VARIABILITY IN *XANTHOMONAS ORYZAE* PV. *ORYZAE*, THE INCITANT OF BACTERIAL BLIGHT DISEASE OF RICE

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Abstract: The virulence pattern of 52 bacterial strains of *Xanthomonas oryzae* pv. *oryzae*, the causal organism of bacterial blight disease of rice was assessed on 41 rice genotypes including five Japanese and five Philippines' differentials. A significant differential interaction observed among the bacterial isolates, the host-genotypes and in their interaction suggested that the host-genotypes differed in vertical resistance and bacterial isolates differed in virulence. The two Japanese differentials Kinmaze and Rantai Emas and two Philippines' differentials IR 8 and IR 20, exhibited highly susceptible reactions against all the 52 bacterial isolates. Five new Indian differentials were selected, one from each of the five clusters of genotypes obtained through hierarchical method of numerical analysis of the virulence pattern of 52 bacterial isolates on 41 host-genotypes. The 52 bacterial isolates could be grouped into six clusters on the basis of their pathogenicity pattern on five new Indian differentials, which were designated as Pathotype-1, 4, 7, 14, 15 and 16, following a standard computer generated virulence pattern chart. These pathotypes were comparable with the Japanese pathotype groups of I, II, III and IV and Philippines' pathotype groups of I, II, III, IV and V. The most virulent pathotype-1 was distributed over four eastern states of India, namely Andhra Pradesh, Orissa, West Bengal and Bihar. In view of the free exchange of genetic material all over the country, continuous monitoring of the prevalence of new pathotypes with the help of the present set of differentials, will ac© ars.

Key words: differential varieties, numerical analysis, pathogenicity pattern, pathotypes

INTRODUCTION

The existence of a broad spectrum of variability in virulence among the bacterial strains of *Xanthomonas oryzae* pv. *oryzae (Xoo)*, the incitant of bacterial blight disease of rice has been a major problem in resistance breeding programme in India. Several attempts have been made worldwide to establish differential host genotypes and group the *Xoo* isolates into pathotypes (Washio *et al*. 1966; Kauffman and Rao 1972; Xia and Fu 1983; Noda *et al*. 1990; Reddy and Reddy 1990; Endo *et al*. 1991; Ogawa 1993 and Kaku 1993). Such attempts resulted in identification of differential host genotypes at IRRI (Mew and Vera Cruz 1979), Japan (Ezuka and Haino 1974) and Indonesia (Yamamoto *et al*. 1977). Rice pathologists in India, have either used these differential host-genotypes or a set of genotypes chosen by them (Rao *et al*. *1971;* Gupta *et al*. 1986; Reddy and Reddy 1990 and Nayak 1996). In view of the fact that the isolates of *Xoo* from South Asian countries are most virulent, Mew *et al*. (1982) stressed the need for identification of differential varieties for each country separately.

Following the reports on existence of physiologic specialization among the Indian isolates (Devadath and Padmanabhan 1969), several attempts have been made by different groups of rice pathologists to classify the bacterial strains into virulence grouping (Rao *et al*. 1971; Kauffman and Pantulu 1972; Gupta *et al*. 1986; Reddy and Reddy 1990; Nayak and Reddy 1993). These virulence groupings are not comparable, since each group of workers used different differential varieties including either Japanese or from Philippines or new differentials selected by them. Since Indian isolates of *Xoo* are most virulent and capable of knocking down some of the Japanese and Philippines differentials, it was felt essential to select a set of new differential varieties which could differentiate Indian isolates possessing broad spectrum of virulence. The main objectives of the present piece of investigation were (I) to select a set of differential varieties those could differentiate Indian isolates of *Xoo*, (II) standardize the methods to identify and designate the pathotypes and (III) study their geographic distribution in India.

MATERIALS AND METHODS

Rice genotypes

Forty one rice genotypes were collected from the International Rice Research Institute, Manila, Philippines; Directorate of Rice Research, Hyderabad, India and the National Genetic Resources, Central Rice Research Institute, Cuttack, India. The origin of these genotypes were Bangladesh, India, IRRI, Philippines, Japan, Indonesia and Vietnam. These genotypes involved IRRI differen-

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tials, Japanese differentials and those used in India by different groups of rice pathologists. Besides, some of the rice genotypes possessed known Xa genes for resistance to bacterial blight disease. Twenty five-day old healthy seedlings were transplanted in well-puddled field with a spacing of 20 cm between plants and 40 cm between rows. The experiment was conducted in a split plot design with four replications. The genotypes were planted as main plot and the bacterial isolates as sub-plots. Fertilizer in the form of urea was applied in three equal split doses; first as basal dose, second at active tillering and third at boot leaf stage, to provide 120 kgN/ha.

Bacterial isolates

The isolates of *Xanthomonas oryzae* pv. *oryzae* were isolated from the diseased leaf samples collected from 52 different locations (provided in table 1, Nayak *et al*. 2006) situated all over the country covering 12 rice growing state namely Andhra Pradesh, Assam, Bihar, Gujarat, Madhya Pradesh, Maharasthra, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal; and the Union Territory of Andaman and Nicobar Islands. The origin of these isolates covered a wide range of host genotypes involving local, high yielding as well as hybrid varieties. The isolations were made on potato-sucrose-agar (PSA) medium. Single cell colonies were cultured on PSA slants and maintained in sterile distilled water at 4°C, as stock culture.

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 48 h-old actively growing bacterial culture of each isolate grown on modified-Wakimoto-agar-medium. A separate pair of sterilized scissors was used for inoculation of each isolate of the causal bacterium. The length of the lesion developed below the point of clipping was measured 21 days after inoculation. The host reaction in terms of lesion development on different rice genotypes was distinguished as resistant (R) or susceptible (S), takings in consideration of both quantitative and qualitative characters of the lesions. The data on the length of lesion developed below the point of inoculation was utilized for quantitative analysis of variance. Dry necrotic lesion progressing upto a maximum of 3–5 cm was considered as resistant. The susceptible reaction was characterized by water soaked lesions initiating within 4–5 days of inoculation followed by rapid progress of there after, with typical yellowish-grey in colour.

Statistical analyses

The significant differential interaction among the isolates, genotypes and their interaction was determined through analysis of variance. The 41 host-genotypes were classified into specific clusters, following Hierarchical method of numerical analysis (Sneath and Sokal 1973) by considering the quantitative reaction of each genotype against 52 isolates of the pathogen as the operational taxonomic units. A set of new differential varieties showing highly specific differential interactions, were selected from among these genotype-clusters. The isolates were grouped into specific pathotypes on the basis of their virulence reaction on Japanese, IRRI as well as new Indian differential varieties, through both conventional method and hierarchical method of numerical analysis.

