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Use of *Pi5(t)* markers in marker-assisted selection to screen for cultivars with resistance to *Magnaporthe grisea*

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Abstract Identification of the PCR markers tightly linked to genes that encode important agronomic traits is useful for marker-assisted selection (MAS). The rice *Pi5(t)* locus confers broad-spectrum resistance to *Magnaporthe grisea*, the causal agent of rice blast disease. It has been hypothesized that the *Pi5(t)* locus carries the same gene as that encoded by the *Pi3(t)* and *Pii(t)* loci. We developed three PCR-based dominant markers (JJ80-T3, JJ81-T3, and JJ113-T3) from three previously identified BIBAC clones—JJ80, JJ81, and JJ113—that are linked to the *Pi5(t)* locus. PCR analysis of 24 monogenic lines revealed that these markers are present only in lines that carry *Pi5(t)*, *Pi3(t)*, and *Pii(t)*. PCR and DNA gel-blot analysis of candidate resistance lines using JJ80-T3, JJ81-T3, and JJ113-T3 indicated that *Tetep* is the likely donor of *Pi5(t)*. Of the 184 rice varieties tested, 34 carried the JJ80-T3-, JJ81-T3-, and JJ113-T3-specific bands. Disease evaluation of those 34 varieties revealed that all conferred resistance to PO6-6. The genomic structure of three of these resistant varieties (i.e., IR72, *Taebaeg*, *Jahyangdo*) is most similar to that of *Pi5(t)*. Our results demonstrate the usefulness of the JJ80-T3, JJ81-T3, and JJ113-T3 markers for MAS for *M. grisea* resistance.

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Introduction

Rice blast, caused by *Magnaporthe grisea*, is one of the most devastating diseases in rice, and its frequent appearance during all stages of plant growth greatly decreases yield and grain quality. Although varietal improvements and disease assessment techniques have been employed since the early 1960s to reduce its occurrence, because of high pathogenic diversity (Ou 1979; Bonman et al. 1986), the resistance of most cultivars is relatively short (Lee and Cho 1990). Consequently, breeding for rice cultivars with durable resistance is a priority in crop improvement programs (Ou 1985; Zeigler et al. 1994; Hittalmani et al. 2000; Conaway-Bormans et al. 2003).

Durable resistance to *M. grisea* is conferred by both major and minor genes (Bonman and Mackill 1988; Zhu et al. 1993; Wang et al. 1994). Extensive genetic studies and quantitative trait locus (QTL) analysis using DNA markers have identified more than 20 major genes and ten QTLs in rice that give rise to resistance (McCouch et al. 1994; Wang et al. 1994; Naqvi and Chattoo 1996; Yu et al. 1996; Chen et al. 1999; Ahn et al. 2000; Chauhan et al. 2002; Jiang and Wang 2002; Zenbayashi et al. 2002). Genes conferring resistance have been identified in the durably resistant rice cultivars *Tetep*, *Pai-Kan-Tao* (PKT), *Moroberekan*, 5173, and LAC23 (Mackill and Bonman 1992; Inukai et al. 1994; Wang et al. 1994). For example, Mackill and Bonman (1992) located the genes *Pi1(t)*, *Pi2(t)*, and *Pi3(t)* as well as two alleles of *Pi4(t)* associated with broad-spectrum resistance from these durably resistant rice cultivars. The resistance locus *Pi5(t)* was

identified by analyzing 281 recombinant inbred lines (RILs) of an F_7 recombinant inbred population of the *Moroberekan*/CO39 cross with 127 restriction fragment length polymorphism (RFLP) markers (Wang et al. 1994). Phenotype assays revealed that RIL125, RIL249, and RIL260, all carrying *Pi5(t)*, displayed resistance to at least six *M. grisea* races belonging to four lineages in the Philippines and also to 26 of the 29 Korean *M. grisea* isolates tested (Wang et al. 1994; Chen et al. 2000; Han 2001).

Genetic analysis indicates that *Pi5(t)* is allelic or closely linked to *Pi3(t)* and that *Pi3(t)* is closely linked to *Pii(t)* (Inukai et al. 1994, 1996). Additional disease assays have shown that the *Pii(t)*, *Pi3(t)*, and *Pi5(t)* genes display a similar reaction when tested against 16 *M. grisea* isolates (Imbe et al. 2000; S.-S. Han et al. unpublished data). Furthermore, we have recently demonstrated that the genomic regions of lines containing *Pi3(t)* and *Pi5(t)* are identical (Jeon et al. 2003). Pan et al. (2003) reported that *Pii(t)*, *Pi3(t)*, *Pi5(t)*, and *Pi15(t)* are all located in the same interval on rice chromosome 9. We have also isolated a 170-kb physical region of *Pi5(t)* from RIL260 (Jeon et al. 2003). Molecular characterization of that region in PKT and *Moroberekan* has revealed that these two lines are not the donors of the respective resistance genes *Pi3(t)* and *Pi5(t)*.

