PN-ABG-099

7.093

PATHOGENIC VARIABILITY AND BACTERIOCIN PRODUCTION PROJECT

FINAL REPORT
1992

USAID OFFICE OF THE SCIENCE ADVISOR
(Grant No. 367-5542-G-00-7020-00)

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(July 1, 1987 - March 31, 1992)

Submitted to the Office of the Science Advisor
U. S. Agency for International Development

PATHOGENIC VARIABILITY OF AND BACTERIOCIN PRODUCTION BY *XANTHOMONAS ORYZAE* PV. *ORYZAE* IN NEPAL

Principal Investigator: Dr. Tika B. Adhikari

Grantee Institution: Department of Plant Pathology
Institute of Agriculture and Animal Science
(IAAS), Central Campus, Rampur, Chitwan,
Nepal

Collaborator: Mr. Sundar M. Shrestha

Institution: IAAS, Central Campus, Rampur, Chitwan, Nepal

Collaborator: Dr. G. L. Shrestha

Institution: National Rice Research Program, Parwanipur, Nepal

Proposal No: 7.093

Project No.: 936-5542

Grant Number: 367-5542-G=00-7020-00

AID Grant Project Officer: Allen Eisenberg and
Mrs. Anjali S. Pradhan

Project Duration: July 1987 to March 1992

Total Grant: US$ 130,000

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Executive Summary

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most destructive diseases of rice in Asia. In Nepal, a severe outbreak of BB occurred in 1979. Many commercially grown rice varieties were susceptible and affected by the disease. This epidemic demonstrates the potential danger from BB in Nepal. Chemical control for this disease is neither feasible nor economical. The deployment of resistant cultivars appears to be the most effective and economical method of controlling the disease. For many years, pathogenic specialization of *X. o.* pv. *oryzae* for rice varieties have not been documented in Nepal. At IAAS in the past 5 years, attempts have been made to examine current situation of the disease, pathogenic specialization, host resistance, bacteriological characteristic and bacteriocin production.

Results of the disease surveys showed that BB was widely distributed in Tarai, Hills and Valleys. The disease was especially severe in Jhapa, Morang, Bara, Parsa and Kanchanpur. Over 1000 local and exotic rice germplasm were evaluated both in greenhouse and fields. Among them IR54 and Laxmi were found resistant. IRRI differentials (IR8, IR20, CAS209, IR1545-339-2-2 and DV85) and three local rices (Himali, Sabitri and Laxmi) were used as panel of differential system and nine pathogenic races were identified. Significant differences on rice cultivars, bacterial strains and cultivar-strain interactions were demonstrated, confirming pathogenic specialization of *X. o.* pv. *oryzae* in Nepal. Analysis of phenotypic features of the 53 strains using unweighed pair-group method revealed that all strains were associated at 82.40% similarity coefficient indicating homogeneous population. Forty-four percent of the strains produced bacteriocinlike substances in peptone sucrose agar medium and six bacteriocin types were distinguished. No correlations between pathogenic races and phenotypic characters or bacteriocin types were observed.

BB caused yield loss of 26% in Nepal. Analysis of epidemiologic parameters such as disease incidence (DI) disease severity (DS) area under the disease progress curves (AUDPC) and apparent infection rates (r) showed that resistant cultivars have significantly lower rates of these parameters than the susceptible cultivar IR24. Additionally, both disease progression over time and lesion expansion were drastically reduced on Laxmi as compared to Sabitri and IR24.

This project was undertaken in collaboration with IRRI, Philippines. Resistant donors obtained from this project are useful to rice scientists working on gene transfer and various national rice improvement programs all over the world. Findings of this project could fulfill the needs of Nepalese farmers and scientists to resolve this problem by developing and including resistant cultivars in an integrated disease management.

Research Objectives

For many years we have not known how variable *X. o.* pv. *oryzae* is in Nepal. The screening of rice cultivars resistant to BB was initiated by National Rice Research Program in 1972. The lack of detailed information on the variability of the *X. o.* pv. *oryzae* has hampered progress. It is therefore important to set up a common base to define the relationship between the resistance of rice cultivars and the virulence of the bacterial strains. The existence of the pathogenic races of *X. o.* pv. *oryzae* were reported on the basis of reactions on the differential varieties. Six and seven pathogenic races of *X. o.* pv. *oryzae* were identified in the Philippines (Mew, 1987; 1989) and Japan (Noda, 1990), respectively.

On the other hand, bacteriocin producer strains have been successfully applied for epidemiology, ecology and controlling the bacterial plant diseases (Echandi, 1976; Kerr, 1980; Vidaver, 1976). The use of a non-pathogenic bacteriocin producer strain 84 of *Agrobacterium radiobacter* is an example of biological control (Kerr, 1980). The knowledge of understanding of the pathogen virulence using bacteriocin typing can provide a basis for determining difference in genotypes of the pathogen. Therefore this project was conducted with the following objectives;

1. To screen Nepalese rice germplasm for obtaining suitable donors in breeding resistance to BB.

2. To investigate pathogenic specialization *X. o.* pv. *oryzae* in Nepal.

3. To develop bacteriocin typing scheme and find out bacteriocin producer strains.

4. To analyze effects of host resistance and environment on BB development.

This project is the first attempt to generate data on the disease in Nepal. So far, there was no information on pathogenic specialization *X. o.* pv. *oryzae*. Our attempt to come up with pathogenic variability and bacteriocin production by *X. o.* pv. *oryzae* in Nepal are quite innovative aspects.

This grant was made to the IAAS, Rampur, Nepal. National Rice Research Program, Parwanipur (Collaborating Institution) has been supported this project by providing greenhouse facilities, rice germplasm and literature related to rice. Likewise, technical advisory role was provided the IRRI. Dr. T. W. Mew, Plant Pathologist and Head, Department of Plant Pathology, IRRI served as consultant in this project. He visited project site at Rampur in April, 1990. Mr. Sundar M. Shrestha (collaborator) visited at IRRI and took short-term training on BB. Winrock International for Agricultural Development, USA assisted in administering the grant and procuring the equipment.

MATERIALS AND METHODS

Distribution and Severity of Bacterial Blight

Systematic disease surveys were conducted in 32 major rice growing districts of Nepal during 1987-1989. In Terai regions, the surveys were conducted along the east-west highway. In hills and valleys, sites were near the river basin. Bacterial blight severity was assessed by examining the percent leaf area affected of 20-25 plants within 1 m^2 area from the center of each site. Two-three diseased leaves were collected from each site (Fig. 1). The specimens were placed in paper bags and labeled with date of collection, name of cultivar and location and brought to the Department of Plant Pathology at IAAS, Rampur, for isolation of the pathogen.

Isolation and Purification

A 2 mm tissue was cut from advancing lesion of a typical bacterial blight symptom. The section was surface sterilized with 70% ethyl alcohol for 10 seconds and was rinsed in sterile distilled water. The leaf tissue was allowed to ooze out into isolation disc for 5 min. A loopful suspension was streaked on peptone sucrose agar (PSA) plates and were incubated at 28 C. After 3 days, a single colony was selected, streaked on PSA slants and again incubated at 28 C. Duplicate test tubes of each strain were prepared and stored at 4 C.

Pathogenicity Test

Pathogenicity of each strain was tested on 35-day old rice cultivar Masuli. Each strain was confirmed to be *X. o.* pv. *oryzae* and pure cultures were made and preserved by freezing in 5% skimmed milk for further studies.

Screening of Rice Germplasm for Resistance

Preliminary screening of large numbers of genetic materials collected from various sources have been carried out in the field during the 1988 season. Over 1000 local and exotic germplasm were first evaluated using single isolate of *X. o.* pv. *oryzae* in Parwanipur and Rampur. The seedlings were transplanted in the experimental plots 28 days after sowing. Plants were clip-inoculated at maximum tillering stage. The disease assessments were made 14 days after inoculation by measuring lesion length in centimeters (cm). Germplasm showing resistance in the field were reevaluated in subsequent years (1989 and 1991). The resistant germplasm were also tested in the greenhouse using representative isolates of *X. o.* pv. *oryzae* from all parts of Nepal.

Pathogenic Variability of *X. o.* pv. *oryzae* in Nepal

<u>Rice cultivars.</u> Eight rice cultivars (five from IRRI, and three from Nepal) were used in this study. Among IRRI cultivars, IR8

Fig. 1. Collection sites of *Xanthomonas oryzae* pv. *oryzae* in Nepal, 1987-1989.

Table 1. Identity of strains of *X. o.* pv. *oryzae* collected in Nepal.

Strain	Location	Variety source	Date of collection
1. NXO101	Jhapa	Masuli	Sept., 1987
2. NXO117	Dang	Simjira	Oct., 1987
3. NXO142	Bara	Dedawa	Oct., 1989
4. NXO146	Bara	Unknown	Oct., 1989
5. NXO147	Parsa	Unknown	Oct., 1989
6. NXO149	Jhapa	B446	Aug., 1989
7. NXO151	Jhapa	Unknown	Aug., 1989
8. NXO152	Jhapa	Unknown	Aug., 1989
9. NXO153	Jhapa	Local	Aug., 1989
10. NXO156	Bara	Unknown	Aug., 1989
11. NXO157	Bara	Unknown	Aug., 1989
12. NXO159	Bara	Unknown	Aug., 1989
13. NXO160	Parsa	Unknown	Aug., 1989
14. NXO161	Parsa	Unknown	Aug., 1989
15. NXO163	Bara	Unknown	Aug., 1989
16. NXO171	Parsa	Farm-45	Aug., 1989
17. NXO174	Parsa	Unknown	Aug., 1989
18. NXO175	Bara	Unknown	Aug., 1989
19. NXO176	Bara	Local	Aug., 1989
20. NXO180	Rautahat	Unknown	Aug., 1989
21. NXO181	Rautahat	Unknown	Aug., 1989
22. NXO188	Gorkha	Himali	Sept., 1989
23. NXO190	Jhapa	Local	Sept., 1989
24. NXO191	Jhapa	Unknown	Sept., 1989
25. NXO194	Jhapa	Unknown	Sept., 1989
26. NXO195	Dang	Bindeshwori	Sept., 1989
27. NXO196	Dang	Bindeshwori	Sept., 1989
28. NXO197	Dang	Unknown	Sept., 1989
29. NXO198	Dang	Unknown	Sept., 1989
30. NXO199	Dang	Unknown	Sept., 1989
31. NXO200	Bhairahawa	Unknown	Sept., 1989
32. NXO201	Bhairahawa	Local	Sept., 1989
33. NXO202	Dang	Bindeshwori	Sept., 1989
34. NXO205	Dang	Local	Sept., 1989
35. NXO207	Butwal	Unknown	Sept., 1989
36. NXO208	Dang	Bindeshwori	Sept., 1989
37. NXO209	Bhairahawa	Kalomasin	Sept., 1989
38. NXO210	Bhairahawa	Himali	Sept., 1989
39. NXO211	Bhairahawa	Unknown	Sept., 1989
40. NXO212	Bhairahawa	Unknown	Sept., 1989
41. NXO213	Bhairahawa	Unknown	Sept., 1989
42. NXO214	Bhairahawa	Local	Sept., 1989
43. NXO215	Butwal	IR20	Sept., 1989
44. NXO216	Bhairahawa	Kabara	Sept., 1989
45. NXO228	Syanja	Karneli	Oct., 1989
46. NXO235	Kaski	Local	Oct., 1989

Contd...

Table 1. Contd...

