RESEARCH ARTICLES
J. Crop Sci. Biotech. 10 : 5 ~ 9

Classification of Korean Rice Cultivars based on Reaction Pattern to Japanese Isolates of Blast Pathogen

Xuan-ji Jin[1], Eun-Jeong Lee[1], and Jae-Eul Choi[1]*

[1]*Division of Plant Sciences & Resources, Chungnam National University, Daejeon 305-764, Korea*

Abstract

Classification of blast resistance type of 129 Korean rice cultivars was carried out based on reaction pattern to 10 Japanese blast pathogen isolates (*Pyricularia oryzae*). The cultivars were divided into 11 groups based on the presumed resistance genes as follows; *Pia* type (19 cultivars), *Pita*-2 type (4), Pik type (3), *Pib* type (5), *Piz* type (11), *Pik-s* type (8), *Pik* and *Pii* type (4), *Pia* and *Pita* type (8), *Pia* and *Pik* type (6), *Pita*, *Pik* and *Pii* type (4) and no-grouping type (57). These results would provide important information to rice breeding for durable and broad resistance to rice blast.

Key words: classification, *Pyricularia oryzae*, rice blast, resistance gene

Introduction

Rice blast disease, caused by *Pyricularia oryzae*, is one of the most widespread and destructive diseases of rice. The disease has been managed by using resistant cultivars, fungicides, and many other cultural practices. The use of resistant cultivars was the most eco-friendly approach for managing blast diseases in rice (Barman et al. 2004).

Genetic analysis of resistance to blast began in the early 1960s, which established the differential system for races of Japanese *P. oryzae*. Kiyosawa (1967; 1969; 1974) grouped Japanese rice cultivars into 16 types and designated the genotypes of representative cultivars based on the reaction patterns to seven strains of blast: Shin2 (*Pik-s*), Aichi-asahi (*Pia*), Ishikari-shiroke (*Pii* and *Pik-s*), Kanto51 (*Pik*), Yashiro-mochi (*Pita*), Pi No.4 (*Pita-2*), Fukunishiki (*Piz*), Toride1 (*Piz-t*), To-to (*Pik* and *Pia*), Shinsetsu (*Pii* and *Pia*), Shimokita (*Pita* and *Pia*), Zenith (*Piz* and *Pia*), K2 (*Pik-p* and *Pia*), K3 (*Pik-h*), BL8 (*Pib*) and K59 (*Pit*) types. Kanto51 type was divided into three genotypes: *Pik, Pii* and *Pik,* and *Pii* and *Pik-m.* To-to type was also divided into three genotypes: *Pia* and *Pik, Pia, Pik* and *Pii,* and *Pia, Pii* and *Pik-m.* Pi No.4 type was divided into two genotypes: *Pita-2,* and *Pia* and *Pita-2* (Ezuka et al. 1969). Recently, 13 dominant resistance genes, *Pish, Pik-h, Pia, Pii, Pik, Piz, Pita, Pita-2, Piz-t, Pik-m, Pib, Pik-p,* and *Pit,* against the Japanese race of the fungus were identified by Kiyosawa and Ling (2001).

In Korea, Cho and Kiyosawa (1973) identified resistance in Korean rice cultivars using Japanese races and the genealogical relationship between parents and Korean-bred cultivars. Kwon and Oh (1978) also identified several types of resistance genes in Korean cultivars. The *Pia* and *Pib* were identified from the *Tongil* cultivar in Korea (Kiyosawa and Cho 1980). Chae et al. (1981) studied the genetic system of the blast resistance in *Tongil*-type varieties. Resistance genes for rice blast have been analyzed and mapped in several rice cultivars through molecular marker technology (Ahn et al. 1997; 2000; Kwon et al. 2002; Cho et al. 2004). Hwang et al. (2004) investigated the genetic diversity of 23 *japonica* cultivars using SSR markers linked to blast resistance genes. In the analysis of resistance genes for 108 rice cultivars consisting of 97 Korean *japonica* and 11 *Tongil*-type using SCAR and SNP markers tightly linked to four genes *Pib, Piz, Pi5(t)* and *Pi9(t),* the *Pib* gene was present in 21.8%, and the *Pi9(t)* in 25.2% of the whole varieties tested, respectively (Cho et al. 2005). Many Korean rice cultivars have been developed using Japanese rice carrying the multiple alleles of resistance genes; blast races have changed as the years go by. Therefore, detection and classification of resistance genes in Korean rice cultivars are very complicated and knowledge on the blast resistance genes of Korean rice cultivars and their parents is limited.