RESULTS

The mean lesion length of 41 rice genotypes averaged over their reactions against 52 bacterial isolates ranged between 3.3 and 15.9 cm. The rice genotypes IR 8, IR 20, IR 36, IR 60, Cemposelek, Rantai Emas and Kinmaze exhibited highly susceptible reaction against all the isolates, while rest of the genotypes showed differential reaction. Among the isolates, CRXoo 26, 28, 31, 38 and 47 exhibited highly virulent reaction, while the rest showed differential reactions. Such differential interactions were evidenced from the analysis of variance (Table 1), which revealed significant differences among the isolates, genotypes and in their interactions, thus suggesting that the host-genotypes differ in vertical resistance and the pathogen isolates differ in virulence.

Table 1. Analysis of variance of 41 genotypes tested for their reaction against 52 isolates of *X. oryzae* pv. *oryzae*

Source	DF	SS	MS	F
Block	3	9.34	3.11	10.29**
Isolate (I)	51	34949.91	685.29	2264.78**
Genotype (G)	40	165083.47	4127.09	13638.69**
Interaction (G x I)	2040	14030.47	6.88	22.73**
Error	6393	1935.34	0.30	

**significant at p = 0.01
DF – degrees of freedom
MS – mean sum of square
SS – sum of square
F – variance ratio

Identification of pathotypes based on Japanese differentials

Attempts to classify the 52 isolates with the help of five Japanese differentials, *viz*. Kinmaze, Kogyoku, Rantai Emas, Wase Aikoku and Java 14 through numerical analysis resulted in identification of four clusters of isolates distinctly depicted in a dendrogram (Fig. 1). Cluster-I constituted of 27 isolates originating from nine states of India and exhibited the virulence pattern of SRSRR on five differential genotypes (Table 2). Cluster-II comprised of nine isolates originating from five states, with a virulence pattern of SSSSR. These two clusters merged together at a similarity level of 0.95 and ultimately merged with cluster-III at similarity level 0.94, which consisted of 11 isolates originating from five states with a virulence pattern of SRSRS. Cluster-IV consisted of five most virulent isolates, originating from three states, exhibited a virulence pattern of SSSSS and maintained its independent identity to merge with the other three clusters at a similarity level of 0.75. These results on the distribution of the same pathotype in different states and the presence of different pathotypes in the same state suggested non-parallelism between the geographical distribution and clustering pattern of the pathogen strains.

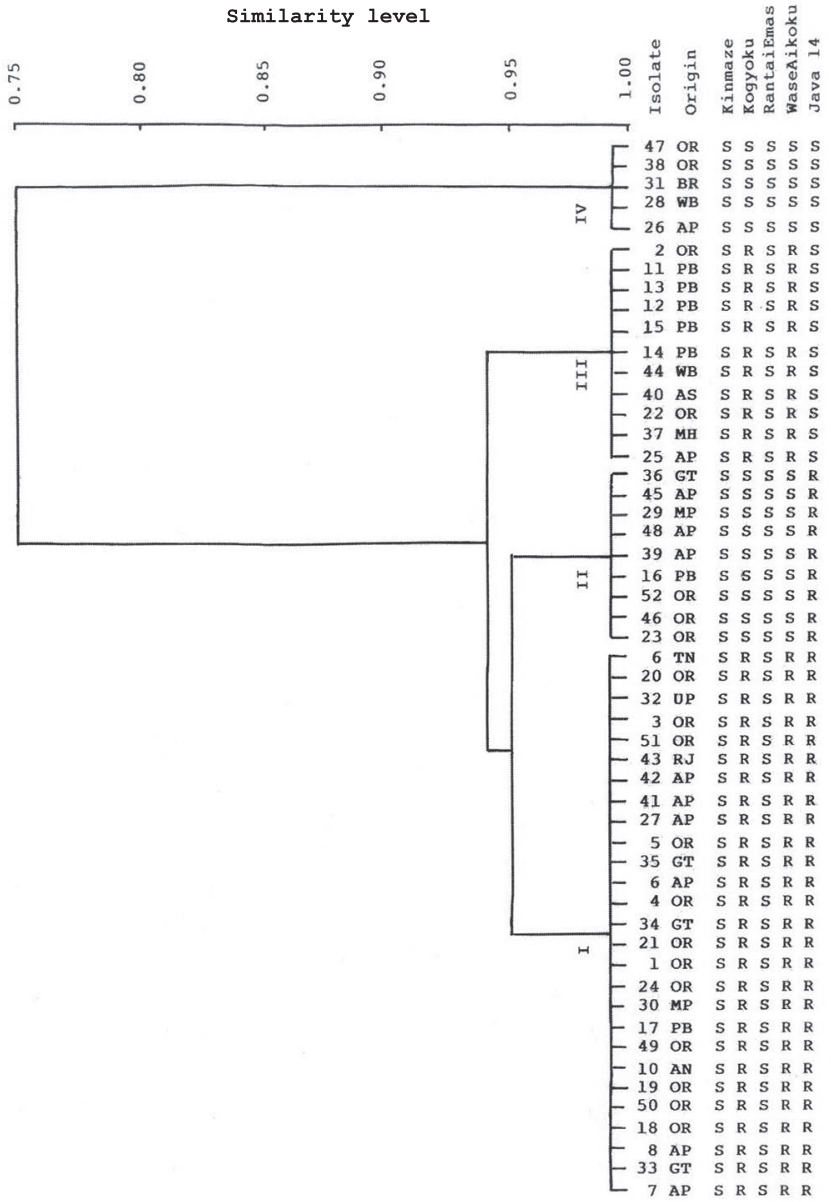

Fig. 1. Dendrogram showing the similarity and successive clustering of 52 isolates of *X. oryzae* pv. *oryzae*, based on their virulence patterns on Japanese differentials

Identification of pathotypes based on IRRI differentials

Pathotyping of the 52 isolates with the help of five IRRI differentials *viz*. IR-8, IR-20, IR-1545-339, DV-85 and Cas-209, through numerical analysis resulted in identification of five clusters of isolates depicted in a dendrogram (Fig. 2). Cluster-I constituted of 27 isolates originating from nine states of India and exhibiting the virulence pattern of SSRRR on five differential genotypes (Table 3). Cluster-II comprised of five isolates originating from three states with a virulence pattern of SSRRR. These two groups of isolates, although exhibited similar qualitative virulence pattern, quantitatively they formed different groups. Cluster-III was composed of 11 isolates, originating from six states and showed a virulence pattern of SSRRS. Clusters-II and III merged into a single group at a similarity level of 0.98, which ultimately blended with the largest group of 27 isolates (cluster-I), at a similarity level of 0.94. These three broad groups jointly merged with cluster-V consisting of four isolates originating from two states with a virulence pattern of SSSSR, at a similarity level of 0.91. Cluster-IV, constituting of five highly virulent isolates with the virulence pattern of SSSSS and originating from four states of India, maintained its independent identity to merge with the other four clusters at a similarity level of 0.75. These results also showed non-parallelism between geographical distribution and clustering pattern. A comparison between the Japanese (Table 3) and IRRI (Table 4) pathotyping methods revealed that the clusters-I, III and IV were identical in both the systems, while cluster-II in Japanese system was divided into clusters-II and IV in IRRI system.

