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HOST-PATHOGEN INTERACTION IN RICE-BACTERIAL BLIGHT PATHOSYSTEM

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Abstract: Virulence pattern among 52 isolates of *Xanthomonas oryzae* pv. *oryzae*, the causal organism of bacterial blight disease was tested on 16 rice genotypes possessing 11 known *Xa* genes conferring resistance. Significant differences among the host genotypes as well as the pathogen isolates and in their interaction, suggested that the host genotypes differed in vertical resistance and the pathogen isolates differed in virulence. None of the genotypes exhibited resistant reaction against all the isolates, while one Japanese and two IRRI differentials were knocked down by all the isolates. The set of 16 rice genotypes possessed the *Xa* genes *viz*. *Xa1, 2, 3, 4, 5, 6, 7, 10, 11, 12* & *13*. The isolates carried 4–11 virulence factors, out of a total number of 11 v-factors that could be evaluated from this set of host genotypes to overcome the resistance offered by the corresponding *Xa* genes. The pattern with virulence to *Xa1, 2, 4* & *11* and avirulence to the genes *Xa6, 7, 5, 13* & *10* was very common. The wide distribution of the virulence factors over different states of India suggested nonparallelism between virulence pattern and geographical distribution of the isolates. The 52 isolates could be classified into five groups using hierarchical agglomerative method of cluster analysis based on the number of v-factors possessed by each of them *viz*. 11, 10, 8, 7 & 4, which were equivalent to the pathotype grouping of 1, 4, 7, 14+15 & 16, respectively. The application of the methods of numerical taxonomy emerged as a valuable tool in classification of bacterial isolates into virulence groupings.

Key words: *Xanthomonas oryzae* pv. *oryzae*, pathotypes, virulence-factors, clustering pattern

INTRODUCTION

The application of gene-for-gene theory (Person 1959) was of great importance for obtaining valuable information on host-pathogen relationship, since it provides specificity in pathogenicity pattern of virulent phenotypes against specific host genotypes

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(Browder *et al*. 1980). Based on the host-specificity of the pathogenicity patterns, Parlevliet (1983) identified three to eight virulence factors out of a total of nine v-factors that could be evaluated from 30 isolates of *Puccinia hordei* causing leaf rust in barley. The genetic similarity among 48 isolates of the lettuce downy mildew agent *Bremia lactucae* was assessed by calculation of similarity coefficients among their 11 virulence phenotypes, through cluster analysis (Lebeda and Jendrulek 1987). The specificity in host-pathogen interaction among the races of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and host genotypes have been reported in rice-bacterial blight pathosystem (Mew and Vera Cruz 1979). Based on the specificity in host-pathogen interaction among 18 isolates of *Xoo* and 10 rice cultivars, Nayak (1986) identified four virulence factors corresponding to *Xa4*, *5*, *11* & *13* genes and concluded that the host-pathogen genetic system in rice-bacterial blight pathosystem is dynamic and genes in one part of the system interact with the corresponding genes in the other part in a manner similar to the gene-for-gene relationship. Till date, 27 *Xa* genes have been identified conferring bacterial blight resistance in rice and efforts are in progress to develop near isogenic lines (NIL) and gene pyramids with two, three or four-gene combinations (Ogawa 1993; Gu *et al*. 2004). These NILs have been tested at different research centres against bacterial strains collected and maintained by the respective pathologists (Adhikari *et al*. 1999; Shanti *et al*. 2001; Shanti and Shenoy 2005; Khare and Thrimurty 2006). No attempt has so far been made to identify the number of virulent factors possessed by the bacterial strains, except Khare and Thrimurty (2006) who reported the existence of seven v-factors to overcome the resistance offered by the genes *Xa1*, *3*, *4*, *5*, *7*, *11* & *14*. The present experiment was therefore aimed at (i) identification of number of virulence factors present in 52 isolates collected from 12 states and one union territory of India, (ii) grouping of isolates on the basis of the v-factors possessed by them and (iii) matching these groups with the pathotypes identified with the help of the new Indian differentials selected by the authors (Nayak *et al*., unpublished).

MATERIALS AND METHODS

Rice genotypes. Sixteen rice genotypes namely IR 8, IR 20, IR 1545, Cas 209, DV 85, Kogyoku, Rantai Emas, Wase Aikoku-3, Java 14, TKM 6, Te-Tep, Semora Mangga, Chinsurah Boro II, BJ 1, Zenith and Malagkit Sung Song were used in the present study. These genotypes were collected from the rice gene bank, International Rice Research Institute, Manila, Philippines and the National Germplasm maintained as the Central Rice Research Institute, Cuttack, India. These genotypes possessed 11 known *Xa* genes for resistance to *Xoo*. They include five IRRI differentials, four Japanese differentials and five new Indian differential varieties selected by the authors (Nayak and Reddy 1993; Nayak *et al*., unpublished). Healthy seedlings of the genotypes were raised in seed beds and 30 day-old seedlings were transplanted in well puddled field with a spacing of 20 x 40 cm between plants and between rows. The experiment was conducted in a split-plot design with four replications. The genotypes were planted as main plot and the isolates as sub-plots. Fertilizer in the form of urea was applied in three equal split doses at basal, active tillering and boot leaf stages to provide a total of 120 kg N/ha.