To provide information for breeding cultivars with broad and durable resistance to blast pathogens, we report the reaction pattern of a number of Korean rice cultivars to ten Japanese standard blast fungus and classification of tested rice cultivars based on the presumed resistance genes.

* To whom correspondence should be addressed
Jae-Eul Choi
E-mail: choije@cnu.ac.kr
Tel: +82-42-821-5729

Materials and Methods

Plant materials

For this study, seeds of 129 Korean cultivars (*japonica*-type) were obtained from National Institute of Crop Science in Korea. The distin-

guishing features of these cultivars are described on the Rural Development Administration server at URL: http://www.rda.go.kr Seeds were sown in plastic cups containing paddy rice bed-soil and grown in greenhouse at 22-28 °C.

Blast isolates

Ten Japanese blast isolates were obtained from National Institute of Agrobiological Sciences (NIAS) in Japan. Ten Japanese races are shown in Table 1.

Table 1. Reactions of known resistance genes to ten Japanese blast isolates used in this study.

Cultivar type	Reaction to isolates											R gene
	Ina86-137	TH68-126	TH68-140	24-22-1-1	Ina168	Ina168	Ken53-33	0528-2	Kyu9439013	Ina93-3	IW81-04	
Aichi Asahi	S[a]	S	R	S	R	R	S	S	S	R	S	*Pia*
Ishikari-shiroke	S	R	S	S	R	R	S	R	S	R	S	*Pii*
Kanto51	R	S	S	S	R	R	S	S	R	R	S	*Pik*
Shinsetsu	S	R	R	S	R	R	S	R	S	R	S	*Pia,Pii*
To-to	R	S	R	S	R	R	S	S	R	R	S	*Pia,Pik*
Shimokita	R	R	R	R	R	R	S	S	R	R	R	*Pia,Pita*
Shin2	S	S	S	S	S	S	S	S	S	S	S	*Pik-s*
Tsuyuake	R	S	S	S	R	R	S	S	R	R	S	*Pik-m*
Yashiro-mochi	R	R	R	R	S	S	S	S	R	S	R	*Pita*
Pi No.4	R	R	R	R	R	R	R	S	R	S	R	*Pita-2*
Fukunishiki	R	R	R	R	R	R	R	R	S	R	R	*Piz*
BL8	R	R	R	R	R	R	R	R	R	R	R	*Pib*

[a] R; resistant, S; susceptible

Inoculum preparation

The ten pathogen isolates tested were grown on PDA medium (potato 300 g, $Na_2H_2PO_4 \cdot 2H_2O$ 2 g, $Ca(NO_3)_2 \cdot 4H_2O$ 0.5 g, peptone 5 g, sucrose 15 g, agar 15 g per 1L, pH 7.0) at 26 °C for 5 days, transferred to rice bran medium (rice bran 20 g, dextrose 10 g, agar 12 g per 1L) and cultured at 28 °C for 12 days. The aerial mycelia of the medium were gently washed off with a water-soaked paint-brush and placed under continuous illumination with fluorescent light at 21 °C for 3~4 days to induce sporulation. To prepare a conidial suspension, the mycelia were scraped and flooded with water containing 0.01% Tween 20. The conidial suspension was filtered through a gauze mesh and adjusted to $1 \times 10^5 \sim 5 \times 10^5$ spores/ml.

Inoculation and classification of disease lesion

Rice seedlings with 4.5 to 5 leaf stages were inoculated by spraying the spore suspension. The inoculated seedlings were immediately placed in a dark chamber with a moisture-saturated atmosphere at 25 °C for 24 h and then transferred to a greenhouse at 20-28 °C. Lesion length from inoculated seedling was measured as milimeters (mm) at 7 days after inoculation. The experiment was conducted at least five times. Disease reaction was categorized according to the classification described by Hayashi et al. (1998): Lesion length from 0 to 0.5 mm was classified as resistant (R) and longer than 2 mm as susceptible (S).

