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Pathogenic Diversity of *Xanthomonas oryzae* pv. *oryzae* Strains from Yunnan Province, China

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Abstract

The strains of *Xanthomonas oryzae* pv. *oryzae* collected in Yunnan province, China, during the period from 1994 to 1996 were polymorphic for virulence to the 12 near-isogenic lines harboring the resistance genes *Xa1*, *Xa2*, *Xa3*, *Xa4*, *xa5*, *Xa7*, *xa8*, *Xa10*, *Xa11*, *xa13*, *Xa14* and *Xa21*, and the 3 check varieties, IR24, Toyonishiki and Sigadagabo. One hundred and thirty-eight strains were classified into 10 pathogenic groups (tentatively designated as pathotypes A to J) based on their pathogenicity. These 10 pathotypes were classified into 3 large groups based on the pathogenic range to the differentials; group 1 with a very narrow pathogenic range was virulent to only 2 differentials, group 2 with an intermediate pathogenic range was virulent to 4 to 7 differentials and group 3 with the widest pathogenic range was virulent to 9 to 12 differentials, respectively. Groups 1 and 2 were distributed in the northern and central parts of Yunnan province where various kinds of Japonica rice varieties are cultivated. On the other hand, Group 3 was distributed mainly in the southern part, where Indica rice varieties including hybrid rice are widely cultivated. In this study, we showed that 2 differentials, IRBB13 (*xa13*) and IRBB21 (*Xa21*), were resistant to all the strains tested, while IRBB5 (*xa5*) was susceptible only to 6 strains (4.3%). These resistance genes could be used for a breeding program for resistance to BLB in Yunnan province.

Discipline: Plant disease
Additional key words: bacterial leaf blight of rice, *Oryza sativa*, pathotype

Introduction

Bacterial leaf blight (BLB) of rice (*Oryza sativa* L.), caused by *Xanthomonas oryzae* pv. *oryzae*, is a major constraint on rice production in tropical Asia. Some chemicals have been developed to control this disease, but none of them have been fully effective in the case of outbreaks. Therefore, the use of resistant cultivars is the most promising method of control of the disease. Genetic analysis of resistance in rice along with the examination of variation in the pathogenicity of the causal bacterium has been extensively conducted in Japan as well as at the International Rice Research Institute (IRRI). Following the initial studies on differential reaction between rice varieties and bacterial strains in Japan carried out by Kuhara et al.[11], a number of reports have been published on the variability of the pathogenicity of the bacterium and on the resistance of rice cultivars in major rice-growing countries [1,2,4,5,7,8,15,18–20,22,28].

Most of the resistance genes provide an effective and stable protection only against subpopulations of the pathogen. Changes in the pathotype structure within the population may result from several factors such as genetic change or migration from other geographic areas. For the selection and development of resistance genes, therefore, the pathogenic specificity and geographical distribution of the pathogen need to be investigated.

BLB is widely distributed in Yunnan province, China, in particular, in the Indica rice cultivation area.

This paper is a part of the report on the joint study on rice breeding in China undertaken by the Yunnan Academy of Agricultural Sciences, China and the Japan International Research Center for Agricultural Sciences (JIRCAS) during the period from 1994 to 1996.
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Table 1. Differential varieties, check varieties, resistance genes and pathogenic strains of *Xanthomonas oryzae* pv. *oryzae* collected in Yunnan province, China, between 1994 and 1997

Differential varieties	Resistance gene	No. of pathogenic strains[a)]	% of pathogenic strains
IRBB1	*Xa1, Xa12*	110	79.7
IRBB2	*Xa2*	74	53.6
IRBB3	*Xa3*	65	47.1
IRBB4	*Xa4*	20	14.5
IRBB5	*xa5*	6	4.3
IRBB7	*Xa7*	27	19.6
IRBB8	*xa8*	52	37.7
IRBB10	*Xa10*	77	55.8
IRBB11	*Xa11*	68	49.3
IRBB13	*xa13*	0	0
IRBB14	*Xa14*	0	0
IRBB21	*Xa21*	74	53.6
IR24	*Xa16, Xa18*	110	79.7
Toyonishiki	*Xa18*	138	100
Sigadagabo	Unknown	138	100

a): Total number of strains, 138.

