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SCREENING OF PAKISTANI RICE (*ORYZAE SATIVA*) CULTIVARS AGAINST *XANTHOMONAS ORYZA* PV. *ORYZAE*

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Abstract

Bacterial blight of rice (*Xanthomonas oryzae* pv. *oryzae*) significantly reduces the yield and quality of rice all over the world. Screening of Pakistani 15 genotypes revealed Kashmir Basmati as a highly resistant genotype and showed ≥ 75% resistance to all the tested strains/isolates, only YR6W14D3 infect the genotype but the severity was not divesting. IR-6, Basmati-370, JP-5 and KSK-370 were ≥ 50% resistant to all the tested strains, while the remaining genotypes were susceptible to all the strains/isolates of *Xanthomonas oryzae* pv. *oryzae*. Among the four strains/isolates LKA4(i) showed 38% severity. As Kashmir Basmati showed resistance to LKA4(i), so plant breeder can easily transfer the gene into high yielding and susceptible genotype to enhance the food quality and quantity.

Introduction

Rice (*Oryzae sativa* L.), a member of family Poaceae is widely grown in tropical and subtropical regions of the world (Ezuka & Kaku, 2000). Rice is one of the major food crops of the world especially of the most Asian countries like Pakistan, Bangladesh, China, Vietnam and Korea. Rice is placed on second position in cereal cultivation around the globe and occupies an important position in the economy of Pakistan as an export item as well as staple food (Zahid *et al*., 2005). Approximately 90% of the world's rice is grown in Asia continent and constitutes a staple food for 2.7 billion people worldwide (Salim *et al*., 2003). Rice as the third largest crop of Pakistan covers about 10% cultivated area and contributes about 17% towards total cereal grains production of the country (Ahmad *et al*., 2005).

It is however unfortunate that such an important crop is attacked by many kinds of diseases including Bacterial leaf Blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama, 1922). Bacterial leaf blight of rice is one of the most destructive diseases of rice throughout the world (Swings *et al*., 1990). BLB is the most serious disease in South Asia (Ou, 1985) and in many Asian countries bacterial leaf blight has become endemic on rice following repeated cultivation (Mew *et al*., 1993). This disease become serious because many improved, high yielding varieties, when managed with high nitrogen levels and close spacing, have inadequate resistance to the pathogen (Eamchit & Mew, 1982). Bacterial leaf blight was first noticed by the farmers in Fukuoka prefecture Kyushu Island, Japan, as early as in 1884-85 (Ezuka & Kaku, 2000). In Pakistan the disease was recorded for the first time by Mew & Majid (1977), latter on Ahmad & Majid (1980) observed it on rice varieties IR-6, Palman, Basmati-198 at Rice Research Institute, Kala Shah Kaku and farmer's fields.

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Bacterial Leaf Blight is a vascular disease. The pathogen normally enters the host through wounds or natural openings such as the water pores on hydathodes (Mew, 1987) and multiplies in the tissues of the epitheme, into which xylem vessels open (Tabei, 1997). After the bacterial multiplication occurs in epitheme, some of the bacterial cells reach the xylem vessels of the vascular system (Li *et al*., 2001). When a bacterial colony has established in the xylem vessels, the bacterial cells grow vigorously and translocate through the network of the vascular system (Ezuka & Kaku, 2000).

Materials and Methods

The research work was conducted at the Department of Biotechnology, University of Malakand, N.W.F.P. Pakistan.

Rice varieties and Bacterial strains used to study bacterial blight: Fifteen different Pakistani rice varieties were selected to study the bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae (Xoo)*. These varieties include Basmati-385, IR-6, Swat-2, Kashmir Basmati, NIAB IR-9, KSK-282, Shahkar, GNY-53, Basmati-370, KSK-133, Pakhal, Swat-1, Fakhre Malakand, JP-5 and Dilrosh. Three local and one exotic isolates/strains of *Xoo* were used to study bacterial blight of rice. These strains/isolates includes LKA4(i),YR6W14D3, PXO379 and LKA4(i)y. Seeds of all the above mentioned varieties and the isolates/strains of *Xoo* were provided by NARC, Islamabad, Pakistan.