Results and Discussion

We investigated the reaction pattern of 129 Korean cultivars against ten Japanese blast races. Based on the resistant or susceptible reaction, the cultivars tested were classified into eleven types. Among the eleven, six types had a single gene, three types contained two genes, one type had three resistance genes, and the other type was a no-grouping type.

Pia type

The reaction pattern of 19 cultivars was similar to that of Aichi Asahi type which has a *Pia* gene (Table 2). Manmibyeo, Daeyabyeo, Daecheongbyeo, Undoobyeo, Unbongbyeo, Hwaseonchalbyeo, Chucheongbyeo, Hwaseongbyeo, Daeanbyeo, Heughyangbyeo, Hwasinbyeo and Shinseonchalbyeo were shown to be susceptible to Ina86-137, TH68-126, 24-22-1-1, Ken53-33, 0528-2, Kyu9439013, and IW81-04, while being resistant to Ina93-3, Ina168, and TH68-140. The other cultivars were not equal to Aichi Asahi type in only one reaction pattern. Geumobyeo, Geumobyeo2, Saechucheongbyeo, Gancheokbyeo, Ilpumbyeo, and Hwajungbyeo were susceptible to Ina93-3 and Seojinbyeo was susceptible to TH68-140. This group consisted of 19 cultivars was designated as *Pia* type.

Pik type

Palgongbyeo and Geumobyeo1 showed susceptible reaction to TH68-126, TH68-140, Ken53-33, 0528-2, 24-22-1-1, and IW81-04, but were resistant to Ina 86-137, Ina168, Kyu9439013, and Ina93-3. The resistance spectrum of the two varieties was the same as Kanto51 type which has a *Pik* gene. The reaction of Dongjinchalbyeo to Ina86-137 was different from Kanto51 type, but the reaction to the other isolates was consistent with those of Kanto51. Therefore, Palgongbyeo, Geumobyeo, and Dongjinchalbyeo probably belonged to Kanto51 type with *Pik* gene (Table 2).

Piz type

Eleven cultivars showed a susceptibility to Kyu9439013, but showed resistance reactions to the other races. These were Jinpumbyeo, Sambaegbyeo, Yeonghaebyeo, Samgwangbyeo, Unjangbyeo, Hojinbyeo, Obongbyeo, Jungsanbyeo, Geumanbyeo, Manhobyeo, and Nonganbyeo. This reaction pattern was the same as that of Fukunishiki which has the gene *Piz*. Therefore, we designated this group as the *Piz* type (Table 2).

Pik-s type

Hwasambyeo, Nagdongbyeo, Samdeogbyeo, and Daeripbyeo1 showed a susceptible reaction to all test races. This reaction pattern was

the same as that of Shin2 with the *Pik-s* gene. Haepyeongbyeo, Manpungbyeo, Heugnambyeo, and Namilbyeo showed one different reaction pattern compared to the Shin2 type. Haepyungbyeo, Manpungbyeo, Heugnambyeo, and Namilbyeo were resistant to Ina168, TH68-140, 0528-2, and IW81-04, respectively. As a result, eight cultivars that showed a very similar pattern to the Shin2 type were designated as the *Pik-s* type (Table 2).

Pita-2 type

Inweolbyeo, Donganbyeo, and Anseongbyeo were susceptible to 0528-2 and Ina93-3, and Odaebyeo was susceptible to Ina93-3. This reaction pattern was very similar to the Pi No.4 type which has the *Pita*-2 gene (Table 2).

Pib type

Five cultivars, Jinmibyeo, Seoganbyeo, Mananbyeo, Naepungbyeo, and Jinbongbyeo, showed resistant reactions to all test races. This reaction pattern was the same as that of BL8 which has the *Pib* gene. Therefore, we designated this group as the *Pib* type (Table 2).

Pia and *Pita* type

Seven cultivars, Janganbyeo, Dongjinbyeo, Jungnambyeo, Namgangbyeo, Gyehwabyeo, Wonhwangbyeo, and Yeongnambyeo, showed resistant reactions to Ina86-137, Ina93-3, Ina168, IW81-04, Kyu9439013, TH68-126, TH68-140, and 24-22-1-1, but showed susceptible reactions to Ken53-33 and 0528-2. This reaction pattern was the same as that of the Shimokita type which has *Pia* and *Pita* genes. The reaction of Daepyeongbyeo to Ina86-137 was different from the Shimokita type, but the reaction to the other races was consistent with Shimokita. Therefore, this group was designated as *Pia* and *Pita* type (Table 3).

