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Notes

Development of Monogenic Lines of Rice for Blast Resistance

Hiroshi Tsunematsu*[1)], Mary Jeanie T. Yanoria[1)], Leodegario A. Ebron[1)], Nagao Hayashi[2,3)], Ikuo Ando[2,4)], Hiroshi Kato[1,5)], Tokio Imbe[1,6)] and Gurdev S. Khush[1)]

[1)] *International Rice Research Institute*, MCPO Box. 3127, Makati 1271, Philippines
[2)] *National Agriculture Research Center*, 3-1-1 Kannondai, Tsukuba, Ibaraki 305-8666, Japan
[3)] Present address; *Aichi-ken Agriculture Research Center, Mountainous region Agricultural Research Institute*, 11 Susogaeto, Inahashi, Inabu, Aichi 441-2513, Japan
[4)] Present address; *Hokkaido National Agricultural Experiment Station*, 1 Hitsujigaoka, Toyohira, Sapporo, Hokkaido 062-8555, Japan
[5)] Present address; *Department of Lowland Farming, Tohoku National Agricultural Experiment Station*, 3 Shimofurumichi, Yotsuya, Omagari, Akita 014-0102, Japan
[6)] Present address; *National Agriculture Research Center*, 3-1-1 Kannondai, Tsukuba, Ibaraki 305-8666, Japan

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Blast, caused by *Pyricularia grisea* (Cooke) Sacc., is one of the most destructive diseases of rice (*Oryza sativa* L.). Information about the virulence of rice blast isolates is important for genetic studies and breeding of resistant varieties. Varieties with a single but different resistance gene are ideal as differentials for pathogenicity tests of a pathogen (Flor 1956). A set of international differential varieties for rice blast which was established by Atkins *et al.* (1967) has been used in various parts of the world and has enabled researchers to compare research information. However, since genetic analysis of the resistance of the varieties is lacking, differentiation of races based on their avirulence could not be carried out.

Two sets of differential varieties (Yamada *et al.* 1976, Kiyosawa 1984) have been used in Japan to identify races of the blast fungus. Each differential variety with one resistance gene effective against Japanese races was selected based on genetic analysis. Shin 2, one of the differentials, carries *Pi k-s*, which is not effective against Japanese races but is effective against a Philippine blast isolate (Kiyosawa 1969a). Then the presence of *Pi k-s* in the differentials was not deemed to be important in Japan. These sets enabled to differentiate blast races based on their avirulence in Japan.

In Shin 2, *Pi sh* was identified using a Japanese isolate (Imbe and Matsumoto 1985). We first inoculated a few hundred blast isolates to Japanese differentials at the International Rice Research Institute (IRRI) in the Philippines. As a result, *Pi k-s* was found to be effective against some of the Philippine isolates and *Pi sh* showed moderately resistant reactions to almost all the isolates used (data not shown). Since *Pi k-s* and *Pi sh* were not included in the differentials for race identification, the Japanese differential sets could not be used in the Philippines. A set of differential lines with single known resistance genes was needed to identify the virulence of rice blast isolates. We therefore developed a set of lines with single but different blast resistance genes as monogenic lines. A set of monogenic lines could be suitable for use as differentials for rice blast because of the superior ability of these lines for race differentiation. The monogenic lines also can be used for genetic analysis of genes for blast resistance.

Pi 19 (t) was identified in the Japanese variety Aichi Asahi (Hayashi *et al.* 1998). This gene could not be incorporated into the monogenic lines at IRRI, as it was not effective against any of the isolates at IRRI. Therefore, selection of *Pi 19* (t) and confirmation of the existence of *Pi a*, *Pi k-s* and *Pi sh* in the lines were carried out at the National Agriculture Research Center (NARC) in Japan through the Japan-IRRI shuttle research project.

