The *Pi40* Gene for Durable Resistance to Rice Blast and Molecular Analysis of *Pi40*-Advanced Backcross Breeding Lines

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ABSTRACT

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Rice blast severely reduces production in both irrigated and water-stressed upland ecosystems of tropical and temperate countries. Nearly 50 blast resistance genes have been identified and some of those are incorporated into several rice cultivars. However, most of the resistance genes break down in a few years because of their race specificity and the rapid change in pathogenicity of the blast fungus (*Magnaporthe grisea*). The objective of this study was to analyze advanced backcross breeding lines (ABL) possessing the gene *Pi40* for durable rice blast resistance. In all, 4 resistant genotypes, 4 japonica cultivars, and 10 monogenic differential rice genotypes with some known resistance genes were bioassayed in the greenhouse using seven sequential plantings and 29 virulent *M. grisea*

isolates of Korea. The genotypes with the *Pi40* gene had <3% diseased leaf area, which was significantly below the disease threshold level of 40% considered for durable blast resistance. Moreover, the genotypes with the *Pi40* gene expressed compatibility with only two to three virulent *M. grisea* isolates supporting durability of resistance, in contrast to susceptible cultivars with >50% diseased leaf area and 10 compatible isolates. Of the 10 known resistance genes tested, *Piz-t*, *Piz-5*, and *Pi9* showed differential reactions to the pathogen isolates in seven plantings. Genotyping of the ABL with 260 simple sequence repeat (SSR) markers revealed rapid conversion toward recurrent parent genotypes with fewer donor chromosomal segments (5.3 to 14.5%). Our study based on a sequential testing and background selection of breeding lines with the resistance gene *Pi40* provided valuable information for durable blast resistance breeding in rice.

Additional keywords: blast disease, japonica rice.

Rice blast disease, caused by *Magnaporthe grisea* (T. T. Hebert) M. E. Barr (anamorph: *Pyricularia grisea* Sacc.), is one of the most devastating diseases of rice in both the tropics and temperate countries. In extreme cases, blast disease severity becomes epidemic and causes significant yield losses (34). Host-plant resistance is one of the best strategies for controlling blast disease. During the past decade, nearly 50 blast resistance genes have been identified, and 8 of these genes have been cloned (9,24). However, most of the known resistance genes are race specific and resistant cultivars possessing these genes break down within 2 to 3 years. The blast fungus generally undergoes a series of well-defined developmental steps during leaf infection, including the formation of elaborate penetration structures. The foliar blast pathogen also invades roots, using a typical root-specific pathway (28). Durable resistance of a gene to a disease is resistance that remains effective during its prolonged and widespread use in an environment favorable to the disease. The most powerful test for the durability of blast resistance occurs when a cultivar is widely grown for a long period in an environment favoring the disease. The test for durable resistance must include two factors: (i) time (a long period) and (ii) area (large) (19). To estimate blast resistance, methods such as a blast nursery test, field screening, and virulent isolate inoculation have been used. However, a suitable method for a durability test of blast resistance is not yet established. A sequential planting method (SPM) has been developed to identify durable blast resistance in Korean rice cultivars

(21). Results from an SPM evaluation conducted in a greenhouse were similar to the results from farmers' field data, suggesting that the current SPM is effective in evaluating the durability of rice blast resistance.

Rice breeders in Korea have developed many japonica cultivars with blast resistance. However, the resistance in most cultivars has broken down within a few years after they were released to farmers because of the spread of new races of *M. grisea*, which became virulent to the resistance genes in the cultivars (11). In the 1970s, Tongil-type cultivars were released for cultivation in >75% of the total rice production area. Subsequently, several japonica-type cultivars were grown in the mid-1980s. The proliferation of diverse cultivars of rice with enhanced disease resistance, however, triggered genetic mutations leading to the differentiation of the blast fungus, *M. grisea*. This development eventually led to the breakdown and loss of blast resistance in Tongil-type rice cultivars. Consequently, rice area under Tongil-type cultivars reduced in Korea starting in 1990 (11). The same virulent *M. grisea* isolates also caused great damage to japonica cultivars, and since 1999, japonica cultivars have been frequently susceptible to leaf and neck blast isolates (11). This may be attributed to the narrow genetic diversity of japonica cultivars and the rapid expansion of cultivation area with a few cultivars. The pathogenicity of *M. grisea* isolates of Korea is highly dynamic and sometimes a small section of the virulent isolates spreads rapidly and overcomes the resistance genes. Nevertheless, the development of a blast-resistant cultivar has still been the most effective and economical breeding strategy to control the spread of the disease (11). Attempts should also be made to accumulate new resistance genes imparting durability of cultivars for many years on a large production region.

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Broad-spectrum durable resistance against multiple rice blast pathogen populations is one of the major objectives of rice breeding programs to contain the damage caused by the rice blast fungus (31). Resistance controlled by single genes sometimes remains effective for many years, but this is typically not durable. However, the genetic and molecular basis of durable resistance is poorly understood. Because durability of resistance is essentially unpredictable, the best way to examine its mechanism is probably to examine a cultivar or genotype that has remained resistant to a highly variable pathogen for a long period of time. Durable resistance to *M. grisea* is also conferred by both major and minor genes (3,31).