Pia and *Pik* type

The reaction pattern of six cultivars was very similar to that of the To-to type which has the *Pia* and *Pik* gene (Table 3). Donghaebyeo and Hwajinbyeo showed a resistant reaction to Ina86-137, Ina93-3, Ina168, Kyu9439013, and TH68-140, but they showed susceptible reactions to IW81-04, Ken53-33, TH68-126, 0528-2, and 24-22-1-1, which was the same as that of the To-to type. Tamjinbyeo, Hwanambyeo, Seolhyangchalbyeo, and Aranghyang-chalbyeo showed a little different reaction pattern compared to the To-to type. Tamjinbyeo and Hwanambyeo were resistant to TH68-126, 24-22-1-1, and IW81-04. Seolhyangchalbyeo andAranghyangchalbyeo were susceptible to Kyu9439103 and Ina168, respectively. However, because these six cultivars showed a similar pattern to the To-to type, we designated this group as the *Pia* and *Pik* type (Table 2).

Pik and *Pii* type

Nampyeongbyeo was susceptible to TH68-140, 24-22-1-1, Ken53-33, and IW81-04, but was resistant to Ina86-137, TH68-126, Ina168, 0528-2, Kyu9439013, and Ina93-3. This reaction pattern suggested that Nampyeongbyeo might have *Pi-k* and *Pi-i* genes. Manchubyeo and Moonjangbyeo were susceptible to 24-22-1-1, Ken53-33, and

Table 2. Korean rice cultivars possessing single resistance genes based on the reactions to Japanese blast isolates.

Cultivar type	Reaction to isolate									
	Ina86-137	TH68-126	TH68-140	24-22-1-1	Ina168	Ken53-33	0528-2	Kyu9439013	Ina93-3	IW81-04
Aichi Asahi (*Pia*) Manmibyeo, Daeyabyeo, Daecheongbyeo, Undoobyeo, Unbongbyeo, Hwaseonchalbyeo, Chucheongbyeo, Hwaseongbyeo, Daeanbyeo, Heughyangbyeo, Hwasinbyeo, Shinseonchalbyeo	Sa)	S	R	S	R	S	S	S	R	S
Geumobyeo, Geumobyeo2, Saechucheongbyeo, Ilpumbyeo, Gancheokbyeo, Hwajungbyeo, Seojinbyeo	S	S	S/R	S	R	S	S	S	S	S
Kanto 51 (*Pik*) Palgongbyeo, Geumobyeo1,	R	S	S	S	R	S	S	R	R	S
Dongjinchalbyeo	S	S	S	S	R	S	S	R	R	S
Fukunishiki (*Piz*) Jinpumbyeo, Sambaegbyeo, Yeonghaebyeo, Samgwangbyeo, Unjangbyeo, Hojinbyeo, Obongbyeo, Jungsanbyeo, Nonganbyeo, Geumanbyeo, Manhobyeo	R	R	R	R	R	R	R	S	R	R
Shin 2 (*Pik-s*) Hwasambyeo, Nagdongbyeo, Samdeogbyeo, Daeripbyeo1	S	S	S	S	S	S	S	S	S	S
Haepyeongbyeo, Manpungbyeo, Heugnambyeo, Namilbyeo	S	S	S/R	S	S/R	S	S/R	S	S	S/R
Pi No.4 (*Pita-2*) Inweolbyeo, Donganbyeo, Anseongbyeo	R	R	R	R	R	R	S	R	S	R
Odaebyee	R	R	R	R	R	R	R	R	S	R
BL 8 (*Pib*) Jinmibyeo, Seoganbyeo, Mananbyeo, Naepungbyeo, Jinbongbyeo	R	R	R	R	R	R	R	R	R	R

a) R; resistant, S; susceptible

Table 3. Korean rice cultivars possessing double resistance genes based on the reactions to Japanese blast isolates.