The disease rapidly spread after introduction of hybrid rice, and the yield reduction was estimated to reach about 25,000 t in 1976[14)]. As described above, host resistance is the only effective method of control of the disease. Therefore, pathogenic differentiation of *X. oryzae* pv. *oryzae* along with the genetic analysis of resistant cultivars has been studied in Yunnan. Based on these previous studies[14)], we predicted that in Yunnan province there would be a high pathogenic diversity of *X. oryzae* pv. *oryzae*. Therefore, we collected BLB samples in Yunnan province during the period from 1994 to 1996, and the populations of *X. oryzae* pv. *oryzae* were further evaluated for virulence on an expanded series of 12 near-isogenic lines and 3 check varieties. The studies reported here were undertaken to provide information on the current population structure of the pathogen in Yunnan province.

Materials and methods

1) Isolation and inoculation of causal bacterium

Rice leaves infected with *X. oryzae* pv. *oryzae* were collected from various areas in Yunnan province, China from 1994 to 1996. Diseased leaf samples were cut into small pieces, about 1 cm in length, with the margin of typical lesions, and were sterilized in 70% ethyl alcohol and 1% sodium hypochlorite solution. Each sample was then homogenized with 1 mL of sterile distilled water. The resulting suspension was diluted with sterile distilled water, and the samples at appropriate dilutions were mixed with nutrient agar medium (Difco) kept at 50ºC in a water bath. The mixture was poured into a plate, and the plates were incubated at 25ºC for 4 days. The resultant viscous and yellow bacterial colonies were transferred to slants of potato semi-synthetic agar (PSA) medium (Wakimoto's medium)[27)] and cultured at 25ºC for 2 days. For preservation, the bacterial suspension in 10% skim-milk containing 0.05% L-glutamic acid was lyophilized. For the preparation of the inoculum, the bacterium was grown on PSA medium at 25ºC for 2 days, then the culture was suspended in sterile distilled water at a concentration of 10^8 cfu/mL.

2) Pathogenicity of BLB strains in Yunnan province

Twelve near-isogenic lines, IRBB1, IRBB2, IRBB3, IRBB4, IRBB5, IRBB7, IRBB8, IRBB10, IRBB11, IRBB13, IRBB14 and IRBB21 which were developed as international differential varieties for BLB strains, and 3 additional varieties, IR24, Toyonishiki and Sigadagabo, were used[15–17)]. The resistance genes of these varieties are shown in Table 1. The test plants were grown in seedling boxes, then transplanted to experimental paddy fields or plastic pots (1/2000 a). Fertilizers were applied according to conventional methods. The uppermost fully opened leaves were inoculated by the clipping method[10)] at the heading stage of the earliest ripening variety among

the test plants. Lesion length on inoculated leaves was measured for each strain at 21 days after inoculation. Disease reactions were categorized according to the lesion length, in which a plant with a leaf lesion 0 to 5 cm in length was classified as resistant (R) (avirulent strain) and more than 5 cm as susceptible (S) (virulent strain). The pathogenicity tests were replicated 2 times.

Results

1) Pathotype diversity in Yunnan province

The strains of *Xanthomonas oryzae* pv. *oryzae* were polymorphic for virulence to the 12 near-isogenic lines harboring the resistance genes *Xa1*, *Xa2*, *Xa3*, *Xa4*, *xa5*, *Xa7*, *xa8*, *Xa10*, *Xa11*, *xa13*, *Xa14* and *Xa21*, and 3 check varieties. One hundred and thirty-eight strains collected in Yunnan province, China, during the period 1994 to 1996 were classified into 10 pathogenic groups (tentatively designated as pathotypes A to J) based on their pathogenicity to the 12 near-isogenic lines and 3 check varieties (Table 2). All the isolates tested were virulent to 2 differentials, Toyonishiki and Sigadagabo, and were avirulent to 2 differentials, IRBB13 and IRBB21 (Table 2). Although IRBB5 was susceptible to 6 strains (4.3%) belonging to pathotype J, the average lesion length for these isolates was only 5.1 cm, which was nearly the border length between R and S (Fig.1).