Development of monogenic lines

To develop a line with a single blast resistance gene, we applied the backcross breeding method. Varieties and lines with known gene(s) for resistance were used as donors. Lijiangxintuanheigu (LTH) was used as a recurrent parent in the backcrossing. LTH, which is a *japonica* variety from Yunnan Province in China, is highly susceptible to rice blast. No isolate incompatible with LTH was found in China (Ling *et al.* 1995) and all of the blast isolates evaluated at IRRI so far were also compatible with the variety. No major resistance gene for rice blast has been identified in LTH. All of the materials were planted under long-day treatment to extend the growing period, because most of the lines probably carry the photosensitivity inherited from LTH. A 30-minute interruption of dark period was performed as a long-day treatment from 21 days after seeding (DAS) to 70-75 DAS.

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*Corresponding author (e-mail: tsunematu@agr.kyushu-u.ac.jp)

To confirm the existence of a single resistance gene, BC_1F_2 populations and their selected progenies were inoculated with suitable isolates. A maximum of 20 pre-germinated seeds of a line was used for the inoculations. Inoculation was perfprmed at the 4.0- to 4.9-leaf seedling stage by using the spraying method (Inukai *et al.* 1994, Hayashi *et al.* 1998). Each seedling was examined 5 to 7 days after inoculation using a modified classification based on a 0-5 scale (Mackill and Bonman 1992). All the isolates of *Pyricularia grisea* used at IRRI were either maintained as stock cultures at the Entomology and Plant Pathology Division of IRRI or were isolated from the blast nursery of IRRI. All the isolates used at NARC were maintained at the Rice Pathology Laboratory in NARC.

Only one resistance gene was transferred from a donor harboring more than one resistance gene. As an example, Aichi Asahi, one of the Japanese differential varieties, carries two blast resistance genes, *Pi a* (Yamasaki and Kiyosawa 1966) and *Pi 19* (t) (Hayashi *et al.* 1998). Aichi Asahi was used as a donor for the two genes. Breeding scheme of monogenic lines having *Pi a* and *Pi 19* (t) is shown in Fig. 1. Aichi Asahi was crossed and backcrossed with LTH. Twenty-four BC_1F_1 plants were selfed. The 24 BC_1F_2 families were inoculated with Ina72 (*Av-a* and *Av-19*+) and CHNOS58-3-1 (*Av-a*+ and *Av-19*) at NARC.

For selecting the monogenic line with *Pi a*, a family that showed segregation for resistance to Ina72 and a susceptible reaction to CHNOS58-3-1 was selected. This family was expected to be heterozygous for *Pi a* but lacked *Pi 19* (t). The same BC_1F_2 families were inoculated with B90002 (*Av-a* and *Av-19*+) and C923-49 (*Av-a* and *Av-19*+) at IRRI. The segregation pattern of *Pi a* in these lines was identical both at NARC and at IRRI. Nine resistant plants in the selected BC_1F_2 family were selfed. Among the nine BC_1F_3 lines, two lines were homozygous resistant to B90002. Thus these two lines were homozygous resistant to *Pi a* but did not carry *Pi 19* (t). From the two lines, seven resistant plants were selected and selfed. All of these BC_1F_4 lines were homozygous resistant to B90002. These BC_1F_4 lines were also inoculated with TH68-140

