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REGULAR ARTICLE

Measurement of genetic diversity of virulence in populations of *Xanthomonas oryzae* pv. *oryzae* in India

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ABSTRACT

This work was designed to ascertain the extent of genetic diversity in the pathogen population of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the causal organism of bacterial blight of rice. The virulence of 52 strains of *Xoo*, collected from 12 rice growing states of India, were clip-inoculated on 16 rice genotypes possessing known genes for resistance. Based on the genetic distance, estimated by multivariate analysis isolates of *Xoo* could be classified into 13 clusters and five broad groups. The genetic variability of virulence in populations of *Xoo* was also measured by estimation of diversity indices viz. Shannon's information function (H'), Levin's diversity index (B), Hill's generalized entropy (H_2), trophic diversity index (D), dominance of virulence factors (λ), the index of evenness or equitability ($J_{H'}$), genetic richness index (d) and normalized pathotypic diversity index (H^n). Wide genetic diversity in the pathogen populations from the country as well as from the eastern and southern regions was indicated by high estimates of H', B, H_2, D, λ, d and low $J_{H'}$. High estimates of H^n were attained for almost all populations of *Xoo*. With regard to the states, genotypic and pathotypic diversities were high in Andhra Pradesh and Orissa, while pathotypic diversity was high in the pathogen population from Gujarat, Punjab, Madhya Pradesh, Uttar Pradesh and Tamil Nadu. The existence of high diversities and the grouping of the pathogen isolates into clusters of similar in virulence facilitated a better understanding of the population structure of *Xoo*, to guide regional rice breeding programs and the deployment of resistance genes in disease control strategy.

Key Words: *bacterial blight; genetic distance; pathogen population; rice; virulence factors.*

INTRODUCTION

Bacterial blight of rice, incited by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Dye, is a serious disease and causes extensive yield losses, which may reach 65-95% during epiphytotic seasons (Reddy, 1980). Host resistance is given priority in disease control strategy, since it is ecofriendly and inexpensive for rice-growing farmers. More than 25 *Xa* genes conferring resistance to *Xoo* have been identified and cultivars possessing such genes have been recommended for cultivation in different areas. Widespread repeated use of a few resistance genes might accelerate the selection of new pathogenic races at a rate equivalent to 1.64 times the increase in specific virulence of the isolates following resistance host-plant selection pressure after a single crop cycle (Nayak, 1986b). This will lead to a change in pathogen population structure through either mutation or recombination to adapt itself to the new resistant host plant or environmental changes. The population structure of the pathogen needs to be monitored and understood, to guide selection and deployment of resistance genes.

Population structure refers to the measurement of pathogenicity association coefficients and virulence association coefficients, the amount of genetic diversity in a population, and the phylogenic relationships and partitioning of the variation in time and space (Leung et al., 1993). Quantification methods of the relationships between a host and a pathogen have been approached by Wolfe et al. (1976), Browder and Eversmeyer (1977) and Lebeda (1982), they solely evaluated the genetic relationships by either the frequency of virulence factors or quantified the degree of association between the resistance factor in the host and the virulence factor in the pathogen population, in a manner similar to the gene-for-gene relationship (Flor, 1955).

Previous studies on phenotypic and genotypic diversity of *Xoo* were based on differential interactions with resistance genes (Adhikari et al., 1994, 1999; Ezuka and Horino, 1974; Gupta et al., 1986; Noda et al., 1990, 1996; Mew et al., 1992). Molecular techniques such as restriction fragment length polymorphism (RFLP) analysis have been successfully used to detect genetic variability in populations of *Xoo* in the Philippines and other Asian countries (Leach et al., 1992; Nelson et al., 1994; Adhikari et al., 1995; Ardales et al., 1996; Kaku et al., 1996; Yashitola et al., 1997; Ochiai et al., 2000). Adhikari et al. (1995) reported regional differentiation among *Xoo* populations and suggested strategies that target regional breeding programs and gene deployment.

Ecologists and mathematicians have suggested a large number of diversity indices (Rao, 1980; Lebeda, 1982). A few indices of genetic diversity such as the Shannon's information function H' (Shannon and Weaver, 1949), Simpson's index on the concentration of dominance of *v*-factors 'λ' (Simpson, 1949), Levin's measure of diversity B (Levin, 1968), the diversity in the form of "generalized entropy" H_2 (Hill, 1973), the index of evenness or equitability of *v*-factors $J_{H'}$ (Sheldon, 1969), the trophic diversity index 'D' (Herrera, 1976) and the genetic richness index 'd' (Margalef, 1958) have been used to quantify and compare the genetic diversity of virulence factors in fungal pathogen populations of *Bremia lactucae* (Lebeda, 1982). These indices give enough weight to the relative abundance of *v*-factors along with species richness but fail to take into account the taxonomic distances between entities (Watve and Gangal, 1996). The use of multivariate analysis methods based on Mahalanobis's D^2 statistics (Rao, 1952) has been used for estimation of genetic diversity among host genotypes for resistance to different components of slow-blasting resistance in rice (Mukherjee et al., 1999). Such analysis measures genetic diversity, inter- and intra-cluster distances between and within clusters of entities i.e. the host genotypes. First attempts at the measurement of genetic diversity in pathogen populations of *Xoo* were by Leach et al. (1992) and Adhikari et al. (1995) through the use of RFLP and virulence analyses.