Disease assays to evaluate resistance to *M. grisea* are well established and can be conducted in blast nurseries or by infecting individual plants under controlled conditions. However, these procedures are time-consuming and labor-intensive and require specialized facilities. With the advances in high-resolution genetic mapping strategies, molecular markers associated with desired agronomic traits, including disease resistance, have now been identified. This marker-assisted selection (MAS) uses tightly linked molecular markers in a low-cost, fast, and efficient approach to breeding for *M. grisea* resistance. We report here the isolation of three PCR-based markers linked to the *Pi5(t)* locus and demonstrate their usefulness for disease-resistance breeding.

Materials and methods

Plant materials and DNA extraction

Rice cultivars *Tetep*, C104PKT, LAC23, and PKT were provided by the National Small Grains Research Facility, USDA-ARS, Idaho. The other cultivars and germplasms used in the experiments were obtained from the rice germplasm center at the National Yeongnam Agricultural Experiment Station (NYAES), Rural Development Administration (RDA), Milyang, Korea, and the National Crop Experiment Station, RDA, Suwon, Korea. Total genomic DNAs for these rice varieties and monogenic lines (Tsunematsu et al. 2000) were extracted from young leaves following the protocol described by Chen and Ronald (1999).

Inoculation and disease evaluation

The *Magnaporthe grisea* isolate PO6-6 was used for each phenotypic analysis. All inoculations and disease evaluations (total of five replications) were conducted at NYAES and at the Kyung Hee University greenhouses according to the methods described by Chen et al. (1996). These experiments were conducted during the wintertime to prevent the risk of field release.

Development of molecular markers

Three PCR markers—JJ80-T3, JJ81-T3, JJ113-T3—were designed from the end sequences of BIBAC clones that comprise the physical map of *Pi5(t)* (Jeon et al. 2003). Three primer sets were used to identify the *Pi5(t)* region (Table 1).

PCR amplification

We carried out PCR using *ExTaq* polymerase (Takara) and the GeneAmp System 9700 (Applied Biosystems, Foster City, Calif.). The PCR conditions included pre-denaturation for 3 min at 94°C, followed by 35 cycles of the polymerization reaction, each consisting of denaturation for 30 s at 94°C, annealing for 30 s at 57°C, and an extension step for 1 min 30 s at 72°C. A final extension step was run for 5 min at 72°C. The PCR product was separated on a 0.8% agarose gel and stained with ethidium bromide to detect the amplicons.

DNA gel-blot analysis

Approximately 3 μg of rice genomic DNA was digested with restriction enzymes and separated by electrophoresis on a 0.8% agarose gel. DNA gel-blot analysis was carried out according to standard procedures under high-stringency hybridization conditions (Sambrook et al. 1989).

Table 1 Primers for *Pi5* PCR markers

Marker	Forward primer (5′→3′)	Reverse primer (5′→3′)	Product size (bp)
JJ80-T3	TTATGAGATTAGGAGTGTAT	ATGTAAAGGCAAAAGCTGAT	442
JJ81-T3	TCTACAAACTCAGTTAAACT	AGCGAAAATCATTTATCACA	343
JJ113-T3	CTCTTGGTGATCTTTGTTAC	GGATGATGTGATCTGCAGAG	484

Results

Identifying PCR markers dominant to *Pi5(t)*

The *Pi5(t)* rice blast resistance gene has been delimited in a 170-kb interval between C1454 and S04G03 on chromosome 9 (Jeon et al. 2003). This resistance gene region consists of a contiguous set of 12 RIL260 genomic DNA clones. We sequenced both end regions of each BIBAC clone and used them for developing the dominant markers in the *Pi5(t)* lines for efficiency in MAS. All sequences were analyzed against *Oryza sativa* L. ssp. *indica* contigs (http://www.ncbi.nlm.nih.gov/BLAST/) to remove repetitive DNAs or multicopy sequences (Yu et al. 2002). Only single or unmatched sequences were then used for developing the PCR primers (Table 1). To determine whether these were suitable for *Pi5(t)* markers, we carried out DNA gel-blot analysis using the sequences amplified by PCR (Fig. 1). Our blots showed three sequences—JJ80-T3, JJ81-T3, and JJ113-T3—as being dominant to *Pi5(t)*.

