Resistance of Rice to *Xanthomonas oryzae* pv. *oryzae* in Nepal

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ABSTRACT

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Resistance to bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* in rice cultivars, was evaluated in the field and under greenhouse experiments from 1987 to 1991. In the field studies, plants were assessed by measuring lesion length (LL) and disease severity (percentage of leaf diseased). The area under the disease progress curves (AUDPC) and LL were used to compare rice cultivars. Rice cultivars BR-34-13, PAU-50-B-25, Laxmi, Sabitri, BW293-21, IR7167-33, Rodina, and Amonghaud had significantly ($P < 0.05$) shorter LL and smaller AUDPC than the susceptible check IR24. In the greenhouse studies, highly significant ($P < 0.01$) cultivar, strain, dose, cultivar × strain, and cultivar × dose effects were observed, indicating a differential host–pathogen interaction. Differences in virulence among bacterial strains and resistance among rice cultivars were observed. Inoculum that contained 10^9 cfu/ml induced larger differences in LL between resistant and susceptible cultivars. Laxmi consistently exhibited the highest level of resistance as indicated by reduced LL and AUDPC, and could be a source of resistance to BB in Nepal.

Additional keywords: dose-response curve, *Oryza sativa*

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (ex Ishiyama) Swings et al (20), is one of the most widespread and destructive bacterial diseases of rice (*Oryza sativa* L.) occurring worldwide (14,17). In Nepal, no chemical control is practiced. A few cultural practices are available to reduce the incidence of BB. The use of resistant cultivars is the most effective method to combat the disease (13,17). The principal criterion used previously for evaluation of rice cultivar–*X. o. oryzae* interaction was lesion length (LL) measured 30–40 days after clip inoculation of the leaves (15,16). One way of evaluating this interaction is to monitor inoculum doses of the pathogen needed to elicit a certain response in the host (8). Rice cultivars vary in their reaction to pathogenic races and inoculum doses of *X. o. oryzae* (7). Further, when infectivity titration was used to study specific rice cultivar–race combinations, differences in the effective doses (ED_{50}) of the rice cultivar Cas209 for compatible and incompatible reactions at different growth stages were obtained (16).

In Nepal, BB causes severe damage in rice production (1,5) and can reduce grain yields by up to 26%, especially during prolonged periods of rainy weather from August to October (2). Pathogenic specialization of *X. o. oryzae* on rice cultivars was observed (4). Several resistant cultivars have been developed and are grown in other rice-growing countries of Asia. Current evaluations of rice cultivars under controlled conditions in Nepal are lacking. To complement accurate evaluations of rice cultivars for sources of resistance to BB, a quantitative measure of the effects of the host's response to inoculum doses and a clear understanding of the pathogenic variability of *X. o. oryzae* are necessary in developing rice cultivars with stable resistance. The main objectives of this study were to evaluate resistance to BB in rice cultivars and to measure the effects of different inoculum doses of *X. o. oryzae* strains on the level of resistance of rice cultivars tested. Preliminary results of this work have been published (3).

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MATERIALS AND METHODS

Field experiments. A total of 980 rice cultivars from the germ plasm collection of the National Rice Research Program, Parwanipur, Nepal, were screened for BB resistance at the Institute of Agriculture and Animal Science (IAAS) farm (lat. 27°37′ N, long. 84°25′ E, 256 m) during the 1987 and 1988 rice growing seasons. The experiment was conducted on sandy-loam soil (pH 5.8, 2.68% organic matter), which had been cropped to winter wheat the previous season. The site has an average annual rainfall of 840 mm. During the summer prior to transplanting, the site was moldboard plowed and disked twice to control weeds. Seeds of each rice cultivar were planted in seed beds in the third week of June. Seedlings were uprooted and transplanted to the field 25 days after sowing. Each rice cultivar was transplanted in three rows (3 m long) and spaced 20 cm apart. The field was fertilized with NH_4SO_4 (21% N) at 120 kg/ha of N. Fertilizer was applied by broadcasting in late July (one-third of total), late August (one-third), and late September (one-third). The BB-infected leaves of rice cultivar Masuli were collected from farmers' fields, and leaves were cut into small segments (2–3 cm long). These tissue sections were soaked in 500 ml of distilled water in a plastic bucket for 30 min, and the resulting bacterial suspension was used as inoculum. Inoculation procedures were adapted from those described by Kauffman et al (12). For each rice cultivar, five plants per row were clip inoculated 45 days after sowing. Disease evaluation was conducted 14 days after inoculation (DAI); the top two leaves of 10 randomly selected plants per row were rated for percentage of leaf diseased (11). Rice cultivars with an average disease severity of 0–25% leaf diseased were classified as resistant, with 25.1–50% as moderately resistant (MR), and above 50% as susceptible. Seeds from moderately resistant to resistant rice cultivars were harvested and air-dried. Seeds were stored (12% moisture) for further use.