Cultivar type	Reaction to isolate									
	Ina86-137	TH68-126	TH68-140	24-22-1-1	Ina168	Ken53-33	0528-2	Kyu9439013	Ina93-3	IW81-04
Pik and Pii Nampyeongbyeo	Ra)	R	S	S	R	S	R	R	R	S
Moonjangbyeo, Manchubyeo, Seopyeongbyeo	Ra)	R	S/R	S	R	S	R	R	R	S/R
Pia and Pita Janganbyeo, Wonhwangbyeo, Jungnambyeo, Namgangbyeo, Dongjinbyeo, Gyehwabyeo, Yeongnambyeo	R	R	R	R	R	S	S	R	R	R
Daepyeongbyeo	S	R	R	R	R	S	S	R	R	R
Pia and Pik Hwajinbyeo, Donghaebyeo	R	S	R	S	S	S	S	R	R	S
Tamjinbyeo, Hwanambyeo, Seolhyangchalbyeo, Aranghyangchalbyeo	R	S/R	R	S/R	S/R	S	S	S/R	R	S/R

a) R; resistant, S; susceptible

Table 4. Korean rice cultivars possessing three resistance genes based on the reactions to Japanese blast isolates.

Cultivar type	Reaction to isolate									
	Ina86-137	TH68-126	TH68-140	24-22-1-1	Ina168	Ken53-33	0528-2	Kyu9439013	Ina93-3	IW81-04
Pik, Pii and Pita Daejinbyeo, Saesangjubyeo, Samcheonbyeo, Sangjubyeo	R[a)]	R	R	R	R	S	R	R	R	R

a) R; resistant, S; susceptible

IW81-04, and Seopyeongbyeo was susceptible to TH68-140, 24-22-1-1, and Ken53-33. But reactions of these two cultivars to the other races were similar to Nampyeongbyeo. Therefore, we designated this group as *Pik* and *Pii* type (Table 3).

Pik, Pii and *Pita* type

Four cultivars, Daejinbyeo, Saesangjubyeo, Samcheonbyeo, and Sangjubyeo, showed a susceptible reaction to Ken53-33, but showed a resistant reaction to the other races. These results were suggested that the four cultivars might have *Pik, Pii,* and *Pita* genes (Table 4).

No-grouping type

We could not identify resistance genes in 57 cultivars because their reaction pattern was very complicated. It was difficult to decide whether the reaction pattern of these 57 cultivars was resistant or susceptible to Japanese races. However, we presumed that these cultivars might have other genes which could not be identified with the ten Japanese races used in this study. This is the first attempt to categorize a large number of Korean rice cultivars into groups on the basis of their reaction to ten Japanese standard races. For the present, it is difficult to distinguish cultivars with blast resistance genes using Korean races because most of blast resistance genes in Korean rice were derived from Japanese cultivars. Therefore, our preliminary experiments will provide important information to develop cultivars of effective resistance in rice breeding in addition to analyzing in detail using molecular markers linked to the resistance genes. On the basis of this study, Korean rice cultivars were divided into eleven groups having blast resistance genes. To date, at least 25 blast resistance genes have been identified and mapped to chromosomes using molecular markers linked to the resistance genes (Wu et al. 2005). Pyramiding major genes for blast resistance is the most promising choice to provide broad spectrum and durable resistance (Suh et al. 1987; McClung et al. 1997; Tabien et al. 2002). Also, detecting and using new blast resistant genes is necessary to manage the problem in rice breeding.

Presently, we are trying to investigate reaction patterns to blast races in more Korean cultivars and the F2 population between Korean cultivars and different Japanese cultivars having a single gene for resistance. Furthermore, more variable pathogens, especially the Korean races, is needed to inoculate rice cultivars in order to obtain more a comprehensive view of the genetics of blast resistance.

Acknowledgement

The authors thank National Institute of Agrobiological Sciences (NIAS), Japan, for generous gifts of blast isolates and Dr. S. H. Choi for valuable discussion and critical reading of the manuscript.