2) Race composition in Yunnan province

More than 30% of the isolates were classified as pathotype G, which was virulent to 10 differentials, but was avirulent to 5 differentials, IRBB4, IRBB5, IRBB7, IRBB13 and IRBB21 (Table 2). Pathotypes A and B accounted for 20.3% and 22.5% of the total strains, respectively; pathogenic characteristics of these 2 pathotypes slightly differed in virulence to 2 differentials, IR24 and IRBB1. Pathotype A was avirulent to all the differentials except for Toyonishiki and Sigadagabo. And IR24, which had been used as a susceptible check in many experiments[2,13,20,21], was resistant to this pathotype.

The degree of resistance reaction of the differentials differed with the combinations of varieties and pathotypes (Fig. 1). Seven differentials, IRBB1, IRBB2, IRBB7, IRBB10, IRBB11, IRBB14 and IR24, could be easily differentiated between R and S. However, for the others the differences between R and S were not clear in some combinations of varieties and pathotypes. Lesion length in 2 differential varieties, IRBB4 and IRBB7, in relation to each pathotype is shown in Fig. 1. It is obvious that IRBB7 was resistant to the pathotypes A, B, E and G, and was susceptible to pathotypes H, I and J. On the other hand, the reactions of IRBB4 to each pathotype were not distinct and the difference between the lesion length in relation to virulent and avirulent pathotypes was not conspicuous.

Table 2. Pathogenic diversity of the 138 strains of *Xanthomonas oryzae* pv. *oryzae* from Yunnan province using 12 near-isogenic lines and 3 check varieties

Differential varieties	Race (Tentative name)									
	A	B	C	D	E	F	G	H	I	J
IRBB1	–[a)]	S[b)]	S	S	S	S	S	S	S	S
IRBB2	–	–	–	–	S	S	S	S	S	S
IRBB3	–	–	–	–	–	S	S	S	S	S
IRBB4	–	–	–	–	–	–	–	S	S	S
IRBB5	–	–	–	–	–	–	–	–	–	S
IRBB7	–	–	S	S	–	S	–	S	S	S
IRBB8	–	–	–	–	S	–	S	–	–	–
IRBB10	–	–	–	S	S	S	S	S	S	S
IRBB11	–	–	–	S	S	–	S	–	S	S
IRBB13	–	–	–	–	–	–	–	–	–	–
IRBB14	–	–	–	–	S	S	S	S	S	S
IRBB21	–	–	–	–	–	–	–	–	–	–
IR24	–	S	S	S	S	S	S	S	S	S
Toyonishiki	S	S	S	S	S	S	S	S	S	S
Sigadagabo	S	S	S	S	S	S	S	S	S	S
No. of strains	28	31	2	3	9	2	43	7	7	6

a): Resistant, b): Susceptible.

