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

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Abstract

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

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

Introduction

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

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

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

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

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

Materials and methods

Evaluation and selection of monogenic lines using standard differential blast isolates

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

Classifications

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

Results and discussion

Selection of representative differential varieties

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

Grouping of differential variety

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

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

Table 1. Rice blast fungus isolates used in the study

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

1: Determined by Japanese 12 differential system

2: National Institute of Agrobiological Sciences Genebank

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

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

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

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

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

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

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

Designation system

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

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

new resistance gene, extensibility is poor.

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

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