Bacterial isolates. Fifty two cultures of *Xoo* were isolated from the diseased leaf samples collected from different locations covering 12 states, namely Andhra Pradesh

(AP), Assam (AS), Bihar (BR), Gujarat (GT), Madhya Pradesh (MP), Maharashtra (MH), Orissa (OR), Punjab (PB), Rajasthan (RJ), Tamil Nadu (TN), Uttar Pradesh (UP), West Bengal (WB) and the Union Territory of Andaman and Niccobar Islands (AN). The isolation was made on potato-sucrose-agar (PSA) medium. Single colonies were picked up on PSA slants and maintained in sterile distilled water at 4°C as stock culture. The details on the origin of the isolates have been provided in Table 1 already published by the authors (Nayak *et al*. 2006).

Inoculation and observation. The rice plants were clip-inoculated (Kauffman *et al*. 1973) at boot leaf stage with a pair of scissors, every time dipped into the bacterial suspension containing 10^9 cells/ml, prepared from a 48h old bacterial culture of each isolate grown on modified Wakimoto-agar-medium (20g, Sucrose; 5g, Peptone; 0.5g, $Ca(NO_3)_2 4H_2O$; 2g, Na_2HPO_4 12 H_2O; 0.5g, $FeSO_4 7H_2O$; 15g, Agar agar; 1000ml, Distilled water; pH, 6.8–7.0). The length of the lesion developed below the point of clipping was measured 21 days after inoculation. The data on the length of the lesion developed below the point of inoculation was utilized for quantitative analysis of variance. The qualitative development of dry necrotic lesions progressing up to a maximum of 3–5 cm was considered as resistant (R), while water-soaked lesions initiating within 4–5 days of inoculation followed by rapid progress thereafter with typical yellowish-grey colour was characterized as susceptible (S) reaction.

Statistical analyses. The virulence phenotype of each isolate was characterized by eleven v-factors *viz*. V-1, 2, 3, 4, 5, 6, 7, 10, 11, 12 & 13 corresponding to the 11 *Xa* genes of *Xa1*, *2*, *3*, *4*, *5*, *6*, *7*, *10*, *11*, *12* & *13*, those could be evaluated through the present set of 16 rice genotypes. So far, 27 *Xa* genes have been identified (*Xa1-27*) and NILs have been developed (Ogawa 1993; Gu *et al*. 2004). The present set of 11 genes was regarded as a model to serve the function of verifying the potential application of cluster analysis methods in the studies on genetic similarity among virulence phenotypes of *Xoo*. The bacterial isolate showing virulence reaction on a specific host-genotype possessing specific *Xa* gene and showing susceptible reaction, was considered to carry a v-factor corresponding to that *Xa* gene. Thus 4–11 v-factors were identified in the set of 52 bacterial isolates tested in the present experiment (Table 3). The similarity among the virulence phenotypes was analyzed by adopting the hierarchical agglomerative method of cluster analysis (Sneath and Sokal 1973), following a series of successive fusions of 52 bacterial strains to form groups. The entire analysis was conducted using the statistical package developed by the Indostat Services, Hyderabad, India.

RESULTS AND DISCUSSION

The 16 rice genotypes possessing known *Xa* genes, exhibited a broad spectrum of resistance against 52 isolates of *Xoo* and the isolates also showed a broad spectrum of virulence on the rice genotypes tested. Analysis of variance revealed significant differences among the host genotypes, the pathogen isolates and also in their interaction (Table 1). This suggested that the genotypes differed in vertical resistance and the isolates differed in virulence.