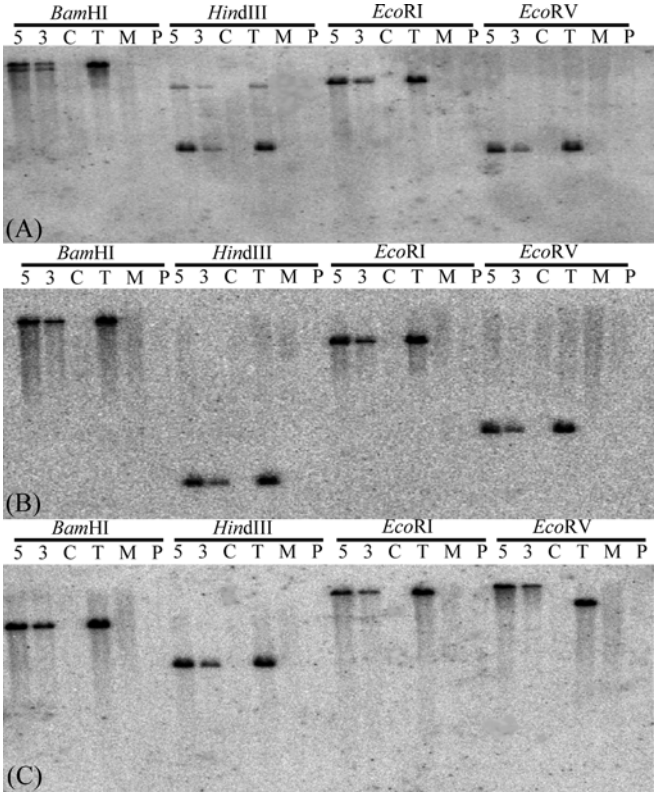

Fig. 1a–c DNA gel-blot analysis of RIL260 (*5*), C104PKT (*3*), CO39 (*C*), *Tetep* (*T*), *Moroberekan* (*M*), and PKT (*P*) genomes. Genomic DNAs digested with *Bam*HI, *Hin*dIII, *Eco*RI, and *Eco*RV were electrophoresed, blotted, and hybridized with the BIBAC-end sequences JJ80-T3 (**a**), JJ113-T3 (**b**), and JJ81-T3 (**c**), respectively, as probes. Note: none of the markers could be hybridized with the CO39, *Moroberekan*, or PKT genomic DNAs

Pi5(t) markers are also dominant in *Pi3(t)* and *Pii(t)* lines

To check whether the JJ80-T3, JJ81-T3, and JJ113-T3 markers were unique to *Pi5(t)*, we used PCR to analyze 24 monogenic lines (Table 2; Imbe et al. 2000; Tsunematsu et al. 2000). This analysis revealed that the three markers were present only in those lines carrying *Pi5(t)*, *Pi3(t)*, or *Pii(t)*. It has been previously demonstrated that the *Pi5(t)* locus is allelic to *Pi3(t)* and that both loci are physically identical (Inukai et al. 1996; Jeon et al. 2003). Therefore, using the three markers, we analyzed two monogenic lines, IRBL5-M and IRBL3-CP4, by DNA gel-blot analysis (Fig. 2). Both lines were generated by back-crossing RIL249 [carrying *Pi5(t)*] and C104PKT [carrying *Pi3(t)*], respectively, to *Lijiangxintuanheigu* (LTH). Our analysis showed that RIL260, IRBL3-CP4, and IRBL5-M were monomorphic at the resistance locus, thereby providing additional evidence that *Pi3(t)* and *Pi5(t)* are identical (Fig. 2).

To determine whether the three markers were linked to the *Pii(t)* locus, we carried out PCR analyses on six *Pii(t)*-containing varieties— *Inabawase*, *Hikurikul1*, *Tarehonami*, *Akitagomachi*, *Fujisaka5*, and *Nongbaeg*—as well as three varieties that carry Pik^s, *Pish*, Pik^m, or $Pita^2$ (see Table 2). The results clearly confirmed that the markers are present in all *Pii(t)*-containing varieties but missing in the others. The *Pii(t)* monogenic line IRBLi-F5, which was generated in BC_1F_9 of the cross between the donor parent