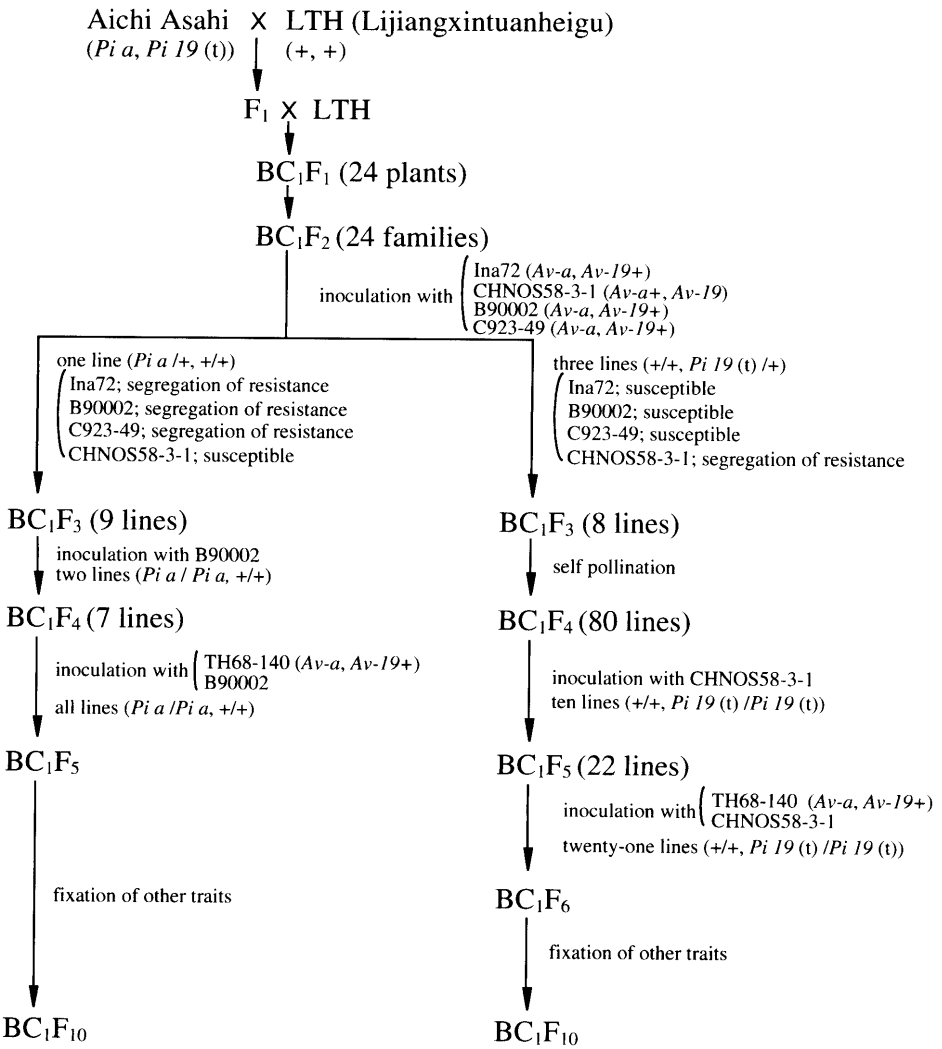

Fig. 1. Breeding scheme of monogenic lines with *Pi a* and *Pi 19* (t)

(*Av-a* and *Av-19*+) at NARC. All of the plants in each line were resistant to TH68-140. The homozygosity of *Pi a* was thus confirmed. These lines were self-pollinated until they were morphologically uniform.

For obtaining a monogenic line with *Pi 19* (t), three BC_1F_2 families that showed segregation for resistance to CHNOS58-3-1 but a susceptible reaction to Ina72 were selected at NARC. These families which were expected to be heterozygous for *Pi 19* (t) but lacked *Pi a* were inoculated with B90002 and C923-49 at IRRI. All the plants in these families were susceptible to both isolates. Eight plants of the selected families were selfed and BC_1F_3 lines were obtained. Ten BC_1F_4 lines were derived from each BC_1F_3 line. A total of 80 BC_1F_4 lines was tested with CHNOS58-3-1 at NARC. Ten lines were found to be homozygous resistant. These lines were homozygous for *Pi 19* (t) but did not have *Pi a*. From the 10 BC_1F_4 lines, 22 plants were randomly selected and self-pollinated. These 22 BC_1F_5 lines were inoculated with CHNOS58-3-1 and TH68-140 at NARC. All the plants of these lines were resistant to CHNOS58-3-1. Twenty-one lines showed a susceptible reaction to TH68-140 and only one line was moderately resistant to this isolate. Two lines that were resistant to CHNOS58-3-1 but susceptible to TH68-140 were selected. These lines were self-pollinated until they were morphologically uniform.

For developing other lines, inoculation and selection with suitable isolates were carried out in the same way as for Aichi Asahi. Some of the lines were also backcrossed to select out unnecessary gene(s). From the 25 resistant donors, a total of 29 lines with single genes was developed (Table 1). Monogenic homozygous lines for 23 known resistance genes were obtained. These lines were designated as IRBL, followed by the resistance gene and an abbreviation for the resistant donor. For example, IRBLa-A is the monogenic line with *Pi a* developed from Aichi Asahi.