Genes conferring resistance have been identified in different indica and japonica rice cultivars such as Tjina (*Pib*), Tetep (*Pib*, *Pi1*, and *Pita*), Pai-Kan-Tao (*Pi3*), Moroberekan (*Pi5* and *Pi7*), 5173 (*Piz-5* = *Pi2*), TKM1 (*Piz-t*), Tadukan (*Pita*), Zenith (*Piz*), and LAC23 (*Pi1*) (5,14,31). The genes *Pia*, *Pii*, *Pik*, and *Pik-m* are identified only in japonica cultivars (7). The resistance gene *Pi9* originated from the wild species *Oryza minuta* (25). However, the blast resistance genes *Pib* (32), *Pita* (4), *Pid2* (6), *Piz-5*, *Piz-t*, *Pi9* (35), *Pi36* (26), and *Pi37* (24) have been cloned using map-based cloning strategies. Some major genes, such as *Pi1*, *Piz-5* (*Pi2*), *Pi5*, *Pi9*, and *Pi40*, are reported to have resistance to a broad spectrum of *M. grisea* isolates (16,17). Most of the Korean japonica cultivars are derived from Japanese cultivars having the blast resistance genes *Pia*, *Pib*, *Pii*, *Pik*, and *Pita* based on pedigree analysis (8). However, the effective use of blast resistance genes has been limited due to the breakdown in blast resistance as a result of the development of new blast races. Therefore, it is imperative to identify broad-spectrum durable blast resistance genes for effective protection against dynamic blast isolates of *M. grisea*.

Of the several known blast resistance genes, *Pi40* has been identified in an indica introgression line that has inherited the resistance gene from an EE genome of wild species *O. australiensis* (17). The *Pi40* gene has been introduced into two susceptible japonica cultivars by marker-assisted backcross breeding. The objective of this study was to analyze advanced backcross breeding lines (ABL) possessing the gene *Pi40* for durable rice blast resistance.

MATERIALS AND METHODS

Plant germplasm. Four ABL derived from a cross between two japonica cultivars (Jinbubyee and Junambyee) and IR65482-4-136-2-2 carrying the *Pi40* gene, and 10 monogenic differential

genotypes (MDG) with known blast resistance genes were used in this study (Table 1). Two japonica cultivars, Ilpumbyee and Palgongbyee, were used as susceptible (S) and resistant (R) checks, respectively, during screening using SPM. These cultivars were also evaluated in the field for their reaction to field blast isolates. Two japonica cultivars, Nagdongbyee and Hopyeongbyee, were also used as S checks in field testing. Seed of Jinbubyee, Junambyee, Ilpumbyee, Nagdongbyee, Hopyeongbyee, and Palgongbyee were obtained from the Rice Research Division of the National Institute of Crop Science (NICS), Rural Development Administration (RDA), Republic of Korea, and seed of MDG were obtained from the Plant Breeding, Genetics, and Biotechnology Division of the International Rice Research Institute (IRRI), Los Baños, Philippines.

***M. grisea* inoculation.** Jinbubyee, Junambyee, IR65482-4-136-2-2, four ABL, and 10 MDG were screened against 29 individual *M. grisea* isolates as well as a mixture of these isolates (Table 2) by spray inoculation in seven sequential plantings following the procedure of Jeung et al. (17). The blast isolates used were collected from Korean paddy fields and blast nurseries from 1984 to 2002 (11,21). The 29 *M. grisea* isolates used in the inoculation test and SPM were the representative isolates from 22 Korean blast races (21). All inoculations and disease evaluations were conducted in the greenhouse of the NICS at Suwon. A single-race spore suspension concentration was adjusted to 1.5×10^5 spores/ml. Seedlings (21 days old) were inoculated with 20 ml of spore suspension by a spray method and were kept for 24 h in darkness inside a dew growth chamber at 25°C and then transferred to a greenhouse with a 12-h-day, 12-h-night photoperiod at 90% relative humidity for 7 days. Disease reactions were scored 7 days post inoculation using a numerical system similar to that of Campbell et al. (5). Percent diseased leaf area (DLA) scoring was obtained from ten random seedlings (1).

Rice blast field test. The test materials and S checks, Nagdongbyee and Hopyeongbyee, were planted in rice blast nurseries at Cheolwon (CW), Suwon (SW), and Jecheon (JC) during the wet season in 2006 and 2007. In all, 50 to 60 seedlings of test genotypes were planted in a complete randomized block design (CRBD) with three replicates. Border rows with a mixture of susceptible cultivars were set up around each replicate to sustain a diverse pathogen population. Lesion type was scored on a scale of 1 (R) to 9 (S) in accordance with the standard evaluation method of IRRI (13). The DLA of plants in the field showing symptoms of leaf blast was measured at the maximum tillering stage in JC and CW experimental plots.