Ninety-three of the 980 rice cultivars that had moderate to high levels of resistance to BB in the preliminary screening experiments were reevaluated for BB resistance as described above at the same site during the 1989 season. Rice seedlings were first raised in nursery beds in late June and fertilized with 60 kg/ha of N as NH_4SO_4 at the time of sowing. In the field, plants were fertilized at the equivalent rate of 120-60-50 kg/ha of N–P–K. The experimental unit consisted of three rows of a rice cultivar. Rows were 3 m long and 20 cm apart and contained approximately 15 plants. The experimental design was completely randomized with two replications. Strain NXO153 isolated from the Jhapa district of Nepal was used in these experiments (Table 1). The bacterium was revived from 5% skim milk, prepared by streaking onto petri dishes containing peptone sucrose agar (PSA) (17) at pH 6.8, and incubated in the laboratory at 28 C for 72 hr. A single colony of the bacterium was transferred to a PSA slant and then incubated for 72 hr. Bacteria were suspended in 10 ml of sterile distilled water and shaken vigorously for 1 min. The inoculum concentration was adjusted to 10^8 cfu/ml using a spectrophotometer

prior to inoculation. Ten randomly selected plants in the center row were tagged and used for disease inoculation and assessment. Two fully expanded leaves of each tagged plant were clip inoculated 35 days after transplanting as described above. Plants were inoculated in the morning (8:00) to reduce the possible effects of high temperature on disease reactions and to favor the entry of bacteria into infection courts in the presence of sufficient moisture on the leaf surface.

The severity of BB was assessed on 10 plants (total of 20 leaves per experimental unit) in the center row 7, 14, 21, and 28 DAI (11). The resistance or susceptibility of each rice cultivar was evaluated by measuring lesion length (LL) in centimeters (14). Disease reactions were classified as resistant to susceptible according to LL, where 0–3 cm was rated as highly resistant (HR), 3.1–6 cm resistant (R), 6.1–9 cm moderately resistant (MR), and those exceeding 9 cm susceptible (S). Data obtained from individual plants of each experimental unit were averaged to obtain a mean score for statistical analysis. Disease severity means were used to calculate area under the disease progress curve (AUDPC) as described by Shaner and Finney (19). AUDPC was calculated for each rice cultivar. Data on LL and AUDPC were analyzed by analysis of variance (ANOVA) using the general linear model procedure of SAS (18), and means were compared by Duncan's multiple range tests ($P < 0.05$).

Greenhouse experiments. Ten strains of *X. o. oryzae* collected from different geographic regions of Nepal in 1991 were used in this study (Table 1). A single colony of each strain was subcultured in PSA slants, and their pathogenicity was verified on the rice cultivar Masuli. In the second experiment, the virulence of *X. o. oryzae* strains was assessed by inoculating five International Rice Research Institute (IRRI) differential rice cultivars (IR20, IR24, IR1545-339-2-2, Cas209, and DV85) possessing different genes for resistance and two Nepalese rice cultivars (Sabitri and Laxmi), as described by Adhikari et al (4).

Bacterial blight progression on IR24, Sabitri, and Laxmi was determined in the greenhouse experiments at 28 ± 2 C. Rice seedlings were raised in plastic trays in the greenhouse. After 20 days, three uniform seedlings of each rice cultivar were transplanted into a 15-cm clay pot. Fertilizer (N–P–K, 20–20–10 g/pot) was applied 1 wk after transplanting. Four bacterial strains, NXO301, NXO305, NXO326, and NXO327, were randomly selected and used in this study (Table 1). Inoculum of each strain was adjusted to 10^8 cfu/ml using a spectrophotometer. Each experimental unit consisted of three plants per pot, and there were four replications per treatment. Ten leaves per pot of each rice cultivar were clip inoculated 35 days after sowing and used for disease scoring as described above. Development of LL on each rice cultivar was measured (in centimeters) 3, 6, 9, 12, 15, and 18 DAI. The experiment was arranged in a split plot design with the rice cultivar as main plot and the bacterial strain as subplot (9). The experiment was performed twice, and means of each treatment for both experiments were averaged to obtain the overall mean.