Table 1. Analysis of variance for virulence of 52 isolates of *Xanthomonas oryzae* pv. *oryzae* on 16 rice genotypes

Source	DF	SS	MS	F
Isolate (I)	51	14002.2	274.5	3660.00**
Varieties (V)	15	66371.2	4424.7	58996.0**
Interaction (I x V)	765	4284.0	5.6	74.7**
Replications	3	2.4	0.8	10.7**
Error	2493	188.9	0.075	

** significant at p = 0.01

The 11 genes conferring resistance in the present set of host genotypes are *Xa1*, *Xa2*, *Xa3*, *Xa4*, *xa5*, *Xa6*, *Xa7*, *Xa10*, *Xa11*, *Xa12* & *xa13*; present either as single or in two to three gene combinations. None of the 16 host genotypes exhibited resistant reaction to all tested isolates. On the other hand, the resistance conferred by *Xa4* gene in IR 20, *Xa11* gene in IR 8 and *Xa1* & *Xa2* genes in Rantai Emas could be knocked down by all the isolates of *Xoo*. Similarly, none of the isolates was avirulent on all the genotypes, while five isolates *viz*. CRXoo 26, 28, 31, 38 & 47 were highly virulent to overcome the resistance offered by all the 11 *Xa* genes (Table 2). The *Xa6* gene in Zenith as well as M Sung-Song, *Xa4* in Semora Mangga, *xa5* in IR-1545 and *xa5*+*Xa7* in DV-85 exhibited resistant reaction against the groups of isolates in pathotypes 7, 14, 15 & 16 and susceptible reaction against those in pathotypes-1 & 4. Uniformly susceptible reaction patterns were exhibited by *Xa1*+*Xa3*+*Xa12* in Kogyoku, *Xa3* in Wase-Aikoku-3, *Xa4* in TKM 6, *Xa1*+*Xa2* in Tetep, *xa5*+*xa13* in BJ-1 and *Xa3*+xa 5+*xa13* in CB.II against the groups of isolates in pathotypes 1, 4 & 7, and resistant reaction patterns by the groups of isolates in pathotypes 14, 15 & 16. The *Xa10* in Cas 209 and *Xa1*+*Xa3* +*Xa12* in Java 14 exhibited susceptible reaction patterns against the groups of isolates in pathotypes 1, 14 & 15 and resistant reaction pattern against those in pathotypes 4, 7 & 16. The *Xa 4* gene in TKM-6 displayed susceptible reaction pattern against the groups of isolates in pathotypes 1, 4 & 7, while *Xa4* in Semora Mangga exhibited susceptible reactions against those in pathotypes 1 & 4, indicating the presence of some unknown genes in the latter variety which is yet to be identified.

Attempts were made to recognize the number of virulence factors (v-factor) present in each isolate corresponding to each resistant gene (R-gene) present in the host genotype. The number of v-factors carried by each isolate corresponding to the R-genes are shown as a numerals of the respective *Xa* gene in each line (Table 3). The isolates carry from four to eleven v-factors out of a total number of eleven that could be evaluated from the present set of host genotypes x pathogen isolate interactions. Bacterial isolates with wide range of virulence are a common occurrence. The pattern with virulence to *Xa1*, *2*, *4* & *11* was very common among all the 52 isolates with a very wide range of distribution over all the 12 states and one Union Territory of India. A group of 25 isolates carry v-factors corresponding to the R-genes of *Xa3* & *Xa12*, while a group of 16 isolates carry v-factors against *Xa10* gene. Similarly, 14 isolates carry v-factors corresponding to *xa5* and *Xa13*, while nine isolates carry v-factors against *Xa6* & *Xa7* genes.

Table 2. The virulence pattern of 52 isolates of *Xanthomonas oryzae* pv. *oryzae* against 15 rice genotypes possessing known *Xa* genes and the number of virulence factors recognized in each isolate

Isolates	Origin	Kogyoku (*Xa1Xa3+Xa12*)	Rantai Emas (*Xa1+Xa2*)	Wase Aikoku-3 (*Xa3*)	Java-14 (Xa1+*Xa3+Xa12*)	IR-8 (*Xa11*)	IR-20 (*Xa4*+Xa1)	IR-1545-339 (*xa5*)	Cas-209 (*Xa10*)	DV-85 (*xa5+Xa7*)	TKM-6 (*Xa4*)	Tetep (*Xa1+Xa2*)	Semora Mangga (*Xa4*)	CB-II (Xa3+xa5+xa13)	BJ-1 (*xa5+xa13*)	Zenith (*Xa6*)	M.Sung Song (*Xa6*)	No.of v-factors	Pathotypes
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	OR	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
2	OR	R	S	R	S	S	S	R	S	R	R	R	R	R	R	R	R	7	14
3	OR	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
4	OR	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
5	0R	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
6	AP	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
7	AP	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
8	AP	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
9	TN	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
10	AN	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
11	PB	R	S	R	S	S	S	R	S	R	R	R	R	R	R	R	R	7	14
12	PB	R	S	R	S	S	S	R	S	R	R	R	R	R	R	R	R	7	14
13	PB	R	S	R	S	S	S	R	S	R	R	R	R	R	R	R	R	7	14
14	PB	R	S	R	S	S	S	R	S	R	R	R	R	R	R	R	R	7	14
15	PB	R	S	R	S	S	S	R	S	R	R	R	R	R	R	R	R	7	14
16	PB	S	S	S	R	S	S	S	R	S	S	S	S	S	S	S	S	10	4
17	PB	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
18	OR	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
19	OR	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
20	OR	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
21	OR	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
22	OR	R	S	R	S	S	S	R	S	R	R	R	R	R	R	R	R	7	15
23	OR	S	S	S	R	S	S	S	R	S	S	S	S	S	S	S	S	10	4
24	OR	R	S	R	R	S	S	R	R	S	R	R	R	R	R	R	R	4	16