Strain	Location	Variety source	Date of collection
47. NXO236	Palpa	Unknown	Oct., 1989
48. NXO237	Syanja	Battisara	Oct., 1989
49. NXO239	Syanja	Unknown	Oct., 1989
50. NXO240	Kaski	Gurdi	Oct., 1989
51. NXO244	Palpa	Masuli	Oct., 1989
52. NXO245	Palpa	Ampjuhtte	Oct., 1989
53. NXO253	Tanahua	Kalnakhari	Oct., 1989
54. NXO256	Lamjung	Sali	Oct., 1989
55. NXO257	Tanahua	Masuli	Nov., 1989
56. NXO259	Tanahua	Battisara	Oct., 1989
57. NXO261	Jhapa	Masuli	Oct., 1989
58. NXO262	Jhapa	Laxmi	Oct., 1989
59. NXO263	Jhapa	Janaki	Oct., 1989
60. NXO264	Jhapa	Unknown	Oct., 1989
61. NXO270	Banke	Salidhan	Nov., 1989
62. NXO274	Kailali	Alka	Nov., 1989
63. NXO275	Bardia	IR64	Nov., 1989
64. NXO282	Banke	Unknown	Nov., 1989
65. NXO269	Banke	Tilka	Nov., 1989
66. NXO301	Jhapa	Kanchhi Masuli	Sept, 1991
67. NXO302	Jhapa	Masuli	Sept. 1991
68. NXO305	Jhapa	Masuli	Sept. 1991
69. NXO319	Kathmandu	Masuli	Sept. 1991
70. NXO327	Chitwan	Masuli	Sept. 1991

has dominant gene, Xa-11 for resistance to BB. IR20 is conditioned by a single dominant gene, Xa-4 and IR1545-339-2-2 (hereafter IR1545) has a recessive gene, Xa-5. DV85 has one recessive gene and one dominant gene, xa-5 and Xa-7. CAS209 has a single dominant gene, Xa-10. Three Nepalese rice cultivars, Himali, Laxmi and Sabitri were released by the National Rice Improvement Program, Parwanipur in 1977, 1975 and 19075, respectively.

Seeds of different rice cultivars were sown in plastic trays. At 20 days after sowing, uniform seedlings were transplanted to clay pots each containing three seedlings. Fertilizers were applied as basal dose at the rate of 120:60:50 kg of NPK/ha.

Bacterial strains. Thirty-five strains were randomly chosen and tested for their virulence on the 8 rice cultivars. The strains were revived on PSA slants and used for inoculation (Table 1).

Inoculum preparation and inoculation. The strains were subcultured on PSA slants and incubated for 48 hr at 28 C. Inoculum was prepared by suspending bacterial mass in 10 ml sterile distilled water. The actual number of colony forming unit/ml (cfu/ml) in the suspension were counted by plating 0.1 ml suspension of each strain into PSA plates. Two young fully expanded rice leaves were clip-inoculated with each strain 2-3 cm from the tip at 35 days after sowing (Kauffman et al, 1973).

Experimental design and disease assessment. The experiment was designed on split-plot with the rice cultivars as main plot and the bacterial strains as subplot (Gomez and Gomez, 1984). The experiment was replicated twice in the greenhouse. The virulence of each strain was characterized by measuring the lesion length in centimeter (cm) from the tip of leaf at 14 days after inoculation. The strain causing >3 cm lesion on a particular rice cultivar was considered as "virulent strain". Analyses of variance were performed using SAS program to determine significant interaction effects between the rice cultivars and the bacterial strains.

Infectivity Titration Test

Cultures of the strains grown on PSA slants were suspended in 5 ml of sterile distilled water, and the suspensions were bulked and shaken vigorously for 5 min. Dilutions of the bulked suspension were prepared and the concentrations of viable cells were determined by dilution plate counts. A series of dilutions from the initial suspension was prepared (10^1, 10^3, 10^5, 10^7 and 10^9 cells/ml) and plated for each strain to determine the actual number of colony forming units (cfu/ml) on PSA medium. Three rice cultivars IR24, Sabitri and Laxmi were clip-inoculated at 35 days after sowing. The order of inoculation was done from the lowest inoculum concentration of each strain to the highest. To test level of resistance of rice cultivars against different strains, a split plot design was used with isolates as the main plot and cultivars as subplots. All experiments had two replications. For each replication, at least 30 leaves were inoculated. Lesion

development on individual leaves was measured in cm at 7 and 14 days after inoculation.

In separate experiment, the three rice cultivars IR24, Sabitri and Laxmi were exposed to high inoculum densities (10^9 cells/ml) and lesion development and rate of lesion expansion were monitored every 3 days after inoculation.

Bacteriological Characteristics of *X. o.* pv. *oryzae*

The fifty-three cultures grown on PSA slants and incubated at 28-30 C for 48 hr were used throughout the experiment (Table 1). All contaminated tubes were ascertained by repeated testing. Uninoculated media were used as control. Unless otherwise specified, all methods described by Dye (1962) were followed.

The following morphological, cultural, biochemical and physiological tests were performed. These were Gram staining; effect of temperature; effect of NaCl concentrations; effect of glucose concentrations; metabolism of glucose, levan production, catalase test, gelatin liquefaction, action on milk, indole production, nitrate reduction, hydrogen sulfide (H_2S) production, starch hydrolysis, hydrolysis of Tween 20 and 80, action on egg yolk, acetoin production, reduction of tetrazolium chloride (2, 3, 5-triphenyl-tetrazolium chloride), growth in the presence of dyes, acid production from carbohydrates and sensitivity to antibiotics.

Numerical analysis of phenotypic features

Results obtained from the standard physiological and biochemical tests were used for the computer-assisted numerical analysis. All tests were scored as either positive (coded 2) or negative (coded 1). Missing and doubtful results were coded as 0 (no comparison). The reference culture were used only in few diagnostic tests and were not included in the calculation. Both positive and negative matches were determined in the calculation by unweighed average pair-group method (Sneath and Sokal, 1973). Similarity coefficients (S_{sm}) were calculated and data were transposed to a dendrogram using a Clustan program (Wishart, 1987).

Bacteriocin Production

<u>Bacterial strains</u>. Forty-five strains were randomly selected and used for bacteriocin production (Table 1).

<u>Media</u>. Media were peptone sucrose agar (PSA), 0.7% water agar (WA) and 0.1% peptone water.

<u>Bacteriocin production and detection</u>. Bacteriocin was detected by the modified methods of Echandi (1976). The producer strains were transferred to PSA slants and incubated for 48 hr at 28 C. The cultures were transferred into 5 ml skimmed milk shaken for 1 min on rotary shaker. Half ml (0.5 ml) suspension of each strain was

placed in a well of ELISA plate with micropipette. The strains were transferred from these plates with a multipoint replicator with 4 mm diameter rods to PSA plates previously dried for 3 days. After 48 hr, the colonies were killed by inverting the plates over 2 ml of chloroform until all chloroform had evaporated (about 60 min). The plates, with the lid off, were then placed in a transfer chamber with continuous air circulation for another 60 min. Indicator strains were selected by reciprocal tests. Presence of inhibition zones around killed bacterial colonies after 24-48 hr indicated bacteriocin production.

Field Experiments

Selection of genotypes. "Genotypes" in this study refer to rice characteristics that are genetically fixed. Six rice genotypes developed at IRRI were selected. IR24 which has no major gene for resistance to the Philippines races of *X. o.* pv. *oryzae*, was used as susceptible check. IR36, IR54, IR60 and IR64 have Xa-4 genes for resistance. IR1695 has Xa-3 gene and an adult plant resistant line resulting from cross made between BPI76 BPI*/Zenith. The following were the reasons for selecting these genotypes.

1. All genotypes are full-season, high yielding and widely grown under tropical environment (eg. Philippines), but little is known about their performance in sub-tropical environment like in Nepal.

2. Despite race-specific resistance Xa-4 gene and compatible race 2, some of these genotypes slowed down the disease epidemics in the farmers' fields in the Philippines. Moreover, these genotypes are being used as sources of resistance in some Asian countries but these have not been evaluated for resistance to BB in Nepal.

Rice seeds were treated at IRRI with 0.1% Benlate [methyl 1-(butylcarbamoyl) benzimidazol-1-ylcarbamate)] and with 0.3% Dithane M-45 [(1, 2-ethanediylbis) carbamodithioatol) (2--) manganese mixture] with [1, 2-ethanediylbis) (carbamodithioatol) (2--) zinc] and brought to Nepal.

Selection of environment. The term "environment" herein is defined as the surrounding conditions such as climatic, edaphic and biotic factors that act upon the plant community. The reasons for selecting these environments were as follows:

1. Only research stations were chosen for safety and management reasons.

2. Both Rampur and Kankai are in major rice growing districts and these are ecologically different. Both locations are BB-prone areas.

Thus, the environments in this study differed from one another in terms of elevation, latitude, longitude, temperature, relative humidity, soil types, agronomic practices and inputs

used (Table 2). The rice genotypes were planted to all experimental environments.

Table 2. Characterization of sites in Nepal where bacterial experiments were conducted.

Character	Site	
	Rampur	Kankai
1. Elevation	256 M	100 M
2. Latitude	27°37' N	26° N
3. Longitude	84°25' E	87°E
4. Organic matter	2.68	1.61
5. Total nitrogen (%)	0.13	0.07
6. Total phosphorus(kg/ha)	53.60	123.60
7. Total potash (kg/ha)	112.80	139.70
8. Soil pH	Acidic	Acidic
9. Soil texture		
Sand (%)	65.60	39.90
Silt (%)	24.40	39.40
Clay (%)	8.70	20.70
10.Soil type	Sandy loam	sandy loam
11.Cropping pattern	Rice-fallow	Rice-rice

Selection of the pathogen. The inoculum was prepared in small bottles containing 100 ml PSA medium. After 3 days of incubation at room temperature, bacterial cells were collected by suspending 100 ml sterile distilled water per bottle. The inoculum was filtered through double layer cheese-cloth and collected in conical flasks. Twenty-day of seedlings were carefully uprooted and soil particles from the roots were washed with tap water. The seedlings were spray-inoculated with bacterial suspension at 2.1 x 10^8 cfu/ml and transplanted to the fields following day.

Crop management. Soil samples were collected from each location. The chemical and physical characteristics of the soils were analyzed in the Division of Soil Science and Agricultural Chemistry at Khumaltar in Nepal. These characteristics of each location are presented in Table 2.

Same crop management and cultural practices were carefully applied in both environments. Land preparation consisted of two plowing by a pair of oxen in mid June, followed by one puddling and leveling just before transplanting. Transplanting dates were July 25 and 27, 1989 at Kankai and at Rampur, respectively. Two-hand-weedings were done. *Echinochloa crusgalli* and *Mononchoria* sp. were the most common weed species. Insect pests were generally not seen as serious problem. The damage by stem borer *Chilo* sp and rice bug (*Leptocorisa* sp.) were noticed in some plots at Rampur and at Kankai, respectively. These insects were controlled by 2 sprayings of 0.2% Dimecron (2-chloro-2-diethylcarbamoyl-1-methyl vinyldimethyl phosphate). No other serious diseases were observed. At Rampur, zinc deficiency was noticed after trans-

planting and corrected with zinc sulfate at the rate of 5 kg/ha. Ammonium sulfate was applied at the rate of 120 kg/ha in two equal split applications. One-half was basally broadcasted and incorporated thoroughly into the mud without standing water and the remaining half was applied at the maximum tillering stage.

Measurement of yield components. Twenty-five to 30 panicles were randomly selected to determine yield components of the genotypes. Recorded yield components were panicle length (cm), total number of filled spikelets per panicle (no.), total number of unfilled spikelets per panicle (no.) and 100-grain weight (g). Yield per m^2 area was converted into metric tons/ha at 14% moisture content. Yield loss due to bacterial blight in each genotype was calculated as:

$$\text{Yield loss (\%)} = \frac{\text{(Yield of healthy)} - \text{(Yield of diseased)}}{\text{Yield of healthy}} \times 100$$

Design of experiments. Each experimental unit consisted of a single rice genotype planted to a plot at 20 x 30 cm spacing with 2 seedlings per hill. The individual plot size was 4 x 4 m. The treatments were replicated 3-4 times depending on the trial. The plots were spaced 1 m between the blocks and 75 cm within the plots. Experiment was designed in split-plot with the inoculated plots as mainplot and price genotypes as subplot. The plots inoculated with distilled water served as control.