To determine the effect of inoculum dose on the reaction of IR24, Sabitri, and Laxmi, they were assessed using the five *X. o. oryzae* strains NXO302, NXO309, NXO314, NXO316, and NXO319. These strains were selected because they were shown to be highly virulent on IRRI differential rice cultivars. The experimental procedures and conditions were the same as described above, except that five inoculum doses of 10^1, 10^3, 10^5, 10^7, and 10^9 cfu/ml were examined for each strain of *X. o. oryzae*. The experiment was conducted using a split-split plot design with the rice cultivar as main plot, the bacterial strain as subplot, and the inoculum dose as subsubplot (9). Each treatment was replicated three times. LLs on 10 leaves per treatment were measured 7 and 14 DAI. LL data were analyzed by ANOVA, and the sources and amount of variation were compared using an F test (18). To compare levels of doses with cultivars, least significant differences ($P < 0.05$) were calculated.

Table 1. *Xanthomonas oryzae* pv. *oryzae* strains from Nepal used in this study

Strain	Origin	Cultivar isolated from	Year of collection
NXO153	Jhapa	Unknown	1989
NXO301	Jhapa	Kanchhi Masuli	1991
NXO302	Jhapa	Masuli	1991
NXO305	Jhapa	Masuli	1991
NXO309	Jhapa	Unknown	1991
NXO314	Jhapa	Unknown	1991
NXO316	Jhapa	IRAT257	1991
NXO319	Kathmandu	Masuli	1991
NXO325	Jhapa	RP1017-76-1-5-2	1991
NXO326	Nawalparasi	Masuli	1991
NXO327	Chitwan	Masuli	1991

RESULTS

Field experiments. During the field screening, no rice cultivar was free of BB. Approximately 91% of the rice cultivars were susceptible to BB (>50% leaf diseased). Only 9% of the rice cultivars were moderately to highly resistant (20–50% leaf diseased). The ranks of rice cultivars for resistance toward BB were consistent as determined by either LL or AUDPC. Significant differences ($P < 0.05$) in LL and AUDPC among rice cultivars were observed. Field assessments of LL and AUDPC indicated that BR-34-13 had the highest levels of resistance to BB, followed by PAU-50-B-25, Laxmi, Sabitri, BW293-21, IR7167-33, Rodina, and Amonghaud (Table 2).

Greenhouse experiments. The majority of *X. o. oryzae* strains were virulent on IR24, IR20, IR1545, Cas209, and Sabitri (*data not shown*). Average LL caused by the 10 strains of *X. o. oryzae* on IR24 was 17.3 cm; on IR20, 12.2 cm; on IR1545, 12.1 cm; on Cas209, 13.2 cm; on DV85, 7.3 cm; on Sabitri, 8.5 cm; and on Laxmi, 2.7 cm.

The disease progress curves for IR24, Sabitri, and Laxmi inoculated with four representative strains of *X. o. oryzae* are shown in Figure 1. No BB development

Table 2. Lesion length (LL) and area under the disease progress curve (AUDPC) for nine rice cultivars inoculated with *Xanthomonas oryzae* pv. *oryzae* in the field[w], Rampur, Nepal, 1989

Cultivar	LL[x]	AUDPC[y]
BR-34-13	2.45 c[z]	410 c[z]
PAU-50-B-25	2.51 b	490 bc
Laxmi	2.89 b	546 bc
Sabitri	2.95 b	580 bc
BW293-21	2.97 b	516 bc
IR7167-33	3.20 b	570 bc
Rodina	3.61 b	671 bc
Amonghaud	5.65 b	710 b
IR24 (susceptible check)	19.36 a	1587 a

[w] Experiments conducted with 93 rice cultivars. Only nine are shown for comparison.

