

Preliminary evaluation of resistance genes in rice against bacterial leaf blight in Guilan Province—Iran

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ABSTRACT

The reactions of rice bacterial leaf blight races were identified in Guilan province—Iran on 12 near-isogenic lines and 14 pyramiding lines from International Network for Genetic Evaluation of rice (INGER) and 8 local and improved Iranian varieties were evaluated under natural photoperiod condition in the field. Inoculation was done at panicle initiation by clipping the sterilized scissors in the bacterial suspension to booting stage. Scoring of inoculated plants was made 21 days after inoculation. Infection levels of pyramiding lines containing two to five resistance genes, except, IRBB53 and IRBB61 with respectively resistance gene combination, Xa5 + Xa13 and Xa4 + Xa5 + Xa7, were not so clear. Among near-isogenic lines IRBB1, IRBB2, IRBB4 and IRBB10 carrying resistance gene Xa1, Xa2, Xa4 and Xa10 were susceptible; IRBB8, IRBB11, IRBB3, IRBB5 and IRBB13 were moderately susceptible; (having resistance gene Xa8, Xa11, Xa3, Xa5 and Xa13) IRBB14, IRBB21 and IRBB7 with respectively resistance gene Xa14, Xa21 and Xa7 were moderately resistance to bacterial blight. Furthermore, most of the time gene combinations support the strategy of pyramiding appropriate resistance gene. Local varieties were more susceptible than improved varieties to leaf blight disease. Among local varieties, Tarom was the most susceptible. And also, there were no significant differences among improved varieties and all of them were moderately resistance.

KEYWORDS

Bacterial Leaf Blight; Iran; Isogenic Lines;

Pyramiding Lines; Resistance Genes; RICE

1. INTRODUCTION

Bacterial leaf blight of rice (*Oryza sativa*), caused by *Xanthomonas oryzae* pv. *oryzae* [1] is a very serious disease in rice growing regions of the world [2]. The disease has become a major rice disease in last three decades because of the introduction of modern cultivars, which is highly responsive to nitrogen fertilizer. Bacterial blight of rice is a widespread and destructive disease in irrigated and rain fed environments of Asia [3,4]. There are no effective ways of protecting rice from the disease other than by the development of resistant cultivars. Host resistance is an important component of an integrated disease management program for bacterial disease [5]. Bacterial leaf blight interacts with rice in a gene—gene manner, which makes it an ideal model for studying plant—pathogen interactions [6,7]. So far, thirty resistance genes for Bacterial Blight have been identified and utilized in rice breeding programs. Five of them, Xa1 [8], Xa21 [9], Xa5 [10], Xa26 [11] and Xa7 [12] have been cloned as full length DNA sequences. The last decade has seen a dramatic advancement in our understanding of the molecular basis of the rice *X. oryzae* pv. *oryzae* interaction, with the cloning of the two rice resistance (R) genes, Xa1 and Xa21 [8,9] the bacterial avirulence (*avr*) genes, *avr-Xa5*, *avrXa7* and *avr-Xa10* [13], the hypersensitive response and pathogenicity (*hrp*) genes [14] and many other virulence related genes [15-21]. Analyses of *X. oryzae* pv. *oryzae* genomic sequences showed that there are 15 alleles in the *avr Bs3/pthA* (*avr/pth*) family of effectors [22]. So far, the functions of *avrXa7* [23], *avr-Xa10* [24,25], *avrXa5* [26] and *avrXa27* have been investigated. However, only the interaction between *avr-Xa27* and Xa27 has been fully characterized [12]. In Myanmar, on the basis of host-pathogen interaction,

Myanmar rice varieties were classified into four groups. Almost of Myanmar cultivars were belonged to Type II and III which were susceptible to Myanmar isolates of *Xanthomonas oryzae* pv. *oryzae* [27].

In Guilan province, previous results on BLB showed that there were four races of *X. oryzae* pv. *oryzae* [28]. The purpose of this study is to identify resistance sources for controlling the rice bacterial blight in Guilan—Iran.