These diversity indices measure different components of genetic diversity such as the genetic distance between clusters of isolates, frequency or relative frequency of occurrence of

virulence factors (*v*-factors), concentration of dominance of *v*-factors, generalized entropy, normalized pathotypic diversity or genetic richness of the pathogen isolates. Diversity measured by one method cannot readily be compared with that measured by another (Watve and Gangal, 1996). However, comparison of diversity values makes it possible to study genetic differences among pathogen populations and to evaluate the genetic structure of virulence in space and time on the basis of single quantitative data (Lebeda, 1982). The exact quantification of genetic diversity of the plant pathosystem gives basic scientific information on epidemiology and population genetics. Genetic diversity analysis of pathogen populations solves problems such as (i) the extent of genetic diversity in the pathogen population, (ii) factors affecting intraspecific diversity of virulence, (iii) the effect of diversified host populations on genetic diversity, (iv) evolution in the genetic structure of virulence in time and space, (v) effects of migration of virulence factors among populations and (vi) the comparison of values for the same population in a time sequence or in different geographical ecosystems/regions. We have attempted to address the first problem. The present experiment was designed to quantify the extent of diversity in the population of *Xoo* collected from rice-growing states of India by estimation and by comparison of diversity indices to understand the population structure in the country in general and in different regions/states in particular.

MATERIALS AND METHODS

The experimental material was 52 isolates of *Xanthomonas oryzae* pv. *oryza*e (*Xoo*), collected from 12 rice-growing states of India (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 Nicobar Islands (AN)). Single cell colonies of each isolate of the bacterial strain were isolated on potato-sucrose-agar (PSA) medium and maintained in sterile distilled water at 4˚C as stock culture. Isolate virulence was tested on 16 rice genotypes, each possessing known genes for resistance, present either as single gene or in combinations of two or three genes. The rice genotypes used are listed in Table 1. Each experimental unit was a single row plot of 10 plants spaced at 20 cm between plants and 40 cm between rows.

The experiment used a randomized complete block design with four replicates. Thirty-day old healthy seedlings were transplanted with a spacing of 40 by 20 cm between rows and plants, respectively. Nitrogen fertilizer (urea), at 120 kg N ha^{-1} was applied in three equal doses, 1st as a basal dressing, 2nd at 30 d after planting and 3rd at flag leaf emergence. The rice plants were clip-inoculated (Kauffman et al., 1973) at flag leaf emergence, with scissors, which were dipped, prior to inoculation, into a bacterial cell suspension containing approximately 10^9 cfu ml^{-1}. The suspension was prepared from 48-h old actively growing cultures of each isolate grown on modified Wakimoto-agar-medium. The length of the lesion developed, below the inoculation point, was measured 21 d after inoculation. The average lesion length of all 10 plants in an experimental unit was considered to be the virulence level of a particular isolate on a specific host genotype for that replicate.

Analysis of genetic divergence among *Xoo* isolates used Mahalanobis D^2 statistics as described by Rao (1952) with the aid of the statistical package, Indostat (2004). Grouping of isolates into different clusters was done following Tocher's method (Rao, 1952). In this method, entities (isolates of the pathogen population) were arranged in order of their relative distances from each other. Two entities having the least distance from each other are considered first; a third entity having the smallest average D^2 value from the first two is added. Similarly, the fourth entity is chosen to have the smallest average D^2 value from the first three and so it goes on. At the stage when the average D^2 of a group from those already listed appears to be high, then that group does not fit in with the former and it is included in

another cluster. The process of grouping is continued till all the entities in the pathogen population are included into one or the other cluster.

Table 1. Analysis of variance for genetic diversity among 52 isolates of *Xoo* for virulence against 16 rice genotypes with known resistance genes.