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
25	AP	R	S	R	S	S	S	S	S	R	R	R	R	R	R	R	R	7	15
26	AP	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	11	1
27	AP	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
28	WB	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	11	1
29	MP	S	S	S	R	S	S	R	R	R	S	S	R	S	S	R	R	8	7
30	MP	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
31	BR	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	11	1
32	UP	R	S	S	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
33	GT	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
34	GT	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
35	GT	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
36	GT	S	S	S	R	S	S	R	R	R	S	S	R	S	S	R	R	8	7
37	MH	R	S	R	S	S	S	R	S	R	R	R	R	R	R	R	R	7	15
38	OR	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	11	1
39	AP	S	S	S	R	S	S	R	R	R	S	S	R	S	S	R	R	8	7
40	AS	R	S	R	S	S	S	R	S	R	R	R	R	R	R	R	R	7	15
41	AP	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
42	AP	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
43	RJ	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
44	WB	R	S	R	S	S	S	R	S	R	R	R	R	R	R	R	R	7	15
45	AP	S	S	S	R	S	S	R	R	R	S	S	R	S	S	R	R	8	7
46	OR	S	S	S	R	S	S	S	R	S	S	S	S	S	S	S	D	10	4
47	OR	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	D	11	1
48	AP	S	S	S	R	S	S	R	R	R	S	S	R	S	S	R	T	8	7
49	OR	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	T	4	16
50	OR	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	T	4	16
51	OR	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	4	16
52	OR	S	S	S	R	S	S	S	R	S	S	S	S	S	S	S	S	10	4
v-frequency		0.27	1.00	0.29	0.31	1.00	1.00	0.15	0.31	0.17	0.27	0.27	0.17	0.27	0.27	0.17	0.17		

Table 3. Isolates of *Xanthomonas oryzae* pv. *oryzae* with origin and the virulence factors present

Isolates	Origin	Virulence factors											Total V-factors	Patho-type*
		1	2	3	4	5	6	7	10	11	12	13		
26	AP	1	2	3	4	5	6	7	10	11	12	13	11	1
8	WB	1	2	3	4	5	6	7	10	11	12	13	11	1
31	BR	1	2	3	4	5	6	7	10	11	12	13	11	1
38	OR	1	2	3	4	5	6	7	10	11	12	13	11	1
47	OR	1	2	3	4	5	6	7	10	11	12	13	11	1
16	PB	1	2	3	4	5	6	7	0	11	12	13	10	4
23	OR	1	2	3	4	5	6	7	0	11	12	13	10	4
46	OR	1	2	3	4	5	6	7	0	11	12	13	10	4
52	OR	1	2	3	4	5	6	7	0	11	12	13	10	4
29	MP	1	2	3	4	5	0	0	0	11	12	13	8	7
36	GT	1	2	3	4	5	0	0	0	11	12	13	8	7
39	AP	1	2	3	4	5	0	0	0	11	12	13	8	7
45	AP	1	2	3	4	5	0	0	0	11	12	13	8	7
48	AP	1	2	3	4	5	0	0	0	11	12	13	8	7
2	OR	1	2	3	4	0	0	0	10	11	12	0	7	14
11	PB	1	2	3	4	0	0	0	10	11	12	0	7	14
12	PB	1	2	3	4	0	0	0	10	11	12	0	7	14
13	PB	1	2	3	4	0	0	0	10	11	12	0	7	14
14	PB	1	2	3	4	0	0	0	10	11	12	0	7	14
15	PB	1	2	3	4	0	0	0	10	11	12	0	7	14
22	OR	1	2	3	4	0	0	0	10	11	12	0	7	15
25	AP	1	2	3	4	0	0	0	10	11	12	0	7	15
37	MH	1	2	3	4	0	0	0	10	11	12	0	7	15
40	AS	1	2	3	4	0	0	0	10	11	12	0	7	15
44	WB	1	2	3	4	0	0	0	10	11	12	0	7	15
1	OR	1	2	0	4	0	0	0	0	11	0	0	4	16
3	OR	1	2	0	4	0	0	0	0	11	0	0	4	16
4	OR	1	2	0	4	0	0	0	0	11	0	0	4	16
5	OR	1	2	0	4	0	0	0	0	11	0	0	4	16
6	AP	1	2	0	4	0	0	0	0	11	0	0	4	16
7	AP	1	2	0	4	0	0	0	0	11	0	0	4	16
8	AP	1	2	0	4	0	0	0	0	11	0	0	4	16
9	TN	1	2	0	4	0	0	0	0	11	0	0	4	16
10	AN	1	2	0	4	0	0	0	0	11	0	0	4	16
17	PB	1	2	0	4	0	0	0	0	11	0	0	4	16
18	OR	1	2	0	4	0	0	0	0	11	0	0	4	16
19	OR	1	2	0	4	0	0	0	0	11	0	0	4	16
20	OR	1	2	0	4	0	0	0	0	11	0	0	4	16
21	OR	1	2	0	4	0	0	0	0	11	0	0	4	16
24	OR	1	2	0	4	0	0	0	0	11	0	0	4	16
27	AP	1	2	0	4	0	0	0	0	11	0	0	4	16
30	MP	1	2	0	4	0	0	0	0	11	0	0	4	16
32	UP	1	2	0	4	0	0	0	0	11	0	0	4	16
33	GT	1	2	0	4	0	0	0	0	11	0	0	4	16
34	GT	1	2	0	4	0	0	0	0	11	0	0	4	16
35	GT	1	2	0	4	0	0	0	0	11	0	0	4	16
41	AP	1	2	0	4	0	0	0	0	11	0	0	4	16
42	AP	1	2	0	4	0	0	0	0	11	0	0	4	16
43	RJ	1	2	0	4	0	0	0	0	11	0	0	4	16
49	OR	1	2	0	4	0	0	0	0	11	0	0	4	16
50	OR	1	2	0	4	0	0	0	0	11	0	0	4	16
51	OR	1	2	0	4	0	0	0	0	11	0	0	4	16
Total		52	52	25	52	14	9	9	16	52	25	14		