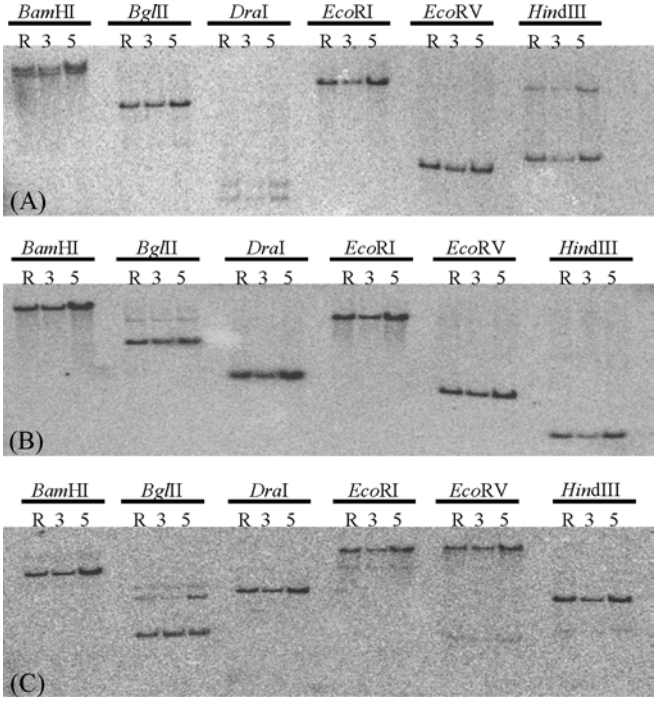

Fig. 2a–c DNA gel-blot analysis of RIL260 (*R*), IRBL3-CP4 (*3*), and IRBL5-M (*5*) genomes. Genomic DNAs digested with *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, and *Hin*dIII were electrophoresed, blotted, and hybridized with the BIBAC-end sequences JJ80-T3 (**a**), JJ113-T3 (**b**), and JJ81-T3 (**c**), respectively, as probes. All lines are monomorphic at the three probe regions tested

Table 2 PCR analysis of 24 monogenic rice lines and nine resistance donors

Line	Resistance gene	Marker		
		JJ80-T3	JJ81-T3	JJ113-T3
IRBLa-A	*Pia*	–	–	–
IRBLa-C	*Pia*	–	–	–
IRBLb-B	*Pib*	–	–	–
IRBLi-F5	*Pii*	+	+	+
IRBLk-Ka	*Pik*	–	–	–
IRBLks-F5	*Pik^s*	–	–	–
IRBLks-S	*Pik^s*	–	–	–
IRBLkh-K3	*Pik^h*	–	–	–
IRBLkp-K60	*Pik^p*	–	–	–
IRBLt-K59	*Pit*	–	–	–
IRBLsh-S	*Pish*	–	–	–
IRBLsh-B	*Pish*	–	–	–
IRBLta-K1	*Pita*	–	–	–
IRBLta-CT2	*Pita*	–	–	–
IRBLz-Fu	*Piz*	–	–	–
IRBLz5-CA	*Piz5(=Pi2)*	–	–	–
IRBLzt-T	*Piz^t*	–	–	–
IRBL1-CL	*Pi1*	–	–	–
IRBL3-CP4	*Pi3*	+	+	+
IRBL5-M	*Pi5*	+	+	+
IRBL7-M	*Pi7*	–	–	–
IRBL9-W	*Pi9*	–	–	–
IRBL12-M	*Pi12*	–	–	–
IRBL19-A	*Pi19*	–	–	–
Inabawase	*Pii*	+	+	+
Hikuriku11	*Pii*	+	+	+
Tarehonami	*Pii*	+	+	+
Akitagomachi	*Pia, Pii*	+	+	+
Fujisaka5	*Pii*, *Pik^s*	+	+	+
Nongbaeg	*Pii*	+	+	+
Koshihikari	*Pik^s*, *Pish*	–	–	–
Tsuyuake	*Pik^m*	–	–	–
Reiho	*Pita^2*	–	–	–

Fujisaka5 and the susceptible line LTH, was analyzed via genomic DNA hybridization using the three dominant markers (Fig. 3). The hybridization band pattern was the same for IRBLi-F5 and *Fujusaka5*, further indicating that both are identical at the *Pii(t)* locus. Interestingly, we found in using markers JJ80-T3 and JJ113-T3 that the two *Pii(t)*-carrying lines were distinguishable from RIL260 carrying *Pi5(t)*. This would suggest that the entire genome structure, including the *Pii(t)* gene, is not identical to that of *Pi3(t)*/*Pi5(t)* and that the origin of *Pii(t)* may differ from that of *Pi3(t)*/*Pi5(t)*. *Pi3(t)* is known to be tightly liked to *Pii(t)* and both genes show very similar resistance spectra to various rice blast isolates (Inukai et al. 1994; Imbe et al. 2000; S.-S. Han et al., unpublished data). Considering the similar spectrum and the nearly identical chromosomal location, it is probable that *Pi3(t)*/ *Pi5(t)* and *Pii(t)* are the same gene and that these three DNA sequences can be utilized as a marker of *Pi3(t)*/*Pi5(t)*/*Pii(t)*.

Identification of the likely donor of *Pi5(t)*

We had observed previously that the *Pi5(t)* genomic region differs remarkably from *Moroberekan*, which had been assumed to be its donor (Jeon et al. 2003). It is probable that the non-parental alleles observed in the *Pi5(t)*-containing lines are due to an outcross with another line. Because *Tetep*, LAC23, and 5173 were used in the same greenhouses simultaneously to generate 22 near-isogenic lines (NILs) for blast resistance (Mackill and Bonman 1992) and because all three varieties confer resistance to *M.grisea* PO6-6 (Mackill and Bonman 1992; data not shown), we considered those lines to be candidates for the *Pi5(t)* donor.