Rice genotype	Mean squares	
	Isolate (df = 51)	Error (df = 153)
BJ-1 (*xa*5 + *xa*13)	28.93**	0.07
Cas-209 (*Xa*10)	18.32**	0.07
CB-II (*Xa*3 + *xa*5 + *xa*13)	28.70**	0.05
DV-85 (*xa*5 + *Xa*7)	16.40**	0.05
IR1545-339 (*xa*5)	17.15**	0.04
IR-20 (*Xa*4)	26.49**	0.12
IR-8 (*Xa*11)	23.90**	0.15
Java-14 (*Xa*1 + *Xa*3 + *Xa*12)	17.65**	0.04
Kogyoku (*Xa*1 + *Xa*3 + *Xa*12)	28.87**	0.04
MSS (*Xa*6)	15.91**	0.04
Rantai Emas (*Xa*1 + *Xa*2)	25.43**	0.13
Semora Mangga (*Xa*4)	17.05**	0.05
Tetep (*Xa*1 + *Xa*2)	21.49**	0.04
TKM-6 (*Xa*4)	27.64**	0.03
Wase Aikoku-3 (*Xa*3)	27.56**	0.21
Zenith (*Xa*6)	17.07**	0.04

** Significant at 0.01 probability level.

Genetic diversity among pathogen strains was also quantified based on virulence factors (*v*-factors) in each of the isolates, derived from the virulence of the isolates on rice genotypes with different *Xa* genes. For virulence analysis and better comparison of divergence among isolates, India was divided into four regions, viz., southern region consisting of Andhra Pradesh, Andaman and Nicobar Islands and Tamil Nadu; eastern region: Assam, Bihar, Orissa and West Bengal; northern region: Punjab, Rajasthan and Uttar Pradesh and western region: Gujarat, Maharashtra and Madhya Pradesh. Besides the present data, the diversity of virulence, with respect to published data in Table 2 of Nayak (1986a), Table 1 of Gupta et al. (1986), Table 4 of Shanti et al. (2001), Table 2 of Shanti and Shenoy (2005) and in Table 7, and Figures 1, 2 and 5 of Nayak et al. (unpublished) were also analyzed to obtain a better comparison of the diversity among the bacterial strains occurring in India.

The pathotypic diversity of *Xoo* within each region was calculated as

$H = -\Sigma\, p_i \ln (p_i)$

where p_i is the frequency of the i^{th} pathotype in a given region. The estimated diversity H was normalized to correct for differences in sample size as

$H^n = H/\ln (S)$

where S is the number of pathotypes in the sample.

Genetic diversity was calculated following the equation of Nei (1987):

$H = [n/(n\text{-}1)]\, (1- \Sigma\, X_i^2)$

where X_i is the proportion of the ith pathotype in a group and n is the number of strains tested in each group.

The genetic diversity of virulence factors was calculated by the Shannon's information function H′ (Shannon and Weaver, 1949) as

$H' = -\Sigma\, p_i \ln (p_i)$

where p_i is the relative frequency of occurrence of virulence factors in the pathogen population sampled.

Simpson's index of diversity 'λ' (Simpson, 1949), which indicates the dominance or concentration of *v*-factors in the population, was calculated as

$\lambda = \Sigma\, p_i^2$.

The reciprocal value of λ is a measure of diversity (Levins, 1968), which is defined as $B = 1 / \Sigma\, p_i^2$.

Diversity in the form of generalized entropy H_2 (Hill, 1973) is a logarithmic form of Levin's measure of B, which is given by:

$H_2 = \ln (B)$

where $B = 1/\lambda$.

The index of evenness, or equitability, $J_{H'}$ is used to confront empirical and maximum theoretical values of H' and is defined as:

$J_{H'} = H'/H_{\max}$

where $H_{\max} = \ln S$, and S = maximum number of virulent factors in the pathogen population (Sheldon, 1969).

The diversity index D is used to compute trophic diversity (Herrera, 1976). This is given by:

$D = -\Sigma \ln p_i$

where p_i is the estimation of relative frequency of occurrence of virulence factors in the pathogen population.

The index of genetic richness (d) of the virulence phenotypes, in the population, was calculated as S/n, where S is the number of determined *v*-factors in the sample and n is the number of theoretical *v*-factors (Margalef, 1958).