Fourteen genes for blast resistance—*Pi a*, *Pi i*, *Pi k* (Yamasaki and Kiyosawa 1966), *Pi ta* (Kiyosawa 1966), *Pi ta-2* (Kiyosawa 1967a), *Pi z* (Kiyosawa 1967b), *Pi k-s* (Kiyosawa 1969a), *Pi k-p* (Kiyosawa 1969b), *Pi k-h* (Kiyosawa and Murty 1969), *Pi z-t* (Yokoo and Kiyosawa 1970), *Pi b*, *Pi t* (Kiyosawa 1972), *Pi k-m* (Kiyosawa 1978), *Pi sh* (Imbe and Matsumoto 1985)—were identified in Japan. Monogenic lines with all of these genes except for *Pi t* were developed. The monogenic line with *Pi t* is under development. In accordance with the naming and symbolization of blast resistance genes, new blast resistance genes are designated as *Pi* followed by a numeral. *Pi 1* to *Pi 20* have been designated (Kinoshita 1998). Monogenic lines with nine of the genes, e.g. *Pi 1*, *Pi 3*, *Pi 5* (t), *Pi 7* (t), *Pi 9* (t), *Pi 11* (t), *Pi 12* (t), *Pi 19* (t) and *Pi 20*, were developed. Mackill and Bonman (1992) developed near-isogenic lines (NILs) of CO39 with *Pi 1* (t), *Pi 2* (t), *Pi 3* (t) *Pi 4-a* (t) and *Pi 4-b* (t). Inukai *et al.* (1994) investigated the relationship between the resistance genes in the NILs of CO39 and the Japanese differentials. It was found that *Pi 1* and *Pi 3* were new genes. *Pi 2* (t) was renamed as *Pi z-5* because it was allelic to the *Pi z*. *Pi 4-a* (t) was found to be identical with *Pi ta*. Wang *et al.* (1994) identified and mapped *Pi 5* (t) and *Pi 7* (t) using recombinant inbred lines derived from the cross between CO39 and Moroberekan. Hayashi *et al.* (1998) identified *Pi 19* (t) in Aichi Asahi and Imbe *et al.* (1997) reported *Pi 20* in IR24. Although *Pi 9* (t), *Pi 11* (t) and *Pi 12* (t) are not registered by the committee on rice gene symbolization of the Rice Genetics Cooperative, monogenic lines with these resistance genes were developed. Brar and Khush (1997) reported *Pi 9* (t) in an introgression line of *Oryza minuta*. Zhu *et al.* (1993) identified *Pi zh* in Zhaiyeqing 8 and it was renamed as *Pi 11* (t) (Kinoshita *et al.* 1994). Inukai *et al.* (1996) reported *Pi 12* (t) in the recombinant inbred line. The remaining resistance genes identified recently have not yet been incorporated into the monogenic lines. Monogenic resistant lines with only the major resistance genes were developed and genes conferring partial resistance were not considered in this study.