All the strains/isolates were in dry preserved form at 4^{o}C in small vials. So to proceed, first these strains/isolates were revived. Bacteria were grown on single media for revival i.e. Yeast Dextrose Calcium Carbonate medium (Table 1).

Strain revival: Three to four ml distilled water was added in vials containing preserved bacterial culture, mixed well and then a loop full of bacterial culture was streaked on the Petri plates with solid Yeast Dextrose Calcium Carbonate medium (YDC). The pouring and inoculation were carried out in laminar air flow. These plates were incubated at 28^{o}C for two to three days depending upon colony development. All the bacterial strains and isolates were revived using the same method (Fig. 1).

Preparation of bacterial suspension: For the preparation of fresh inoculum, 10 ml of sterile distilled water was added to the 48 hours culture of *Xanthomonas oryzae* pv. *oryzae* in the slants for the preparation of bacterial suspension (Fig. 2).

Pathogenicity Test: For pathogenicity test, clip method was used for the inoculation of the rice plants with *Xanthomonas oryzae* pv. *oryzae.* These tests were conducted on leaf flag stage (80-90 days after sowing).

Clip method: Kauffman *et al*., (1973) reported the clip method. In clip method sterilized surgical scissors dipped in bacterial suspension were used for inoculation. For this purpose a pair of scissors was dipped in bacterial suspension. Leaves of all the three plants in a pot were grasped in one hand and the top 1-3 inches of three leaves were clipped off simultaneously. The same procedure was followed for inoculation of the different strains and isolates to each variety of rice. The inoculum should be used within two hours after preparation as *Xanthomonas oryzae* pv. *oryzae* quickly losses its viability. A control of each variety was also maintained, by using scissors dipped in sterile water for clipping off the leaves. The pathogenicity of each strain was tested on 11-12 weeks old rice plants of all varieties at 35^{o}C in glass house (Fig. 3).

Table 1. Composition of YDC medium.

Composition	Quantity
Yeast extract	10g
Dextrose	20g
Calcium carbonate	20g
Agar	20g
Distilled water	1liter

a **b** **c** **d**

Fig. 1. Photographic presentation of slants used for inoculation (a), (b), (c), (d) 48 hours culture of *Xanthomonas oryzae* pv. *oryzae* on slants used for inoculum preparation.

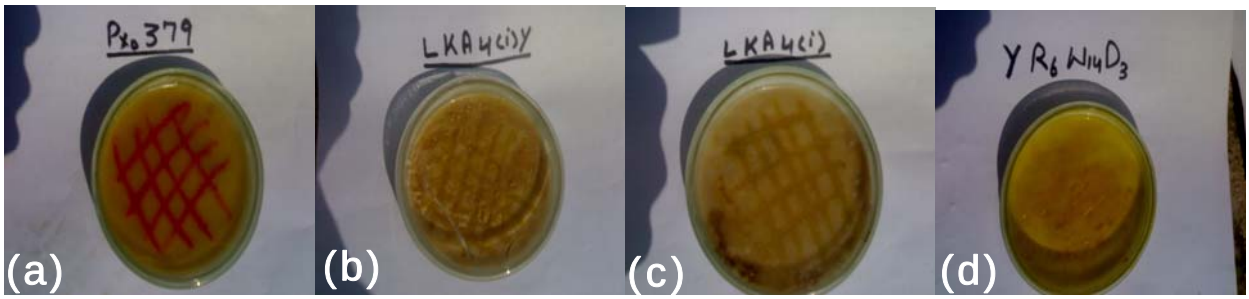

Fig. 2. Photographic presentation of pure culture of Xa*nthomoas oryzae* pv. *oryzae* (Xoo).
(a) Pure culture of PXO379 strain of *Xoo*, (b) Pure culture of LKA4(i)y isolate of *Xoo*,
(c) Pure culture of LKA4(i) isolate of *Xoo*, (d) Pure culture of Y R6 W14 D3 isolate.