RESULTS AND DISCUSSION

Analysis of variance showed significant differences among the 52 isolates of *Xoo* for all characters, i.e. the *Xa* genes (resistance factors) present in the 16 rice genotypes (Table 1). This indicated the existence of wide genetic variability in the pathogen population. Based on the relative magnitude of D^2 estimates, the 52 isolates of *Xoo* could be grouped into 13 clusters. Among them, the first broad group of clusters-I, II, IV and V constituted of isolates possessing four virulence factors (*v*-1, 2, 4 and 11) each belonging to pathotype-16 distributed over eight states and one Union Territory of India (Table 2). The second broad group of clusters-III, VIII, IX and XIII were isolates each of which possessed 7 *v*-factors (*v*-1, 2, 3, 4, 10, 11 and 12) belonging to pathotypes 14 and 15 distributed over six states (Table 2). The third broad group of cluster-VII constituted of isolates with eight *v*-factors each (*v*-1, 2, 3, 4, 5, 11, 12 and 13) belonging to pathotype 7 distributed over the states of Andhra Pradesh, Gujarat , and Madhya Pradesh. The fourth broad group of clusters X and XII were isolates belonging to pathotype-4 and possessed 10 *v*-factors each (*v*-1, 2, 3, 4, 5, 6, 7, 11, 12 and 13) distributed over Orissa and Punjab. The fifth broad group of most virulent clusters VI and XI, were isolates of pathotype-1 and possessed all 11 *v*-factors (*v*-1, 2, 3, 4, 5, 6, 7, 10, 11, 12 and 13), were distributed over the states of Andhra Pradesh, Bihar, Orissa and West Bengal (Table 2). Each of these five broad groups contained the majority of strains from specific pathotypes. These findings on groupings, based on the number of *v*-factors corroborate the report of Nayak et al. (unpublished). They also identified these groups of isolates as belonging to pathotypes 1, 4, 7, 14, 15, and 16 respectively.

The relative contribution of characters, towards genetic diversity, plays a major role in grouping isolates into clusters and the detection of diagnostic strains of the pathogen. In this

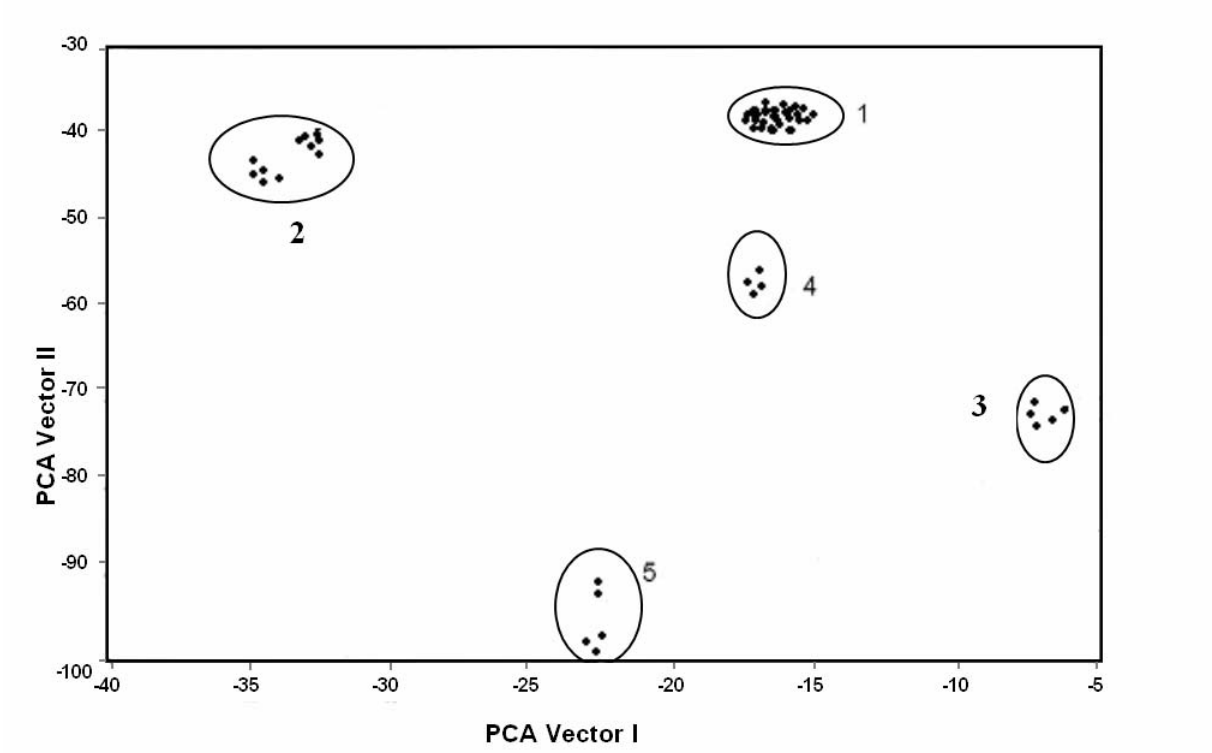
study, the characters are the resistance factors (*r*-factors), present in the host genotypes, corresponding to the *v*-factors present in the pathogen isolates. Among the *r*-factors, *Xa*4 present in TKM-6 exhibited highest contribution, followed by *Xa*1 + *Xa*3 + *Xa*12 in Java-14, *Xa*4 in Semora Mangga, *Xa*1 + *Xa*2 in Tetep and *xa*5 in IR 1545-339 (Table 4). These four host genotypes contributed 95% of the divergence, while the two genotypes (TKM 6 and Semora Mangga) possessing the *Xa* 4 gene, contributed 50% of the divergence. This might be due to the fact that the *Xa* 4 gene was widely used by the rice breeders for transfer of resistance in their breeding programs. The r-factors *Xa*3 in Wase Aikoku-3, Xa11 in IR-8, Xa10 in Cas 209, *xa*5 + *Xa*7 in DV-85 and *xa*5 + *xa*13 in *BJ*-1 exhibited the least contribution towards diversity.