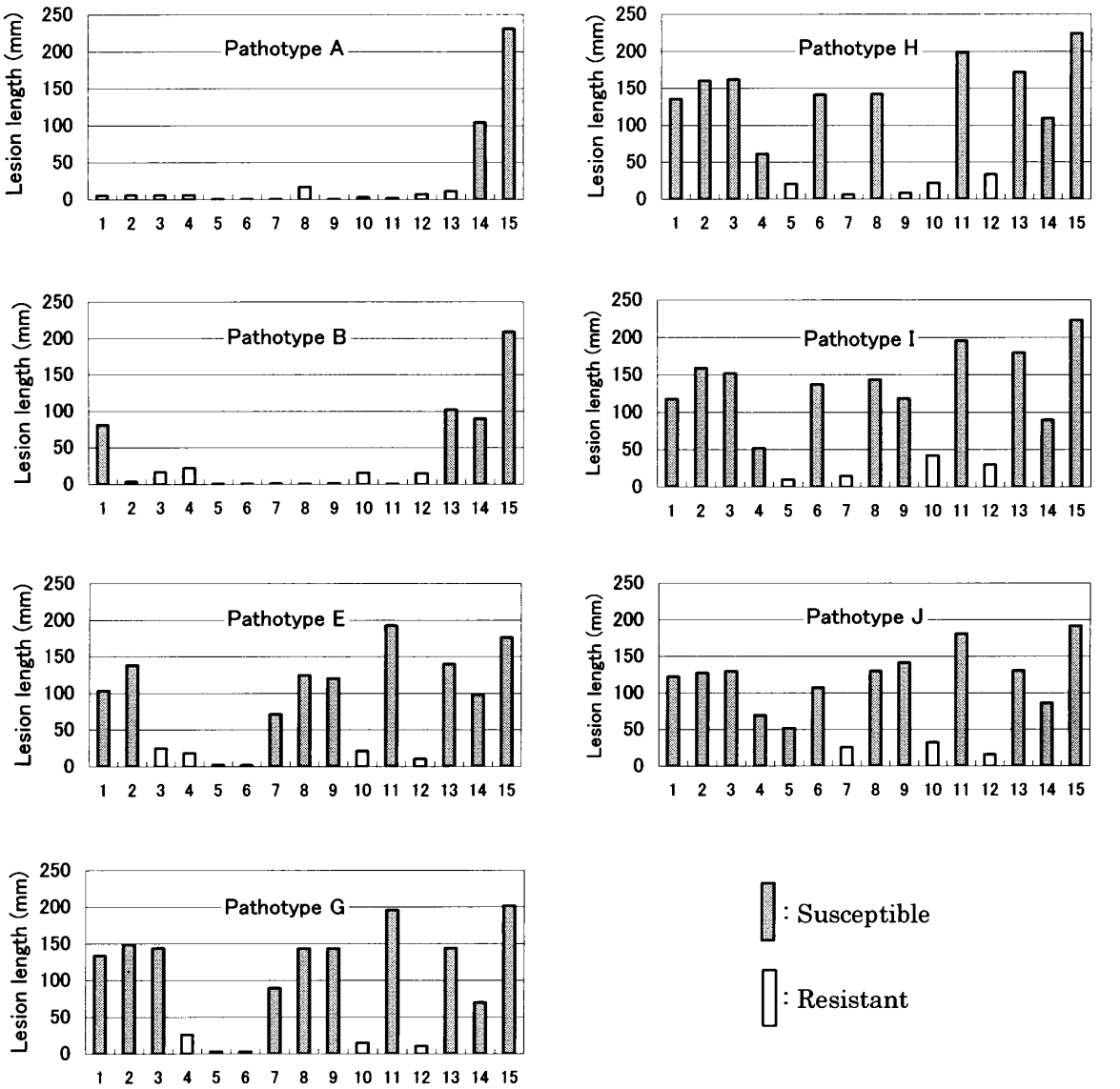

Fig. 1. Reaction of differential varieties to predominant pathotypes of *Xanthomonas oryzae* pv. *oryzae* collected in Yunnan province, China

3) Race distribution in Yunnan province

Among the 138 strains of *Xanthomonas oryzae* pv. *oryzae* tested, 10 pathotypes (A to J) were identified in Yunnan province. We classified these races into 3 large groups based on the pathogenic range to the differentials; Group 1 contains Pathotype A, Group 2 contains Pathotypes B, C and D, and Group 3 contains Pathotypes E, F, G, H, I and J. Group 1 with a very narrow pathogenic range was virulent to only 2 differentials, Group 2 with an intermediate pathogenic range was virulent to 4 to 7 differentials and Group 3 with the widest pathogenic range was virulent to 9 to 12 differentials, respectively. Table 3 and Fig. 2 show the distribution of the strains belonging to each large pathogenic group in 11 counties of Yunnan province. It was obvious that the 3 large pathogenic groups were distributed in different cultivation areas. Groups 1 and 2 were collected in the northern and the central parts of Yunnan province where many kinds of Japonica rice varieties are cultivated. On the other hand, only Group 3 was distributed in 3 counties,

Table 3. Race distribution in each county of Yunnan province, China

Location (County)	No. of races[a] distributed in each county										
	A	B	C	D	E	F	G	H	I	J	Total
Lijiang	2	5	–[b]	–	–	–	–	–	–	–	7
Dali	6	1	1	–	–	–	–	–	–	–	8
Chuxiong	10	10	–	–	–	–	–	–	–	–	20
Baoshan	5	–	–	–	1	–	–	–	2	2	10
Kunming	–	8	1	3	–	–	1	–	–	–	13
Dehong	–	–	–	–	4	2	2	7	5	–	20
Yuxi	4	5	–	–	1	–	12	–	–	4	26
Honghe	–	2	–	–	3	–	–	–	–	–	5
Wenshan	1	–	–	–	–	–	–	–	–	–	1
Simao	–	–	–	–	–	–	10	–	–	–	10
Xishuangbanna	–	–	–	–	–	–	18	–	–	–	18

a): Tentative name, b): Not detected.