Environmental factors. Meteorological factors such as air temperature (C), relative humidity(%) and rainfall (mm) were continuously monitored for each location. Data were obtained from the weather station of each test location. Hygrothermographs served as partial backup units.

Quantification of the disease progression. At the center of each plot, 1 m^2 nondestructive area was separated. Twenty-five to 30 hills were randomly tagged outside this area before disease scoring. The disease was sampled in W-shaped pattern according to block sampling (Gomez and Gomez, 1984). The disease progression in each genotype was quantified using the following epidemiologic parameters.

$$\text{I. Disease incidence (DI)} = \frac{\text{No. of infected leaves/hill}}{\text{Total number of leaves/hill}} \times 100$$

II. Disease severity (DS), expressed as percent leaf area infected/hill. At least 10 leaves/hill were sampled for disease severity.

III. Area under the disease progress curve (AUDPC) was calculated according to Shaner and Finney (1979).

$$AUDPC = \sum_{i-1}^{n} [(Y_{i+1} + Y_i)/2] [(T_{i+1} - T_i)]$$

Y_i = Disease severity (%) at ith observation
T_i = Time (days) of the ith observation
n = Total number of observation

VI. Apparent infection rate (r) was calculated using logistic model of Vanderplank (1963).

$$r = \frac{2.3}{T_2 - T_1} \left(\text{Log } 10 \frac{X_2}{1 - X_2} - \text{Log } 10 \frac{X_1}{1 - X_1}\right)$$

T = Time (days)
X = Amount of the disease

Visual ratings were made at 10 days interval between 30-90 days after transplanting to determine disease progression. Six scoring were made from each environment.

Disease progress curves (BB severity versus time) were plotted for each genotype. A computer software program was written to calculate AUDPC and r from disease severity values for each genotype. The r expressed in units per day, was calculated by first converting disease severity values to a proportion on a scale of 0-1 and then transforming the data into logits, ln [Y/(1-Y)], where Y represents the proportion of infected tissue and 1-Y represents the proportion of healthy tissue remaining in the plant. In addition, AUDPC and r values for the corresponding time period were likewise pooled for calculations of total AUDPC and r for each environment.

<u>Data analysis</u>. Analysis of variance was used to determine significant differences ($P < 0.05$ or 0.01) in DI, DS, AUDPC, and r. Duncan's multiple range test (DMRT) was used to determine significant differences among genotypes ($P < 0.05$). Regression analysis was used to determine the relationships between DI and DS and the goodness of fit was evaluated by examining coefficient of determination (R^2). Correlation coefficients were calculated to test the relationships among epidemiologic parameters DI, DS, AUDPC, r and yield loss. The relationships between percent yield reduction and AUDPC were analyzed using regression analysis.

Grain yield data were separately analyzed to determine genotypic differences. A partitioned analysis of variance was run to detect genotype-environment interaction. The combined analyses of variance on environmental factors, minimum and maximum temperatures (c), relative humidity (%) and rainfall (mm) and AUDPC were computed using general linear models of SAS package. Simple correlations between environmental factors and AUDPC for each

genotype were determined and the probability of obtaining significant correlation values were tested at 1% and 5% level of significance.

RESULTS AND DISCUSSION

Pathogenic Variability of *X. o.* pv. *oryzae* in Nepal

Distribution and disease severity. Bacterial blight was prevalent throughout the Terai, Valleys and Hills (Fig. 2). Disease severity was high (> 50%) in Jhapa, Morang, Bara, Parsa and Kanchanpur and low (< 10%) in Gorkha and Nuwakot districts. The average disease severity ranged from 30-40% in Terai and 15-20% in Hills and Valleys. The occurrence of the disease was earlier in the Eastern Terai than other parts of the country. Masuli, a high yielding variety was susceptible in 23 surveyed sites. Other improved varieties, CH-45, Sarju-49 and Bindeshwori were also highly susceptible to the disease. Laxmi and Sabitri were moderately resistant. Among the IR varieties, IR24 was susceptible in two surveyed sites.

Virulence analysis on IRRI differential and local cultivars. All strains of *X. o.* pv. *oryzae* were pathogenic to all rice cultivars used, but differences in virulence, as measured by the lesion length was apparent (Fig.3). Significant difference on cultivars, strains, and cultivar-strain interaction were demonstrated. Nineteen of the 35 strains were virulent to all test cultivars (Appendix 1). Length of lesions ranged from 2.07-19.66 cm on IR8, 4.80-19.08 cm on IR20, 3.25-19.33 cm on IR1545, 0.08-22.25 cm on CAS209, 0.27-15.99 cm on DV85, 0.05-20.24 cm on Himali, 0.05-14.75 cm on Laxmi and 0.26-14.66 cm on Sabitri. The strains collected from Terai were predominant, however, no associations between virulence and location or rice cultivar were observed. Strains, NXO207 and NXO147 collected from Butwal and Parsa were the most and least virulent on the test cultivars, respectively. Rice cultivars also differed significantly in their degree of resistance of *X. o.* pv. *oryzae*, although none were immune. Three Nepalese cultivars, Laxmi, Sabitri and Himali showed differential reactions to the 18 strains. DV85 was resistant to the 6 strains. CAS209 was resistant to only 1 strain, IR8 was resistant to the 2 strains. IR20 and IR1545 were susceptible to all strains (Fig. 4).

Characterization of races. Only three IRRI differential cultivars, IR8, DV85 and CAS209 could differentiate these strains into 4 pathogenic races. Using these three IRRI differentials and Nepalese rice cultivars, the 35 strains were classified into nine pathogenic races (Fig. 4 and 5). Race 1 consisted of strains, NXO117, NXO211, NXO188, NXO153, NXO171, NXO202, NXO213, NXO175, NXO212, NXO213, NXO175, NXO212, NXO208, NXO201, NXO200, NXO191, NXO216, NXO207, NXO157, NXO157, NXO152, NXO214, and NXO1215. These strains were virulent on all rice cultivars. Race 2 was composed of strains, NXO190, NXO149, NXO180 and NXO147. These strains were virulent on all rice cultivars except Sabitri. Race 3 strains, NXO156, NXO142, and NXO209, were only avirulent or

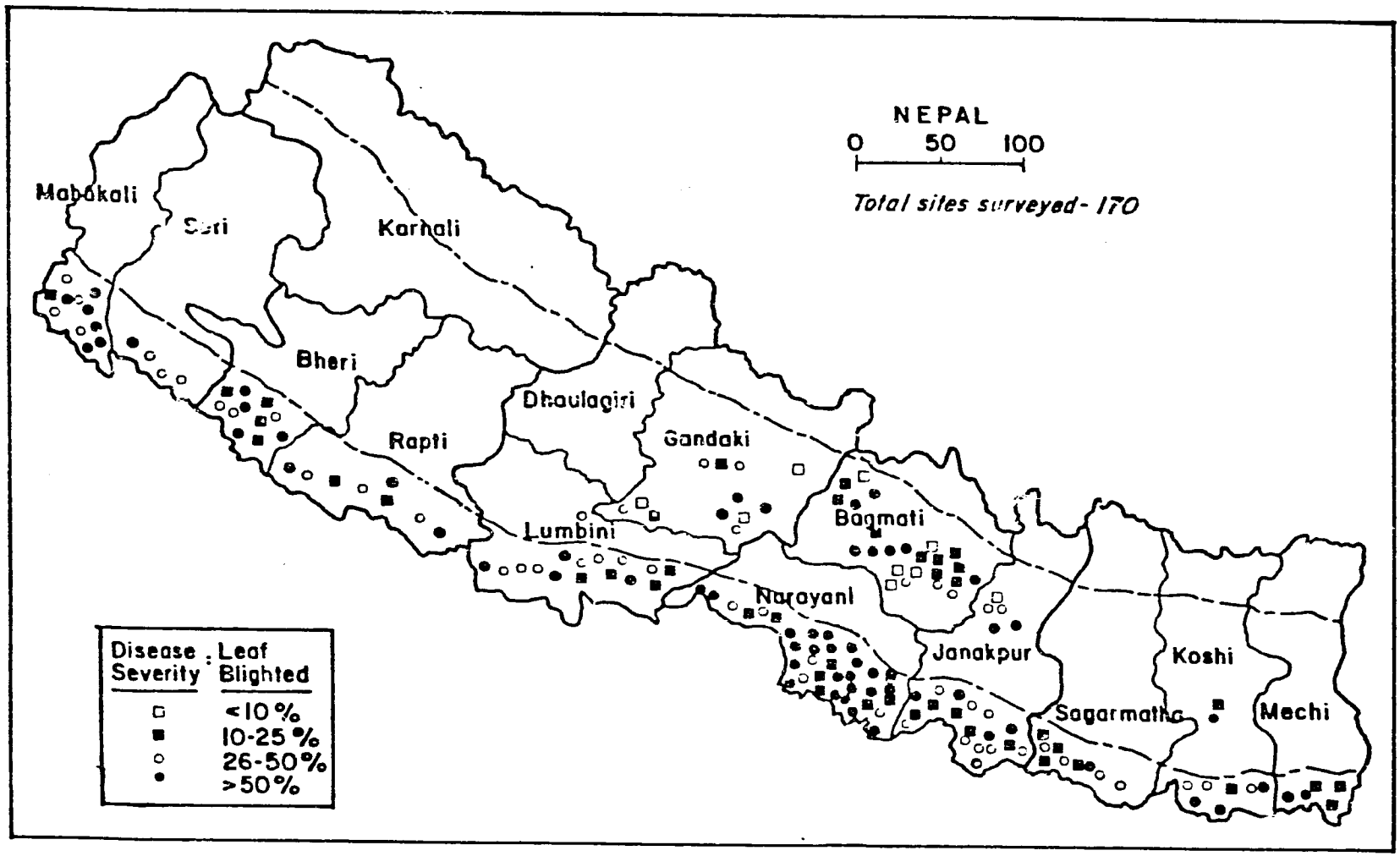


Fig. 2. Distribution and severity of bacterial blight in Nepal.