In a PCR analysis the three primer pairs from JJ80-T3, JJ81-T3, and JJ113-T3 amplified PCR products in C104PKT carrying *Pi3(t)*, in three RILs (RIL125, RIL249, and RIL260) carrying *Pi5(t)*, and in *Tetep*; they did not amplify any products in *Moroberekan*, LAC23, PKT, or 5173 (data not shown). Because *Tetep* produced amplicons dominant in the *Pi3(t)* and *Pi5(t)* lines, we further investigated whether it contained a genomic structure similar to the *Pi3(t)*/*Pi5(t)* locus. Likewise, in the DNA gel-blot analysis with the three dominant *Pi5(t)* markers, hybridizing bands were detected only in

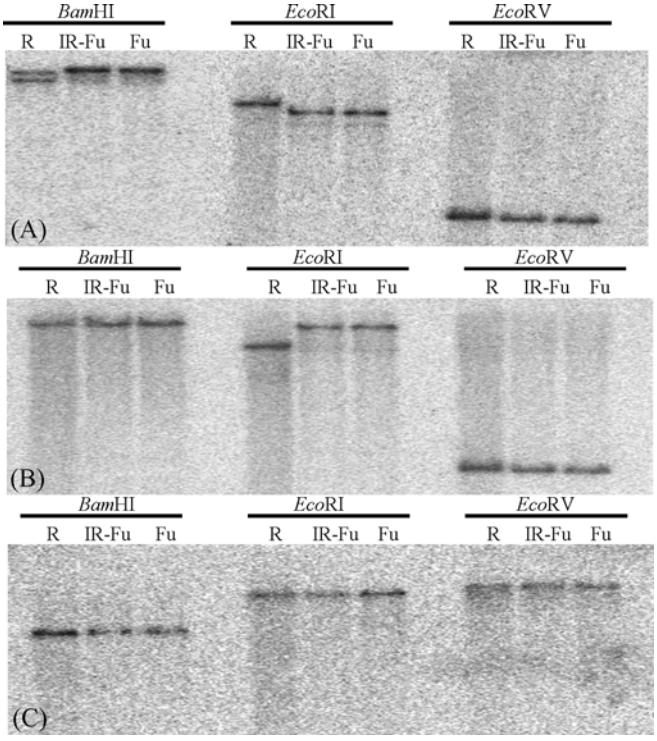

Fig. 3a–c DNA gel-blot analysis of RIL260 (*R*), IRBLi-F5 (*IR-Fu*), and *Fujisaka5* (*Fu*). Genomic DNAs digested with *Bam*HI, *Eco*RI, and *Eco*RV were electrophoresed, blotted, and hybridized with the BIBAC-end sequences JJ80-T3 (**a**), JJ113-T3 (**b**), and JJ81-T3 (**c**), respectively, as probes

C104PKT, RIL260, and *Tetep* but not in *Moroberekan*, C039, and PKT (Fig. 1). The physical region of C104PKT and RIL260 was identical to that of *Tetep* around the JJ113-T3 marker, which is located at the center of the *Pi5 (t)* locus (Fig. 1b). When JJ80-T3 and JJ81-T3, which are located at distal portions of the 170-kb *Pi5(t)* locus, were used as probes, three of the four restriction enzymes tested displayed identical hybridizing band patterns among C104PKT, RIL260, and *Tetep* (Fig. 1a,c). A polymorphism between C104PKT/RIL260 and *Tetep* was observed in the *Bam*HI- and *Eco*RV-digested DNA for the JJ80-T3 and JJ81-T3 probes, respectively (Fig. 1a,c). If we consider that both markers are close to the flanking markers C1454 and S04G03, this polymorphism might have resulted from a genetic recombination generated while backcrossing the line of C104PKT to CO39 six times. These data suggest that *Tetep* could be a genetic source of resistance for *Pi3(t)* in C104PKT and for *Pi5(t)* in the RILs.

Mackill and Bonman (1992) reported that PKT is susceptible to rice blast PO6-6, a finding that further supports our hypothesis that its genome is not the source of *Pi3(t)*. Moreover, it is unlikely that the *Pi5(t)* resistance gene was created by a recombination event in the *Moroberekan*/CO39 cross because we have observed identical, non-parental alleles in RIL125, RIL249, RIL260, and C104PKT (Jeon et al. 2003) and because many dominant sequences were missing in *Moroberekan* and PKT.