Fig. 3. Photographic presentation of plants 21 days after inoculation (DAI), vascular bundles in the meristem region killed with bacteria the plant begins to wilt and after 30 days (DAI) died.
(a) (Control rice plant), (b) (Infected with LKA4(I)), (c) (Infected with PXO379), (d) (Infected with YR6W14D3), (e) (Infected with LKA4(i)y). Picture taken 30 Days after inoculation

Glasshouse test: Following the inoculation, the plants were surveyed after every 24h time interval to note the appearance of disease symptoms and final data was recorded after 14 days (2 weeks) of inoculation. Percent disease incidence was calculated by the help of following formula (Gnanamanickam *et al*., 1999):

$$\% \text{ Disease incidence} = \frac{\text{Lesion length}}{\text{Total leaf length}} \times 100 \quad \text{(Gnanamanickam } et\ al\text{., 1999)}$$

The score chart given in Table 2 was used to evaluate the response of host plant (Anonymous, 1996).

Confirmation of the bacterial blight in infected rice leaves: The leaves of rice plants inoculated with *Xanthomonas oryzae* pv. *oryzae,* showing the symptoms of bacterial blight i.e. yellow lesions were used for isolation of the bacteria in order to confirm the bacterial blight. The infected leaves were cut with the help of a pair of scissors from plants grows in the glass-house and taken into the lab. These leaves were then washed with autoclaved distilled water for 2-3 times. After that, the isolation of the bacteria was done by using the following methods:

1. Direct plating of infected leaves.
2. Plating inoculated rice leaves using dilation method.

Records: After 48h, plates were observed for presence of *Xanthomonas oryzae* pv. *oryzae*. The same procedure was followed for all the rice varieties inoculated with different strains of *Xanthomonas oryzae* pv. *oryzae* showing the visual symptoms of blight.

Results

Three local and one exotic isolates/strains of *Xanthomonas oryzae* pv. *oryzae* were tested for pathogenicity to check their virulence on the fifteen different rice varieties. We got the following results.

The isolates/strains of *Xanthomonas oryzae* pv. *oryzae (Xoo)* were incubated on YDC medium for the revival as well as inoculum preparation. After 24 hr yellow, smooth, and viscous colonies appeared which became somewhat irregular after 48 hr due to viscous fluid secreted by the bacteria.

Development of symptoms: Disease symptoms first appeared 3 days after inoculation in most varieties. The initial symptoms were leaf curling near the cut-off portion. Soon after curling, water-soaked lesions were developed from the cut surface and advanced down the leaf. Fourteen days after inoculation (DAI), vascular bundles in the meristem region got filled with bacteria and the plant begins to wilt and after 30 days (DAI) plants were completely dead.

Length of lesion caused by *Xanthomonas oryzae* pv. *oryzae (Xoo)* isolates/isolates: In each variety, lesions developed uniformly downward from the point of inoculation. However, the length from the leaf tip varied, from variety to variety and isolate to isolate (Fig. 4).

Table 2. Scale for bacterial blight (Anon., 1996).

Infection %	Score	Host response
0 %	0	Highly resistant (HR)
> 1-10 %	1	Resistant (R)
> 10-30 %	3	Moderately resistant (MR)
> 30-50 %	5	Moderately susceptible (MS)
> 50-75 %	7	Susceptible (S)
> 75-100 %	9	Highly susceptible (HS)

Fig. 4. Reaction of bacterial blight disease on rice plants. Leaves were taken 2 weeks after inoculation

Pathogenicity of *Xanthomonas oryzae* pv. *oryzae (Xoo)* at leaf flag stage: The results of pathogenicity test are summarized in Table 3. Almost all varieties showed moderate resistance.

Detection of bacteria from infected rice leaves: To confirm that the symptoms showed by the inoculated rice plants were due to the presence of *Xanthomonas oryzae* pv. *oryzae (Xoo)*, diseased leaves were plated on YDC medium. Plates were monitored between 72-98 hr for bacterial colonies. The colonies produced were similar to the bacterial colonies used for inoculation, i.e. yellow, smooth and viscous.

Statistical analysis

Genetic sensitivity/resistance of Pakistani Rice genotypes/cultivars: Among the 15 genotypes, Kashmir Basmati showed ≥ 75%, IR-6, KSK-370 and JP-5 showed ≥ 50% resistance to all the tested strains/isolates, while the remaining genotypes were proven susceptible to all bacterial strains/isolates (Table 4, Fig. 5). The genome of these genotypes needs much more attention. Kashmir Basmati was specifically also resistant to isolate LKA4(i) which showed more then 38% disease severity across the genotypes. It was concluded that Kashmir Basmati should be used for breeding programs.