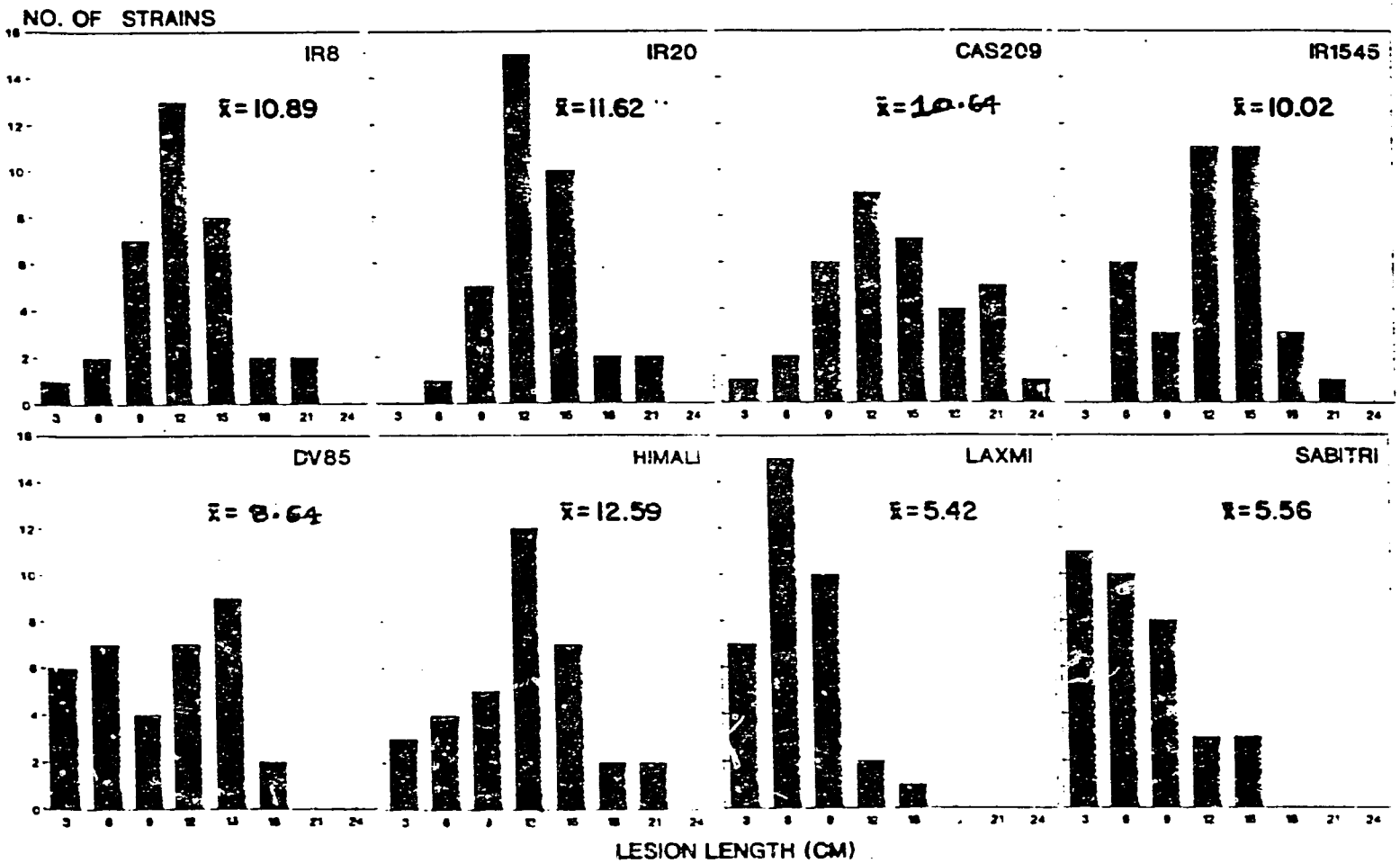


Fig. 3. Frequency distribution of lesion length caused by the 35 strains of *X. o.* pv. *oryzae* on IRRI differentials and local rice cultivars, Nepal, 1989. Lesion lengths of each strain was measured at 35 days after sowing.

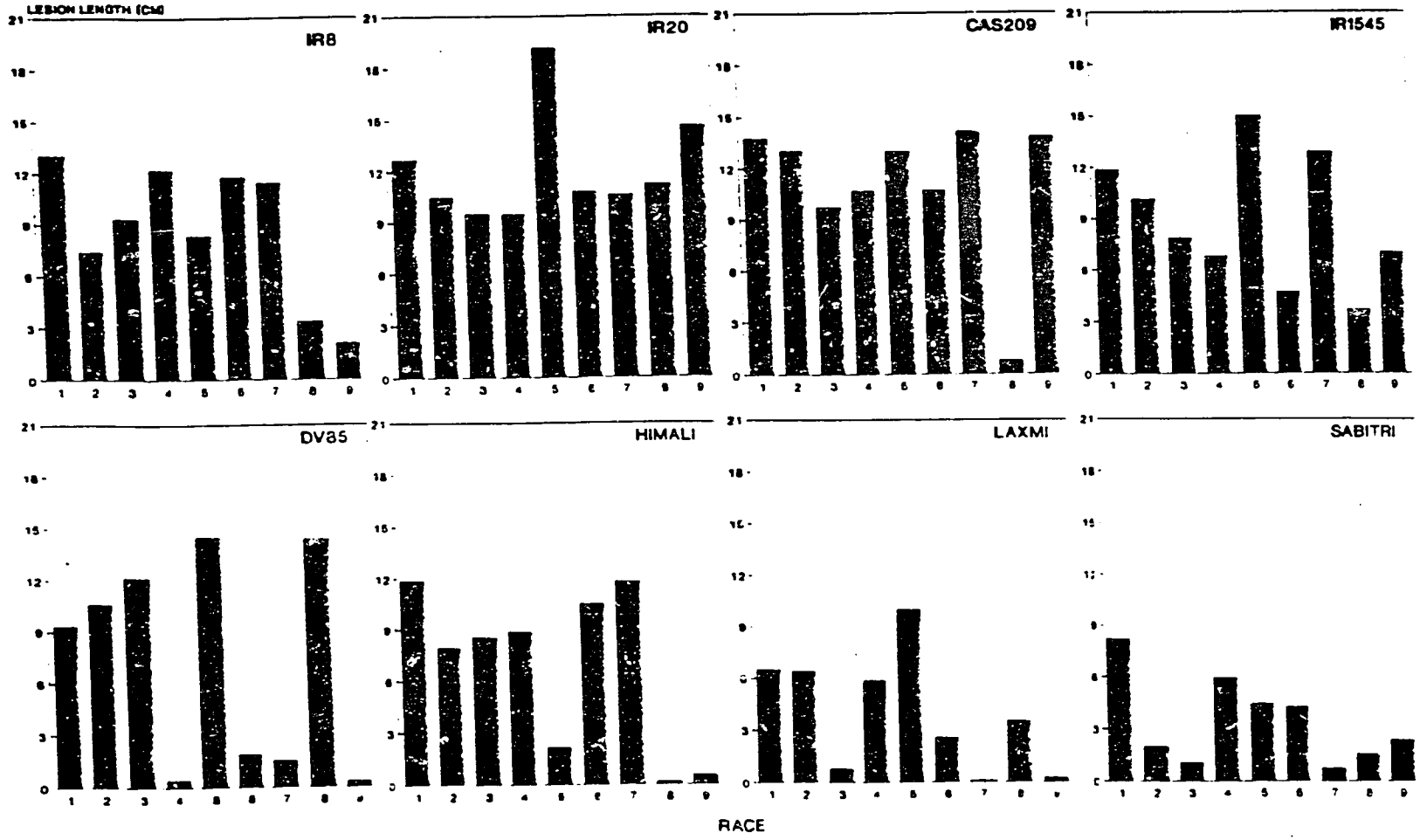


Fig. 4. Differential reactions of rice cultivars to nine races of *X. o.* pv. *oryzae* in Nepal, 1989. Mean of lesion lengths of two replications recorded at 35 days after sowing.

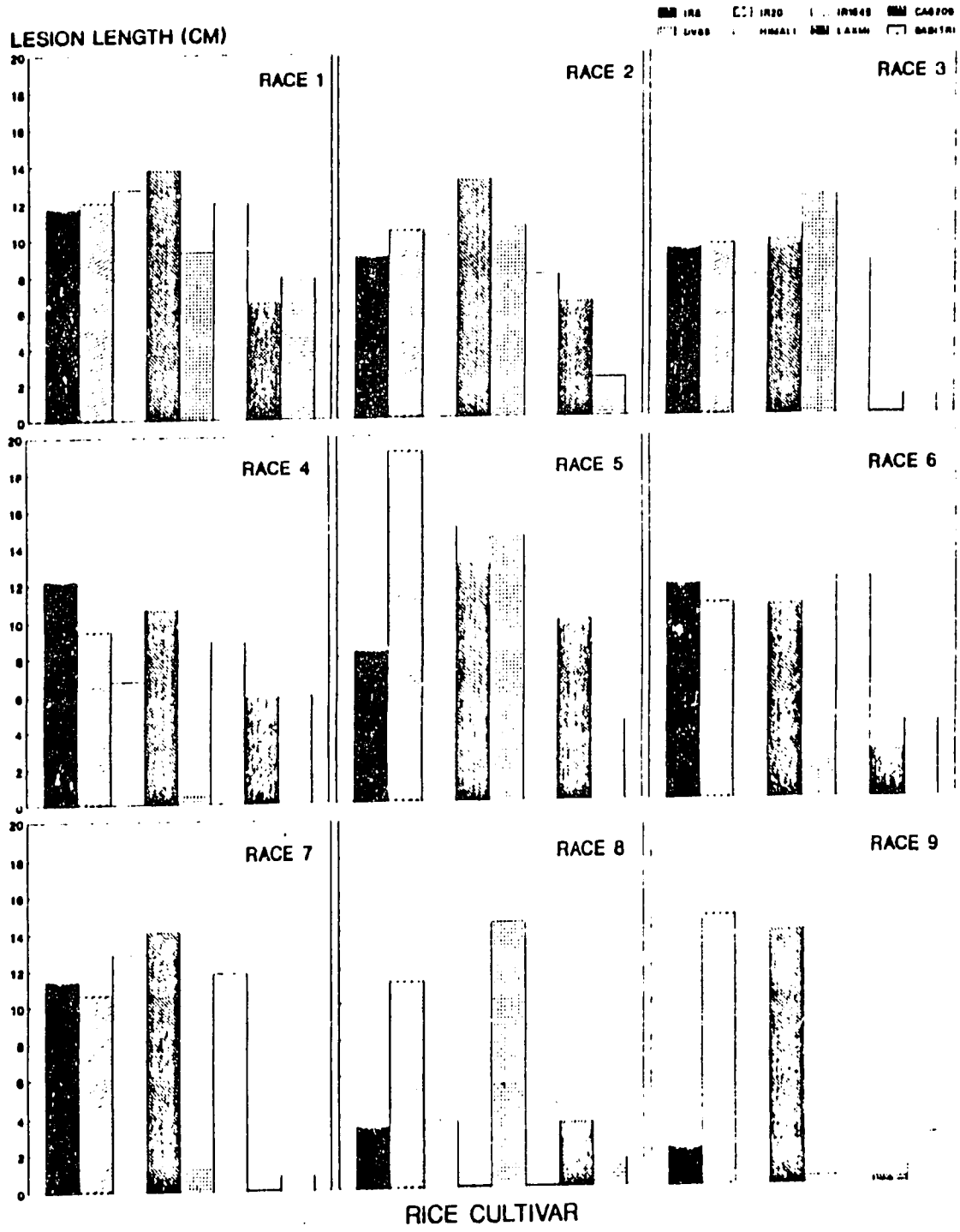


Fig. 5. Interactions between nine races of *X. o.* pv. *oryzae* and rice cultivars differing in resistance. Mean of lesion lengths of two replications recorded at 35 days after sowing.

Laxmi and Sabitri. Race 4 consisted of NXO198, and NXO210 and, was avirulent only on DV85. Race 5 contained only NXO151, which was avirulent on Himali but virulent on other cultivars. Race 6 strains, NXO195, and NXO159, were found avirulent on DV85 and Laxmi. Race 7 strain NXO181 was avirulent on DV85, Laxmi, and Sabitri but highly virulent on other cultivars. Race 8 strain NXO199 caused shorter lesion on CAS209, Himali, and Sabitri. Race 9 strain, NXO205 was avirulent on IR8, DV85, Himali, Laxmi and Sabitri only.

Infectivity Titration Test

Laxmi had significantly shorter lesion as compared to IR24 and Sabitri even at high inoculum doses (10^8 - 10^9 cells/ml) (Fig. 6). Lesion expansion was also progressively reduced on Laxmi (Fig. 7).

The disease survey results suggested that many commercially grown rice cultivars such as Masuli, Bindeshwori, CH-45 and Sarju-49 were highly susceptible to the disease in the Terai regions of Nepal. The sudden appearance of the disease in the Terai could be coincided with the introduction of these rice cultivars susceptible to bacterial blight from 1970 to 1980 and with the increase in double cropping. The combined effects of host, environment and cultural practices possibly exerted a high selection pressure on the pathogen. Consequently, the disease became severe due to rapid adaptation of the pathogen to a particular host cultivar (Nelson, 1973).

The significant strain cultivar interaction in this study suggested pathogenic specialization of *X. o.* pv.*oryzae* in Nepal. IRRI differential cultivars alone can not be used for identifying pathogenic variation of *X. o.* pv. *oryzae* in Nepal. Using three Nepalese rice cultivars, Himali, Sabitri and Laxmi and IRRI differentials, IR8, CAS209, and DV85, the 35 strains of *X. o.* pv. *oryzae* were classified into nine pathogenic races. The strains differed widely in pathogenicity and should be considered to represent "pathogenic races". Specificity in host-pathogen relationships was often indicated by significant strain-cultivar interactions in the analysis of variance of an experiment where a number of pathogen genotypes were tested in all possible combinations of a set of host genotypes (Vanderplank, 1968; 1978). The pathogenic specialization of *X. o.* pv. *oryzae* was also reported from other rice growing countries on the basis of specificity on differential cultivars possessing specific genes fro resistance (Mew and Vera Cruz, 1979; Emachit and Mew, 1982; Noda et al, 1990).