Using *Pi5(t)* markers in MAS to identify rice varieties displaying resistance to PO6-6

Three *Pi5(t)* dominant markers—JJ80-T3, JJ81-T3, and JJ113-T3—were used to pre-screen the rice varieties, which were then tested for their resistance response to the rice blast isolate PO6-6. Of the 184 varieties evaluated (Table 3), we found 34 that produced PCR products for all three markers. The size of the PCR bands generated by primers for JJ80-T3, JJ81-T3 and JJ113-T3, respectively, were the same among the PCR-positive cultivars. In the PCR analysis two co-dominant *Pi5(t)* markers, C1454 and JJ39, were used as controls for the reaction (Jeon et al. 2003). To determine whether the PCR-positive varieties were associated with that response, we performed an

Table 3 Rice varieties tested for value in marker-assisted selection. Primers for JJ80-T3, JJ81-T3, and JJ113-T3 were used in the PCR analyses

Ecotype	PCR-positive varieties (34 varieties)	PCR-negative varieties (150 varieties)
Japonica	*Akitagomachi, Ansan, Aya, Baekjinju, Dondokui, Fukei166, Heughyang, Hitomebore, Hoshinoyume, Hoshiyutaka, Hwacheong, Hwacheongdu-6, Hwacheong wx, Ilpum*, IR68997-20-1-2-2-2, *Jinbuchal, Jinmi, Joiku 414, Joryeong, Lemont, Manan, Manchu, Mangeum, Nampyeong, Seolgeang, Shingeumo, Sobi, Yeongnam*	*Akenohoshi, Akihikari, Anseong, Aranhyangchal, Bukrukbanna*, C418, *Chucheong, Congshengla, Daesan, Dogye517, Dongan, Donghae, Dongjin, Donjin1, Donjinchal, Geumgang, Geuru, Goami, Gwangmyeong, Haepyeong, Hapcheon1, Heugjinju, Heugnam, Hoan, Hojin, Hwaan, Hwabong, Hwadong, Hwanam, Hwasam, Hwaseonchal, Hwaseong, Hwayeong, Hyangmi2, Hyangnam, Iksan423, Ilmi, Italica livorno, Jangan, Jeogjinju, Jinbong, Jinbu, Jinpum, Jongnam, Juan, Junam, Jungan, Junghwa, Jungsan, Kanto PL7, Keumo, Keumo1, Keumo2, Koshihikari, Kyehwa*, LA1, LA2827-2-1, LGC1, LS3313-2, M202, M401, *Manpung, Manweol, Mihyang, Milky queen, Mu95-22, Munjang, Naepung, Nagdong, Namgang, Namweon, Newbonnet, Nipponbare, Nongan, Nongho, Norin6, NorinPL9, Odae*, Ou244, Ou316, Ou349, *Palgong, Saechucheong, Saegaehwa, Saesangju, Sambaeg, Samcheon, Samnam, Sampyeong, Sanghaehyanghyeolna, Sangju, Sangjuchal, Sangmi, Sangnambat, Sangsan, Sasanishiki, Seolhyangchal, Seokjeong, Seomjin, Shennung258, Shennung89-366, Shindongjin, Shinseonchal, Shinunbong, Sobaeg, Sugary* (IT 212585), *Sujin, Sura, Sx864, Taebong, Tamjin, TN-1, Tohoku149, Unbong, Undu, Unjang, Weonhwang*, Wx154-134-40-1-1, *Yeonghae, Yeonhwa5, Yeoungan, Yongmun*, YR13616Acp1, YR15965Acp33, YR17664Acp4, YR20429Acp 87, *Yumenohatamochi*, 9516
Indica	*Guichao2*, IR72, IR841-76-1, *Jahyangdo*, CPSLO17	*Dinurado, Hangangcha*l, IR36, IR50, IRAT13, *Mashuri*, PSBRc4, *Takanari*
Tongil	*Taebaeg*	*Anda, Areum, Cheongcheong, Dasan, Gaya, Jangseong, Jungweon, Namcheon, Namyeong, Pungsan, Samgang, Shinkwang, Sujeong, Yongju*