TABLE 1. List of four advanced backcross breeding lines, 10 monogenic differential genotypes, donor parent, and four japonica rice cultivars used in this study

Cultivar, breeding line ^y	Description, genes ^z	Remarks
Jinbubyee	Recurrent parent	Korean elite japonica cultivar
Junambyee	Recurrent parent	Korean elite japonica cultivar
IR65482-4-136-2-2	Donor parent	<i>Oryza australiensis</i> introgression line with <i>Pi40</i> gene
SR30062-2-1-12-5-1-7-B	ABL1	Jinbubyee genetic background
SR30070-1-1-1-5-B	ABL2	Junambyee genetic background
SR30070-1-1-7-2-1-4-B	ABL3	Junambyee genetic background
SR30070-2-10-5-2-1-B	ABL4	Junambyee genetic background
Palgongbyee	Control	Japonica cultivar resistance to blast
Ilpumbyee	Control	Japonica cultivar susceptible to blast
IRBLi-F5	<i>Pii</i>	LTH monogenic differential genotype
IRBLz-Fu	<i>Piz</i>	LTH monogenic differential genotype
IRBLz5-CA	<i>Piz-5</i>	LTH monogenic differential genotype
IRBLzt-T	<i>Piz-t</i>	LTH monogenic differential genotype
IRBLb-B	<i>Pib</i>	LTH monogenic differential genotype
IRBL1-CL	<i>Pi1</i>	LTH monogenic differential genotype
IRBL5-M	<i>Pi5</i>	LTH monogenic differential genotype
IRBL9-W	<i>Pi9</i>	LTH monogenic differential genotype
IRBLta-K1	<i>Pita</i>	LTH monogenic differential genotype
Lijiangxintuanheigu	LTH	Japonica cultivar with no blast resistance gene

^y IRBL: International Rice Research Institute-bred blast resistance lines.

^z ABL: advanced backcross breeding lines having *Pi40* gene.

Sequential planting method of evaluation. The SPM was used for a durability test of blast resistance genes (21). In this method, blast resistance level was investigated by a series of seven plantings with 2-week intervals and plants were inoculated at the four- to five-leaf stage by a spray method with a mixture of 29 blast isolates in the first planting. In total, 10 g of seed per 19 genotypes was sown in 40-by-30-by-10-cm plastic trays for each of the seven plantings. Following inoculation, the seedlings were kept for 24 h in the dark inside a dew growth chamber at 25°C and then transferred to a greenhouse with 90% relative humidity for 7 days. From the second to seventh sequential planting, test seedlings were put at the side of the previous planting that had full disease symptoms and was infected naturally in a temperature-controlled greenhouse. The seedlings were covered with polyvinyl sheets overnight and misted with water three to four times each day during the daytime to maintain high humidity. The DLA was estimated visually following the standard evaluation system for rice (13) and scored 14 days after natural infection for every sequential planting. A CRBD with three replications was used. The criteria to determine durable blast resistance were the presence of compatible isolates and disease severity <40% of the DLA (21).

Molecular analysis for gene diagnosis and genotyping of ABL. The presence or absence of eight major blast resistance genes (*Pib*, *Pia*, *Piz*, *Piz-t*, *Pi9*, *Pi5*, *Pita*, and *Pi40*) was validated using foreground selection with gene-specific DNA markers in the parents and ABL (Table 3). The 260 simple-sequence repeat (SSR) markers of known chromosomal positions distributed evenly on the 12 chromosomes with an average interval of 5.9 centimorgans (cM) were used in a genome-wide survey to identify the chromosome segment substitution locations in the four ABL compared with the donor line. The length of substituted chromosome segments in ABL was estimated based on the graphical genotyping procedure of Xi et al. (33). A chromosome segment flanked by homozygous marker alleles of the donor parent was considered to be a 100% donor type, a chromosome segment

flanked by homozygous marker alleles of the recipient parent was considered to be a 0% donor type, and a chromosome segment flanked by one marker allele of the donor parent and another marker allele of the recipient parent was considered to be a 50% donor type.

Polymerase chain reaction (PCR) was performed in a total volume of 20 µl containing 40 ng of DNA template, 10 pmole of each primer, 1.5 mM MgCl₂, 0.2 mM dNTP, and 1 U of *Taq* polymerase. The PCR amplification condition was one cycle at 95°C for 4 min; followed by 35 cycles at 95°C for 30 s, at 55°C for 30 s, and 72°C for 1 min; with a final extension at 72°C for 10 min (PTC-200 Thermocycler; Bio-Rad, Germany). The PCR products were detected using a 4% denaturing polyacrylamide gel electrophoresis with silver staining. The SSR analysis was conducted as described in McCouch et al. (27).