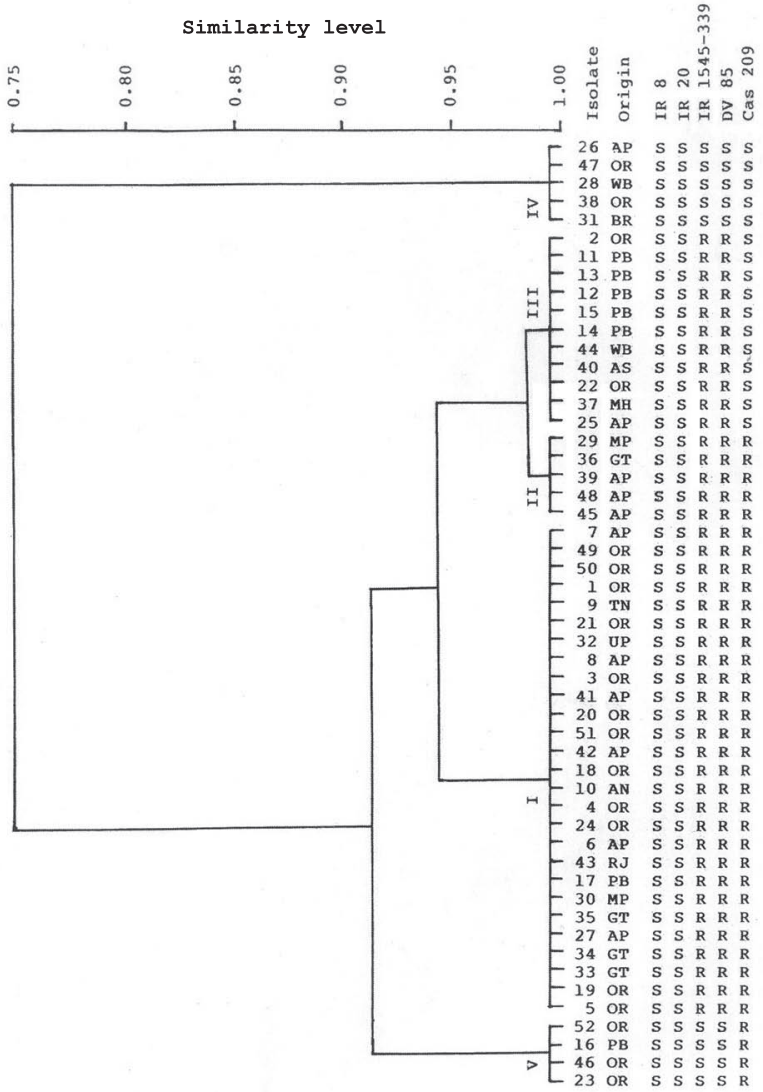

Fig. 2. Dendrogram showing the similarity and successive clustering of 52 isolates of *X. oryzae* pv. *oryzae,* based on their virulence patterns on IRRI differentials

Table 2. Grouping of Indian isolates of *X. oryzae* pv. *oryzae* according to Japanese differentials based on numerical analysis

Groups	No. of isolates	Isolates	Origin
I	27	1, 3, 4, 5, 6, 7, 8, 9, 10, 17, 18, 19, 20, 21, 24, 27, 30, 32, 33, 34, 35, 41, 42, 43, 49, 50, 51	Andaman and Nicobar Islands, Andhra Pradesh, Gujarat, Madhya Pradesh, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh
II	9	16, 23, 29, 36, 39, 45, 46, 48, 52	Andhra Pradesh, Gujarat, Madhya Pradesh, Orissa, Punjab
III	11	2, 11, 12, 13, 14, 15, 22, 25, 37, 40, 44	Andhra Pradesh, Assam, Maharashtra, Orissa, Punjab, West Bengal
IV	5	26, 28, 31, 38, 47	Bihar, Orissa, West Bengal

Table 3. Grouping of Indian isolates of *X. oryzae* pv. *oryzae* according to IRRI differentials based on numerical analysis

Groups	No. of isolates	Isolates	Origin
I	27	1, 3, 4, 5, 6, 7, 8, 9, 10, 17, 18, 19, 20, 21, 24, 27, 30, 32, 33, 34, 35, 41, 42, 43, 49, 50, 51	Andaman and Nicobar Islands, Andhra Pradesh, Gujarat, Madhya Pradesh, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh
II	5	29, 36, 39, 45, 48	Andhra Pradesh, Gujarat, Madhya Pradesh
III	11	2, 11, 12, 13, 14, 15, 22, 25, 37, 40, 44	Andhra Pradesh, Assam, Maharashtra, Orissa, Punjab, West Bengal
IV	5	26, 28, 31, 38, 47	Andhra Pradesh, Bihar, Orissa, West Bengal
V	4	16, 23, 46, 52	Orissa, Punjab

Table 4. Clustering pattern of 41 rice genotypes, based on virulence pattern of 52 isolates of *X. oryzae* pv. *oryzae*

Cluster	Genotypes	Origin	Cluster mean [cm]	Range [cm]
I	**TKM 6*,** Kogyoku, Tetep, BJ 1, Wase Aikoku, CNGS 20083, Saket 4, ARC 10464, HKR 120, IR 64, IS 134, IR 50, Chinsurah Boro-II	India, IRRI, Japan, Vietnam	5.35	3.6–12.2
II	**DV 85,** IR 1545-339, PR 109, Semora mangga, Zenith, Malagkit Sung Song	Bangladesh, India, Indonesia, IRRI	3.50	2.3–8.5
III	**PN 13,** PN 56, PN 239, IR 54, Cas-209, PN 82, Java 14, PN 225	India, IRRI, Japan	3.97	2.6–8.0
IV	**IET 8585,** IET 4141, IET 9910, IET 7029, IET 8319, IET 9263	India	4.91	3.1–9.1
V	**IR 8,** Rantai Emas, Cemposelek, IR 20, IR 32, Kinmaze, IR 60, IR 36	IRRI, Japan	15.59	13.8–21.6

*selected differential varieties are printed in bold letters

Selection of new Indian differentials and identification of pathotypes

The 41 rice genotypes of diverse origin could be grouped into five clusters through cluster analysis done by considering their reactions against each of the 52 isolates as the operational taxonomic units (OTU). The clustering pattern depicted in the dendrogram (Fig. 3) clearly revealed that each group of genotypes joined together to construct independent clusters at a similarity level of 0.96. Clusters-III and IV merged into a single group at a similarity level of 0.91 which in turn merged with cluster-II at similarity level of 0.89 and cluster-I at a close similarity level of 0.88. A group of eight genotypes constituting cluster-V maintained its independent identity, which ultimately merged with the blended group of clusters-I, II, III and IV at a similarity level of 0.50. The extract from the dendrogram (Table 4) revealed that cluster-I was composed of 13 genotypes originating from widely different eco-geographical regions involving India, Japan, Vietnam and Philippines, with mean lesion length ranging from 3.6 to 12.2 cm. Cluster-II was composed of six genotypes originating from five countries *viz*. India, Bangladesh, Indonesia, Philippines and USA; and mean lesion length ranging from 2.3 to 8.5 cm. The eight genotypes originating from three countries namely India, Japan and Philippines and the mean lesion length ranging from 2.6 to 8.0 cm constituted of cluster-III. Cluster-IV consisting of six genotypes, all originating from India, exhibited their reaction against 52 isolates ranging from 3.1 to 9.1 cm. It is interesting to note that cluster-V was composed of eight genotypes, originating from two east Asian countries of Japan and Philippines and exhibited highest lesion length ranging from 13.8 to 21.6 cm. Among them, IR-8 and IR-20 are IRRI differentials and Kinmaze and Rantai Emas are Japanese differentials, all exhibiting highly susceptible reactions against all the 52 isolates used in the present investigation.