Table 1. Monogenic lines with different genes for blast resistance

Line	Resistance gene	Donor	Generation
IRBLa-A	*Pi a*	Aichi Asahi	BC_1F_{10}
IRBLa-C	*Pi a*	CO39	BC_1F_{10}
IRBLi-F5	*Pi i*	Fujisaka 5	BC_1F_{10}
IRBLks-F5	*Pi k-s*	Fujisaka 5	BC_1F_{10}
IRBLks-S	*Pi k-s*	Shin 2	BC_1F_{10}
IRBLk-Ka	*Pi k*	Kanto 51	BC_1F_9
IRBLkm-Ts	*Pi k-m*	Tsuyuake	BC_1F_6
IRBLkp-K60	*Pi k-p*	K60	BC_1F_8
IRBLkh-K3	*Pi k-h*	K3	BC_1F_8
IRBLz-Fu	*Pi z*	Fukunishiki	BC_1F_{10}
IRBLz5-CA	*Pi z-5* (=*Pi 2* (t))	C101A51	BC_3F_8
IRBLzt-T	*Pi z-t*	Toride 1	BC_1F_{10}
IRBLta-K1	*Pi ta* (=*Pi 4* (t))	K1	BC_2F_8
IRBLta-CT2	*Pi ta*	C105TTP2L9	BC_3F_8
IRBLta-CP1	*Pi ta*	C101PKT	BC_5F_6
IRBLta2-Pi	*Pi ta-2*	Pi No. 4	BC_1F_5
IRBLta2-Re	*Pi ta-2*	Reiho	BC_1F_6
IRBLb-B	*Pi b*	BL1	BC_1F_8
IRBLsh-S	*Pi sh*	Shin 2	BC_1F_{10}
IRBLsh-B	*Pi sh*	BL1	BC_1F_8
IRBL1-CL	*Pi 1*	C101LAC	BC_3F_8
IRBL3-CP4	*Pi 3*	C104PKT	BC_2F_8
IRBL5-M	*Pi 5* (t)	RIL249[1)]	BC_3F_8
IRBL7-M	*Pi 7* (t)	RIL29[1)]	BC_3F_8
IRBL9-W	*Pi 9* (t)	WHD-IS-75-1-127	BC_3F_8
IRBL11-Zh	*Pi 11* (t)	Zhaiyeqing 8	BC_2F_8
IRBL12-M	*Pi 12* (t)	RIL10[1)]	BC_2F_8
IRBL19-A	*Pi 19* (t)	Aichi Asahi	BC_1F_{10}
IRBL20-IR24	*Pi 20*	ARL24[2)]	BC_1F_6

[1)] Recombinant inbred lines from a cross of CO39 and Moroberekan (Wang *et al.* 1994)

[2)] Recombinant inbred line from a cross of Asominori and IR24 (Tsunematsu *et al.* 1996)

Reactions of the monogenic lines to blast isolates

The monogenic lines, their donors and some of the differential varieties were inoculated with 12 representative

Table 2. Reactions of monogenic lines, their donors and differentials to selected Philippine blast isolates