Table 2. Clustering pattern of 52 isolates of *Xoo* based on D^2 values.

Cluster	Number of isolates	Isolate name of *Xoo*	*v*-factors	State of isolate origin	Pathotypes
I	7	CR *Xoo* 17, 18, 19, 20, 21, 24, 27	4 = *v*-1, 2, 4, 11	Andhra Pradesh, Punjab, Orissa	16
II	6	CR *Xoo* 41, 42, 43, 49, 50, 51	4 = *v*-1, 2, 4, 11	Andhra Pradesh, Orissa, Rajasthan	16
III	6	CR *Xoo* 11, 12, 13, 14, 15, 22	7 = *v*-1, 2, 3, 4, 10, 11, 12	Orissa, Punjab	14, 15
IV	9	CR *Xoo* 1, 3, 4, 5, 6, 7, 8, 9, 10	4 = *v*-1, 2, 4, 11	Andhra Pradesh, Andaman, Orissa, Tamil Nadu	16
V	5	CR *Xoo* 30, 32, 33, 34, 35	4 = *v*-1, 2, 4, 11	Gujarat, Madhya Pradesh, Uttar Pradesh	16
VI	2	CR *Xoo* 26, 28	11 = *v*-1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 13	Andhra Pradesh, West Bengal	1
VII	5	CR *Xoo* 29, 36, 39, 45, 48	8 = *v*-1, 2, 3, 4, 5, 11, 12, 13	Andhra Pradesh, Gujarat, Madhya Pradesh	7
VIII	1	CR *Xoo* 25	7 = *v*-1, 2, 3, 4, 10, 11, 12	Andhra Pradesh	15
IX	3	CR *Xoo* 37,40, 44	7 = *v*-1, 2, 3, 4, 10, 11, 12	Assam, Maharashtra, West Bengal	15
X	2	CR *Xoo* 46, 52	10 = *v*-1, 2, 3, 4, 5, 6, 7, 11, 12, 13	Orissa	4
XI	3	CR *Xoo* 31,38, 47	11 = *v*-1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 13	Bihar, Orissa	1
XII	2	CR *Xoo* 16, 23	10 = *v*-1, 2, 3, 4, 5, 6, 7, 11, 12, 13	Orissa, Punjab	4
XIII	1	CR *Xoo* 2	7 = *v*-1, 2, 3, 4, 10, 11, 12	Orissa	14

Canonical analysis extracted Eigenvalues, Eigenvectors, and percent variation explained by each of the four vectors. The first three vectors accounted for 96 % of the variation present in the communality with vector 1 = 59 %, vector 2 = 28% and vector 3 = 9% variability. Such high percent variability values indicate that the graphic representation of the first two canonical variables is appropriate to visualize the relationships among groups and members

within each group. The ordination of the individual isolates on the first two principal component axes in a 2D plot on PCA-1 and PCA-2 (Figure 1) grouped the 52 isolates into the five groups, as mentioned above. Grouping the isolates revealed that the patterns in the observed variations are not congruent with geographical origin. Such non-parallelism between the clustering pattern and geographic origin of the isolates might be due to the resistant host plant selection pressure (Nayak, 1986b), since the pathogen encounters thousands of local, high-yielding varieties as well as wild rices. The wide spectrum of agro-ecosystems the crop is grown in and the pathogen encounters could also be the reason for nonparallelism.



Isolates (*CRXoo*) within each group:
Group 1: *CRXoo*. 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;
Group 2: *CRXoo*. 2, 11, 12, 13, 14, 15, 22, 25, 37, 40, 44; Group 3: *CRXoo*. 29, 36, 39, 45, 48;
Group 4: *CRXoo*. 16, 23, 46, 52; Group 5: *CRXoo*. 26, 28, 31, 38, 47.

Figure 1 Two dimensional principal coordinate plot of 52 isolates of *Xoo* on the first two ordinates (PCA-1, PCA-2) accounting for 58.7 and 28.2 % of the variation present in the community.