2. MATERIALS AND METHODS

2.1. Rice Varieties and Preparation of Plants

Seeds of rice near-isogenic and pyramiding lines were obtained from the International Network for Genetic Evaluation of Rice (INGER) (Table 1). This Lines with local and improved Iranian varieties used to evaluation of resistance genes in rice plant against bacterial blight in Guilan province at Rice Research Institute of Iran. The 26 differential rice lines with a single gene or pyramid lines of two to five genes and 8 local and improved varieties were tested in this study. Rice Seeds were sown in nursery in natural condition in wet season. Individual seedlings transplanted in the field (twenty-one days after sowing). Spacing between and within rows is 20 cm × 20 cm. The length of rows is 3 m. Separate plots were used for different isolates. The fertilizer was applied according to the fertility status of the soil. The cultivation and the management of rice in the field proceeded as usual.

2.2. Inoculum Preparation and Inoculation

Bacterial isolates that were maintained at -80°C were

revived on NA (nutrient agar) slants at 27°C for 48 h. Each isolates was transferred to NA slants and incubated an additional 24 h at 27°C. Each bacterial colony on the slants were suspended with sterilized distilled water and adjusted to concentrations of approximately 10⁹ cfu/ml prior to inoculation (29). Inoculation was don at panicle initiation to booting stage by clipping the sterilized scissors in bacterial suspension and by clipping off the leaves 2 - 3 cm from leaf tip [30]. To reduce the possible effects of high temperature on disease reactions, favors the entry of bacteria into infection courts in the presence of sufficient moisture on the leaf surface and maintain inoculation time consistency, the inoculation was conducted in the morning.

2.3. Disease Reaction

Disease severity was rated by measuring the lesion length from the leaf tips. Scoring was done at 21 days after inoculation (DAI). The scales to be used in scoring were those from the 4th edition of the Standard Evaluation System for Rice [31]. The data analyzed for the main effect and interaction effect between cultivars and bacterial isolates.

3. RESULTS

3.1. Reactions of Near-Isogenic Lines

Interactions between near-isogenic lines and races of *X. oryzae* pv. *oryzae* are shown in Table 2. The degree of disease reaction in this study showed different relationship between combination of rice varieties and bacterial races. Race 4 showed the most susceptible and Race 1

Table 1. Crosses and Resistance genes of differential rice lines used to characterize virulence of *Xanthomonas oryzae* pv. *oryzae* isolates in Iran.

Rice line	Resistance gene(s)	Cross	Rice line	Resistance gene(s)	Cross
IRBB1	<i>Xa1</i>	IR24*5/KOGYOKU	IRBB51	<i>Xa4</i> + <i>Xa13</i>	IRBB4/IR66699-9-1-1-5-2
IRBB2	<i>Xa2</i>	IR24*5/TETEP	IRBB52	<i>Xa4</i> + <i>Xa21</i>	IRBB4/66700-3-3-3-4-2
IRBB3	<i>Xa3</i>	IR24*5/CHUGOKU 45	IRBB53	<i>Xa5</i> + <i>Xa13</i>	IRB4/IR66699-9-1-1-5-2
IRBB4	<i>Xa4</i>	IR24*5/IR20	IRBB56	<i>Xa4</i> + <i>Xa5</i> + <i>Xa13</i>	AY4 + 5/IR68311-13-3-42
IRBB5	<i>Xa5</i>	IR24*5/IR1545-339	IRBB57	<i>Xa4</i> + <i>Xa5</i> + <i>Xa21</i>	AY4 + 5/IR66700-4-2-9-5-2
IRBB7	<i>Xa7</i>	IR24*5/DV85	IRBB58	<i>Xa4</i> + <i>Xa13</i> + <i>Xa21</i>	NH11-35/NH9-53
IRBB8	<i>Xa8</i>	IR24*5/P1231129	IRBB59	<i>Xa5</i> + <i>Xa13</i> + <i>Xa21</i>	NH11-35/NH9-53
IRBB10	<i>Xa10</i>	IR24*5/CAS209	IRBB60	<i>Xa4</i> + <i>Xa5</i> + <i>Xa13</i> + <i>Xa21</i>	NH11-35/NH9-53
IRBB11	<i>Xa11</i>	IR24*5/IR8	IRBB61	<i>Xa4</i> + <i>Xa5</i> + <i>Xa7</i>	IR-BB7/IR-BB60
IRBB13	<i>Xa13</i>	BJ1/5*IR24	IRBB62	<i>Xa4</i> + <i>Xa7</i> + <i>Xa21</i>	IR-BB7/IR-BB60
IRBB14	<i>Xa14</i>	TAICHUNG NATIVE 1/5*IR24	IRBB64	<i>Xa4</i> + <i>Xa5</i> + <i>Xa7</i> + <i>Xa21</i>	IR-BB7/IR-BB60
IRBB21	<i>Xa21</i>	IR24*8/O BARTHII	IRBB65	<i>Xa4</i> + <i>Xa7</i> + <i>Xa13</i> + <i>Xa21</i>	IR-BB7/IR-BB60
IRBB50	<i>Xa4</i> + <i>Xa5</i>	-	IRBB66	<i>Xa4</i> + <i>Xa5</i> + <i>Xa7</i> + <i>Xa13</i> + <i>Xa21</i>	IR-BB7/IR-BB60