The existence of significant differential interaction among the host genotypes, the pathogen isolates and in their interaction was evidenced from the analysis of variance (Table 1). A critical insight into the host pathogen interaction data revealed that specific genotypes exhibited differential reactions against specific isolates. Such differential reactions could be detected in 10 genotypes *viz*. IR-8, TKM-6, DV-85, IET-8585, CB-II, Cas-209, PN-13, Semora Mangga, Malagkit Sung Song and BJ-1. Drawing of a total number of 45 scattered diagrams involving each permutation and combinations of these 10 genotypes revealed highly specific differential interactions among the genotypes *viz*. IR-8, TKM-6, DV-85, PN-13 and IET-8585. Among them IR-8 is a high yielding, semi-dwarf, photo insensitive variety with 130 days duration possessing Xa-11 gene against a group of Philippines isolates. It develops typical susceptible lesions with water soaking and longitudinal folding of the infected leaves. It was chosen as a susceptible check against all the bacterial isolates tested and would help identification of new weakly virulent pathotypes in future. The second differential TKM-6, a tall *indica*, 130 days duration, possessing Xa-4 gene, has been used as a donor in many crossing programmes at IRRI. It exhibited resistant reactions to all the isolates except CRXoo 16, 23, 26, 28, 29, 31, 36, 38, 39, 45, 46, 47, 48 and 52; characterized by yellowish brown, without water soaking lesions with restricted growth. The third differential DV-85, carrying Xa-5 and Xa-7 genes, produced resistant reaction against all isolates, except CRXoo-16, 23, 26, 28, 31, 38, 46, 47 and 52; characterized by small restricted yellowish brown lesions without water soaking. Both IR-8 and DV-85 are also IRRI differential genotypes. The fourth differential, PN-13 (cross between Pankaj/Nagariboa bred at CRRI) exhibited both resistant and susceptible reaction against different groups of bacterial isolates with mean lesion length ranging from 2.7 to 8.4 cm. The resistant reaction was marked by the expression of brown necrotic lesions progressing very slowly, while the susceptible reaction was expressed by typical yellow or yellowish brown lesions without water soaking produced by the isolates CRXoo 2, 11, 12, 13, 14, 15, 22, 26, 28, 31, 38 and 47. The fifth differential IET-8585 [{(IR 8 x BJ 1) / IR 22} / CR- 98-7216] developed slight water soaking pale yellow type of susceptible lesions against the isolates CRXoo 22, 25, 26, 28, 29, 31, 36, 37, 38, 39, 40, 44, 45, 47 and 48. The typical resistant reactions marked brown necrotic lesions and delayed initiation of the symptoms.

Pathogenicity pattern of the isolates on new set of Indian differentials

All the 52 isolates were highly virulent on the rice genotype IR-8, exhibiting susceptible reaction (Fig. 4). The second differential TKM-6 exhibited susceptible reaction against CRXoo 16, 23, 26, 28, 29, 31, 36, 38, 39, 45, 46, 47, 48 and 52. The third differential DV-85 exhibited

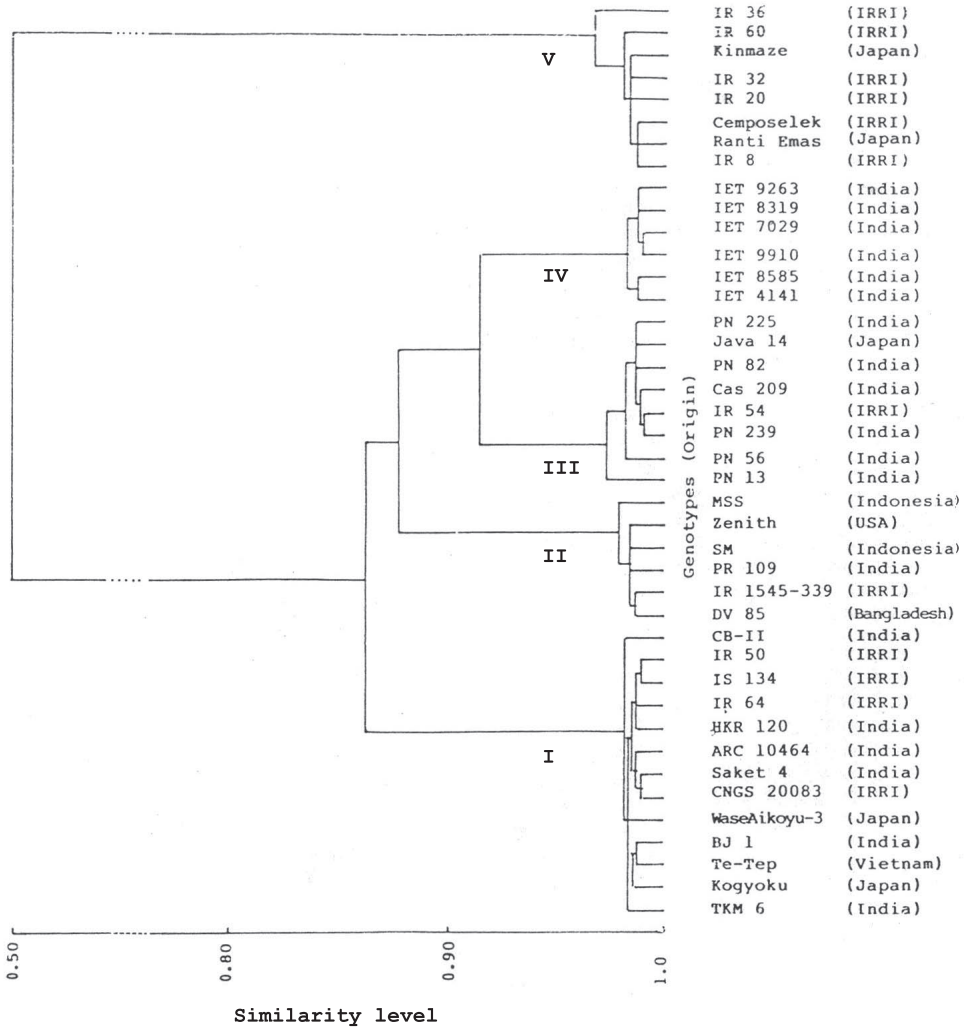

Fig. 3. Dendrogram showing the similarity and successive clustering of 41 rice genotypes on the basis of their reactions against 52 isolates of *X. oryzae* pv. *oryzae*