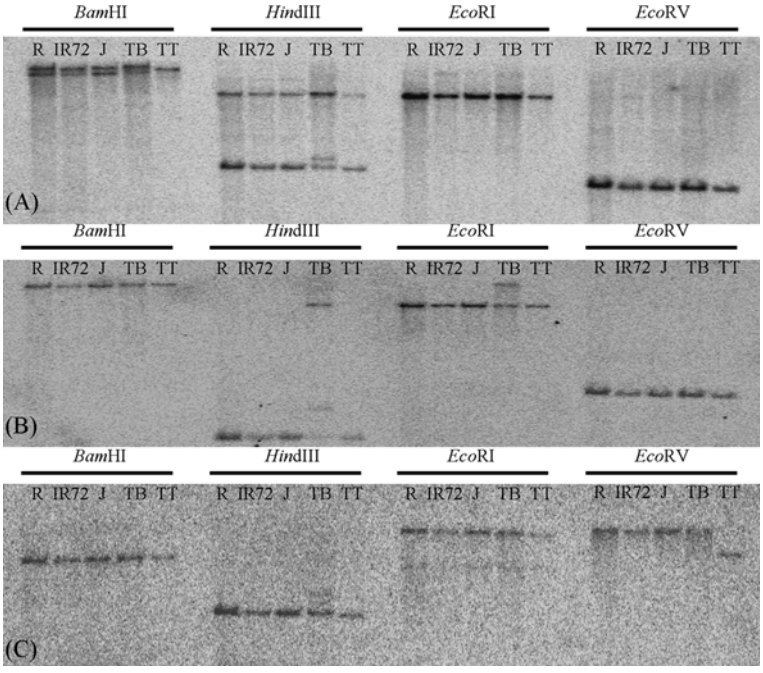

Fig. 4a–c DNA gel-blot analysis of RIL260 (*R*), IR72, *Jahyangdo* (*J*), *Taebaeg* (*TB*), and *Tetep* (*TT*). Genomic DNAs digested with *Bam*HI, *Hin*dIII, *Eco*RI, and *Eco*RV were electrophoresed, blotted, and hybridized with the BIBAC-end sequences JJ80-T3 (**a**), JJ113-T3 (**b**), and JJ81-T3 (**c**), respectively, as probes

inoculation experiment that included the 34 PCR-positive varieties, 11 PCR-negative varieties, a resistant control (RIL260), and a susceptible control (CO39). Except for *japonica* variety *Baekjinju* and *indica* variety IR841-76-1, which showed only moderate resistance (lesion type 2–3), the remaining 32 PCR-positive varieties were completely resistant (lesion type 0–1) (Table 4). These results indicate that PCR markers JJ80-T3, JJ81-T3, and JJ113-T3 are suitable for pre-screening resistant varieties for rice blast isolate PO6-6.

Characterization of the *Pi5(t)* genomic region in the newly identified resistant varieties

We further analyzed those 34 PCR-positive varieties (Table 2) using the cleaved amplified polymorphic sequence (CAPS) markers 40N23r and 76B14f (Jeon et al. 2003). The markers mapped within the 170-kb *Pi5(t)* genomic region were developed from sequences showing similarity to a 'nucleotide binding site plus leucine-rich repeat' (NBS-LRR) motif. Polymorphism analysis with these CAPS markers revealed that, of the 34 varieties, IR72, *Taebaeg*, and *Jahyangdo* were monomorphic to RIL260 carrying *Pi5(t)* in the 40N23r and 76B14f regions (data not shown). To determine whether those same three varieties were identical to RIL260 at the *Pi5(t)* locus, we carried out DNA gel-blot analyses using the JJ80-T3, JJ81-T3, and JJ113-T3 probes (Fig. 4). Here, the genomic structures of all three matched that of RIL260 carrying *Pi5(t)*. Therefore, all these data suggest that the genomic regions of IR72, *Taebaeg*, and *Jahyangdo* are quite similar to that of RIL260 at the *Pi5(t)* locus.

Table 4 Disease evaluation of 45 selected rice varieties for resistance to the rice blast (*Magnaporthe grisea*) PO6-6 isolate

Variety	Phenotype[a]	Variety	Phenotype	Variety	Phenotype	Variety	Phenotype	Variety	Phenotype
Akitagomachi	R	*Hitomebore*	R	IR841-76-1	M	*Mangeum*	R	*Geumgang*[b]	S
Ansan	R	*Hoshinoyume*	R	*Jahyangdo*	R	*Nampyeong*	R	*Hwayeong*[b]	S
Aya	R	*Hoshiyutaka*	R	*Jinbuchal*	R	*Seolgeang*	R	IR36[b]	S
Baekjinju	M	*Hwacheong*	R	*Jinmi*	R	*Shingeumo*	R	IR50[b]	S
CPSLO17	R	*Hwacheongdu-6*	R	*Joiku 414*	R	*Sobi*	R	*Kanto PL7*[b]	S
Dondokui	R	*Hwacheong wx*	R	*Joryeong*	R	*Taebaeg*	R	*Koshihikari*[b]	S
Fukei166	R	*Ilpum*	R	*Lemont*	R	*Yeongnam*	R	M202[b]	S
Guichao2	R	IR68997-20-1-2-2-2	R	*Manan*	R	*Areum*[b]	S	*Namcheon*[b]	S
Heughyang	R	IR72	R	*Manchu*	R	*Dongjin*[b]	S	*Yongju*[b]	S

[a]R, Resistant rice varieties, with scores of 0–1 on a scale of 0–5 with repeated inoculations; S, susceptible varieties, with scores of 4–5; M, moderately resistant varieties, with scores of 2–3
[b]PCR-negative varieties that did not produce PCR products for primers JJ80-T3, JJ81-T3, and JJ113-T3

Discussion

The chromosomal location of the *Pi5(t)*, *Pi3(t)*, and *Pii(t)* loci has been debated ever since Kiyosawa (1967) identified a linkage relationship between the *Pii(t)* gene and the *Piz(t)* gene on chromosome 6. Analysis of their genetic linkage and resistance profiles has suggested that these three genes might be allelic or closely linked (Inukai et al. 1994, 1996; Imbe et al. 2000). Most recently, fine mapping has demonstrated that all three loci are tightly linked on chromosome 9 (Jeon et al. 2003; Pan et al. 2003). In the present study, we confirmed this by using three PCR-dominant markers to show that the genomic structures of *Pi5(t)*, *Pi3(t)*, and *Pii(t)* are alike. These results indicate that these markers are good candidates for use in MAS for the *Pi5(t)*, *Pi3(t)*, and *Pii(t)* resistance genes.