The phenotypic distances represent the index of genetic diversity among the clusters of entities (the bacterial isolates in the present context). Application of multivariate analysis based on Mahalanobis's D^2 statistics (Rao, 1952) proved to be a potential tool in differentiating the 52 isolates into 13 clusters (Table 3). Bacterial isolates belonging to the clusters with maximum inter-cluster distances are genetically more divergent. The average genetic distance between clusters ranged between 11.89 (clusters-III and VIII) and 70.45 (clusters-IV and XI), while within clusters (intra-cluster) distances ranged from 2.79 (cluster-VI) to 11.51 (cluster-XI). The results further showed involvement of bacterial isolates in cluster-XI possessed all 11 *v*-factors with groups of isolates under clusters-I, II, III, IV, V, VIII, IX and XIII possessed 4 to 7 *v*-factors, showed high genetic divergence. There was low genetic divergence between clusters-III and VIII (11.89). The involvement of isolates possessing 4 and 7 *v*-factors resulted in these low genetic diversities. The intra-cluster

distance was high for cluster-XI (11.51) and VII (10.85), while it was lowest for in clusters VI (2.79) and V (3.44). Intra-cluster distances were zero for clusters VIII and XIII, since both were single-isolate clusters.

Estimates of cluster means of characters in different clusters of isolates give useful information on the population structure in general and virulence of the pathogen population under a particular group of isolates on host resistance, in particular. Among the 13 clusters of bacterial strains identified in this study, cluster-XI with all 11 *v*-factors showed the highest cluster means for most of the genes/gene combinations, followed by cluster-VI (Table 5).

Table 3. Average intra-cluster (bold) and inter-cluster distance (D^2) values.

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
I	5.62	25.82	19.20	15.81	12.71	58.27**	30.23*	20.34	28.27*	46.10**	65.21**	36.46**	26.07
II		6.70	36.31**	40.50**	14.11	60.69**	24.72	28.91*	21.19	36.27**	62.84**	44.79**	47.52**
III			6.49	19.86	25.58	54.60**	35.51**	11.89	26.62*	51.67**	62.12**	39.12**	13.36
IV				5.07	27.22**	61.41**	40.98**	27.44	39.96**	56.24**	70.45**	38.55**	18.18
V					3.44	58.00**	24.43	21.18	21.62	39.65**	62.75**	38.50**	35.56**
VI						2.79	45.18**	51.65**	53.55**	38.69**	25.31	32.47**	57.27**
VII							10.85	28.21*	24.89	28.07*	44.85**	32.91**	44.61**
VIII								0.00	15.69	45.25**	57.04**	39.06**	23.25
IX									5.38	39.76**	55.69**	44.32**	38.63**
X										6.12	35.22**	29.96*	59.84**
XI											11.51	39.09**	66.67**
XII												7.33	41.25**
XIII													0.00

*, ** significant at 0.05 and 0.01 probability level, respectively, based on a χ^2 test

Table 4. Relative contribution of characters (rice genotypes) towards genetic diversity among 52 isolates of *Xoo*.

	Contribution (%)	Rank
TKM-6 (*Xa*4)	28.89	1
Java-14 (*Xa*1 + *Xa*3 + *Xa*12)	28.67	2
Semora Mangga (*Xa*4)	21.33	3
Tetep (*Xa*1 + *Xa*2)	16.22	4
IR1545-339 (*xa*5)	2.00	5
IR-20 (*Xa*4)	1.11	6
Zenith (*Xa*6)	0.67	7
MSS (*Xa*6)	0.44	8
Kogyoku (*Xa*1 + *Xa*3 + *Xa*12)	0.22	9
Rantai Emas (*Xa*1 + *Xa*2)	0.22	9
CB-II (*Xa*3 + *xa*5 + *xa*13)	0.22	9
Wase Aikoku-3 (*Xa*3)	0.00	10
IR-8 (*Xa*11)	0.00	10
Cas-209 (*Xa*10)	0.00	10
DV-85 (*xa*5 + *Xa*7)	0.00	10
BJ-1 (*xa*5 + *xa*13)	0.00	10