Line/variety	Resistance gene	Reaction to isolate[1)]											
		1	2	3	4	5	6	7	8	9	10	11	12
IRBLa-A	*Pi a*	S	S	S	S	S	S	R	R	S	S	S	S
IRBL19-A	*Pi 19 (t)*	S	S	S	S	S	S	S	S	S	S	S	MS
Aichi Asahi	*Pi a, Pi 19* (t)	S	S	S	S	S	S	R	R	S	S	S	S
IRBLa-C	*Pi a*	S	S	S	S	S	S	R-M	R	S	S	S	S
CO39	*Pi a*	S	S	S	S	S	S	R	R	S	S	S	MS
IRBLi-F5	*Pi i*	M	S	R-M	S	R	S	S	S	R	S	S	S
IRBLks-F5	*Pi k-s*	S	S	S	S	S	S	S	S	R	R	S	S
Fujisaka 5	*Pi i, Pi k-s*	R-M	S	R	S	R	S	MS	S	R	R	S	M-S
IRBLks-S	*Pi k-s*	M	S	S	S	S	S	MS-S	S	R	R	S	S
IRBLsh-S	*Pi sh*	R-M	M-MS	R-M	R-M	R	R-M	R-M	M	M	R-M	R-M	M-MS
Shin 2	*Pi k-s, Pi sh*	M	M	M	R-M	R	R	M	M-MS	R	R	M-MS	M
IRBLk-Ka	*Pi k*	R	R	S	R	R	S	R	R	R	R	S	R
IRBLkp-K60	*Pi k-p*	R	R	S	R	R	S	R	R	R	R	S	R
IRBLkh-K3	*Pi k-h*	R	R	S	R	R	S	R	R	R	R	S	R
IRBLkm-Ts	*Pi k-m*	R	R	S	R	R	S	R	R	R	R	S	R
Kanto 51	*Pi k*	R	R	S	R	R	S	R	R	R	R	S	R
IRBLz-Fu	*Pi z*	R-M	M	M	R-M	S	R	R-M	M	MS	M	M	MS
Fukunishiki	*Pi z, Pi sh*	R	M	R	R	R	R	R	R	R	R-M	R-M	R
IRBLz5-CA	*Pi z-5*	R-M	R-M	R-M	R-M	R	R	R-M	R	R-M	R-M	R-M	S
C101A51	*Pi z-5, (Pi a)*	R	R	M	R-M	R	R	R	R	R	R	R	M-MS
IRBLzt-T	*Pi z-t*	S	S	R-M	S	S	S	R	R	S	R	R	S
Toride 1	*Pi z-t, (Pi sh)*	M	M	R	M	M	R-M	R	R	M	R	R	M
IRBLta-K1	*Pi ta*	S	S	R-M	M-S	S	S	R	S	R	S	R	S
IRBLta-CT2	*Pi ta*	S	S	MS-S	S	S	S	R	S	R	S	R	S
IRBLta-CP1	*Pi ta*	S	S	R	S	S	S	M	S	R	S	R-M	S
Yashiromochi	*Pi ta*	S	S	R	MS	S	R-M	R	S	R	S	R	S
IRBLta2-Pi	*Pi ta-2*	S	S	R	R	R	R	R	S	R	R	R	R
IRBLta2-Re	*Pi ta-2*	S	S	R	R	R	R	R	S	R	R	R	R
Reiho	*Pi ta-2, Pi a*	S	S	R	R	R	R	R	R	R	R	R	R
IRBLb-B	*Pi b*	S	S	S	R	S	S	R	R	S	R-M	S	R
IRBLsh-B	*Pi sh*	M	M-S	M	M	M	M	M	S	M	R-M	MS	MS
BL1	*Pi b, Pi sh*	R	R	R-M	R	R	R	R	R	R	R	M	R
IRBL1-CL	*Pi 1*	R	R	S	R-M	R	S	R	R	R	R	S	R
C101LAC	*Pi 1, (Pi a)*	R	R	S	R	R	S	R	R	R	R	S	R
IRBL3-CP4	*Pi 3*	R	S	R	S	R	S	S	S	R	S	S	MS
C104PKT	*Pi 3, (Pi a)*	R	S	M	S	R	MS-S	R	R	R	S	S	M-MS
IRBL5-M	*Pi 5* (t)	S	S	R	S	R	S	M-MS	MS-S	R	MS-S	S	MS
RIL 249	*Pi 5* (t)	MS-S	MS-S	R	R	R	M	R	R	R	MS	S	R
IRBL7-M	*Pi 7* (t)	R	R	S	R	R	S	R	R	R	R	S	R
RIL 29	*Pi 7* (t)	R	R	S	R	R	MS	R	R	R	R	S	R
IRBL9-W	*Pi 9* (t)	R	R	M	R	R	R	R	R	R	R-M	R	R
WHD-IS-75-1-127	*Pi 9* (t)	R	R	R-M	R	R	R	R	R	R-M	R	R-M	R
IRBL11-Zh	*Pi 11* (t)	S	S	S	R	S	S	R-M	R	S	M	S	R
Zhaiyeqing 8	*Pi 11* (t)	M	MS-S	R	R	MS	M-MS	R	R	R	R	R	R
IRBL12-M	*Pi 12* (t)	S	S	S	R	S	S	R-M	R	S	MS-S	S	R
RIL 10	*Pi 12* (t)	S	S	S	R	S	MS-S	R	R	M-MS	M	S	R
IRBL20-IR24	*Pi 20*	S	S	S	S	R	R	R	R	S	R	S	S
ARL 20	*Pi 20*	S	S	S	S	R	R	R	R	S	R	S	S

[1)] Isolate; 1: PO6-6, 2: Ca89, 3: IK81-25, 4: BN209, 5: BN111, 6: M36-1-3-10-1, 7: B90002, 8: C923-49, 9: V850196, 10: V86010, 11: M64-1-3-9-1, 12: M101-1-2-9-1

Reactions; R: resistant, M: moderately resistant, MS: moderately susceptible, S: susceptible

blast isolates at IRRI (Table 2).