Table 3. Comparison of % disease incidence of three local and one exotic strains /isolates of *Xanthomonas oryzae* pv. *Oryzae (Xoo)* on 15 different rice cultivars at leaf flag stage.

Strains/isolates	LKA4(i)	YR6W14D3	PXO379	LKA4(i)y	Avg.
Basmati-385	26.70	44.50	13.00	21.80	26.50
IR-6	16.00	15.60	25.00	20.50	20.70
Swat-2	25.20	28.60	24.70	31.00	27.27
Kashmir Bas.	15.40	21.50	19.00	8.60	16.10
NIAB-1R9	19.50	30.00	32.60	24.00	26.50
KSK-282	31.40	27.20	25.00	26.90	27.70
Shahkar	32.80	30.60	37.00	15.80	29.00
GNY53	36.80	20.00	30.00	31.90	29.70
Bas-370	27.20	26.70	36.00	16.90	26.80
KSK-370	24.00	19.00	39.13	13.34	23.90
Pakhal	10.00	20.00	27.00	29.00	21.50
Swat-1	35.60	10.00	30.00	33.30	27.22
Fakhre mala.	31.00	22.70	26.00	28.30	27.15
JP-5	86.40	16.30	14.20	28.70	36.60
Dilrosh	19.60	25.00	30.60	20.90	24.20

Table 4. Comparison of cultivars resistance to disease incidence (Tabulated representation).

Cultivars/isolates	LKA4(i)	YR6W14D3	PXO379	LKA4(i)y
Basmati-385	1*	1	0**	1
IR-6	0	0	1	1
Swat-2	1	1	1	1
Kashmir bas	0	1	0	0
NIAB-1R9	0	1	1	1
KSK- 282	1	1	1	1
Shahkar	1	1	1	0
GNY53	1	1	1	1
Basmati-370	1	1	1	0
KSK-370	1	0	1	0
Pakhal	0	1	1	1
Swat-1	1	0	1	1
Fakhar Malakan	1	1	1	1
JP-5	1	0	0	1
Dilrosh	0	1	1	1

* = Susceptible cultivars, ** = Resistant cultivars

Strain severity: The severity of four strains/isolates LKA4(i), YR6W14D3, PXO379 and LKA4(i)y were tested on 15 different rice cultivars. Among the strains/isolates LKA4(i) showed 37.7%**,** YR6W14D3 showed 28.57%, PXO379 showed 21.43% and LKA4(i)y showed 21.43% disease severity (Table 5, Fig. 6). Isolate LKA4(i) showed the most divesting effect and maximum level of severity**.** While YR6W14D3 isolate also showed significant damage to the genotypes. Further more it was concluded that the genotypes resistant to isolate LKA4(i) should be considered genetically resistant genotype and can be used as resistant cultivar for breeding programs.