Similarly, isolates in clusters-X and XII, each possessing 10 of the 11 *v*-factors, showed the next higher cluster means against the majority of the gene(s) combinations. This was followed by cluster-VII with 8 *v*-factors; clusters-III, IV, VIII and IX, each with 7 *v*-factors; and clusters-I, II and V each with 4 of the 11 *v*-factors. There was a highly significant correlation between the number of *v*-factors present in each cluster and the average cluster means (r =0.88**). This suggests that the number of *v*-factors in any particular cluster is strongly associated with virulence of the constituent isolates onto the host genotypes. Highest protection from the disease was from the *Xa*4 gene in Semora Mangga, *xa*5 in IR 1545-339, *xa*5 + *Xa*7 in DV-85, *Xa*6 in Zenith and MSS which exhibited resistant reactions against 9 of the 13 isolate clusters. This was followed by Cas209 (*Xa*10) and Java-14 (*Xa*1+*Xa*3+*Xa*12), which showed resistance against 7 of the 13 clusters of isolates and Wase Aikoku-3 (*Xa*3) and Kogyoku (*Xa*1+*Xa*3+*Xa*12) showing resistance against 5 of the 13 isolate clusters. On the contrary, *Xa*1+*Xa*2 in Rantai Emas, *Xa*4 in IR 20 and *Xa*11 in IR 8 did not provide any protection against all the 13 clusters of isolates.

Table 5. Cluster mean (cm) of each group of isolates of *Xoo* for their virulence on 16 rice genotypes possessing known resistance genes.

Characters (Genotypes)	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
1545-339 (*xa*5)	2.40	2.71	2.69	2.50	**2.36**	**8.53**	2.84	2.70	2.79	7.53	8.26	7.40	2.98
BJ-1 (*xa*5 + *xa*13)	4.06	3.92	3.92	**3.90**	4.09	12.14	7.50	4.15	4.18	8.26	**12.27**	8.55	3.98
Cas-209 (*Xa*10)	2.89	2.90	7.12	**2.84**	2.94	8.31	3.96	7.35	7.40	3.25	**8.37**	3.33	7.00
CB-II (*Xa*3+*xa*5+ *xa*13)	4.05	**3.83**	4.08	3.88	3.92	**12.19**	7.73	4.38	4.27	8.13	12.15	8.38	4.35
DV-85 (*xa*5 + *Xa*7)	2.69	2.66	2.80	2.61	2.61	**8.40**	2.93	2.70	**2.55**	7.44	8.30	7.53	2.98
IR-20 (*Xa*4)	**13.65**	13.72	17.24	13.76	13.82	21.48	16.80	18.30	18.24	14.89	**21.77**	14.74	17.50
IR-8 (*Xa*11)	**13.91**	14.11	17.38	13.92	14.02	21.25	17.05	18.50	18.33	14.95	**21.53**	15.01	17.50
Java-14 (*Xa*1+*Xa*3+*Xa*12)	2.92	2.98	7.00	2.96	**2.82**	**8.45**	4.00	7.30	7.47	3.40	8.16	3.10	7.08
Kogyoku (*Xa*1+*Xa*3+ *Xa*12)	**3.89**	4.00	3.98	3.89	3.92	**12.34**	7.58	4.48	4.40	8.23	12.13	8.40	4.03
MSS (*Xa*6)	2.50	2.35	2.54	2.43	2.45	7.99	2.48	2.43	**2.21**	7.04	**8.14**	6.99	2.98
Rantai Emas (*Xa*1+*Xa*2)	14.01	**13.82**	16.96	14.03	13.94	21.59	16.85	18.40	18.33	14.80	**22.08**	15.13	17.43
Semora Mangga (*Xa*4)	**2.39**	2.63	2.79	2.57	2.67	8.35	2.80	2.50	2.44	7.59	**8.50**	7.13	2.70
Tetep (*Xa*1 + *Xa*2)	4.02	**3.94**	4.06	4.04	4.01	4.40	7.70	4.40	4.23	8.50	**12.24**	8.25	4.20
TKM-6 (*Xa*4)	**3.90**	3.94	3.99	4.01	3.91	12.00	7.74	4.20	4.23	8.04	**12.04**	8.15	4.30
Wase Aikoku-3 (*Xa*3)	4.05	3.90	4.05	3.89	3.98	**12.11**	7.41	3.95	4.37	8.00	12.06	8.23	**3.55**
Zenith (*Xa*6)	**2.49**	2.53	2.62	2.64	**2.49**	8.35	2.93	2.60	2.74	7.44	**8.45**	7.48	2.95
Overall mean	5.24	5.25	6.45	5.24	5.25	11.74	7.39	6.77	6.76	8.59	12.28	8.61	6.59
Number of *v*-factors	4	4	7	4	4	11	8	7	7	10	11	10	7

Bold figures indicate the highest and lowest values (lesion length in cm.) for each character (specific gene/gene combinations).