Table 5. Analysis of variance of five differential varieties tested for their response against 52 isolates of *X. oryzae* pv. *oryzae*

Source	DF	SS	MS	F
Replications	3	37.02	12.34	0.70
Isolate (I)	51	25080.69	491.78	27.76**
Varieties (V)	4	117.94	29.49	1.66*
Interaction (I x V)	204	8298.64	40.68	2.30**
Error	777	13762.99	17.71	
Total	1039	47297.29	45.52	

* and **significant at p = 0.05 and 0.01 levels
DF – degrees of freedom; MS – mean sum of square; SS – sum of square; F – variance ratio

susceptible reaction to nine isolates *viz*. CRXoo 16, 23, 26, 28, 31, 38, 46, 47 and 52. The fourth differential PN-13 exhibited susceptible reaction against 11 isolates *viz*. CRXoo 2, 11, 12, 13, 14, 15, 26, 28, 31, 38 and 47. Similarly, 15 isolates namely CRXoo 22, 25, 26, 28, 29, 31, 36, 37, 38, 39, 40, 44, 45, 47 and 48 exhibited highly virulent reaction on the fifth differential genotype IET 8585. Thus the new differential genotypes exhibited specific differential reactions against the 52 isolates of the pathogen. The analysis of variance between 52 isolates and five new Indian differential genotypes (Table 5) revealed significant differences among the isolates, differential genotypes as well as in their interactions. This suggested that the isolates differed in virulence and the host-genotypes differed in vertical resistance.

Standard virulence pattern for numbering the pathotypes

The isolates could be numbered according to the standardized system developed on the basis of the virulence pattern on the five differential host genotypes. Based on the qualitative reaction pattern (R = resistant and S = susceptible) of the five differentials, a maximum number of 32 pathotypes can be differentiated as per the computer generated output of the host response in all possible permutations and combinations (Table 6). The 32 pathotypes would consist of all the group of pathogen strains with the virulence pattern ranging from SSSSS to SRRRR on the five differential host genotypes in the order of IR-8, TKM-6, DV-85, PN-13 and IET-8585. Any bacterial strain exhibiting highly virulent reaction (S) on IR-8, would come under pathotype groups of 1 to 16. The designation of the pathotype will however depend on its virulence pattern on the five differential host genotypes in different

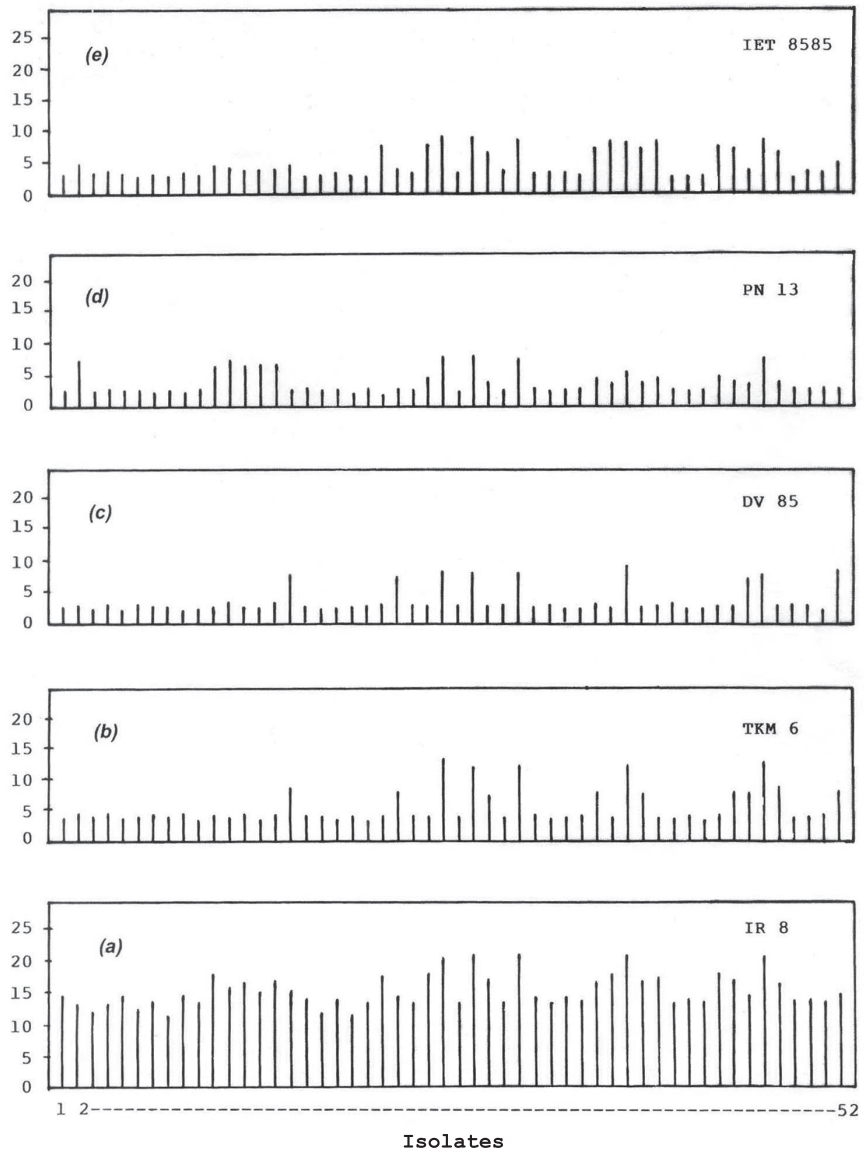

Fig. 4. Virulence levels of 52 isolates of *X. oryzae* pv. *oryzae* on a set of five new Indian differentials

permutation combinations (Table 6). Any bacterial strain exhibiting highly virulent (S) reaction on the second differential genotype TKM-6 but 'R' reaction exhibited by the first differential IR-8, would be designated with a pathotype number between 17 to 24 depending upon the reaction of other three differential genotypes i.e. DV-85, PN-13 and IET-8585. The bacterial strain exhibiting virulent reaction (S) on the third differential genotype, DV-85 with a resistant reaction shown by the first and second differentials IR-8 and TKM-6, would be designated by a number between 25 to 28, depending upon the reaction pattern on PN-13 and IET-8585 in different combinations. When the fourth differential host-genotype PN-13 exhibits highly susceptible reaction with a corresponding highly resistant reaction shown by the first three differentials (IR-8, TKM-6 and DV-85), the pathogen strain would be designated with a number either 29 or 30 depending upon the reaction pattern on IET 8585. The last differential genotype IET-8585 showing highly susceptible reaction with a corresponding resistant reactions shown by the first four differentials (IR-8, TKM-6, DV-85 and PN-13) against a particular bacterial isolate, the pathotype number 31 would be assigned to that strain. The most weakly virulent strain, which produces typical necrotic lesions on all the five differential genotypes resulting in a virulence pattern of RRRRR, would be designated as pathotype-32.