Data analysis. The linkage and orientation of SSR markers were assigned following the SSR map constructed by McCouch et al. (27) and as depicted in Gramene (www.gramene.org/). MapChart 2.0 was used for the graphical presentation of the linkage genetic map between SSR markers (30). The least significant difference (LSD) and Duncan's multiple range test were used for multiple mean comparisons using the SAS statistical software package (version 8.2; SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Pooled reaction of *M. grisea* isolates on rice genotypes in the sequential planting method. We examined the DLA of plants at each time point of sequential plantings of four ABL, parents, and check cultivars, including that of MDG at each planting of SPM (Table 4). The *Pi40* donor genotype, IR65482-4-136-2-2, expressed strong resistance to Korean *M. grisea* isolates in all sequential plantings. Cv. Palgongbyeon is known to have durable resistance to blast. However, it had maintained a small number of susceptible lesions not exceeding 30% DLA, whereas Ilpumbyeon continuously showed a higher rate of DLA (>60%) in all

TABLE 2. Twenty-nine *Magnaporthe grisea* isolates used for testing reaction to rice blast of Korean differential cultivars

Race no.	Isolate no.	Reaction of Korean differential cultivars ^z							
		A	B	C	D	E	F	G	H
KI-101	04-205	S	S	S	S	S	S	S	S
KI-1113	04-053	S	R	R	R	S	S	S	S
KI-1117	86-228	S	R	R	R	S	R	S	S
KI-1117	03-123	S	R	R	R	S	R	S	S
KI-1119	97-277	S	R	R	R	S	R	R	S
KI-165	04-005	S	R	S	S	S	S	S	S
KI-197	R93-093	S	R	R	S	S	S	S	S
KI-209	97-268	R	S	S	S	R	S	S	S
KI-307	87-108	R	R	S	S	S	R	R	S
KI-309	97-254	R	R	S	S	R	S	S	S
KI-313	R88-002	R	R	S	S	R	R	S	S
KI-315	85-242	R	R	S	S	R	R	R	S
KI-401	04-004	R	R	R	S	S	S	S	S
KI-405	05-043	R	R	R	S	S	R	S	S
KI-409	93-456	R	R	R	S	R	S	S	S
KJ-101	R90-059	R	R	R	R	S	S	S	S
KJ-101	04-030	R	R	R	R	S	S	S	S
KJ-101	03-085	R	R	R	R	S	S	S	S
KJ-103	93-072	R	R	R	R	S	S	R	S
KJ-105	03-177	R	R	R	R	S	R	S	S
KJ-105	02-319	R	R	R	R	S	R	S	S
KJ-107	03-129	R	R	R	R	S	R	R	S
KJ-107	97-134	R	R	R	R	S	R	R	S
KJ-201	02-039	R	R	R	R	R	S	S	S
KJ-201	05-120	R	R	R	R	R	S	S	S
KJ-203	93-064	R	R	R	R	R	S	R	S
KJ-301	R97-057	R	R	R	R	R	R	S	S
KJ-301	02-013	R	R	R	R	R	R	S	S
KJ-401	89-010	R	R	R	R	R	R	R	S

^z A: Tetep, B: Taebaegbyeon, C: Tongil, D: Yusin, E: Kanto 51, F: Nongbaeg, G: Jinheung, H: Nagdongbyeon; R: resistant, S: susceptible.

sequential plantings. The DLA of Jinbubyeo was 43 to 67% up to the seventh planting and that of Junambyeo was >40% from the first to second sequential plantings but, after the third planting, DLA was <10%. However, the four ABL showed 0 to 3% DLA during the entire sequential plantings. Therefore, considering 40% DLA as a disease threshold index, Palgongbyeo and ABL exhibited durable resistance to Korean *M. grisea* isolates because these cultivars showed a continuous resistance spectrum to blast infection. Junambyeo demonstrated a moderate level of resistance, and Jinbubyeo and Ilpumbyeo were susceptible (Table 4; Fig. 1).

The MDG (Table 1) possessing the genes *Pii*, *Piz*, *Pib*, and *Pita*, and Lijiangxintuanheigu (LTH) showed >50% DLA throughout the duration of sequential plantings (Table 4). The infected plant parts of MDG displayed a higher compatibility to *M. grisea* isolates, suggesting that these resistance genes had no durability to blast resistance. The genes *Piz-t* and *Pil* exhibited >40% DLA from the first to fourth plantings of the SPM but a lower rate of <10% in later plantings. This result indicated that *Piz-t* and *Pil* had a weak resistance to *M. grisea* isolates of Korea, predicting nondurability of these resistance genes. The gene *Pi5* showed 30% DLA in the first planting but displayed a rapid decrease in infection (1%) from the second planting of SPM, suggesting moderate durability to Korean *M. grisea* isolates. However, the genes *Pi9* and *Piz-5* showed the lowest DLA (2 to 4%) during the sequential plantings, suggesting that these genes might have durability and comparatively stable resistance against Korean *M.*

grisea isolates. The improved SPM (21) used in this study can be used as a model system for durable blast resistance breeding. It was interesting to identify the differential development of DLA in tested genotypes. IR65482-4-136-2-2 and four ABL consistently expressed resistance to *M. grisea* isolates in entire plantings, in contrast to *Pi9* and *Piz-5* that had moderate resistance. A very low DLA in ABL may be attributed to a loss of *Pita* and some unknown genes with minor effect due to recombination, which might have some cumulative effect for strong resistance in the donor genotype carrying the *Pi40* gene.