0 = V-factor absent, 1–13 = V-factors present

*Based on the data presented by Nayak *et al*. (unpublished)

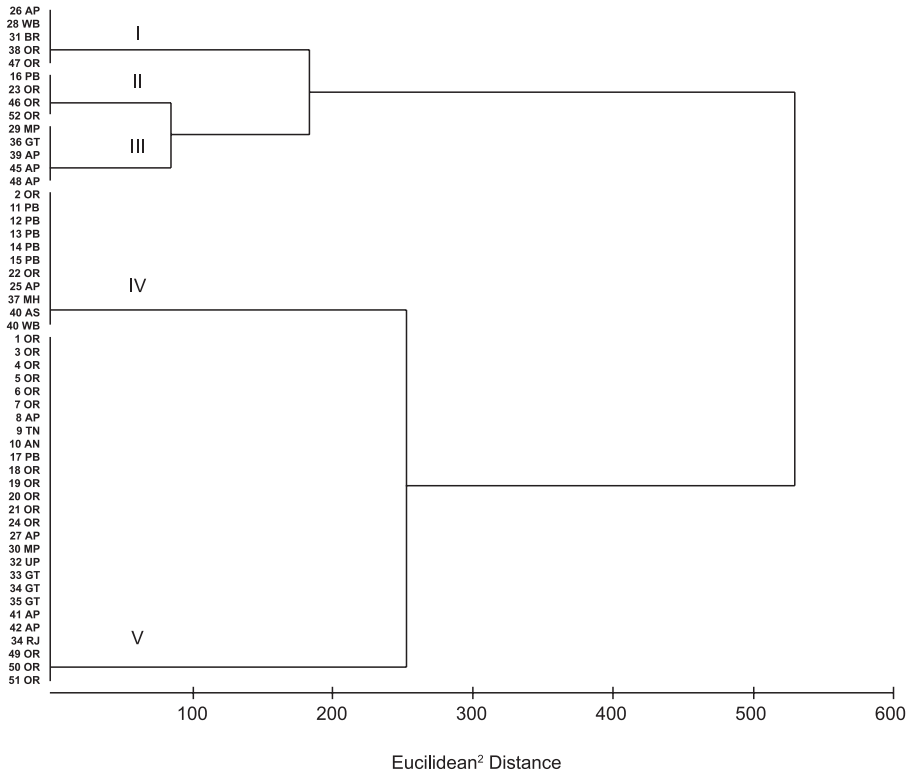

Fig. 1. A dendrogram showing the similarity and successive clustering of 52 isolates of *Xanthomonas oryzae* pv. *oryzae*, based on the number of virulence factors carried by each of the isolates