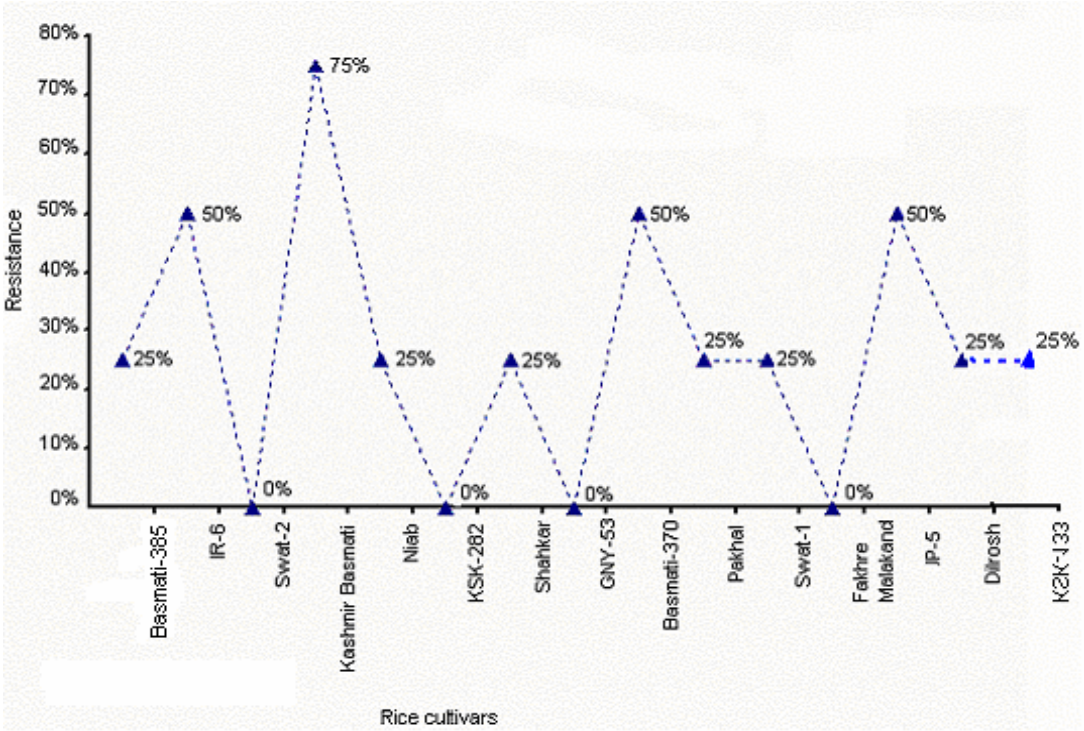

Fig. 5. Comparison of cultivars resistance to disease incidence (Graphic representation).

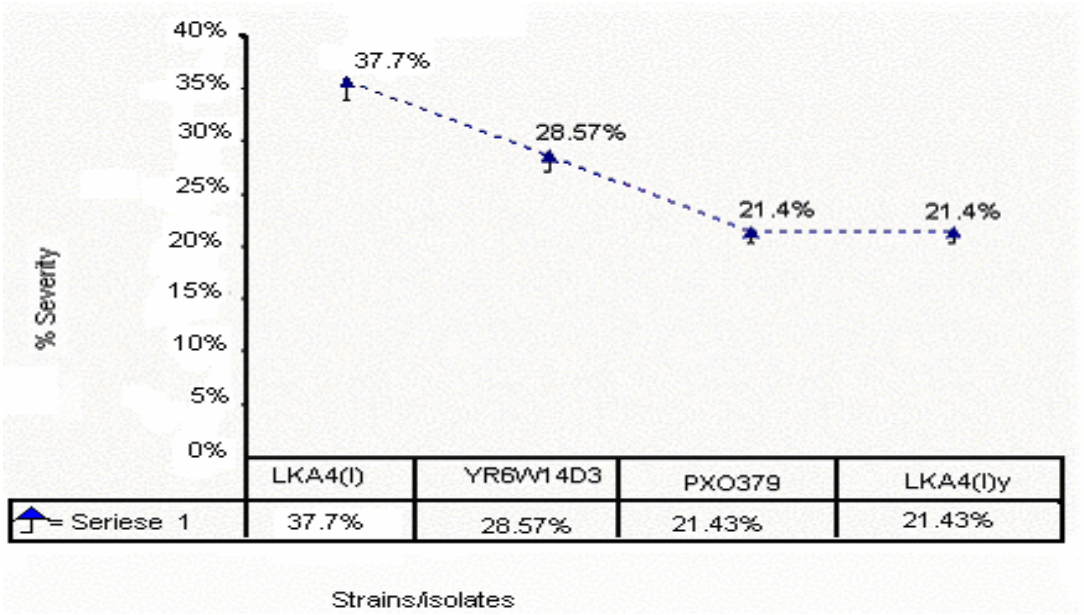

Fig. 6. Comparison of percent disease severity of strains/isolates of *Xanthomonas oryzae* pv. *Oryzae (Xoo)* (Graphic representation).

Table 5. Comparison of percent disease severity of strains/ isolates of *Xanthomonas oryzae* pv. *Oryzae (Xoo)* (Tabulated representation).

Strains/isolates	Percent severity
LKA4(i)	37.7%
YR6W14D3	28.57%
PXO379	21.43%
LKA(i)y	21.43%

Table 6.Comparison of % disease incidence and genotypes resistance of cultivars.