The MDG with *Piz-t* and *Pil* genes with high DLA in the first four plantings and low DLA in the fifth, sixth, and seventh plantings may be attributed to strong interaction between the *R* gene of the host and avirulence gene of the pathogen in which the *M. grisea* isolates lost their virulence or aggressiveness, similar to the fitness loss hypothesis predicted by Vera Cruz et al. (29) for durable resistance to *Xanthomonas oryzae* pv. *oryzae*. The other possibility may be due to epistatic interaction of some unknown resistance genes in the host affecting the virulence of *M. grisea* isolates in later plantings. Further studies are underway to find out the cause of susceptibility of *Piz-t* and *Pil* genes in early plantings of the SPM.

Relationship between compatible isolates and durability of blast resistance. The relationship between compatible blast isolates and resistance genes provided useful information on the genes for durable blast resistance. In our study, four ABL displayed their compatibility with two or three of the 29 isolates.

TABLE 3. Gene-specific polymerase chain reaction primers used for the identification of major blast resistance genes

Resistance genes	Chr. no. ^z	Marker name	Primer sequences used for gene detection		Expected size (bp)	Band type	Reference
			Forward (5'–3')	Reverse (5'–3')			
<i>Pib</i>	2	NSb	atcaactctgccacaaaatcc	cccatatcaccactgtgtcccc	629	Dominant	7
<i>Piz</i>	6	z4792	cgcacgctttccgaactactccgct	agtgtgtgctgcacgtgctttg	340	Dominant	12
<i>Piz-t</i>	6	zt6057	gaagctcaactaggaactgacgc	actggaagtcctctatatgcc	360	Dominant	12
<i>Pi9</i>	6	pBA14	tggtgcactcagaagaa	gcagtgtcatctgtctcc	480	Dominant	25
<i>Pi40</i>	6	9871.T7E2b	caacaaacgggtcgacaaagg	ccccaggctcgtgataccttc	642	Co-dominant	17
<i>Pi5</i>	9	JJ817	gatatggttgaaaagctaactca	atcattgtccttcataatcagagt	1,450	Dominant	22
<i>Pia</i>	11	yc72	aggagaagaagccacaagg	gagctgccacatcttctt	635	Co-dominant	7
<i>Pita</i>	12	YL153/YL154	caacaatttaatcatacag	atgacacctgcgatgcaa	440	Dominant	18

^z Chromosome number.

TABLE 4. Rice blast disease severity and presence of compatible *Magnaporthe grisea* isolates in seven sequential plantings of advanced backcross breeding lines, japonica cultivars, and monogenic differential genotypes evaluated in the greenhouse at Suwon during 2006 and 2007

Cv., line ^x	Diseased leaf area (%) ^y							Compat. ^z
	1st	2nd	3rd	4th	5th	6th	7th	
Jinbubyeo	53.3 d	66.7 e	63.3 cd	60.0 d	63.3 cb	43.3 c	50.0 c	7
Junambyeo	66.7 c	53.3 f	3.0 f	8.7 f	2.0 f	0.4 d	0.1 e	11
IR65482-4-136-2-2 (<i>Pi40</i>)	0.0 f	0.0 h	0.0 f	0.0 g	0.0 f	0.0 d	0.0 f	0
ABL1	0.4 f	0.2 h	0.1 f	0.1 g	0.0 f	0.0 d	0.0 f	2
ABL2	2.0 f	1.3 h	0.3 f	0.0 g	0.0 f	0.0 d	0.0 f	3
ABL3	1.3 f	0.1 h	0.1 f	0.0 g	0.0 f	0.0 d	0.0 f	3
ABL4	3.3 f	2.3 h	0.7 f	0.3 g	0.1 f	0.0 d	0.0 f	3
<i>Pi9</i>	2.0 f	0.4 h	0.1 f	0.1 g	0.3 f	0.0 d	0.0 f	7
<i>Piz-5</i>	2.3 f	4.0 h	1.7 f	1.0 g	0.2 f	0.0 d	0.0 f	9
<i>Piz</i>	80.0 b	83.3 b	80.0 a	70.0 b	63.3 cb	56.7 b	60.0 b	13
<i>Piz-t</i>	90.0 a	66.7 e	60.0 d	46.7 e	4.3 f	0.2 d	0.0 f	4
<i>Pi5</i>	30.0 e	0.8 h	0.3 f	0.3 g	0.1 f	0.1 d	0.0 f	6
<i>Pii</i>	90.0 a	90.0 a	80.0 a	81.7 a	60.0 cd	46.7 c	60.0 b	18
<i>Pib</i>	90.0 a	73.3 d	70.0 b	63.3 cd	80.0 a	70.0 a	60.0 b	28
<i>Pil</i>	53.3 d	43.3 g	40.0 e	48.3 e	13.3 e	1.0 d	0.2 d	17
<i>Pita</i>	90.0 a	80.0 cb	66.7 cb	63.3 cd	56.7 d	56.7 b	60.0 b	15
Lijiangxintuanheigu	90.0 a	76.7 cd	60.0 d	66.7 cb	66.7 b	70.0 a	60.0 b	29
Ilpumbyeo	80.0 b	76.7 cd	66.7 cb	63.3 dcd	63.3 cb	60.0 b	70.0 a	26
Palgongbyeo	30.0 e	5.0 h	1.3 f	2.0 g	4.0 f	0.2 d	0.1 e	10

^x Cultivar or breeding line. ABL1: advanced backcross breeding lines having *Pi40* gene in Jinbubyeo genetic background; ABL2, -3, and -4: advanced backcross breeding lines having *Pi40* gene in Junambyeo genetic background.