The grouping of the isolates based on the number of v-factors carried by each of the 52 isolates (Table 3) through hierarchical agglomerative method of cluster analysis resulted in a dendrogram (Fig. 1) showing five distinct clusters. Cluster-I consisted of the isolates CRXoo 26, 28, 31, 38 & 47, originating from four eastern states of the country, namely Andhra Pradesh, Bihar, Orissa and West Bengal, were found to be most virulent possessing all the 11 v-factors corresponding to 11 *Xa* genes included in this study. Cluster-II was composed of four isolates namely CRXoo 16, 23, 46 & 52; originating from two widely apart localized states of Punjab and Orissa and was the next virulent group possessing 10 out of 11 v-factors corresponding to *Xa1, 2, 3, 4, 5, 6, 7, 11, 12* & *13* genes. A group of five isolates namely CRXoo 29, 36, 39, 45 & 48, originating from three states of Andhra Pradesh, Madhya Pradesh and Gujarat possessing eight of 11 v-factors corresponding to *Xa1, 2, 3, 4, 5, 11, 12* & *13* genes constituted of Cluster-III. Similarly, Cluster-IV consisting of 11 isolates *viz*. CRXoo 2, 11, 12, 13, 14, 15, 22, 25, 37, 40 & 44 originating from six states *viz*. Andhra Pradesh, Assam, Maharashtra, Orissa, Punjab and West Bengal, carried seven v-factors corresponding to *Xa1, 2, 3, 4, 10, 11* & *12* genes. This group of isolates did not carry v-factors operative against *xa5, 6, 7* & *13*. The largest group of 27 isolates originating from

eight states and one Union Territory namely Andhra Pradesh, Andaman & Niccobar Islands, Gujarat, Madhya Pradesh, Orissa, Punjab, Rajasthan, Tamil Nadu and Uttar Pradesh; carried only four v-factors corresponding to *Xa1, 2, 4* & *11* genes, constituted Cluster-V. This group of isolates were weakly virulent and carried no v-factors operative against the R-genes *Xa3, 5, 6, 7, 10, 12* & *13*. Thus five clusters of isolates carrying 11/11, 10/11, 8/11, 7/11 and 4/11 v-factors could be recognized through the present set of host-genotype x pathogen-isolate combinations. The compiled data on the grouping of 52 isolates based on the number of v-factors (Table 4) clearly revealed that these five clusters of isolates correspond to the equivalent pathotypes 1, 4, 7, 14+15 and 16, respectively. The only deviation for the Cluster-IV, carrying 7 v-factors, being grouped into pathotype 14 and 15 together, possibly due to their specific reaction on the two new differentials PN-13 and IET 8585 whose genetic constitutions are not yet known. The present findings corroborate with the previous findings reported by Nayak *et al.* (2008a), on grouping of pathogen isolates into clusters through genetic diversity analysis of host pathogen interactions.

Table 4. The constituent number of isolates, total number and name of virulence factors present in each of the five clusters and the corresponding pathotype

Cluster	No. of isolates	Name of isolates	States of origin	No. of v-factors present	Virulence factors	Cluster Mean (cm)	Pathotype Designate*
I	5	CRXoo 26, 28, 31, 38, 47	AP, BR, OR, WB	11	v-1, 2,3, 4, 5,6, 7, 10,11, 12, 13	11.67	1
II	4	CRXoo 16, 23, 46, 52	OR, PB	10	v-1, 2, 3, 4, 5, 6, 7, 11, 12, 13	9.39	4
III	5	CRXoo 29, 36, 39, 45, 48	AP, GT,MP	8	v-1, 2, 3, 4, 5, 11, 12, 13	8.59	7
IV	6	CRXoo 2, 11, 12, 13, 14, 15	OR, PB	7	v-1, 2, 3, 4, 10, 11, 12	7.94	14
IV	5	CRXoo 22, 25, 37, 40, 44	AP,AS,MH, OR, WB	7	v-1, 2, 3, 4, 10, 11, 12	7.51	15
V	27	CRXoo 1, 3, 4, 5, 6, 7, 8, 9, 10, 17, 18, 19, 20, 21, 24, 27, 30, 32, 34, 35, 41, 42, 43, 49, 50, 51	AN, AP,GT, MP, OR, PB, RJ, TN, UP	4	v-1, 2, 4, 11	8.05	16

*Based on the data presented by Nayak *et al*. (unpublished)

Certain important points need special mentionning here. Firstly, there existed a fairly good agreement between the clustering of the isolates based on the number of v-factors and those of pathotype identification through pathogenicity pattern

(Table 4). Secondly, the extent of virulence of the isolates was closely associated with the number of v-factors in terms of mean lesion length produced by each cluster of isolates. Thirdly, the geographical distribution of the v-factors did not play any role in grouping of the isolates or according to the number of v-factors or the virulence patterns as evidenced from wide distribution over different states of India. Similar nonparallelism between the clustering pattern and geographic distribution of the isolates of *Xanthomonas oryzae* pv. *oryzae*, was also reported by Nayak *et al*. (2008a).