Clustering pattern and identification of pathotypes

The 52 isolates could be grouped into six clusters through hierarchical method of numerical analysis depicted in a dendrogram (Fig. 5). The extract from the dendrogram on the clustering pattern; identified and numbering of pathotypes based on the standard chart compared with those through Japanese land IRRI pathotyping are presented in Table 7. Cluster-I constituting of largest number of 27 isolates with a common reaction pattern of SRRRR was identified as pathotype-16. Cluster-II, composed of six isolates originating from two states, with a common reaction pattern of SRRSR against the five differentials, was designated as pathotype-14. Similarly, five isolates namely CRXoo 22, 25, 37, 40 and 44 with a common virulence pattern of SRRRS constituted of cluster-III, which was designated as pathotype-15. Cluster-IV constituting

Table 6. Standard chart for numbering of pathotypes on the basis of qualitative reaction of *X. oryzae* pv. *oryzae* on five Indian differentials

Pathotype group	Indian differentials				
	IR 8	TKM 6	DV 85	PN 13	IET 8585
1	S	S	S	S	S
2	S	S	S	S	R
3	S	S	S	R	S
4	S	S	S	R	R
5	S	S	R	S	S
6	S	S	R	S	R
7	S	S	R	R	S
8	S	S	R	R	R
9	S	R	S	S	S
10	S	R	S	S	R
11	S	R	S	R	S
12	S	R	S	R	R
13	S	R	R	S	S
14	S	R	R	S	R
15	S	R	R	R	S
16	S	R	R	R	R
17	R	S	S	S	S
18	R	S	S	S	R
19	R	S	S	R	S
20	R	S	S	R	R
21	R	S	R	S	S
22	R	S	R	S	R
23	R	S	R	R	S
24	R	S	R	R	R
25	R	R	S	S	S
26	R	R	S	S	R
27	R	R	S	R	S
28	R	R	S	R	R
29	R	R	R	S	S
30	R	R	R	S	R
31	R	R	R	R	S
32	R	R	R	R	R

R – resistant; S – susceptible

Table 7. Grouping of *X. oryzae* pv. *oryzae* isolates according to Indian differentials compared with IRRI and Japanese differential varieties

Groups/ Clusters	No. of isolates	Isolates	Origin (states)	Cluster mean [cm]	Reaction pattern	Designated pathotype group	No. of v-factors	IRRI pathotype group	Japanese pathotype group
I	27	CRXoo1, 3, 4, 5, 6, 7, 8, 9, 10, 17,18,19, 20, 21, 24, 27, 39, 32, 33, 34, 35, 41, 42, 43, 49, 50, 51	Andaman and Nicobar Islands, Andhra Pradesh, Gujarat, Madha Pradesh, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh	5.38	SRRRR	16	4	I	I
II	6	CRXoo 2, 11, 12, 13, 14, 15	Orissa and Punjab	6.94	SRRSR	14	7	III	III
III	5	CRXoo 2, 25, 37, 40, 47	Aandhra Pradesh, Assam Maharashtra, Orissa, West Bengal	7.88	SRRRS	15	7	III	III
IV	5	CRXoo 29, 36, 39, 45, 48	Andhra Pradesh, Gujarat, Madhya Pradesh	7.99	SSRRS	7	8	II	II
V	4	CRXoo 16, 23, 46, 52	Orissa and Punjab	7.77	SSSRR	4	10	V	II
VI	5	CRXoo 26, 28, 31, 38, 47	Andhra Pradesh, Bihar, Orissa, West Bengal	12.13	SSSSS	1	11	IV	IV

R – resistant
S – susceptible

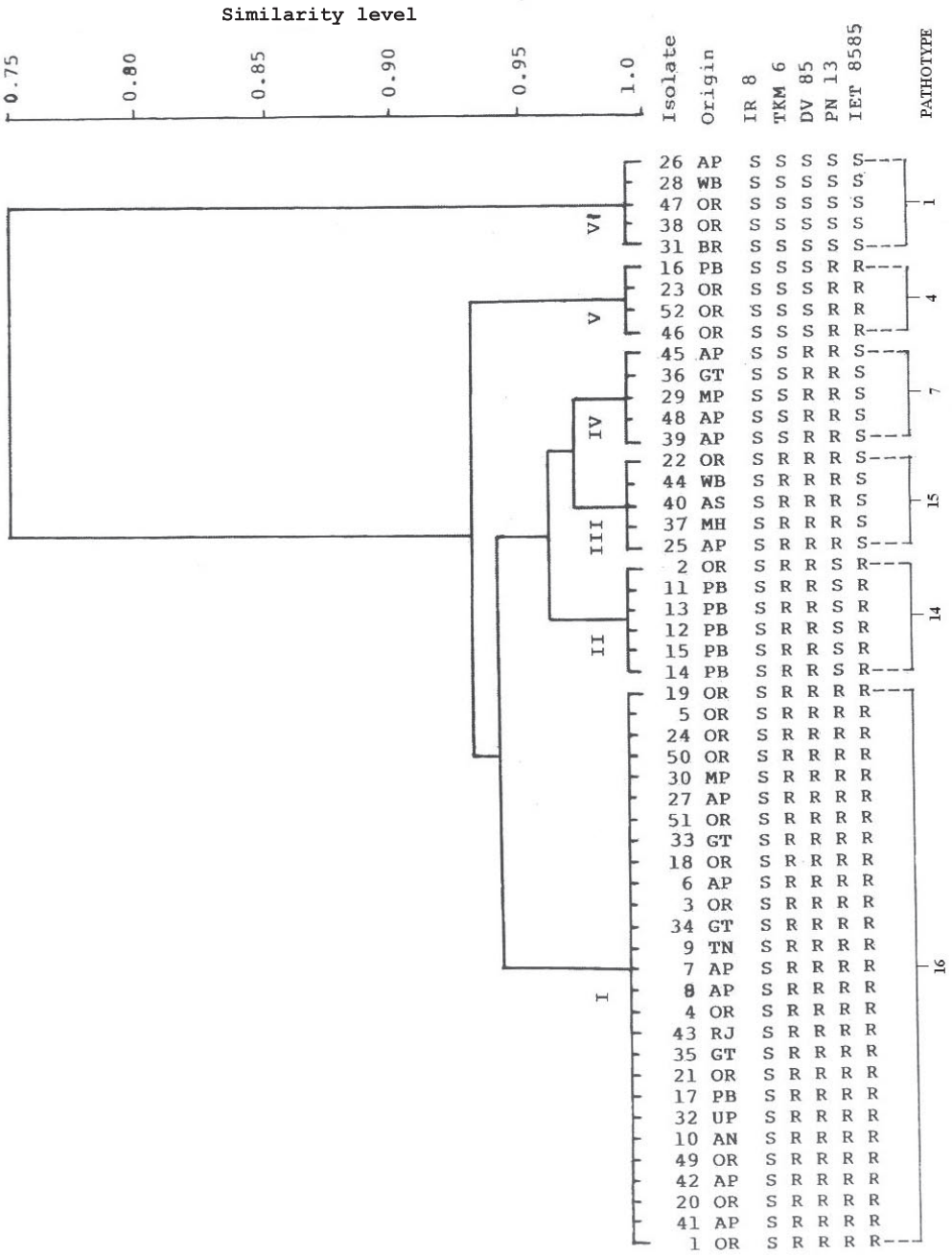

Fig. 5. Dendrogram showing the similarity and successive clustering of 52 isolates of *X. oryzae* pv. *oryzae*, based on their virulence patterns on selected Indian differentials