[x] Plants were clip inoculated 35 days after transplanting. Experimental unit consisted of 10 randomly selected plants in the center row. Lesion length on two leaves per plant (total of 20 leaves/experimental unit) was assessed 14 days after inoculation (DAI) where lesion length 0–3 cm = highly resistant (HR), 3.1–6 cm = resistant (R), 6.1–9 cm = moderately resistant (MR), and above 9 cm = susceptible (S). Data are means of two replications.

[y] AUDPC = $\Sigma_{i=1}^{n} [(Y_{i+1} + Y_i)/2] [T_{i+1} - T_i]$, where Y_i = bacterial blight severity (in percent) at the i^{th} observation, and n = total number of observations were four. Disease severity values were estimated between 21 and 28 DAI according to IRRI (11) and were used to calculate AUDPC for each rice cultivar. Data are means of two replications.

[z] Within a column, means followed by same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

was observed in any cultivar–strain combinations until 4 DAI. Water-soaked lesions were seen in the susceptible cultivar IR24 5 DAI. In all cultivar–strain combinations, the highest disease level was reached 18 DAI. Disease progress curves for each cultivar varied with the strains used. The development of BB symptoms was most rapid and severe on IR24. The disease progress curves for Sabitri were markedly steeper than those for IR24. Laxmi was resistant, as disease progress determined by LL was less than 3 cm even 18 DAI.

With inoculum concentrations of 10^5–10^9 cfu/ml, necrosis of the inoculation site on IR24 was observed about 4–5 DAI. LLs on IR24 enlarged more rapidly in all dose combinations than those on Sabitri and Laxmi. Higher inoculum doses resulted in longer LLs. Rice cultivars inoculated with 10^7–10^9 cfu/ml had greater differences in LL between resistant and susceptible than did those inoculated with 10^1–10^3 cfu/ml. Sabitri showed intermediate reaction. LLs on Laxmi were consistently shorter than those on IR24 and Sabitri (Fig. 2). Analysis of variance of the LL showed significant ($P < 0.05$) effects of strain, cultivar, dose, cultivar × strain, and cultivar × dose on disease expression 7 and 14 DAI (Table 3).