Table 2. Reaction of near-isogenic lines to races of *X. oryzae* pv. *oryzae*.

Rice line	Resistance gene(s)	Reaction			
		R ₁	R ₂	R ₃	R ₄
IRBB1	<i>Xa1</i>	MS	HS	S	HS
IRBB2	<i>Xa2</i>	MS	HS	S	HS
IRBB3	<i>Xa3</i>	S	MS	MS	MR
IRBB4	<i>Xa4</i>	MS	HS	S	HS
IRBB5	<i>Xa5</i>	MR	MR	S	S
IRBB7	<i>Xa7</i>	R	MR	MS	MR
IRBB8	<i>Xa8</i>	MS	S	MR	HS
IRBB10	<i>Xa10</i>	MS	HS	S	HS
IRBB11	<i>Xa11</i>	S	MS	MR	S
IRBB13	<i>Xa13</i>	R	S	MR	MS
IRBB14	<i>Xa14</i>	MR	MR	MS	MR
IRBB21	<i>Xa21</i>	MR	MR	MS	MR

R1-R4: Race 1-Race 4; S: Susceptible; MS: Moderate Susceptible; HS: High Susceptible; R: Resistance; MR: Moderate Resistance.

expressed the lowest susceptible among the races. Hashemi Cultivar (maybe has not major functional gene for resistance to Iranian races) was used as susceptible check. However, none of NILs was highly resistant or resistant to races of *X. oryzae* pv. *oryzae* in Guilan province.

3.2. Reaction of Pyramiding Lines

Interactions of pyramiding lines expressed in **Table 3**. Pyramiding lines containing two to five resistance genes except, IRBB53 and IRBB61 were significantly more resistant to the most races than their respective individual *Xa* gene, IRBB53 and IRBB61 with respectively resistance gene combination *Xa5* + *Xa13* and *Xa4* + *Xa5* + *Xa7* were moderately resistance to bacterial races. Khazar Cultivar (maybe has major functional gene for resistance to Iranian races) was used as resistant check. High level of resistance was observed in combination of four and five dominant resistance genes *Xa4* + *Xa5* + *Xa13* + *Xa21*, *Xa4* + *Xa5* + *Xa7* + *Xa21* and *Xa4* + *Xa5* + *Xa7* + *Xa13* + *Xa21* (IRBB60, IRBB64 and IRBB66 respectively).

3.3. Reaction of Local and Improved Varieties

Iranian varieties were tested for their resistance to the four races (**Table 4**). Local varieties were more susceptible than improved varieties to leaf blight disease. Among local varieties, Tarom was the most susceptible. Between

Table 3. Reaction of pyramiding lines to races of *X. oryzae* pv. *oryzae*.