The results demonstrated that IR1545 was susceptible to the strains tested. Likewise, DV85 was resistant to the six strains of *X. o.* pv. *oryzae*. Mew (1987) reported that IR1545 (xa-5) and DV85 (xa-5, Xa-7) were resistant to all the races of *X. o.* pv. *oryzae* in the Philippines and Japan. IR20 (Xa-4) was resistant to the prevalent races of *X. o.* pv. *oryzae* in east and southeast Asia (Ogawa and Khush, 1989) but not to those in Nepal. Similar-

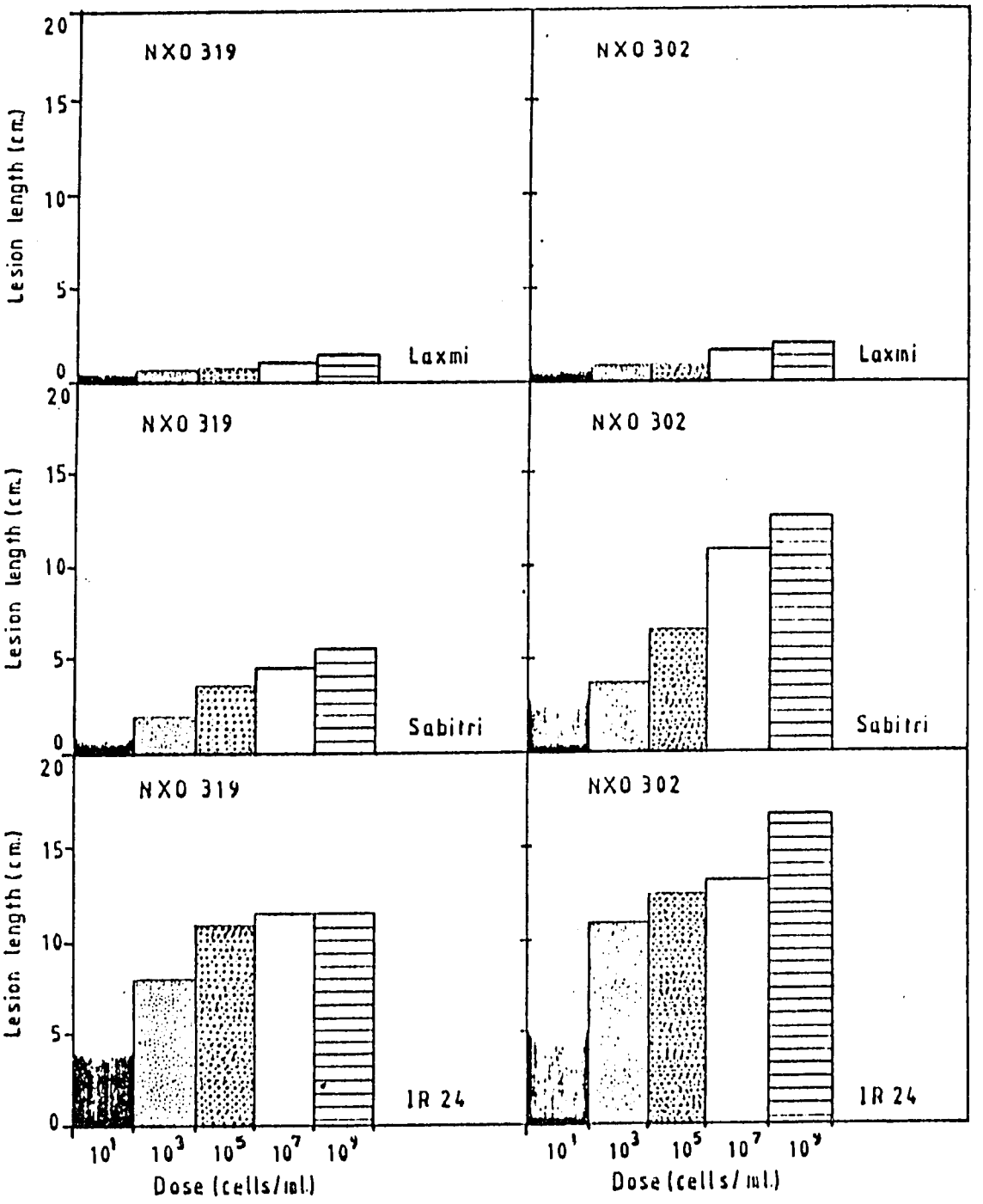
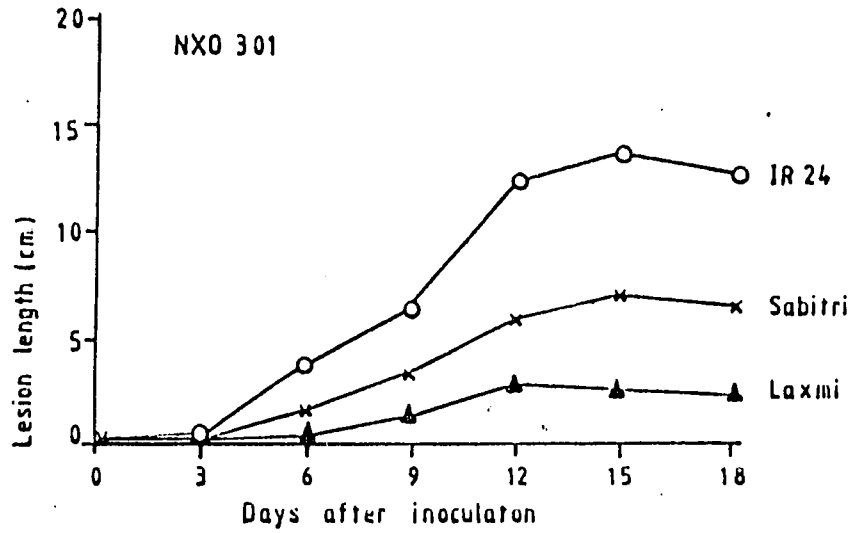


Fig. 6. Dose-response relationship of bacterial blight development on three rice cultivars during 1991 at IAAS Rampur.



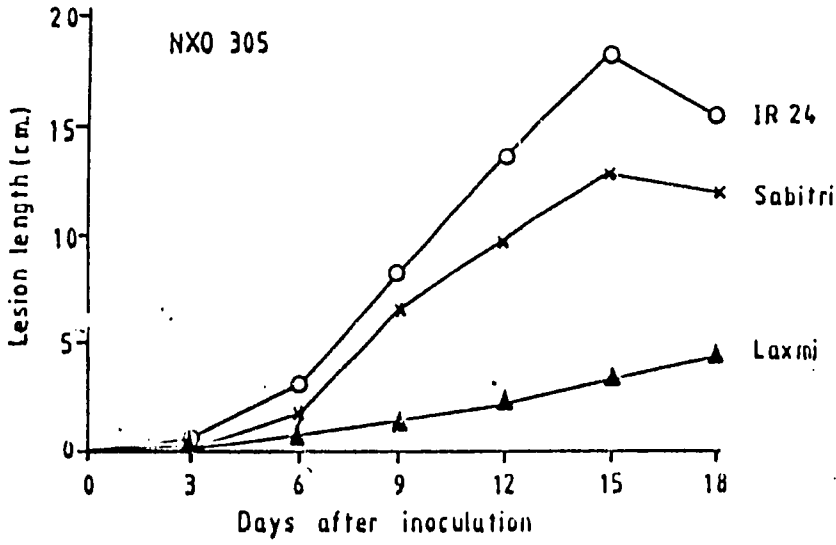


Fig. 7. Progression of bacterial blight on three rice cultivars in the greenhouse during 1991 at IAAS, Rampur.

ly, susceptibility of IR20 (Xa-4) and IR1545 (xa-5) to the pathotypes of *X. o.* pv . *oryzae* had been reported in northwestern India (Gupta et al., 1986). Three IRRI differential cultivars, IR8 (Xa-11), CAS209 (Xa-10), LV85 (xa-5, Xa-7) could be useful panels of differential system along with Nepalese rice cultivars, Himali, Sabitri and Laxmi for monitoring pathogenic variation of *X. o.* pv. *oryzae* in Nepal.

The apparent pathogenic specialization of *X. o.* pv. *oryzae* in Nepal may complicate epidemiologic studies and development of disease management strategies. To resolve this problem, it would be advisable to use the most widely representative virulent strain in breeding rice for resistance to bacterial blight. Moreover, the availability of DNA probes (Leach and White, 1991) could be useful tools to characterize different pathogen populations from broad geographic areas and to understand the diversity of the pathogen populations.

Bacteriological Characteristics of *X. o.* pv. *oryzae*

Clustering of the strains. The phenotypic similarities among the 53 strains of *X. o.* pv. *oryzae* illustrated in the dendrogram (Fig. 8). All strains were associated at 82.40% similarity coefficient (S_{sm}). The thirteen features were positive to all 53 strains tested. All strains were aerobic, catalase positive, grew at 10 C, 20 C, and 30 C, hydrolyzed starch, egg yolk, and Tween 80, produced levan, liquefied gelatin, reduced and peptonized litmus milk, and produced acid from glucose.

The following tests were negative to the 53 strains: Gram stain, acetoin production, growth at 5 C and 40 C, anaerobic condition, nitrate reduction, oxidase activity, growth in the presence of 0.01% (w/v) and 0.05% (w/v) tetrazolium chloride salt, hydrolysis of Tween 20, oxidase activity, indole production, alkalinization of litmus milk, acid production from maltose, lactose, growth in the presence of tetracycline (30 mcg), Penicillin (10 u), Cloxacillin (5 mcg), and 0.05% (w/v) crystal violet.

Several tests such as growth on glucose concentrations, basic fuchsin, 0.1% (w/v) tetrazolium salt, crystal violet, acid production from carbohydrate and sensitivity to Sisomycin (10 mcg), Cotrimoxazole (25 mcg), Ampicillin (10 mcg) and Erythromycin (15 mcg) varied with the strains.