IRBL9-W, with *Pi 9* (t) from WHD-IS-75-1-127, showed a resistant reaction to almost all the isolates used. IRBL19-A, which carries *Pi 19* (t) from Aichi Asahi, showed a susceptible reaction to all of the isolates. IRBLk-Ka, IRBLkp-K60, IRBLkh-K3, IRBLkm-Ts, IRBL1-CL and IRBL7-M showed almost the same reaction pattern to all the isolates used. Multiple alleles at the *Pi k* locus (*Pi k*, *Pi k-p*, *Pi k-h* and *Pi k-m* except for *Pi k-s*), *Pi 1* and *Pi 7* (t) could not be differentiated using these isolates. IRBLi-F5 and IRBL3-CP4 showed almost the same reaction pattern to the isolates used. The isolates could not differentiate between *Pi i* and *Pi 3*. Both IRBLsh-S from Shin 2 and IRBLsh-B from BL1 for *Pi sh* showed a moderately resistant reaction to the isolates used and the lines displayed a broad spectrum of resistance to the Philippine isolates. It is thus considered that *Pi sh* could be useful as a source of durable resistance to blast in the Philippines.

The reaction patterns of almost all of the donors and differential varieties could be explained on the basis of the reaction patterns of the monogenic lines or combination of the lines. However, resistant reactions of some of the donors were higher than expected. For example, BL1 has *Pi b* (Yokoo *et al.* 1978) and *Pi sh* (Imbe and Matsumoto 1985). It was used as a donor for these genes. IRBLb-B for *Pi b* and IRBLsh-B for *Pi sh* were derived from BL1. Resistance pattern of BL1 was different from that expected from the combined resistance of *Pi b* and *Pi sh*. BL1 was resistant to the isolates PO6-6, Ca89, BN111, M36-1-3-10-1 and V850196 whereas IRBLsh-B was moderately resistant to these isolates and IRBLb-B was susceptible, presumably due to complementation of *Pi b* and *Pi sh*, or to the presence of another gene(s) in BL1.

RIL249 is one of the recombinant inbred lines with *Pi 5* (t) derived from a cross between CO39 and Moroberekan (Wang *et al.* 1994). RIL249 may also have inherited *Pi a* from CO39. RIL249 was resistant to the isolates B90002 and C923-49, to which IRBL5-M was susceptible. IRBLa-C was resistant to these isolates. From the reaction patterns of RIL249, IRBL5-M and IRBLa-C, we conclude that RIL249 has *Pi a*. Resistant or moderately resistant reactions of RIL249 to the isolates BN209, M36-1-3-10-1 and M101-1-2-9-1 could not be explained by the combined reaction of *Pi a* and *Pi 5* (t). The higher resistance of RIL249 can be ascribed to either complementation of *Pi a* and *Pi 5* (t) or to the presence of an additional gene or genes for resistance besides *Pi a* and *Pi 5* (t).

Zhaiyeqing 8 is the donor of *Pi 11* (t) in IRBL11-Zh (Zhu *et al.* 1993). This donor showed a resistant or moderately resistant reaction to the isolates PO6-6, IK81-25, V850196 and M64-1-3-9-1, which were compatible with IRBL11-Zh. These results indicated that Zhaiyeqing 8 might harbor a resistance gene or genes in addition to *Pi 11*(t).

The monogenic lines we developed could be useful for studying the pathogenicity of blast isolates and this information may contribute to the elucidation of genetic aspects of rice blast. A set of NILs is the most suitable material for race differentiation. We have begun developing NILs with single resistance genes. However, it may take time to establish them. A set of monogenic lines could be suitable for use as differentials for rice blast until a set of NILs is completed. These monogenic lines will be distributed as candidates for blast differentials to scientists working on rice blast in collaboration with the International Network for Genetic Evaluation of Rice (INGER). Global applicability of the monogenic lines as differentials for blast will be evaluated through international collaboration.

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