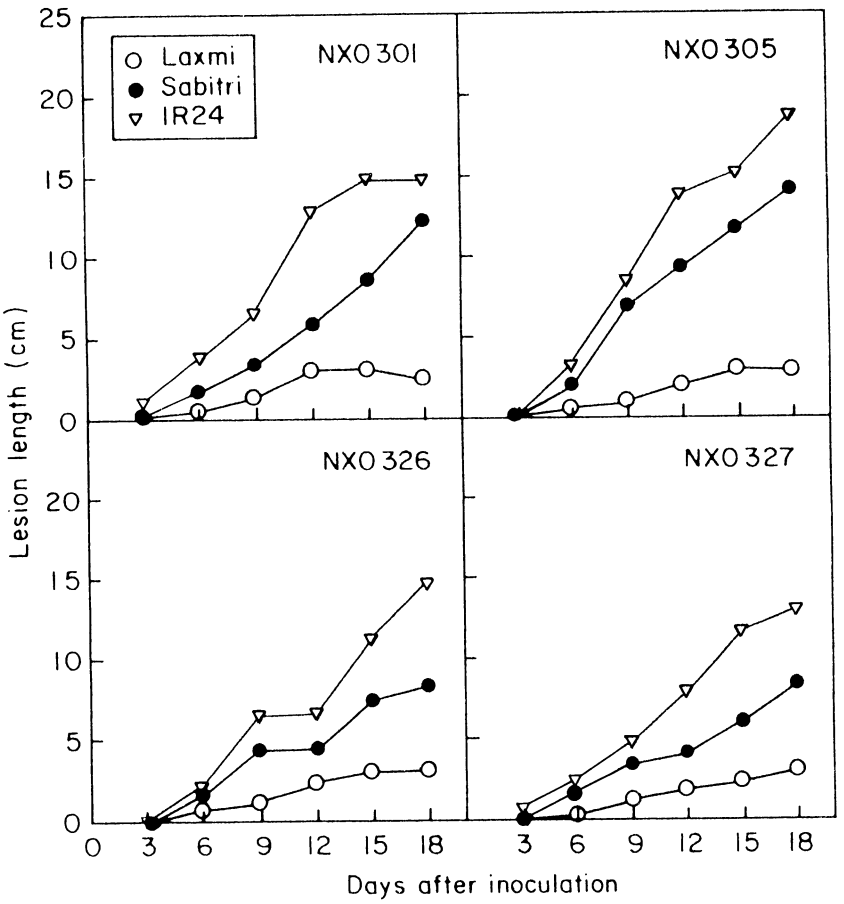

Fig. 1. Disease progress on leaves of three rice cultivars inoculated with four strains of *Xanthomonas oryzae* pv. *oryzae* in the greenhouse, Rampur, Nepal, 1991. Plants were inoculated with concentration of 10^8 cfu/ml 35 days after sowing. Lesion length (cm) measured from 10 leaves per treatment and plotted points are means of four replications.

Table 3. Analysis of variance for lesion length (cm) on three rice cultivars inoculated[y] with five strains of *Xanthomonas oryzae* pv. *oryzae* 7 and 14 days after inoculation (DAI) in the greenhouse, Rampur, Nepal, 1991

Source of variation	Degrees of freedom	Mean of square	
		7 DAI	14 DAI
Strain (I)	4	25.6**[z]	117.4**
Cultivar (V)	2	122.5**	953.3**
I × V	8	5.6*	14.9*
Dose (D)	4	26.5**	237.1**
V × D	8	4.4**	20.7**

[y]Plants were inoculated 35 days after sowing. Lesion lengths (cm) on 10 leaves were measured 7 and 14 DAI. Each treatment was replicated three times.
[z]* = F test significant at $P < 0.05$, ** = F test significant at $P < 0.01$.

DISCUSSION

Between 1970 and 1980, hundreds of rice cultivars were evaluated for resistance to BB at the National Rice Research Program, Parwanipur, Nepal. Most of the rice cultivars were susceptible, but a few had reactions low enough to be considered resistant. Our data confirm earlier findings (10,13) that rice cultivars with some resistance to *X. o. oryzae* can be identified in field nurseries. High levels of BB resistance were detected in breeding lines BR-34-13 and PAU-50-B-25, and Laxmi. Resistance to BB can be assessed by reductions in LL, disease incidence, disease severity, and AUDPC (2,14). For resistance described here, LL and AUDPC consistently reflected the reactions of rice cultivars under field and greenhouse conditions. Resistance of rice to *X. o. oryzae* was apparent as a reduction in LL per leaf. A reduction in LL could be attributed to a decrease in the rate of bacterial multiplication within the plant (6). Highly significant ($P < 0.05$) cultivar, strain, and cultivar × strain effects were found, indicating a differential host–pathogen interaction. This further confirms the existence of pathogenic specialization of *X. o. oryzae* in Nepal. Recently, nine pathogenic races of *X. o. oryzae* have been described in Nepal on the basis of differential reactions to rice cultivars (4).

Our results indicate that differences in resistance of rice to *X. o. oryzae* were related to disease development over time and inoculum doses. In susceptible reactions, disease development was faster and plant response exhibited typical BB disease symptoms at lower inoculum doses. In resistant reactions, disease progression was slow, and BB disease development was only observed at high inoculum doses. With each strain–dose combination, IR24 was most susceptible and Laxmi was resistant. Laxmi is a breeding line, IR2061-628-1-6-4-3 (IRRI accession IRGC 39290), which was introduced to Nepal as a commercial variety in 1975. The exact nature of inheritance of resistance in Laxmi to *X. o. oryzae* has not been studied. It would be worthwhile to perform a genetic analysis of resistance in this cultivar.

LL, AUDPC, disease development over time, and dose–response curves reflected differences in resistance of IR24, Sabitri, and Laxmi. Previous studies also suggested that an appropriate dose of inoculum and the test of a rice cultivar at the proper growth stage is important in evaluating rice cultivar resistance to BB (15,16). The strains of *X. o. oryzae* from Nepal are virulent on most of the resistance genes that are currently used in rice breeding programs