of five isolates with a common virulence pattern of SSRRS was designated as pathotype-7. Similarly, four isolates, originating from the states of Orissa and Punjab, with a common virulence pattern of SSSRR, was composed of cluster-V which was designated as pathotype-4. Cluster-VI was composed of five most virulent isolates namely CRXoo 26, 28, 31, 38 and 47, originating from four different states and exhibiting a common virulence pattern of SSSSS was designated as pathotype-1. It is of interest to note here that pathotype-1 was equivalent to IRRI as well as Japanese pathotype group-IV and pathotype-16 was equivalent to IRRI as well as Japanese pathotype group-I. Pathotypes-4 and 7 were equivalent to IRRI pathotype groups-V and II and Japanese pathotype group-II. Pathotypes-14 and 15 together were equivalent to IRRI as well as Japanese pathotype group-III.

Geographical distribution

The geographic distribution of the six identified pathotypes in different states of India (Fig. 6), revealed that the most virulent pathotype-1 was distributed over four eastern states namely Andhra Pradesh, Bihar, Orissa and West Bengal, while the two pathotypes-4 and 14 were spread over two states *viz*. Orissa and Punjab. Pathotype-7 was distributed over the states of Andhra Pradesh, Gujarat and Madhya Pradesh, while the pathotype-15 was

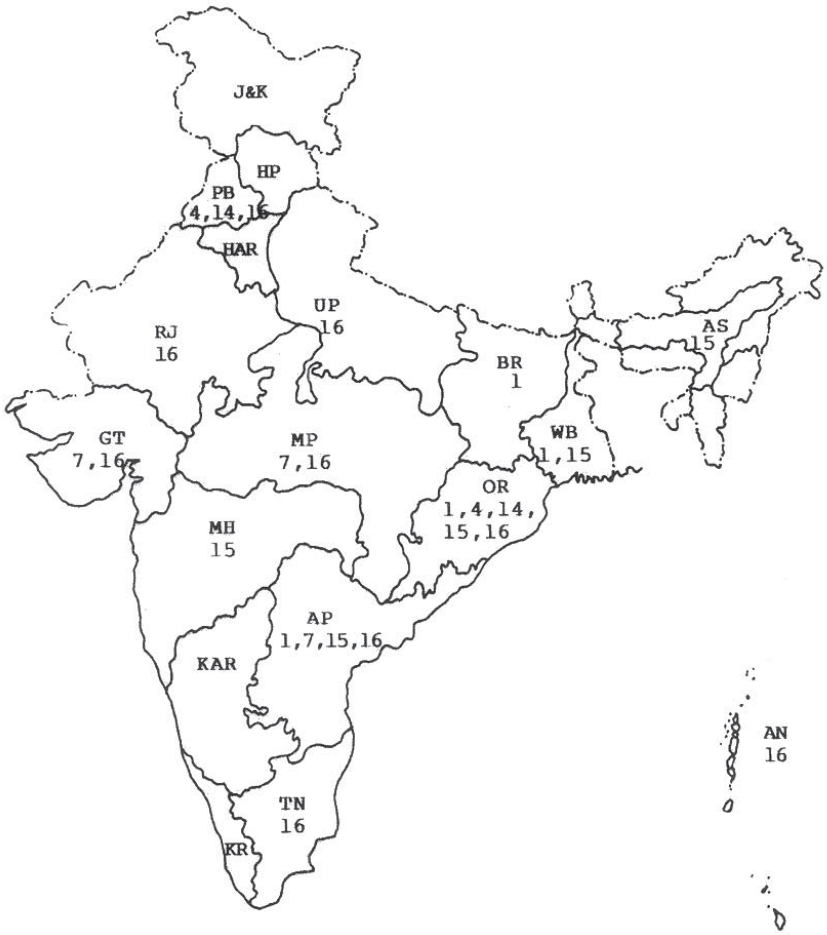

Fig. 6. Geographical distribution of six identified pathotypes of *X. oryzae* pv. *oryzae* in India

spread over five states *viz*. Andhra Pradesh, Assam, Maharashtra, Orissa and West Bengal. The pathotype-16, constituting of a maximum number of 27 bacterial isolates, was spread over eight states and the Union Territory of Andaman and Nicobar Islands, all of which are situated in widely different eco-geographical regions of the country. Among the states, a maximum number of five pathotypes (pathotypes 1, 4, 14, 15 and 16) were present in Orissa, followed by four (pathotypes-1, 7, 15 and 16) in Andhra Pradesh and three (pathotypes-4, 14 and 15) in Punjab state. Thus the pathotypes were widely distributed all over the country.

DISCUSSION

The host pathogen interaction in any biological system is the mathematical expression of the response between the host genotypes and the pathogen strains a phenomenon, which can be analyzed through analysis of variance (ANOVA). Characterization of the race specific/non-specific resistance in the host and cultivar specific/non-specific virulence in the pathogen can be made from the data on ANOVA by presence or absence of significant differential interactions between the host genotypes and pathogen strains. The presence of a significant differential interaction accompanied by significant differences among the host-genotypes as well as among the pathogen strains signifies that the genotypes differ in vertical resistance and the pathogen strains differ in virulence (van der Plank 1968). The existence of a significant differential interaction between the 41 host genotypes and 52 pathogen strains observed in the present investigation (Table 1) clearly demonstrated a differential response of the host genotypes to the pathogen strains, there by proving the presence of physiologic specialization in *Xanthomonas oryzae* pv. *oryzae*. Such phenomenon of the presence of physiologic specialization in *Xoo* has been demonstrated by Devadath and Padmanabhan (1969), Ezuka and Horino (1974), Reddy and Ou (1976), Nayak (1986) and Noda *et al*. (1990). On the contrary, Ou *et al*. (1971) could not find any distinct interaction between 24 rice cultivars and 50 isolates of *Xoo,* which was attributed to be due to the much wider range of pathogenicity of the isolates on some cultivars than others.

The existence of a significant differential interaction between the host genotypes and pathogen isolates (Table 1), the grouping of 41 host genotypes into five distinct clusters even at a similarity level of 0.96 (Fig. 3) and a specific differential response of certain genotypes to specific isolates resulted in identification of five host genotypes namely IR-8, TKM-6, DV-85, PN-13 and IET 8585 for use as differentials to classify Indian isolates into pathotypes.