Rice line	Resistance gene(s)	Reaction			
		R ₁	R ₂	R ₃	R ₄
IRBB50	<i>Xa4</i> + <i>Xa5</i>	HR	MR	MR	R
IRBB51	<i>Xa4</i> + <i>Xa13</i>	MS	MR	MR	MR
IRBB52	<i>Xa4</i> + <i>Xa21</i>	MR	MR	R	MR
IRBB53	<i>Xa5</i> + <i>Xa13</i>	MR	S	MR	S
IRBB56	<i>Xa4</i> + <i>Xa5</i> + <i>Xa13</i>	MR	MS	MR	HR
IRBB57	<i>Xa4</i> + <i>Xa5</i> + <i>Xa21</i>	MR	MR	MR	HR
IRBB58	<i>Xa4</i> + <i>Xa13</i> + <i>Xa21</i>	R	MR	MR	MS
IRBB59	<i>Xa5</i> + <i>Xa13</i> + <i>Xa21</i>	R	MR	MR	R
IRBB60	<i>Xa4</i> + <i>Xa5</i> + <i>Xa13</i> + <i>Xa21</i>	R	HR	R	R
IRBB61	<i>Xa4</i> + <i>Xa5</i> + <i>Xa7</i>	MS	MR	MS	R
IRBB62	<i>Xa4</i> + <i>Xa7</i> + <i>Xa21</i>	MR	MS	MR	MR
IRBB64	<i>Xa4</i> + <i>Xa5</i> + <i>Xa7</i> + <i>Xa21</i>	MR	HR	R	R
IRBB65	<i>Xa4</i> + <i>Xa7</i> + <i>Xa13</i> + <i>Xa21</i>	MR	R	MS	R
IRBB66	<i>Xa4</i> + <i>Xa5</i> + <i>Xa7</i> + <i>Xa13</i> + <i>Xa21</i>	R	R	HR	R

Table 4. Reaction of pyramiding lines to races of *X. oryzae* pv. *oryzae*.

Rice line	Type	Reaction			
		R ₁	R ₂	R ₃	R ₄
Tarom	Local	MS	MS	S	S
Hashemi	Local	MS	MS	MS	S
Hasansaraei	Local	MS	MS	MS	MS
AliKazemi	Local	MR	MS	MS	MR
Khazar	Improved	MR	R	MR	MR
Fajr	Improved	MR	MR	MR	MR
Dorfak	Improved	MR	MR	MR	MR
Gohar	Improved	MR	MR	MR	MR

improved varieties weren't very important differential and all of them were moderately resistance.

4. DISCUSSION

The major goals of this project was to develop rice cultivars with effective resistance genes to *Xanthomonas oryzae* pv. *oryzae*. In this study, we establish the level of resistance to the bacterial blight pathogen conferred by a single gene individually and multiple gene combinations. Previous results on the basis of host-pathogen interaction

showed that there were four bacterial leaf blight races in Iran [28]. These races were tested on 12 international differentials of near-isogenic lines and 14 varieties of pyramided lines and 8 local and improved varieties to support a gene deployment approach to managing the disease using resistant cultivars. The phenotype reactions between the single-gene lines and isolates were clear and easily classified into virulence or avirulence patterns. Among 12 near-isogenic lines with single gene, IRBB1 (Xa1) was low resistance and IRBB7 (Xa7) was high resistance on the basis of percentages of the compatible strains, also IRBB7 (Xa7) was not resistance to all tested isolates. These results indicate that IRBB21 (Xa21), IRBB14 (Xa14) and especially IRBB7 (Xa7) was resistant to the most isolates. Among Iranian varieties were tested in this project. Local varieties were more susceptible than improved varieties to leaf blight disease. Among local varieties, Tarom was the most susceptible. And also, there were no significant differences among improved varieties and all of them were moderately resistance.

Generally, in comparison between near-isogenic and pyramiding lines, the percent lesion area on pyramiding lines was significantly fewer than near-isogenic lines with the single resistant gene. But the Xa5 and Xa13 combination in IRBB53 was less resistant than gene Xa13 in IRBB13. As a result, none of the near-isogenic lines was resistant to all the races tested. IRBB7 (Xa7), IRBB14 (Xa14) and IRBB21 (Xa21) had a good resistant to the most of the races, but pyramiding lines, expect, IRBB53 and IRBB61 were significantly more resistant to the most races. Therefore a breeding program should be initiated to transfer these resistance genes from the differential varieties to high quality Iranian rice varieties to control the disease effectively.

There are obvious differences in the field-testing of the rice with the bacterial blight from different countries due to different agroecosystems [32]. Except for cultivar types, different terrains, climates, periods of rice planting and other factors may be associated with the population diversity and virulent variation of *Xanthomonas oryzae* pv. *oryzae*.

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