The genetic system between the host and the pathogen are dynamic in nature and genes in one part of the system interact with the corresponding genes in the other part, each with widely different magnitude of variability. As a whole, the pattern is race-specific resistance and cultivar-specific virulence, which can be demonstrated by the presence or absence of significant differential interactions between the host genotypes and the pathogen strains. van der Plank (1968) proposed the definition that races which interact differentially with the varieties of the host be said to vary in virulence and the host genotypes which interact differentially with the races of the pathogen be said to vary in vertical resistance. In other words, if there is any significant differential interaction between races and varieties, the significant differences among races involve differences in virulence and significant differences among the varieties involves differences in vertical resistance. A significant difference among the 16 host genotypes and 52 pathogen isolates as well as in their interaction observed in the present experiment (Table 1) suggest that the host genotypes differed in vertical resistance and the pathogen isolates differed in virulence. Such a significant differential interaction among the host genotypes and Indian isolates of *Xoo* was reported by Nayak (1986), Shanti and Shenoy (2005), Nayak *et al*. (unpublished) and also among the Japanese isolates by Ezuka and Horino (1974). On the contrary, Ou *et al*. (1971) could not find distinct interaction between 24 rice cultivars and 50 isolates of *Xoo*, which was attributed to be due to a much wider range of pathogenicity of isolates on some cultivars than others.

During the course of host-pathogen interaction, genes in one part of the system interact with the corresponding genes in the other part of the system in a manner similar to race-specific resistance and cultivar-specific virulence and the resultant effects being expressed either as resistant or susceptible reactions in the host genotypes. Thus the resistance in the host genotype is closely associated with avirulence in the pathogen strain and conversely, the virulence in the pathogen strain is closely associated with the susceptibility expressed in the host genotypes (Flor 1955). Bacterial blight pathosystem is no exception to such type of specificity in host x pathogen interactions (Mew and Vera Cruz 1979; Nayak 1986). Based on specificity in host-pathogen interaction among 18 isolates of *Xoo* and 10 rice cultivars, Nayak (1986) concluded that the host-pathogen genetic system in rice bacterial blight pathosystem is dynamic and genes in one part of the system interact with corresponding genes in the other part, in a manner similar to gene-for-gene relationship.

The present set of 16 rice genotypes possessed 11 *Xa* genes conferring resistance to *Xoo*. A total number of 11 v-factors *viz*. V-1, 2, 3, 4, 5, 6, 7, 10, 11, 12 & 13 were identified in this group of 52 isolates corresponding to the respective *Xa* genes present in the host genotypes. A close insight into the reanalysis of the data presented by Shanti and Shenoy (2005) revealed the existence of 11 v-factors among 10 isolates collected from Andhra Pradesh, Orissa and Tamil Nadu to overcome resistance offered by *Xa1, 3, 4,*

5, 7, 8, 10, 11, 13, 14 & *21* genes. Khare and Thrimurty (2006) reported seven v-factors in one virulent isolate of Chhatishgarh alone to overcome resistance offered by *Xa1, 3, 4, 5, 7, 11* & *14* genes., Data presented by Nayak (1986) revealed the existence of five v-factors in 18 isolates collected from six states of India to overcome resistance offered by *Xa4, 5, 7, 11* & *13* genes. The existence of nine v-factors in 13 isolates collected from the state of Punjab and Haryana alone to overcome resistance offered by *Xa1, 2, 3, 4, 5, 7, 10, 12* & *14* genes was revealed from the data presented by Gupta *et al*. (1986). Compilation of these data indicate that the Indian isolates of *Xoo* tested so far against known *Xa* genes for resistance, carry the 14 v-factors designated as V-1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14 & 21 corresponding to the respective *Xa* genes. In other words, 14 out of 27 *Xa* genes identified so far, are compatible with the Indian isolates of *Xoo*. The data presented by Mew (1987) reveals the presence of nine v-factors to overcome resistance offered by *Xa1, 2, 3, 4, 5, 7, 10, 11* & *12* genes; involving 1–5 from Japan, 4–8 from Philippines, 3–8 from Indonesian, 5–8 from Thailand and 7–9 from Indian races of *Xoo*. These findings and those reported by Vera Cruz and Mew (1989) clearly indicate that Indian isolates of *Xoo* are the most virulent. Such a high level of virulence and compatibility of the bacterial strains with most of the *Xa* genes, raises a question on the utilization of the NILs as differentials for race identification programme as suggested by Ogawa (1993). It is therefore felt essential that breeders and pathologists in a joint effort should make attempts to identify new genes for resistance to the virulent Indian isolates of *Xoo* and develop new NILs which could be utilized as differentials in race identification programme as well as breeding resistant varieties. Continuous attempts to detect such compatible pathogen strains (Nayak *et al.* 2008b), will provide basic information on genetics of host-pathogen interaction leading to identification and incorporation of resistant genes into varietal background in the lines of development of NILs. The present findings and those reported earlier; further indicate that the virulence factors of *Xoo* races are widely distributed over different states of India as well as in different rice growing countries in Eastern and South Eastern countries of Asia. This might be due to the free exchange of genetic material for multi-locational trials over different countries as well as different states of India.