^y Means followed by the same letter are not significant at the 5% significance level by the least significant difference test (LSD = 0.05).

^z Number of compatible isolates.

IR65482-4-136-2-2 with the *Pi40* gene alone was compatible with none of the *M. grisea* isolates tested, whereas Jinbubyeo and Junambyeo (recurrent parents) showed compatibility with 7 and 11 *M. grisea* isolates, respectively (Table 4). The resistance gene *Piz-t* had four compatible *M. grisea* isolates and *Pi5*, *Pi9*, *Piz-5*, and Palgongbyeo exhibited a compatible reaction with 6 to 10 *M. grisea* isolates. However, *Piz*, *Pii*, *Pil*, and *Pita* showed compatibility with 13 to 18 *M. grisea* isolates and *Pib*, LTH, and Ilpumbyeo had compatibility with most of the *M. grisea* isolates (Table 4). In another study, we tested four ABL carrying the *Pi40* gene with 78 *M. grisea* isolates selected from 40 Korean *M. grisea* races that detected only two to three compatible isolates (J. P. Suh et al., unpublished). Detection of 2 to 3 compatible isolates may be considered to be broad-spectrum durable resistance compared with resistant cv. Palgongbyeo, which had 10 compatible isolates.

A previous study (21) proposed that a testing model for durable resistance should have five or more compatible isolates and the

Fig. 1. Progress of rice blast reaction in seven sequential plantings in the greenhouse. ABL: advanced backcross breeding lines having *Pi40* gene, donor parent: IR65482-4-136-2-2.

magnitude of DLA until the seventh planting should be <40%. In this study, three MDG with genes *Pi5*, *Pi9*, *Piz-5* (*Pi2*), and Palgongbyeo had 6 to 10 compatible *M. grisea* isolates in contrast to the ABL carrying *Pi40*, which had only 2 to 3 compatible isolates. To have a more reliable model system for durable blast resistance, we propose here two to three compatible isolates and 20% DLA for durable blast resistance. In this model, Korean cv. Palgongbyeo develops moderate resistance because it shows higher DLA and more compatible isolates in contrast to *Pi40*-derived ABL, which have the lowest DLA (<3%). Additionally, we are constructing MDG carrying the *Pi40* gene in an LTH genetic background to understand more about *Pi40*-derived *R*-gene interaction with a wide range of *M. grisea* isolates.

Disease reaction of resistance genes in blast nurseries. Cvs. Jinbubyeo and Junambyeo displayed an average score of 3.8 and 1.8, respectively (Table 5). IR65482-4-136-2-2 and four ABL possessing the *Pi40* gene expressed complete resistance to *M. grisea* isolates in all field blast nurseries in both years. Most of the MDG showed a differential reaction in blast nursery tests across the regions and years. The resistance genes *Pi5*, *Pi9*, *Piz-5*, and *Piz-t* and cv. Palgongbyeo, with an unknown resistance gene, showed stable resistance across the environment, with an average score of 1.7 to 3.0, and *Pil* showed a moderate resistance score of 4.0 in both years. However, *Piz*, *Pii*, *Pib*, *Pita*, LTH, and Ilpumbyeo had an average score of >7.0, indicating susceptibility in the three blast nurseries (Table 5).

Status of known genes and genetic background selection of ABL. Rice blast *R* genes *Pib*, *Pita*, *Piz-t*, and *Pi40* were detected in the donor parent, IR65482-4-136-2-2, and *Piz* and *Pib* were identified in Jinbubyeo and Junambyeo, respectively (Fig. 2). Four ABL developed from the crosses between Jinbubyeo, Junambyeo, and IR65482-4-136-2-2 had marker alleles specific to *Pib*, *Piz-t*, and *Pi40*. *Pib*, *Piz-t*, and *Pi40* were inherited from the donor parent, IR65482-4-136-2-2 (Fig. 2). The gene-specific DNA markers for *Pi9*, *Pi5*, and *Pia* did not produce any positive band in the parents and ABL. Ilpumbyeo showed positive bands with *Pib* and *Pii* gene-specific markers, and Palgongbyeo showed bands specific to the genes *Pib* and *Pia*.