Rice varieties	Germination time (in days)	Genotypes resistance	% Disease incidence
Basmati 385	17	25%	26.50
IR-6	26	50%	20.70
Swat-2	11	0%	27.27
Kashmir Basmati	19	75%	16.10
NIAB-1R9	22	25%	26.50
KSK-282	9	0%	27.70
Shahkar	14	25%	29.00
GNY-53	21	0%	29.70
Basmati 370	18	50%	26.80
KSK-133	14	25%	23.90
Pakhal	17	25%	21.50
Swat-1	14	25%	27.22
Fakhre Malakand	21	0%	27.15
JP-5	21	50%	36.60
Dilrosh	11	25%	24.20

Discussion

The present study was conducted to evaluate the resistance of fifteen different verities of rice to three local and one exotic isolates/strains of *Xanthomonas oryzae* pv. *oryzae* and a comparison of virulence of *Xoo* between Pakistani and Philippines strains/isolates were made.

For the growth of rice plants in glass house, the dry seeds of each variety were sown in small separate pots. Singh *et al., (2000)* reported that the favorable temperature for the growth of rice plants is 25-35°C. YDC medium was used for the revival of the bacterial strains and later for inoculum preparation on slants, the incubation temperature for the bacterial growth was maintained at 28°C for 48 h. Similarly Leach *et al.*, (1992) used 28°C incubation temperature for the growth of *Xoo* on culture medium, while Khan *et al.* (2000) incubate bacterial culture at 37°C for 48h for screening rice varieties against bacterial blight. After 24h of incubation, yellow, smooth, convex and circular colonies were observed which become somewhat irregular after 48h due to viscous fluid secreted by the bacteria. Similar types of culture and viscous colonies on potato semi synthetic agar medium were reported by Wakimoto (1954) and Khan *et al*., (2000).

Bacterial culture suspended in 25 ml of sterile distilled water was used to inoculate rice plants. Khan *et al*., (2000) used bacterial culture suspended in 10 ml sterile distilled water for screening rice varieties against bacterial blight. The clipping method of artificial inoculation was used to evaluate the resistance of the varieties against bacterial blight (BB) as it was convenient for the inoculation of rice plants in green house studies. Kauffman *et al*., (1973) used the clipping method for evaluating resistance of rice varieties against *Xoo* and they stated that clipping inoculation score and natural infection score were highly correlated. Initial symptoms in the present study were leaf curling which appeared after 5-7 days in moderately susceptible varieties. This was supported by the work of Kaufinan *et al*., (1973) as they reported that the disease symptoms first appear 4-5 days of inoculation in the form of leaf curling near the cut off portion when they evaluated the resistance of IR8 and IR20 against PXO25.

Percent disease incidence calculated for evaluated rice plants on the basis of lesion length was used to evaluate the pathogenicity of the bacterial strains to rice varieties and scores were assigned to these (0-9) according to the standard evaluating system for rice. Ganamanickam *et al*., (1999) also used percentage disease in a series of experiments to evaluate the performance of *Pseudomonas putida* strain V14i as a bio-control agent to suppress bacterial blight disease in IR24. Mew and Khush (1981) used standard evaluation system (0-9 scale) to evaluate the resistance of different rice varieties to PXO61.

From our results it was obvious that most of the rice varieties were moderately susceptible to local isolates LKA4(i), LKA4(i)Y, and YR6W14D3 and one exotic strain PXO379 of *Xoo*. Among the 15 genotypes Kashmir Basmati showed ≥ 75, Basmati-370, KSK-370 and JP-5 showed ≥ 50% resistance to all the tested strains/isolates while the remaining genotypes were proven susceptible to all the bacterial strains. The genome of these genotypes needs much more attention. Kashmir basmati was specifically resistant to isolate LKA4(i) which showed more then 38% disease severity across the genotypes. It was concluded that Kashmir Basmati should be used for breeding programs.

The severity of four strains LKA4(i), YR6W14D3, PXO379, LKA4(i)y was tested. Among the strains LKA4(i) showed 37.7%, YR6W14D3 showed 28.57%, PXO379 showed 21.43% and LKA4(i)y showed 21.43% disease severity. Isolate LKA4(i) showed the most divesting effect and maximum level of severity (38%) while strain YR6W14D3 also showed significant damage to the genotypes. Further more it was concluded that the genotypes resistant to isolate LKA4(i) should be considered genetically resistant genotype and can be used as a resistant cultivar.

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