The phenotypic dendrogram of the 53 strains of *X. o.* pv. *oryzae* collected in Nepal was mainly structured on biochemical, physiological and nutritional properties. No striking differences were observed in biochemical and physiological traits among the strains, confirming the existence of homogeneous populations. The results of this study were similar to the earlier reports of bacterial properties of *X. o.* pv. *oryzae* (Vera Cruz et al, 1984; Reddy and Ou, 1976). Variation among strains in terms of acid production, growth on 0.005% tetrazolium chloride and 0.05% crystal violet and sesitivity towards antibiotics were observed

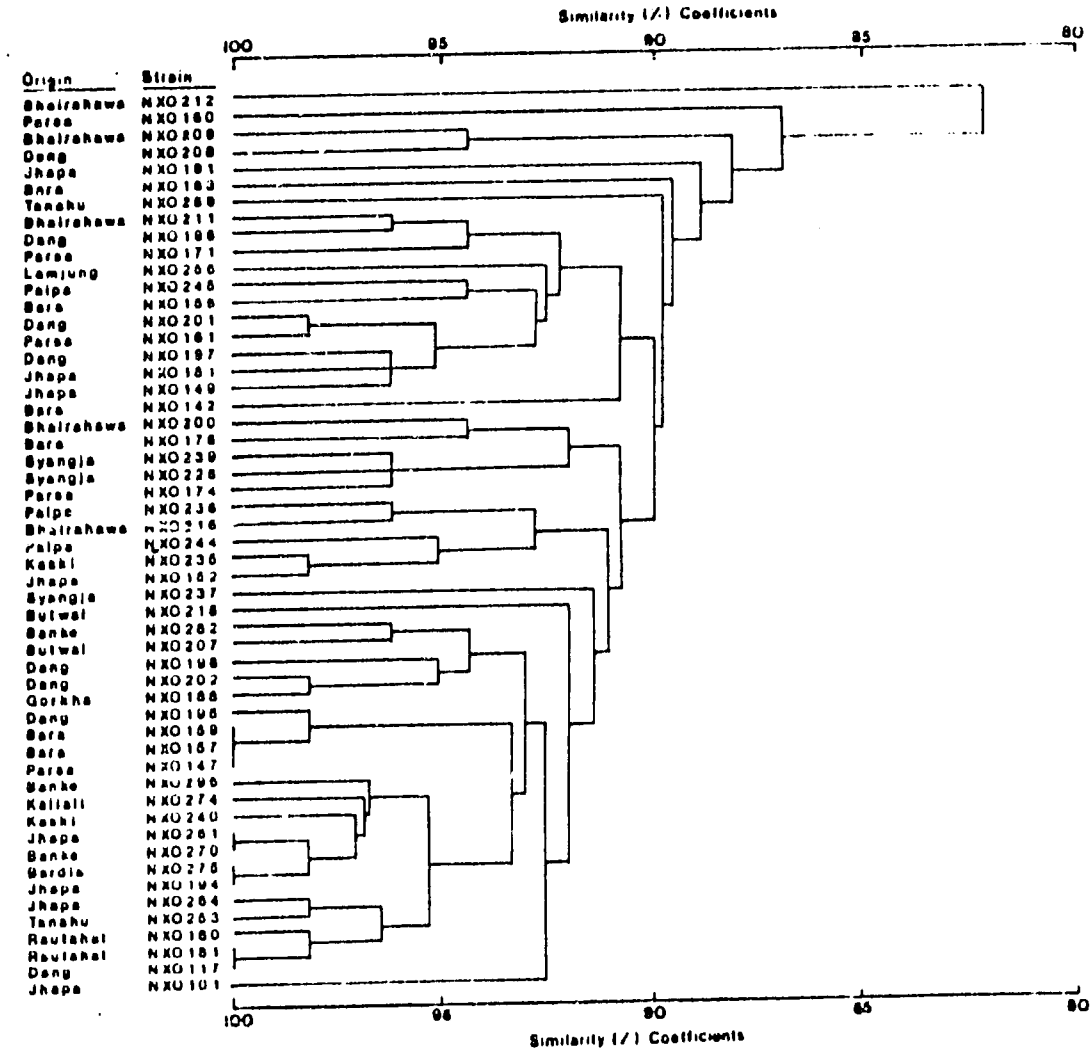


Fig.8 . Dendrogram of similarities coefficients clustered by unweighted average pair-group method showing phenotypic similarities among 53 strains of X. o. pv. oryzae collected in Nepal, 1987-89.

in this study. These phenotypic strains however, did not correlate with location, original host and pathogenicity.

Bacteriocin Production and Typing

Bacteriocinogenicity was presumed when inhibition zones formed around killed bacterial colonies. The strains of *X. o.* pv. *oryzae* differed substantially in bacteriocin production and sensitivity (Fig. 9). Approximately 44% of the strains produced bacteriocin. Four strains, NXO181, NXO276, NXO240, and NXO211 were selected as indicator strains on the basis of cross reactions and sensitivity patterns among the strains. It was therefore possible to distinguish the 45 strains of *X. o.* pv. *oryzae* into 6 bacteriocin types. Eight strains were in type A, two in type B, three in type C, four in type D, 3 in type E and twenty-five strains belonged to type F (Fig. 10).

Comparison between pathogenic races and bacteriocin types. The strains of *X. o.* pv. *oryzae* compared for pathogenicity indicated that there was a wide range of variation in the pathogen populations within a rice growing district of Nepal (Fig. 11). Few strains produced bacteriocin but they were also pathogenic to the rice cultivars tested.

Although different bacteriocin types were present, more strains from various geographic locations need to be characterized as they may be useful in epidemiologic and ecological studies. There was no correlation between bacteriocin types and virulence to rice cultivars or bacteriological properties. Similar findings were reported from *Pseudomonas spp* Vidaver et al, 1972) and *Corynebacterium michiganense* (Echandi, 1976).

Prospects for control of phytopathogenic bacteria by bacteriocins were well-documented in some bacterial diseases (Vidaver, 1976; Kerr, 1980). The strains tested in this study showed little promise for direct use in biological control because most of the bacteriocin producer strains were pathogenic to the test rice cultivars. However, this limitation might be partially overcome by the use of modern biotechnological techniques to develop a non-pathogenic bacteriocin producer mutant (Daniels et al, 1988) or to combine genes for bacteriocin production (Kado, 1977). Confirmation and extension of this study would be worthwhile in supplementing bio-control agent in disease management.

Effects of Environment on the Disease

Disease progress curves of the 6 rice genotypes infected with *X. o.* pv. *oryzae* under each environment are illustrated in Fig. 12. Only very few diseased leaves were found during initial observations. Disease progression was initially faster in susceptible IR24 than the other genotypes but increased rapidly 30 days after transplanting.

The four measures to assess host resistance were calculated for each genotype (Table 3). Disease incidence (DI), disease

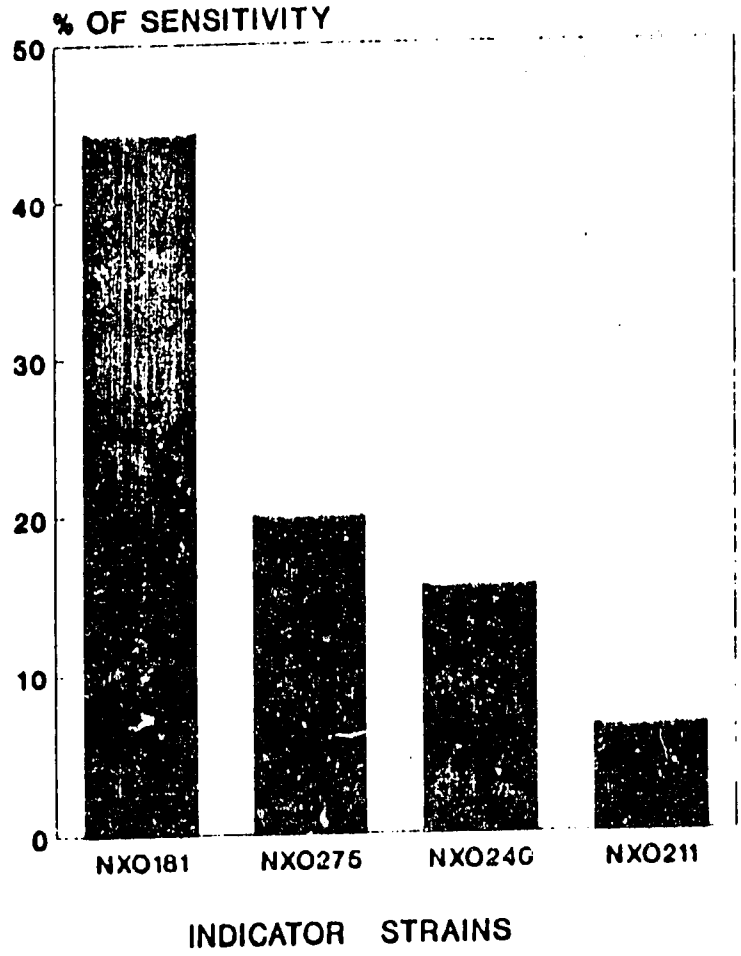


Fig. 9 : Sensitivity of the 45 strains of Xoo to four indicator strains in Nepal, 1989.

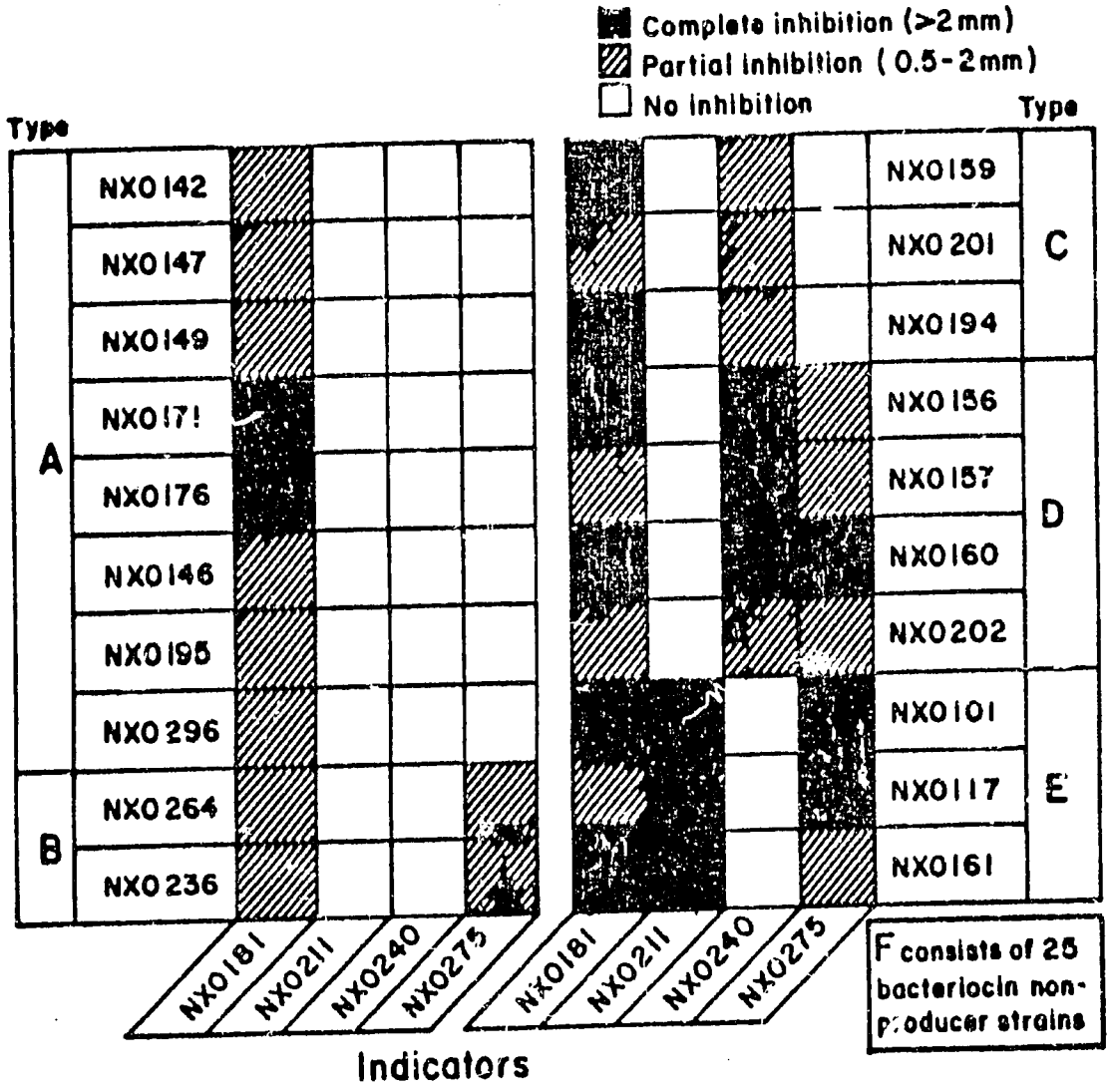


Fig.10. Test of the 45 strains of *X. o.* pv. *oryzae* for bacteriocin activity against 4 indicators on PSA medium, Nepal, 1989.