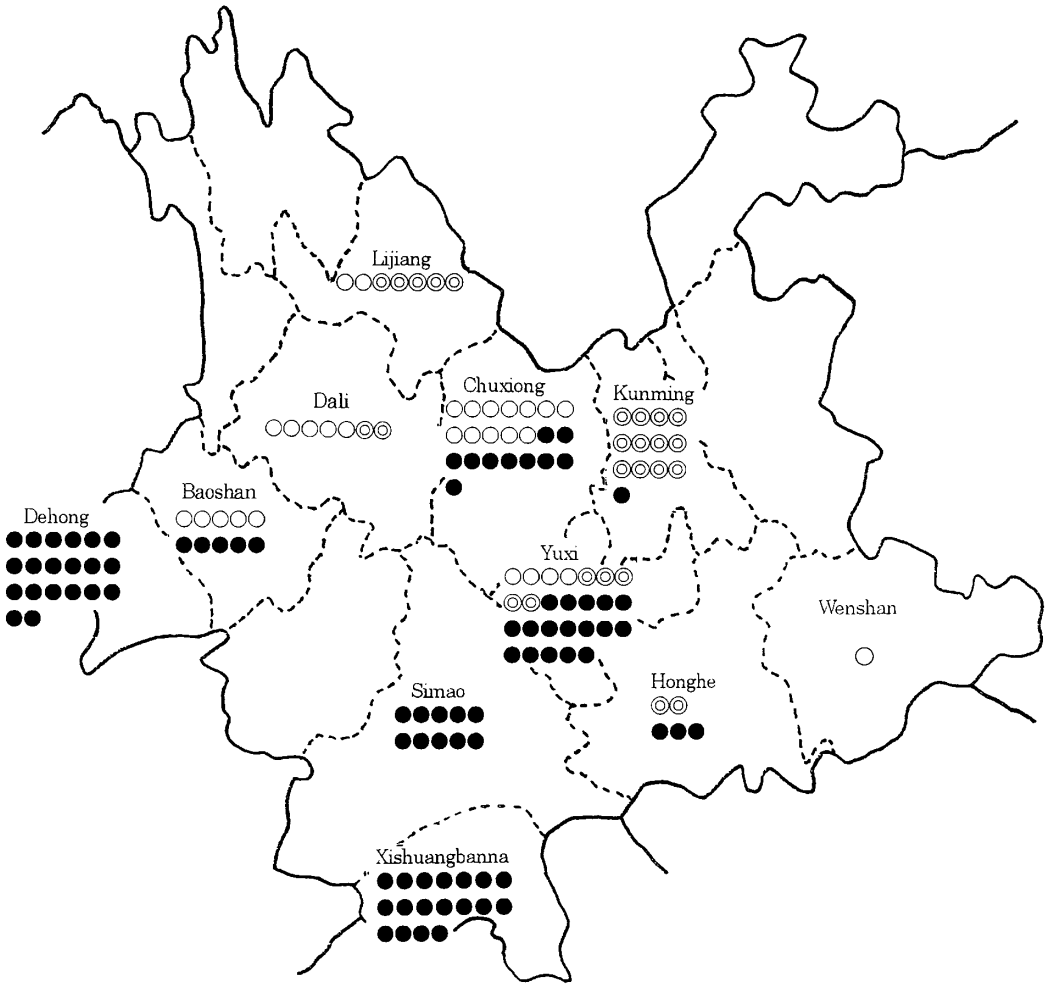

Fig. 2 Distribution of 3 pathogenic groups of *Xanthomonas oryzae* pv. *oryzae* in Yunnan province, China
Pathogenic group 1（○）comprises Pathotype A, Pathogenic group 2（◎）comprises Pathotypes B, C and D, and Pathogenic group 3（●）comprises Pathotypes E, F, G, H, I and J.

Xishuanbanna, Simao and Dehong. These counties are located in the southern and the western lowland areas where Indica rice varieties including hybrid ones are widely cultivated.