In total, 260 SSR markers were used for background selection of the four ABL along with the blast-resistant donor line, and a

TABLE 5. Rice blast reactions of advanced backcross breeding lines, japonica cultivars, and monogenic differential genotypes in three field blast nurseries at Cheolwon, Suwon and Jecheon in 2006 and 2007

Cv., line ^x	<i>R</i> genes ^y	Reaction to rice blast (1–9) ^z								
		2006			2007			Average		
		CW	SW	JC	CW	SW	JC	2006	2007	Overall
Jinbubyeo	<i>Piz</i>	4	3	5	5	2	4	4.0	3.7	3.8
Junambyeo	<i>Pib</i>	3	1	2	1	1	3	2.0	1.7	1.8
Donor	<i>Pi40</i> , <i>Piz-t</i> , <i>Pib</i> , <i>Pita</i>	1	1	1	1	1	1	1.0	1.0	1.0
ABL1	<i>Pi40</i> , <i>Piz-t</i> , <i>Pib</i>	1	1	1	1	1	1	1.0	1.0	1.0
ABL2	<i>Pi40</i> , <i>Piz-t</i> , <i>Pib</i>	1	1	1	1	1	1	1.0	1.0	1.0
ABL3	<i>Pi40</i> , <i>Piz-t</i> , <i>Pib</i>	1	1	1	1	1	1	1.0	1.0	1.0
ABL4	<i>Pi40</i> , <i>Piz-t</i> , <i>Pib</i>	1	1	1	1	1	1	1.0	1.0	1.0
IRBL5-M	<i>Pi5</i>	2	4	4	1	3	1	3.3	1.7	2.5
IRBL9-W	<i>Pi9</i>	4	2	4	3	1	3	3.3	2.3	2.8
IRBLz5-CA	<i>Piz-5</i>	4	1	3	2	3	5	2.7	3.3	3.0
IRBLz-Fu	<i>Piz</i>	9	6	8	8	7	7	7.7	7.3	7.5
IRBLzt-T	<i>Piz-t</i>	3	1	2	1	1	2	2.0	1.3	1.7
IRBLi-F5	<i>Pii</i>	7	8	8	7	8	7	7.7	7.3	7.5
IRBLb-B	<i>Pib</i>	8	8	9	7	9	9	8.3	8.3	8.3
IRBL1-CL	<i>Pil</i>	5	3	5	2	4	5	4.3	3.7	4.0
IRBLta-CT2	<i>Pita</i>	7	7	6	7	9	8	6.7	8.0	7.3
LTH	None	9	8	8	9	9	9	8.3	9.0	8.7
Ilpumbyeo	<i>Pib</i> , <i>Pii</i>	7	7	8	8	6	7	7.3	7.0	7.2
Palgongbyeo	<i>Pib</i> , <i>Pia</i>	3	3	3	4	1	3	3.0	2.7	2.8

^x Cultivar or breeding line. Donor: IR65482-4-136-2-2; ABL1 and ABL2, -3, and -4: advanced backcross breeding lines having *Pi40* gene in Jinbubyeo and Junambyeo genetic backgrounds, respectively; IRBL: International Rice Research Institute-bred blast resistance line; LTH: Lijiangxintuanheigu.

^y Resistance (*R*) genes were evaluated by using DNA markers reported as linked to eight blast resistance genes: *Pib*, *Piz*, *Piz-t*, *Pi9*, *Pia*, *Pi5*, *Pita*, and *Pi40*.

^z CW: Cheolwon; SW: Suwon; JC: Jecheon; blast nursery test: resistance (1–3), moderate resistance (4–6), and susceptibility (7–9).

Fig. 2. Analysis of the presence of *Pi40* and other known genes in advanced backcross rice breeding lines. Polymerase chain reaction profiles generated using dominant DNA markers for *Pib*, *Piz*, *Piz-t*, and *Pita* genes and co-dominant DNA markers (*Pi40*-specific). RP1 (recurrent parent 1): Jinbubyeo, RP2 (recurrent parent 2): Junambyeo, DP (donor parent): IR65482-4-136-2-2, ABL: advanced backcross breeding lines in Jinbubyeo and Junambyeo genetic background. CK (check): *Pib* (IRBLb-B), *Piz* (IRBLz-Fu), *Piz-t* (IRBLzt-T), *Pita* (IRBLta-K1), and *Pi40* (IR65482-4-136-2-2).

genetic map covering a 1,512.4-cM region of the *O. sativa* genome was constructed (Fig. 3). The marker polymorphisms between Jinbubyeo, Junambyeo, and IR65482-4-136-2-2 were 76.2 and 74.1%, respectively. Each ABL contains an SSR marker-defined chromosome segment from the donor in the genetic background of the recurrent parents, Jinbubyeo and Junambyeo. The percentage of substituted chromosome segments in ABL1 ranged from 0 to 48.8%, with an average of 14%. Chromosomes 7 and 12 were completely converted to the Jinbubyeo parent genotype without any introgressed segments from IR65482-4-136-2-2. The average percentage of donor parent chromosome substitution in ABL2, ABL3, and ABL4 was 5.3, 14.5, and 11%, respectively (Table 6). Chromosomes 1 and 2 did not inherit any substituted chromosome segments from the donor parent in ABL2, ABL3, and ABL4. Interestingly, ABL2 was indeed free from introgression of donor chromosome segments on chromosomes 1, 2, 3, 4, 5, 7, and 8. The substituted chromosome segments were also distributed around the region of *Pi40* located on chromosome 6 in the genetic background of all ABL.

In an efficient breeding program using marker-assisted selection, it is necessary to determine the genetic background of the progenies having chromosome segments substituted from donor genotypes contributing resistance genes (10,33). In our study, ABL2 inherited the smallest size (5.3%) of the substituted chromosome segments from the donor genotypes and had phenotypic

Fig. 3. Substituted chromosome segments in four advanced rice backcross breeding lines (ABL) in japonica genetic background. A, B, C, and D are ABL1, ABL2, ABL3, and ABL4, respectively. The black box indicates substituted chromosome segments of the donor parent in ABL.