The method of cluster analysis was proposed to distinguish between similarities among the wheat cultivars for their resistance to *Puccinia striiformis* (Priestley *et al*. 1984). It has opened up new ways of exactly expressing genetic similarity among virulence phenotypes (Lebeda and Jendrulek 1987). The technique of cluster analysis can be used for natural data clustering for simplify description of vast various data sets and to generate hypothesis concerning the nature of the data. The application of the method to the data on the number of virulence-factors carried by each of the 52 isolates of *Xoo* in the present experiment, resulted in five clusters comprising of 11, 10, 8, 7 & 4 v-factors (Fig. 1) which were comparable with the pathotypes 1, 4, 7, 14+15 and 16, respectively (Table 4) identified by the authors (Nayak *et al*. unpublished). Thus the specificity in pathogenicity by each group of bacterial isolates could be expressed by means of analysis of v-factors. Similar expression of specific pathogenicity pattern (Browder *et al*. 1980; Nayak *et al*. unpublished), genetic structure of pathogen population (Browder and Eversmeyer 1977; Lebeda 1981) and genetic intra-specific diversity of virulence (Lebeda 1982) were reported by application of cluster analysis.

The specificity in host-pathogen interaction in rice bacterial blight pathosystem, expressed through determination of v-factors and matching the groupings of bacterial strains with those based on the pathogenicity pattern, provides useful scientific

information on the host-pathogen interaction. Although inclusion of the 11 phenotypes in the present experiment served as a model host-pathogen interaction, a complete set of the phenotypes involving 27 *Xa* genes (the NILs) as well as gene pyramids would definitely throw more light on the host-pathogen interactions in the future. In view of the wide geographical distribution of v-factors over different rice growing states of India, a continuous joint cooperative research effort on detection of more such compatible virulent strains, identification of new races and v-factors through the use of existing NILs as well as development of new NILs for inclusion as differentials in the lines suggested by Ogawa (1993), need to be taken up on priority basis in India. The application of the methods of numerical analysis is expected to play a major role in these areas of research in the future. Such efforts would lead towards breeding for high yielding cultivars possessing resistance to bacterial blight disease.

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POLISH SUMMARY

WSPÓŁDZIAŁANIE ROŚLINY ŻYWICIELSKIEJ Z PATOGENEM W PATOSYSTEMIE RYŻ-ZGORZEL BAKTERYJNA

Testowano wzorce wirulencji u 52 izolatów sprawcy zgorzeli bakteryjnej *Xanthomonas oryzae* pv. *oryzae* na 16 genotypach ryżu posiadających 11 znanych genów *Xa* warunkujących odporność. Istotne różnice występujące wśród genotypów rośliny żywicielskiej i wśród izolatów patogena oraz ich współdziałanie sugerowały,

że genotypy żywiciela różniły się odpornością pionową, a izolaty patogena wirulencją. Żaden z genotypów żywiciela nie wykazał odporności na wszystkie izolaty patogena, a jedna japońska odmiana różnicująca i dwie odmiany różnicujące IRRI były porażane przez wszystkie izobaty. Zestaw 16 genotypów ryżu posiadał geny *Xa* 1, 2, 3, 4, 5, 6, 7, 10, 11, 12 i 13. Izolaty bakterii miały 4–11 czynników wirulencji z ogólnej liczby 11 znanych, które można było określić wykorzystując zestaw genotypów żywiciela. Wzorzec wykazujący wirulencję w stosunku do genów *Xa* 1, 2, 4 i 11 i awirulencję w stosunku do genów *Xa* 6, 7, 5, 13 i 10 był bardzo pospolity. Szerokie rozprzestrzenienie czynników wirulencji w różnych stanach Indii sugerowało brak zgodności pomiędzy wzorcem wirulencji a rozprzestrzenieniem geograficznym izolatów. Pięćdziesiąt dwa izolaty mogły być zaliczne do 5 grup przy wykorzystaniu hierarchicznej metody aglomeracji analizy skupień, w oparciu o liczbę obecnych czynników wirulencji wynoszącą 11, 10, 8, 7 i 4, co odpowiada grupom patotypów 1, 4, 7, 14+15 i 16. Metody taksonomii cyfrowej okazały się wartościowe przy zaliczaniu izolatów bakterii *X. oryzae* pv. *oryzae* do grup wirulencji.