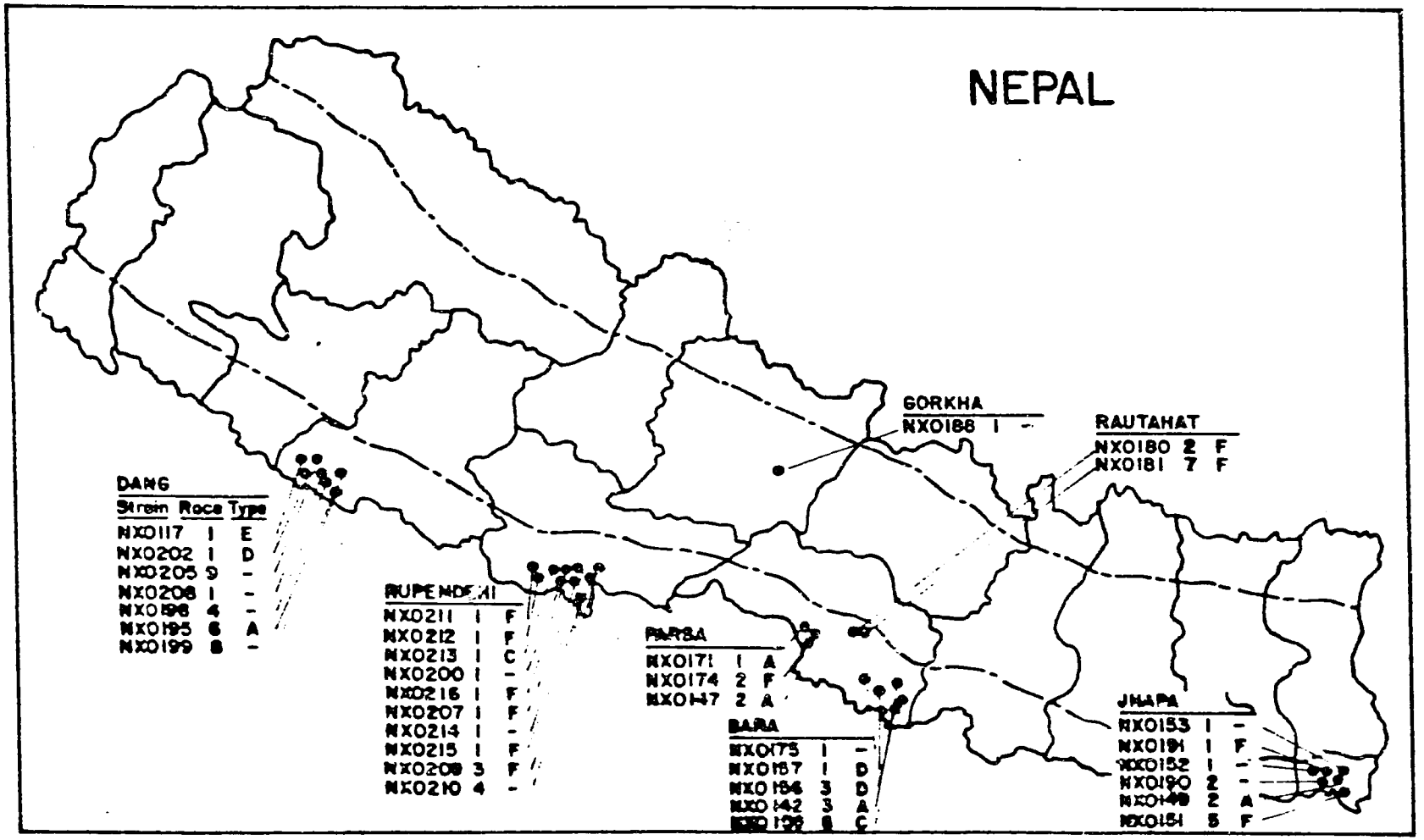


Fig. 11 .. Distribution and relationships between pathogenic races and bacteriocin types of *X. o.* pv. *oryzae* in Nepal, 1989.

severity (DS), area under the disease progress curves (AUDPC) and apparent infection rate (r) were significantly greater in IR24 than in other genotypes. At Rampur, means of DI, DS, AUDPC and r in IR36, IR54, IR60, IR64 and IR1695 were lower than at Kankai.

There were significant (P > 0.05) correlations between epidemiologic parameters and BB progression in Nepal. In both environments, DI was highly correlated with DS and AUDPC (Table 4).

Table 3. Mean disease incidence (DI), disease severity (DS) area under the disease progresss curve (AUDPC) and apparent infection rate (r) caused by BB in Nepal.

Environment	Genotype	Disease progress measurement			
		DI[1] (%)	DS (%)	AUDPC	r (Unit/day)
Rampur	IR24	24.65 a[2]	48.80 a	1649.29 a	0.052 a
	IR36	14.88 b	29.38 b	1249.96 b	0.007 b
	IR54	12.63 b	28.37 b	1243.40 b	0.030 ab
	IR60	14.13 b	31.02 b	1330.12 b	0.013 b
	IR64	13.26 b	29.72 b	1275.17 b	0.013 b
	IR1695	14.71 b	28.73 b	1248.02 b	0.005 b
Kankai	IR24	49.71 a	47.76 a	1922.67 a	0.078 a
	IR36	15.00 b	42.13 b	1290.27 b	0.018 b
	IR54	9.83 c	43.11 b	1294.73 b	0.018 b
	IR60	10.19 c	38.87 b	1306.68 b	0.020 b
	IR64	10.27 c	29.18 c	1203.73 c	0.020 b
	IR1695	12.79 b	42.73 b	1288.92 b	0.010 b

[1]Mean values were taken between 60-70 days after transplanting. Total AUDPC and r were calculated using disease severity; r values were estimated between 60-70 days after transplanting.

[2]In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

The combined analyses of variance for AUDPC, used as a measure of the disease progression and the environmental factors indicated significant location-genotype interactions. The mean temperature at Rampur (27-29 C) has higher than at Kankai (24-25 C). Relative humidity was almost same in both locations in Nepal. However, rainfall recorded at Kankai (133-245 mm) was higher than at Rampur (43-136 mm). Likewise, AUDPCs were also higher at Kankai than at Rampur.

At Rampur, AUDPC was highly correlated with temperature in all genotypes. IR24 was more affected by rainfall in this location. At Kankai, significant correlations between AUDPC and rainfall were observed in all genotypes except in IR64 (Table 5).

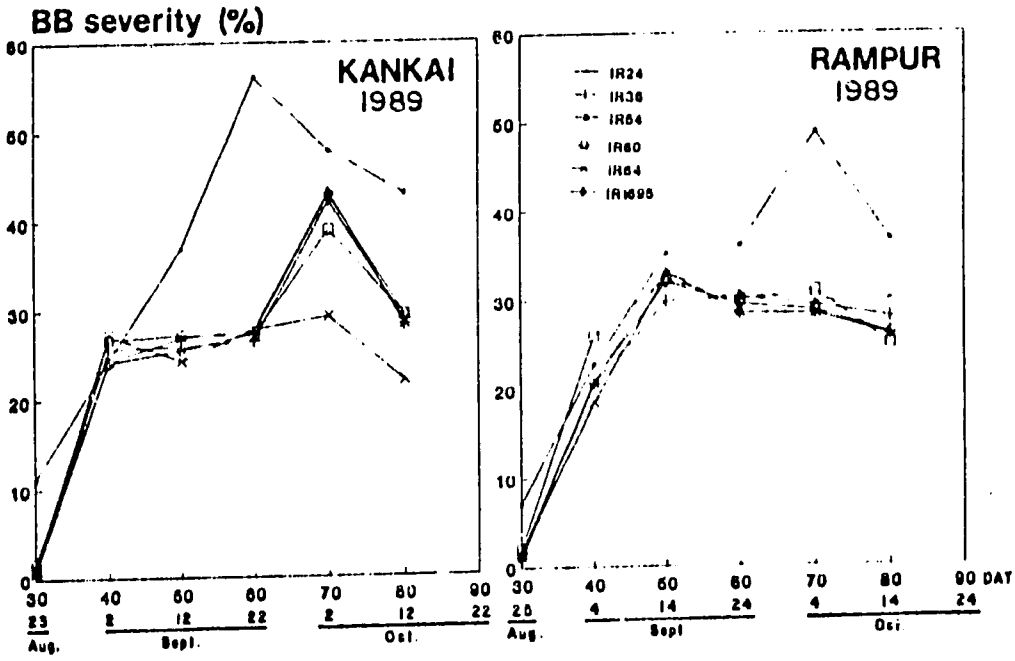


Fig. 12. Disease progress curves for six rice genotypes infected by *X. o.* pv. *oryzae* in Nepal

Table 4. Correlation coefficients between epidemiologic parameters and yield loss (%) in Nepal.

Environment	Measure of Resistance	Epidemiologic parameters DI (%)	DS (%)	r (unit/day)	AUDPC	Yield loss (%)
Rampur	DI	1.000				
	DS	0.798**	1.000			
	r	0.492*	0.578**	1.000		
	AUDPC	0.769**	0.857**	0.437*	1.000	
	Yield loss	-0.115ns	0.109ns	-0.221ns	-0.037ns	1.000
Kankai	DI	1.000				
	DS	0.526**	1.000			
	r	0.849*	0.377ns	1.000		
	AUDPC	0.961**	0.597**	0.805*	1.000	
	Yield loss	0.441*	0.383ns	0.465*	0.439ns	1.000

Table 5. Environmental factors which were significantly correlated with area under the disease progress curve (AUDPC) in Nepal.

Environment	Genotype	Simple correlation coefficient Mean Temp[1]	RH	Rain
Rampur	IR24	0.781**	0.677**	0.654**
	IR36	0.734**	0.438ns	0.429ns
	IR54	0.689**	0.452*	0.286ns
	IR60	0.670**	0.3386	0.257ns
	IR64	0.752**	0.406ns	0.427ns
	IR1695	0.735**	0371ns	0.354ns
Kankai	IR24	0.414ns	0.732**	0.468*
	IR36	0.185ns	0.178ns	0.670**
	IR54	0.094ns	0.214ns	0.717**
	IR60	0.014ns	0.338ns	0.527*
	IR64	0.067ns	0.550*	0.237ns
	IR1695	0.110ns	0.257ns	0.672**

1. Mean temperature (C) = (Minimum + Maximum temperature)/2: RH - relative humidity (%); Rain = rainfall (mm)
2. * = Significant at 5%; ** = highly significant at 1%

Grain yield was reduced by 12% and 26% at Kankai and Rampur, respectively (Table 6). Lower yield reduction was exhibited in IR36 for other genotypes. IR54 sustained yield reduction. IR60 and IR64 showed a modest yield reduction. Significant ($P < 0.05$) to highly significant ($P < 0.01$) correlations between epidemiologic parameters and percent yield reduction was demonstrated.

The percent yield reduction of each genotype was positively related with DI, DS and AUDPC.

Table 6. Grain yield (t/ha) and percent yield loss of six rice genotypes grown in healthy and infected plots in Nepal.

Environment	Genotype	Grain yield (t/ha)		
		Healthy	Infected	Yield loss (%)
Rampur	IR24	3.26 b[1]	2.87 b	11.96
	IR36	3.60 ab	3.50 a	2.78
	IR54	3.90 a	3.58 a	8.20
	IR60	3.77 a	3.38 a	10.34
	IR64	4.05 a	3.65 a	9.87
	IR1695	3.73 a	3.52 a	5.63
Kankai	IR24	3.51 b	2.61 d	25.43
	IR36	3.58 b	3.35 a	6.42
	IR54	3.61 a	3.41 a	5.54
	IR60	3.37 b	3.16 a	6.23
	IR64	3.31 b	3.01 b	9.06
	IR1695	3.38 b	2.81 c	17.16

[1]In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Rice genotypes differed in levels of resistance to *X. o.* pv. *oryzae*. Disease incidence (DI), disease severity (DS) and area under the disease progress curves (AUDPC) were the best variables for detecting genotyes differences between the locations. Infection rates (r) had not widely used to describe disease progression caused by *X. o.* pv. *oryzae* possibly due to uncertainty regarding simple versus compound interest (Vanderplank, 1963).