References

Ahn SN, Kim YK, Hong HC, Han SS, Choi HC, McCouch SR, Moon HP. 1997. Mapping of genes conferring resistance to Korean isolate of rice blast fungus using DNA markers. Korean J. Breed. 29: 416-423

Ahn SN, Kim YK, Hong HC, Han SS, Kwon SJ, Choi HC, Moon HP, McCouch SR. 2000. Molecular mapping of new gene for resistance to rice blast (*Pyricularia grisea*). Euphytica. 116: 17-22

Barman SR, Gowda M, Venu RC, Chattoo BB. 2004. Identification of a major blast resistance gene in rice cultivar "Tetep". Plant Breeding 123: 300-302

Chae YA, Park SI, Ha SB. 1981. Studies on genetic of blast resistance in rice blast fungus and relationship between their resistance and II, VIII, XI and XII linkage groups in some rice varieties. Korean J. Crop Sci. 26: 32-39

Cho CI, Kiyosawa S. 1973. Blast resistance in Korean rice varieties. Res. Rept. Office Rural Dev. 15: 83-88

Cho YC, Choi IS, Baek MK, Yanoria Mary JT, Suh JP, Roh JH, Kim YC, Jena KK, Choi HC. 2004. Analysis of resistance genes to blast (*Magnaporthegrisea*) in the Japonica rice, Ilpumbyeo. Korean J. Breed. 36: 81-89

Cho YC, Choi IS, Kim YG, Yang CI, Jeon JS, Hwang HG, Lee YT. 2005. Identification of major blast resistance genes in Korean rice varieties. In Proceeding of The 2005 Korean Breeding Society of Annual Meeting & International Symposium, June 16-18, Jeju, Korea. pp 229

Ezuka A, Yunoki T, Sakurai Y, Shinoda H, Toriyama K. 1969. Studies on the varietal resistance to rice blast. 1. Tests for genotype of true resistance. Bulletin of the Chugoku Agricultural Experiment Station (Japan) E 4: 1-32

Hwang HG, Kwon SJ, Cho YC, Ahn SN, Suh JP, Moon HP. 2004. Genetic diversity of high-quality rice cultivars based on SSR markers linked to blast resistance genes. Korean J. Crop. Sci. 49: 251-255

Hayashi N, Ando I, Imbe T. 1998. Identification of a new resistance gene to a Japanese rice cultivar Aichi Asahi. Phytopathology 88: 822-827

Kiyosawa S. 1967. The inheritance of resistance of the Zenith type varieties of rice to the blast fungus. Japan. J. breeding 17: 99-107

Kiyosawa S. 1969. Inheritance of blast-resistance in West Pakistani rice variety Pursur. Ibid. 19: 121-128

Kiyosawa S. 1974. Inheritance of blast resistance of a US rice vari ety, Dawn. J. J. Breeding 24: 117-124

Kiyosawa S, Cho CI. 1980. Identification of blast-resistance genes in Korean rice variety, Tongil. Japan J. Breed. 30: 73-82

Kiyosawa S, Ling Z. 2001. Genetics and breeding of blast relationships. In: Sreenivasaprasad S, Johnson R (eds) Major fungal disease of rice. Kluwer Academic, Dordrecht. pp 145-162

Kwon SH, Oh J. 1978. Genetic study on the resistance of blast disease in the rice variety Tongli Korean. Plant port. 15: 163-169

Kwon SH, Ahn SN, Hong HC, Cho YC, Suh JP, Kim YG, Kang KH, Han SS, Choi HC, Moon HP, Hwang HG. 2002. Identification of DNA markers linked to resistance genes to rice blast (*Pyricularia grisea* Sacc.). Korean J. Breed. 34: 105-110

McClung AM, Marchetti MA, Webb BD, Bollich CN. 1997. Registation of 'Jefferson' rice. Crop Sci. 37: 629-630

Suh HS, Heu MH, Nuque FL. 1987. Pyramiding resistance genes to leaf blast in rice through F1 hybrid. Korean J. Crop Sci. 32: 462-465

Tabien R, Li Z, Paterson AH, Marchetti MA, Stansel JW, Pinson SRM. 2002. Mapping QTLs for field resistance to the rice blast pathogen and evaluating their individual and combined utility in improved varieties. Theor. Appl. Genet. 105: 313-324

Wu JL, Fan YY, Li DB, Zhang KL, Leung H, Zhung JY. 2005. Genetic control of rice blast resistance in the durably resistant cultivar Gumei2 against multiple isolates. Theor. Appl. Genet. 111: 50-56