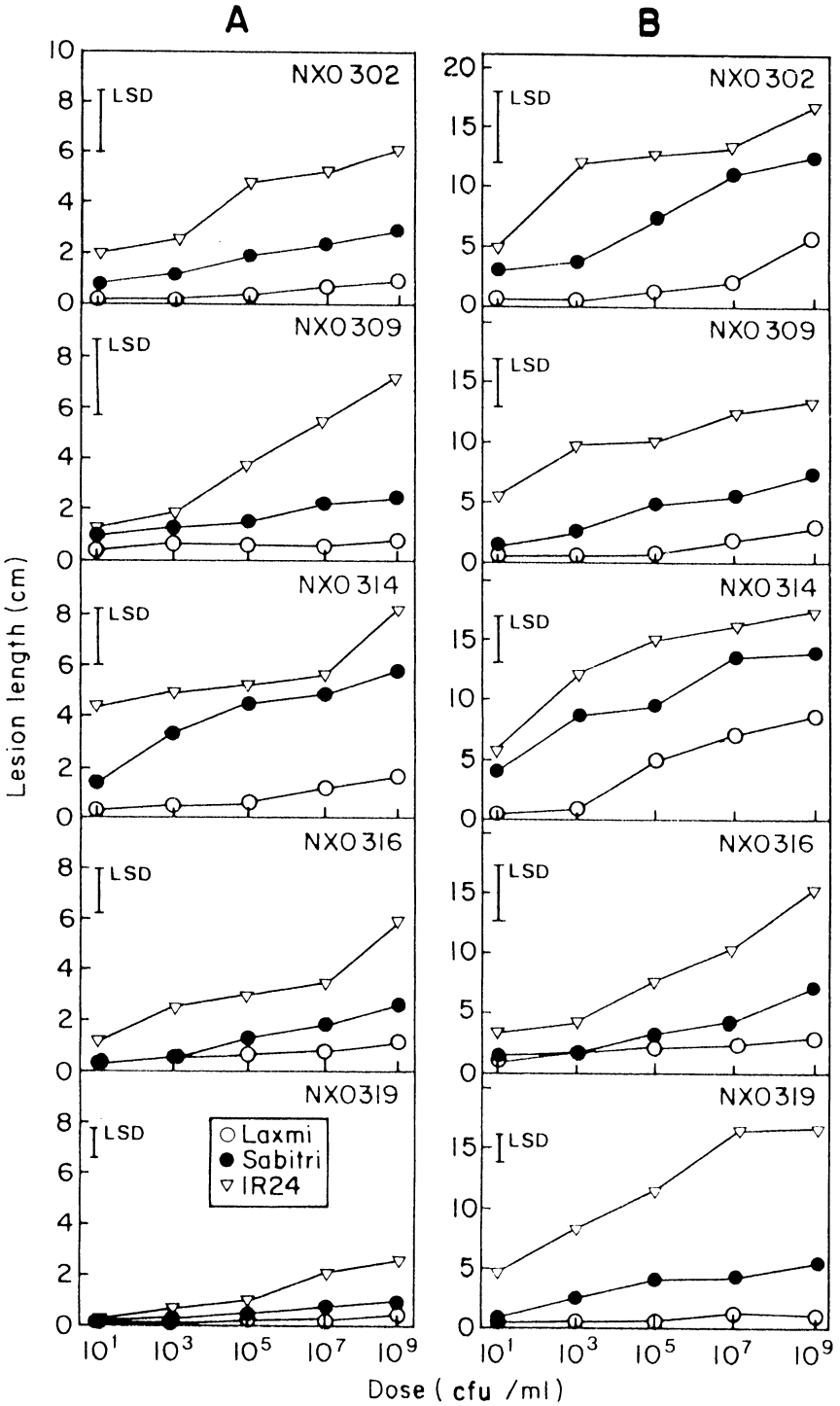

Fig. 2. Dose-response curves for three rice cultivars inoculated with five strains of *Xanthomonas oryzae* pv. *oryzae* in the greenhouse, Rampur, Nepal, 1991. Plants were inoculated with concentrations of 10^1, 10^3, 10^5, 10^7, and 10^9 cfu/ml 35 days after sowing. Lesion length (cm) on ten leaves per treatment was measured **(A)** 7 days after inoculation and **(B)** 14 days after inoculation. Vertical bars indicate least significant difference values to compare effects of inoculum doses on rice cultivars. Data are means of three replications.

at IRRI (4). Thus, identification of the resistant rice cultivar Laxmi, as described here, could provide a possible source of resistance to *X. o. oryzae* in Nepal.

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