Among them, IR 8 and DV 85 are also IRRI differentials, of which DV 85 has been reported to be resistant to all the pathotypes from Japan and Philippines (Horino *et al*. 1980), while IR 8 is used as a susceptible check. TKM-6 possessing Xa-4 gene has been widely used as one of the parents in many crosses made at IRRI as well as India. Although the genic constitution of PN 13 and IET 8585 are not known, each of them has the distinct characteristics of differentiating Indian isolates of *Xoo*. The differential variety IR 8, even though exhibited susceptible reaction to all the 52 isolates tested, would help identification of weakly virulent pathotypes in future. In view of the broad spectrum of variability among the bacterial strains collected from different countries, it is difficult to apply a single international differential system and hence scientists in each country have developed their own differential sets. It is an established fact that Indian isolates of *Xoo* are most virulent and are capable of knocking down many IRRI and Japanese differentials. The highly susceptible reaction of the Japanese differentials Kinmaze and Rantai Emas and the IRRI differentials IR 8 and IR 20 against all the 52 isolates tested in the present experiment are the bright examples. The present set of differentials have special significance since they exhibited distinct differential interaction against 52 isolates collected from widely different rice growing states of the country. However, any differential system can not be claimed as permanent, unless it is capable of differentiating strains collected from various agro-ecological regions. Although the present set of 52 *Xoo* strains have been collected from 12 rice growing states and one Union Territory, there is a need for further improvement of the differential system through testing of more number of strains as well as addition or deletion of new genotypes based on their genetic constitution, which will be our next attempt.

Ogawa (1993) suggested the strategy for monitoring the race distribution of *Xoo* by using near-isogenic lines (NIL) and proposed a set of differentials possessing known genes for resistance to *Xoo*. Unfortunately, certain Indian isolates possess the virulent factors to overcome the R-genes present in these NILs *eg*. Xa-1, 2, 3, 4, 5, 7, 10 and 12 (Gupta *et al*. 1986), Xa-1, 3, 4, 5, 7 and 11 (Khare and Thrimurty 2006), Xa-1, 3, 4, 5, 7, 10, 11, 13 and 21 (Shanti and Shenoy, 2005), Xa-1, 2, 3, 4, 5, 6, 7, 10, 11, 12 and 13 (Nayak *et al*. unpublished). Hence, there is a need to identify new genes governing resistance against most virulent Indian strains of the bacterium and develop NILs possessing such genes, before their utilization as differentials. Till then the present system of identification of races of *Xoo* has to be continued.

The 52 isolates of *Xoo* could be classified into six pathotypes with the help of these five differential varieties which were designated as pathotypes-1, 4, 7, 14, 15 and 16 following the standard computer generated virulence patterns. Among them, pathotype-1 constituting of five isolates was most virulent and possessed 11 matching v-factors to overcome the R-genes of Xa 1, 2, 3, 4, 5, 6, 7, 10, 11, 12 and 13, while pathotype-16 was least virulent consisting of a maximum number of 27 isolates possessing four v-factors to overcome the R-genes Xa-1, 2, 4 and 11 and was widely distributed over eight states and one Union Territory of India. Such classification was also confirmed through the number of V-factors present in specific isolates corresponding to the R-genes present in the host genotypes (Nayak *et al*. unpublished). Although the distribution of the most virulent pathotype-1 is at present limited to the eastern states of Andhra Pradesh, Orissa, West Bengal and Bihar; collection and testing of more number of isolates from each state may reveal a different picture. The free exchange of genetic material through various testing programmes of All India Coordinated Trials would effectively spread the virulent pathotypes into other states. Hence, a continuous country-wide monitoring of the prevalence of pathotypes is felt essential.

Following the first report on the clear-cut differential reaction of nine rice genotypes against nine isolates of *Xoo.* (Devadath and Padmanabhan 1969), several workers in India have identified pathotypes using either Philippines or Japanese differentials or through a set of differential varieties selected by each group (Rao *et al*. 1971; Kauffman and Pantulu 1972; Gupta *et al*. 1986; Reddy and Reddy 1990; Nayak and Reddy 1993). The present set of differential varieties are capable of differentiating Indian isolates possessing wide range of virulence levels. The grouping of 52 isolates into six pathotypes with the help of the present set of differentials are also comparable with Japanese pathotype groups-I, II, III and IV and IRRI pathotype groups of I, II, III, IV and V. The numbering system of the pathotypes will have an additional advantage of uniformity all over the country so that in future if new pathotypes are identified those will be comparable with the already identified pathotypes. The available information on host-pathogen interaction indicates considerable variation in virulence among the bacterial strains in Asian countries, but the lack of a functional differential system and uniform criteria to score the reaction have made the progress slow (Mew 1987). It is expected that the present techniques of clustering rice genotypes, identification of differential genotypes, clustering of pathogen isolates on the basis of their virulence pattern, development of a method to designate the clusters of bacterial strains into pathotypes and finally confirmation of this method of classification with those on the basis of V-factor present in the pathogen strains will fill up the gap in research on pathotype identification.

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POLISH SUMMARY

ZMIENNOŚĆ *XANTHOMONAS ORYZAE* PV. *ORYZAE*, CZYNNIKA WYWOŁUJĄCEGO BAKTERYJNE ZAMIERANIE RYŻU

Model wirulencji 52 szczepów bakterii *Xanthomonas oryzae* pv. *oryzae*, czynnika sprawczego bakteryjnego zamierania ryżu, badano na 41 genotypach ryżu, wykorzystując pięć japońskich i pięć filipińskich odmian różnicujących. Wśród izolatów bakterii zaobserwowano istotną reakcję różnicującą, a ich interakcja z genotypami żywiciela sugerowała, że genotypy te różniły się pionową odpornością, a izolaty bakterii różniły się wirulencją. Dwie japońskie odmiany różnicujące IR8 i IR20, wykazywały wysoce wrażliwe reakcje przeciwko wszystkim 52 izolatom bakterii. Wybrano pięć nowych indyjskich odmian różnicujących, po jednej z każdego skupienia genotypów wybranych przy zastosowaniu metody hierarchii analizy cyfrowej modelu wirulencji 52 izolatów bakterii na 41 genotypach żywiciela. 52 genotypy bakterii mogły być zgrupowane w sześciu skupieniach na podstawie ich modelu patogeniczności na pięciu nowych, indyjskich odmianach różnicujących, które określono jako patotypy 1, 4, 7, 14, 15 i 16, wykorzystując standardową, uzyskaną przy pomocy komputera mapę. Te patotypy były porównywane z japońskimi grupami patotypów I, II, III, IV i grupami filipińskimi patotypów I, II, III, IV i V. Najbardziej wirulentny patotyp-1 był rozprzestrzeniony w czterech wschodnich stanach Indii, mianowicie w Andora Pradesh, Orisa, Bengal Zachodni i Bihar. Z punktu widzenia wolnej wymiany materiału genetycznego w kraju, ciągłe monitorowanie występowania nowych patotypów przy wykorzystaniu obecnego zestawu odmian testowych przyspieszy program hodowli i pomoże zwalczać chorobę poprzez wprowadzenie specyficznych dla lokalizacji odmian odpornych.