Discussion

Generally, Yunnan province in China has been considered to be one of the centers of diversity of Asian cultivated rice, *Oryza sativa* L., due to the abundance and diversity of rice germplasm resources. Yunnan province has a very complex climate and topography, with the rice-growing regions covering a range of altitudes from 76 to 2,700 m. We could predict that strains of the pathogens would be genetically and geographically diverse under the cultivation conditions mentioned above. BLB is the most serious bacterial disease of rice in Yunnan and the pathogenic diversity of *X. oryzae* pv. *oryzae* and the varietal resistance to BLB have been studied since the 1960s[14]. In this study, to investigate the recent pathotype diversity and regional distribution of *X. oryzae* pv. *oryzae* collected in Yunnan province, we analyzed the virulence using 12 near-isogenic lines and 3 check varieties.

The population structure of *X. oryzae* pv. *oryzae* in Yunnan province varied with the cultivation area, that is, strains with a wide host range were distributed in the southern part of Yunnan province where BLB outbreaks are usually more severe than in the northern part. The predominant Pathotype A, which was collected in the northern and the central parts of Yunnan province, was avirulent to all the differentials including IR24 used as a susceptible check variety, except for 2 check varieties, Toyonishiki and Sigadagabo. On the other hand, Group 3, which displayed a wide host range, was distributed mainly in the southern part of Yunnan province. It is possible that a high frequency of mutation had introduced new pathogenic characteristics under severe outbreak conditions. Based on these results, we suggest that *X. oryzae* pv. *oryzae* has acquired new pathogenic characters with its migration from the northern part to the southern part of Yunnan through water systems.

In 1995, Japonica rice varieties were cultivated mainly in the northern and the central parts of Yunnan province (accounting for about 50% of the total rice cultivation area), and Indica rice varieties including hybrid rices were cultivated mainly in the southern part of Yunnan province (about 40%)[14]. It is also suggested that host genetic diversity could be a determining factor of pathogenic diversity of *X. oryzae* pv. *oryzae*. However, Adhikari et al.[2] reported that host diversity did not affect the pathogenic diversity because the diversity of *X. oryzae* pv. *oryzae* populations collected from traditional and improved rice cultivars was similar in Nepal. Ardales et al.[3] also suggested that host diversity did not affect appreciably the pathogen diversity based on a comparison of different agroecosystems and cultivars in the Philippines.

In this study, we observed that 2 differentials, IRBB13 (*xa13*) and IRBB21 (*Xa21*), were resistant to all the strains tested, while IRBB5 (*xa5*) was susceptible only to 6 strains (4.3%). These resistance genes could be used in a breeding program as resistance gene sources to BLB in Yunnan province. Resistance gene *Xa21* conferred a resistance to strains of *X. oryzae* pv. *oryzae* from many countries and strain virulence to plants harboring this gene was not detected in our limited samples from Yunnan province. These results are similar to those recorded in strains from Vietnam[20] and Sri Lanka[21], namely only one strain collected in each country was virulent to plants harboring *Xa21*. However, recent studies have shown that strains virulent to the plants with this gene were widely distributed in Korea[13] and Nepal[2]. It was reported that the introduction of the *Xa21* gene into cultivated rice should be based on a detailed characterization of *X. oryzae* pv. *oryzae* populations in a given region.

In Asia, previous studies relating to the pathotypic and genetic diversity of *X. oryzae* pv. *oryzae* were based on differential interactions with resistance genes[1,2,4,5,7,8,17–20,22–25,28], restriction fragment length polymorphism (RFLP) using repetitive DNA elements (insertion sequences) as probes[1,3,9,12,16,29] and polymerase chain reaction (PCR)-based DNA fingerprinting[2,6,26]. Since these DNA-based studies did not enable to identify pathogenic variations in the *X. oryzae* pv. *oryzae* populations in detail, it is necessary to use DNA fragments related to the virulence or avirulence mechanism of *X. oryzae* pv. *oryzae*. Due to the diversity of the distribution of the bacterial pathotypes, a genetic and pathological approach to the use of horizontal resistance should be considered to avoid damage due to the breakdown of pathotype-specific resistance. The host-parasite relationship in BLB should be elucidated in greater detail.

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