TABLE 6. Simple sequence repeat markers with polymorphism between recurrent parent and donor parent and substituted chromosome segments from donor parent in the advanced backcross breeding lines of rice

Chr. no. ^u	Markers ^v	Chr. length (cM) ^w	Interval (cM) ^x	Polymorphism (%) ^y		Chromosome segments of DP (%) ^z			
				RP1/DP	RP2/DP	ABL1	ABL2	ABL3	ABL4
1	34	181.8	5.3	76.5	76.5	21.9	0	0	0
2	29	157.9	5.4	79.3	75.9	10.2	0	0	0
3	26	164.4	6.3	84.6	80.8	4.9	0	18.5	28.6
4	21	129.6	6.2	57.1	66.7	5.8	0	3.4	3.4
5	22	122.0	5.5	59.1	59.1	3.6	0	6.6	15.7
6	22	124.4	5.7	77.3	63.6	23.8	13.7	33.4	20.5
7	21	118.3	5.6	85.7	76.2	0	0	6.5	0
8	19	119.9	6.3	84.2	78.9	6.3	0	20.9	34.8
9	15	92.1	6.1	73.3	80.0	1.7	16.7	0	0
10	15	83.8	5.6	86.7	80.0	41.2	7.0	64.8	7.0
11	20	115.1	5.8	75.0	70.0	48.8	3.5	3.5	0
12	16	103.1	6.4	75.0	81.3	0	22.3	16.9	22.6
Total	260	1,512.4	5.9	76.2	74.1	14.0	5.3	14.5	11.0

^u Chromosome number.

^v Number of markers.

^w Chromosome length in centimorgans (cM).

^x Average marker interval.

^y RP1 (recurrent parent 1): Jinbubyeo, RP2 (recurrent parent 2): Junambyeo, DP (donor parent): IR65482-4-136-2-2.

^z ABL1 and ABL2, -3, and -4: advanced backcross breeding lines in Jinbubyeo and Junambyeo genetic background, respectively.

traits similar to those of the recurrent parent. It was converted completely with the recurrent parent genome, except for chromosomes 6, 9, 10, 11, and 12, suggesting marker-assisted backcross breeding as a powerful tool for novel gene introgression and selection of an improved genotype in a short time (15). However, ABL1 had 14% substituted chromosome segments in the genetic background of Jinbubyeo and exhibited a variable phenotypic performance compared with Jinbubyeo. The substituted chromosome segments of the donor parent were distributed on all chromosomes except for chromosomes 7 and 12, which might be linked with quantitative trait loci for either agronomic traits or the inhibition of crossovers on those chromosomal regions. Accumulation of donor-derived segments on chromosome 6 in all ABL could be attributed to the selection of genotypes with blast resistance using foreground selection of resistance gene *Pi40*.

The survey of four ABL with DNA markers specific for the resistance genes *Pib*, *Piz*, *Piz-t*, *Pi9*, *Pi5*, *Pia*, *Pita*, and *Pi40* revealed the presence of only three genes: *Pib*, *Piz-t*, and *Pi40*. This indicates that *Piz* and *Pita* were lost during the recombination process in segregating generations. Additionally, the *Pi40* gene had already been differentiated from *Piz*, *Piz-5*, *Pi9*, and *Piz-t* by haplotyping and pathotyping assays with virulent *M. grisea* isolates (17). Four ABL showed strong resistance to *P. grisea* isolates during entire plantings and blast nurseries in contrast to MDG with *Piz-t* and *Pib* genes that was susceptible. This result confirms that *Pi40* is differentiated from *Piz-t* and *Pib* and has expressed durable resistance to blast. However, the cumulative effect of *Pib*, *Piz-t*, and *Pi40* might have an advantage for durable resistance breeding, which needs further research.

Durable resistance to blast is often considered to be associated with only partial resistance (31). Some cultivars with major resistance genes have been used in rice production for a long time without a complete loss of resistance to blast (2,20). For example, *Piz-5* and *Pi9* confer broad-spectrum resistance to diverse *M. grisea* isolates and *Piz-5*, especially, has been used for improving durable resistance to blast in rice breeding programs for >10 years (25).

Durable resistance to blast is sometimes reported as quantitative in nature even though there is no successful development of blast-resistant cultivars with genes of quantitative inheritance. However, some detailed studies have been conducted using several candidate defense response (DR) genes such as oxalate oxidase, chitinase, PR1, and so on, and it is concluded that a DR gene such as oxalate oxidase may colocalize and may not actually contribute to blast resistance (23). In this context, it is evident

from our study that *Pi40* has broad-spectrum durable resistance to Korean *M. grisea* isolates. *Pi40* is indeed a major genetic factor, with 71.3% phenotypic variation for quantitative blast resistance (17). In the future, the *Pi40* gene will be cloned and the resistance gene product will provide information on the mechanism of durable blast resistance in rice.

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