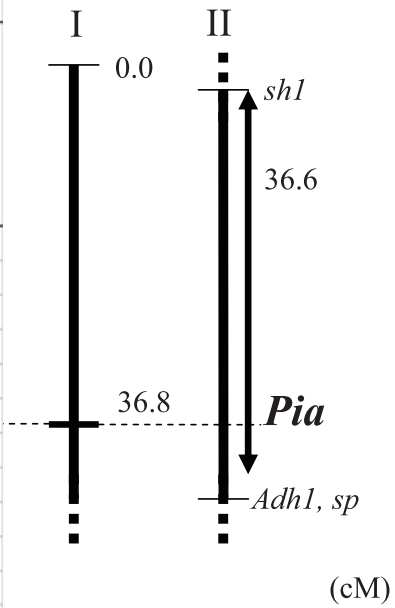

Fig. 9. Graphical genotypes of chromosome 11 of differential varieties containing *Pia*

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	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]
		Differential variett																																											
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

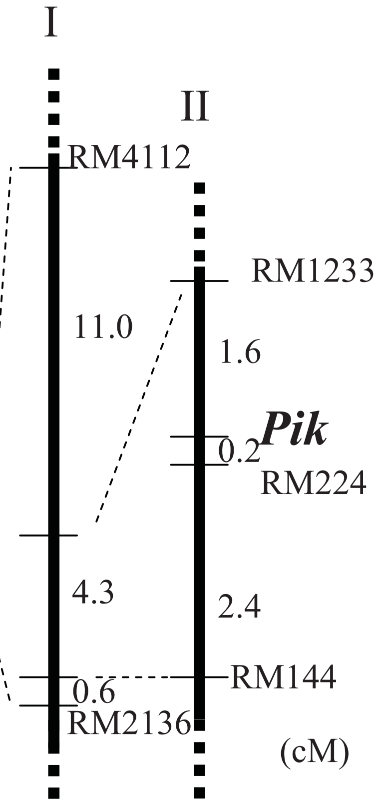

Fig. 10. Graphical genotypes of chromosome 11 of differential varieties containing *Pik* alleles and *Pi7*(t)

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

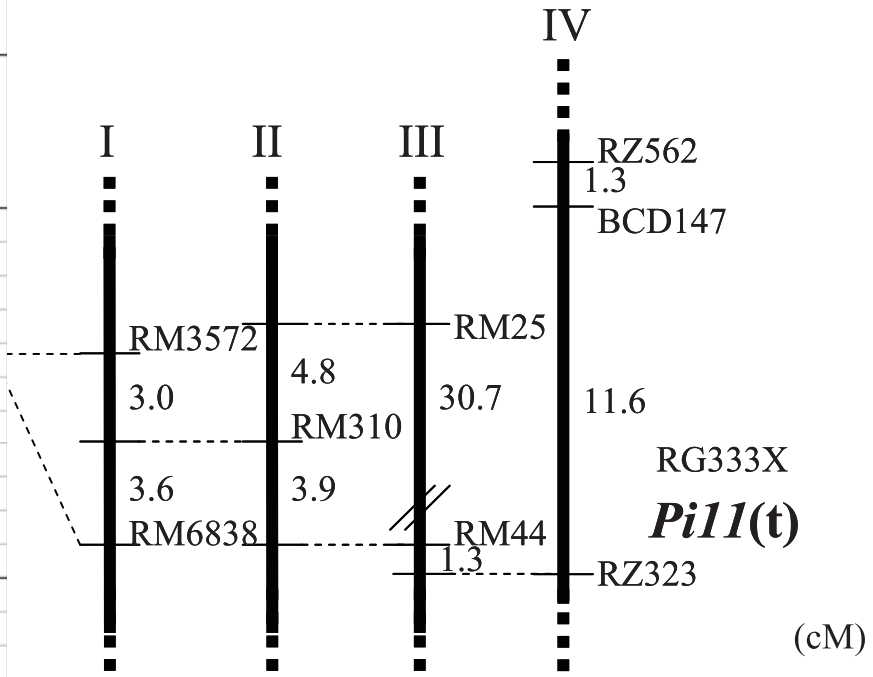

Fig. 12. Graphical genotypes of chromosomes 2 and 8 of differential varieties containing *Pi11*

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

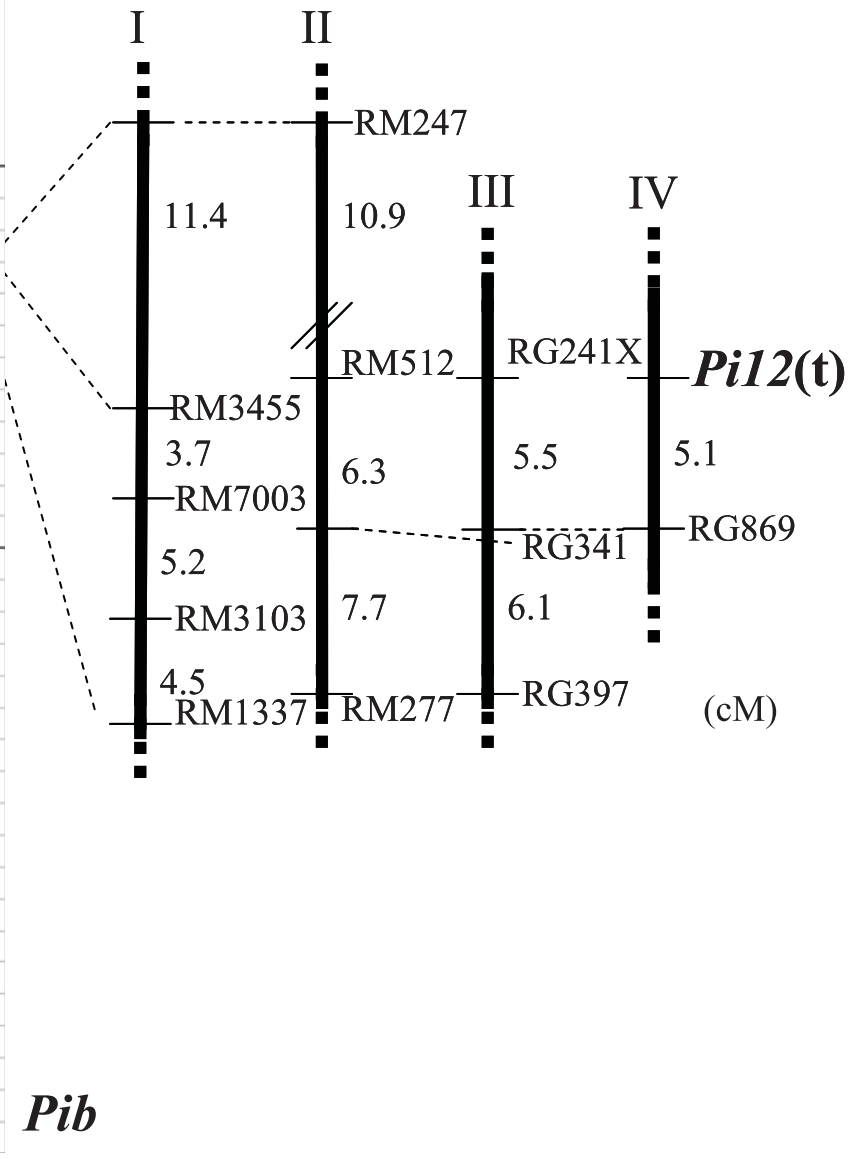

Fig. 13. Graphical genotypes of chromosomes 2 and 12 of differential varieties containing *Pi12*(t)

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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