Final DI and DS values were often considered to represent the summation of host-pathogen-environment interactions over the course of a season (Vanderplank, 1963; Zadoks and Schein, 1979). In the Philippines, differences among some IRRI rice genotypes in terms of low percent of infection and reduced lesion expansion were characterized by quantitative resistance (Koch, 1990). Despite race-specific resistance Xa-4 gene and a compatible race, some IRRI varieties slowed down BB epidemics in the farmers' fields in the Philippines. The quality of such resistance was tmed as background resistance (Mew and Vera Cruz, 1988) and an attribute of durability (Johnson, 1979). In Nepal, Xa-4 gene (IR20) was also found susceptible to many strains tested in the greenhouse but the genotypes possessing Xa-4 such as IR36, IR54, IR60 and IR64 were moderately resistant in the fields. The resistance gene Xa-4 might be interacted with environment in the field and thus causing disease variation. Additionally, several factors such as methods of inoculation, amount of inoculum depos-

ited at the infection court, crop growth and environmental factors might have caused the disease variation between the greenhouse and the fields.

Environmental factors had great influence on BB development because DI, DS, AUDPC and r were significantly different between locations. Kankai environment appeared to be more conducive for bacterial blight development than Rampur environment. AUDPC was highly correlated with rainfall at Kankai. Warm days tented to favor the disease development at Rampur. Mizukami and Wakimoto (1969) reported correlation between disease development and temperature, and emphasized the effect of rainfall and typhoons in the rice growing season.

Significant differences in yield reduction were demonstrated among genotypes. Yield loss due to the disease was higher at Kankai than Rampur. The range of yield loss found in this study was comparable to those other studies (Exconde et al, 1971; Rao and Kauffman, 1977). The positive correlation of AUDPC and yield loss in this study confirmed Vanderplank's (1963) hypothesis that this was an accurate method for estimating yield loss.

These results have direct implications for a rice improvement program. There is an urgent need to improve bacterial blight resistance in rices. The information on pathogenic specialization, effects of host genotypes and environmental factors should take into consideration in formulating disease management strategies in Nepal.

IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER

This project has relevance to development because BB causes substantial damage of rice crop. Yield losses in infected fields can cause up to 26%. Therefore the project has been implemented well and significant results have been obtained in: (i) specialization of the bacterial blight pathogen and its distribution in the country, (ii) resistance of the rice varieties, both local and IRRI to the prevalent races of *X. o.* pv. *oryzae* was relatively well understood and (iii) the strains of the bacterial blight pathogen were also well characterized for the phenotypic features, pathogenicity and bacteriocin production. These information provide an important database of the bacterial blight in Nepal.

Since the current project has provided seed money for Lab upgrading and construction of the greenhouse, these facilities are readily available to other scientists at IAAS working on similar problems in other cereals and vegetables too. Results obtained from this project are first hand information in the country. Therefore National Rice Research Program can collaborate with P. I. because the information eventually can accelerate the rice improvement program. Rice varieties identified as "resistant donors" are planned to include in International Rice Bacterial Blight Nursery (IRBBN coordinated by IRRI) and National Rice Bacterial Blight Nursery (NRBBN by National Rice Research Pro-

gram). These resistant donors will be testing along with others at different rice ecosystems. Seeds are being distributed to the farmers through minikit programs. Results are in the process of publishing in American Journal such as Phytopathology and Plant Disease soon.

Pathogenic specialization of *X. o.* pv. *oryzae* in Nepal appeared different (more virulent) from southeast Asia, as evidenced by the high proportion of susceptibility of cultivars: CAS209 (Xa-10) and IR1545-339-2-2 (xa-5) with genes conferring resistance to the most races *X. o.* pv. *oryzae* in the Philippines, but these were susceptible to all Nepali races. Rice cultivars carrying (Xa-4) and (xa-5, Xa-7) genes from IR20 and DV85 are being used as resistance donors to BB at IRRI, were also moderately resistance to Nepali races of *X. o.* pv. *oryzae*. P. I. had completed his Ph. D. degree at IRRI under the guidance of Dr. T. W. Mew. He had also used some of the results of this project in his dissertation. P.I. is well-qualified to undertake this endeavor.

PROJECT OUTPUTS

a. Advanced training and Ph. D. degree at IRRI

Adhikari, T. B. 1991. Effects of Rice Genotype and Environment on Bacterial Blight Progression. Ph. D. Dissertation submitted to Graduate School, UPLB, Philippines, 143 p.

b. Conference/meeting attended and paper presented

1. Adhikari, T. B. and T. W. Mew. 1988. Research on bacterial blight of rice in Nepal. Page 98 in Proceeding of the Fifth International Congress of Plant Pathology, Kyoto, Japan.

2. International Workshop on Bacterial Blight of Rice held from March 14-18 at IRRI, Philippines.

3. Adhikari, T. B., T. W. Mew and J. E. Leach. 1992. Bacterial blight of rice in Nepal. Paper to be presented at the VIIIth International Conference on Plant Pathogenic Bacteria to be held in France from June 9-12, 1992.

4. Adhikari, T. B., T. W. Mew and J. E. Leach. 1992. Reaction of rice cultivars to different inoculum densities of *Xanthomonas oryzae* pv. *oryzae*. Paper to be presented at the 1992 Annual Meeting of the American Phytopathological Society, Portland, Oregon, USA.

5. Adhikari, T. B. 1992. Research on bacterial blight of rice in Nepal. Paper presented at the 17th Summer Crop Workshop held from Jan 26-31, 1992 at National Maize Research Program, Rampur, Nepal.

c. Publications

1. Adhikari, T., and S. M. Shrestha. 1990. Distribution of bacterial blight in Nepal IRRN 15:22-23.

2. Adhikari, T. B., T. W. Mew and P. S. Teng. 1990. Distribution and severity of bacterial blight of rice in Nepal. Phytopathology 80 (11): 347 (Abstr.).

3. Adhikari, T. B. and S. M. Shrestha. 1992. Distribution and severity of bacterial blight disease of rice in Nepal. J. Inst. Agric. Anim. Sci. 10:31-38.

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PROJECT PRODUCTIVITY

Biocontrol agents like nonpathogenic bacteriocin-producing organisms appear to be attractive alternative in terms of cost and safety to the user and to the environment. In view of this we proposed the exploration of bacteriocin producer strain and its application to BB management. We found many strains of the bacterium producing bacteriocinlike substances in agar medium. At the same time these strains were also pathogenic to the rice plants tested. Thus direct uses of these strains in disease management complicates the problem. However, a more straightforward approach will be to clone the bacteriocin producer gene (s) from these strains and to introduce it to an epiphyte. Likewise, the recent reports on successful introduction of bacterial gene like *Bacillus thuringiensis* endotoxin gene to the crops themselves opens to the possibility of the incorporation of the bacteriocin genes to the rice plants for the scientists working in the developed countries. These long-term goals require the genetic analysis of bacteriocin production of *X. o.* pv. *oryzae*.

FUTURE WORK

In view of substantial progress made and available facilities established under the current grant, future work will enable P. I. to look into population structure and genetic diversity of the bacterial pathogen and its distribution with reference to rice planted in the country. More importantly, how the population changes over time and space in response to the deployment of improved varieties are our primary concerns in Nepal. Genetic

basis of comparison of the Nepali strains with south and southeast Asian strains of *X. o.* pv. *oryzae* is warranted. Collaboration is planned with IRRI, Philippines and Kansas State University, USA to investigate all these aspects in future.

ACKNOWLEDGEMENTS

P. I. would like to gratefully acknowledge the following individuals and organizations who assisted in various ways to successfully complete this project:

o Science Advisor, Office of the Science Advisor, AID/SCI, Washington, D.C., USA.

o Dr. T. W. Mew, Plant Pathologist and Head, Division of Plant pathology, IRRI, Philippines.

o Drs. John DeBoer and G. Gill, Winrock International Institute for Agriculture Development, USA and Nepal, respectively.

o Dean of the Institute of Agriculture and Animal Science (IAAS), Central Campus, Rampur, Chitwan, Nepal.

o Dr. Tim Harris and Mrs. Anjali Pradhan, PPD Office, USAID/Mission Kathmandu, Nepal.

o All staff and collaborators of the Bacterial Blight Project at IAAS, Nepal.

o Mr. R. B. Bhujel, Computer Center, IAAS, Rampur, Nepal.

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Appendix 1. Virulence of representative strains of *X. o.* pv. *oryzae* on eight rice cultivars[1] with specific gene for resistance, Nepal during 1989.

STRAIN	RACE	LESION LENGTH (CM) ON RICE CULTIVAR							
		IR8 (Xa-11)	IR20 (Xa-4)	IR1545 (xa-5)	CAS209 (Xa-10)	DV85 (xa-5,Xa-7)	HIMALI (Unknown)	LAXMI (Unknown)	SABITRI (Unknown)
NXO171	1	9.83 a[2]	10.75 cd	19.33 a	11.20 bcd	14.55 ab	12.16 b	5.83 abc	7.37 b
NXO207	1	9.33 ab	18.33 ab	16.00 ab	20.49 a	15.99 a	20.24 a	6.0 abc	13.83 a
NXO149	2	9.87 a	10.02 cd	11.30 bcd	12.80 bcd	9.19 b	8.92 bc	6.83 ab	0.29 d
NXO174	2	9.33 ab	10.55 cd	9.45 cde	8.26 bcd	10.80 ab	3.13 cde	5.52 abc	2.45 bcd
NXO156	3	11.08 a	12.83 bcd	11.79 bcd	10.71 bcd	10.49 ab	12.10 b	0.37 bc	1.6 bcd
NXO209	3	8.65 ab	8.85 cd	4.13 e	7.69 cd	1.74 c	8.41 bcd	1.89 bc	0.26 d
NXO198	4	12.37 a	12.60 bcd	10.35 bcd	14.50 b	0.69 c	10.63 b	5.11 abc	7.12 bc
NXO210	4	12.03 a	6.42 d	3.25 e	6.87 d	0.27 c	7.16 bcd	6.63 abc	4.80 bcd
NXO151	5	8.33 ab	19.16 a	15.00 abc	13.00 bcd	14.50 ab	2.61 de	10.00 a	4.44 bcd
NXO195	6	12.83 a	11.35 cd	10.74 bcd	11.37 bcd	2.50 c	10.96 b	2.87 bc	4.91 bcd
NXO159	6	10.71 a	10.25 cd	3.43 e	10.04 bcd	1.33 c	10.06 c	2.23 bc	3.64 bcd
NXO181	7	11.41 a	10.60 cd	12.87 bcd	14.12 bc	1.54 c	11.80 b	0.05 c	0.76 cd
NXO199	8	3.36 bc	11.22 cd	3.65 e	0.08 e	14.37 ab	0.05 e	3.50 bc	1.55 bcd
NXO205	9	2.07 c	14.61 abc	6.99 de	13.83 bc	0.37 c	0.56 e	0.27 bc	2.35 bcd

1. 40 days after sowing.
2. In each column, means followed by a coommon letter are not significantly different at 5% by DMRT.

Appendix 2. Bacteriocin typing of 45 strains of *X. o.* pv. *oryzae* when tested against four indicators strains in Nepal.

TYPE	SENSITIVITY AGAINST INDICATORS				TOTAL STRAIN	STRAIN DESIGATION
	NX0181	NX0211	NX0240	NX0275		
A	+[1]	-	-	-	8	NX0142, NX0147, NX0149, NX0171, NX0176, NX0146, NX0195, NX0296.
B	+	-	-	+	2	NX0264, NX0236.
C	+	-	+	-	3	NX0159, NX0201, NX0194.
D	+	-	+	+	4	NX0156, NX0157, NX0160, NX0202.
E	+	+	-	+	3	NX0101, NX0117, NX0161.
F	-	-	-	-	25	NX0207, NX0212, NX0174, NX0163, NX0209, NX0239, NX0244, NX0240, NX0215, NX0253, NX0256, NX0259, NX0275, NX0282, NX0216, NX0261, NX0151, NX0228, NX0180, NX0191, NX0211, NX0237, NX0197, NX0181, NX0274.

1. + = Sensitive; - = Insensitive to indicator strain.