We have previously reported that *Moroberekan*, the presumed donor of *Pi5(t)*, and PKT, the presumed donor of *Pi3(t)*, have no markers associated with the *Pi5(t)/Pi3(t)/Pii(t)* locus (Jeon et al. 2003). DNA gel-blot analysis however, revealed that the genomic region of the *Pi5(t)* locus in RIL260 is identical to that of the *Pi3(t)* genomic region in C104PKT. In the present study, we showed that the corresponding genomic region in *Tetep* is similar to that of the *Pi3(t)/Pi5(t)/Pii(t)* locus in RIL260 and C104PKT for all of the markers tested. It is probable that *Tetep* was outcrossed to CO39 to produce C104PKT and that this *Tetep*/CO39 line was then used as a donor to generate the *Pi5(t)* locus in RIL260. In support of this hypothesis, we have also observed the C104PKT genomic region in about 10% of the *Moroberekan*/CO39 RIL population when we used the 40N23r as the marker (Jeon et al. 2003). In contrast, the *Pi5(t)* RILs can be distinguished from *Tetep* and CO39 by the hybridization pattern of a few markers, such as JJ80-T3, JJ81-T3, 17I18-12, and 34E14-10 (Fig. 1; data not shown). This suggests that a separate *Tetep*/CO39 outcross was not performed to generate the *Pi5(t)* locus. It is also possible that C104PKT, as derived from a *Tetep*/CO39 cross, contaminated the cross between *Moroberekan* and CO39 and that C104PKT, carrying *Pi3(t)*, is the *Pi5(t)* donor.

Tetep is a well-documented source of durable and broad-spectrum resistance to rice blast (Ahn 1994, 2000). This genome carries at least three resistance genes—*Pi1(t)*, *Pi4*^{a}*(t)*, and a resistance gene in C105TTP-4L23 (Inukai et al. 1994). Kiyosawa (1973) reported that *Tetep* carries *Pik*^{h}*(t)* gene that might be allelic to *Pik(t)*. Because C104PKT, which carries *Pi3(t)*, displays a unique resistance spectrum (Mackill and Bonman 1992), *Pi3(t)* appears to be distinct from *Pi1(t)*, *Pi4*^{a}*(t)*, and C105TTP-4L23.

In the past, researchers needed both a good breeding strategy and a good sense for selecting desirable phenotypes. However, the evaluation of disease resistance or other traits often requires specialized techniques. Recent molecular-marker technology, especially that based on PCR analysis, can greatly reduce the amount of labor needed for evaluating phenotypes by prescreening with MAS. The usefulness of MAS can be increased by creating markers tightly linked to a target gene.

In this study, we developed three dominant DNA markers (JJ80-T3, JJ81-T3 and JJ113-T3) that were evenly distributed within the 170-kb physical region of the *Pi5(t)* locus (Jeon et al. 2003). All were able to amplify positive bands in 34 of the 184 randomly selected rice varieties. Because the expected bands could not be amplified from the remaining varieties, we suggest that this 170-kb interval, including the three DNA markers, is well conserved among these positive varieties. The 34 rice varieties identified through our prescreening process (including *Baekjinju* and IR841-76-1, which displayed moderate resistance to *M. grisea* PO6-6) manifested a resistance phenotype that perfectly matched our PCR-positive genotypes. *Baekjinju* was isolated from the *N*-methyl-*N*-nitrosourea-treated *Ilpum* mutant population (Choi et al. 2002). Because *Ilpum* was resistant and *Baekjinju* was moderately resistant to PO6-6, it is likely that a mutated gene in *Baekjinju* caused the reduced resistance. Future studies are needed to uncover the genetic basis of this interesting phenotype. Using CAPS and DNA gel-blot analysis, we showed that of these 34 PCR-positive varieties, IR 72, *Taebaeg*, and *Jahyangdo* were most similar to *Pi5(t)* at the resistance locus. This suggests that these varieties, together with *Tetep*, could serve as donors of resistance to *M. grisea* isolate PO6-6. Moreover, *Taebaeg*, a *Tongil*-type rice variety, is known to have resistance to 35 *M. grisea* isolates and is classified as a durable resistance cultivar in Korea (Han 2001). Therefore, we propose that the three DNA markers presented here can be useful in MAS for resistance to *M. grisea* isolate PO6-6.

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