Selection pressure imposed by resistant hosts in any plant-pathosystem influences the genetic diversity and population structure of the pathogen (Leonard and Czochor, 1980; Burdon and Jerosz, 1990; Marshall, 1977) in addition to the diverse agro-climatic conditions encountered by the pathogen. Multivariate analysis using Mahalanobis D^2 statistics (Rao,

1952) is a method of classificatory analysis, which measures the genetic distance between and within the groups of entities and classifies them into clusters with similarity in genotypic or pathotypic or geographic diversity. However, it does not provide a measure of comparison of diversity among populations reported by other researchers. A number of diversity indices have been suggested and used by ecologists and mathematicians (Rao, 1980; Lebeda, 1982).

Among different populations of *Xoo*, high diversity values of the indices H', B, H_2 and D were determined for pathogen populations from the eastern and southern regions as well as India, as a whole, for population-1 (Table 6), while those for the eastern region and India for population-2. The values for the other regions, in both populations 1 and 2, were low and non significant. Highest diversity values in respect of all four indices were determined for the populations dealt with by Shanti et al. (2001) and Shanti and Shenoy (2005). The value of the dominance index λ accompanied by the highest value of the evenness or equitability index $J_{H'}$ for these two populations is due to the low concentration of dominance of one or few *v*-factors and high equitability. These findings support the theoretical assumption that H' and λ have a reciprocal relationship. No such marked differences were found among the remaining populations. This was also evident from comparison of the genetic richness ('*d*') of the virulent phenotypes in the populations, which was high (0.79) for the total population of the country as well as those from southern, eastern regions (population-1, Table 6) and also those from Orissa, Madhya Pradesh and Uttar Pradesh (Shanti et al., 2001) as well as Andhra Pradesh, Orissa, Punjab and Tamil Nadu (Shanti and Shenoy, 2005). The estimate of '*d*' was also high (0.71) for the population of pathogens from the northern region (population-1, Table 6), 0.64 for the population for southern, eastern regions and the total population for the country (population-2) and also for the pathogen population-4 from Punjab and Haryana states (Gupta et al., 1986). Estimates of '*d*' were lowest for population-3 (Nayak, 1986a). The highest λ value and lowest H' for population-4 from Punjab and Haryana (Gupta et al., 1986) might be due to the high dominance concentrations of the *v*-factors v-1, 2, 3, 4, 5, 7, 10, 12 and 14. Further, the lowest value of $J_{H'}$ shows little variation among rest of the populations. However, the differences indicate the differential concentration of dominance of the *v*-factors among the populations.

The genetic diversity approach of Nei and Tajima (1981), expressed by the diversity index H, was used to analyze the population structure of *Xoo*. The genotypic diversity (H) of the total population of *Xoo* in India was estimated to be 0.70, which was similar to that in the eastern region. This was followed by the diversity values of 0.67 in the western region, 0.64 in the northern region, and 0.61 in the southern region. The genotypic diversities estimated for four states, from where considerably higher numbers of isolates were collected, revealed highest diversity (0.70) among isolates from Andhra Pradesh, followed by Orissa (0.59), Punjab (0.53) and Gujarat (0.50).

High, normalized pathotypic diversity estimates (H^n) ranging from 0.73 to 0.86 were obtained for the total population of *Xoo* in India as well as for the four regions of the country (population-1, Table 6). Pathotypic diversity ranged from 0.61 to 0.86 for the pathogen population from the four regions and the total population from India, as a whole, when tested against 15 differentials (population-2). Lowest pathotypic diversity (0.27) was estimated for the Punjab and Haryana (population-4, Table 6) despite the presence of a high number of 9 *v*-factors in the isolates. This might be due to the high number of pathotypes identified by these authors (Gupta et al. 1986) with the help of 14 different varieties from three countries. In the present study the pathotypic diversity for isolates collected from the Punjab was estimated at 0.72 due to the presence of 10 *v*-factors. High estimates of normalized pathotypic diversity were recorded for Andhra Pradesh (0.80), Orissa (0.70), Punjab (0.72) and Gujarat (0.81). Similar high pathotypic diversity values were reported by Shanti et al. (2001) for 450 isolates collected from Orissa (0.87), Madhya Pradesh (0.79) and Uttar Pradesh (0.77). Adhikari et al. (1995) evaluated the population structure of 308 strains

of *Xoo* using RFLP and virulence analysis and reported high genetic diversity of 0.92 for isolates from India compared with those from Nepal (0.98), the Philippines (0.96), China (0.91), Indonesia (0.87), Korea (0.83) and Malaysia (0.23). High genetic diversity (0.93) of the total population of *Xoo* collected from the Philippines and those collected over 15 years from 1972-86 (0.89 – 0.92) was reported by Leach et al. (1992).

Table 6. Genetic diversity of virulence factors in different populations of *Xanthomonas oryzae* pv. *oryzae*.

Populations	Region/State*	H'	B	H_2	D	λ	$J_{H'}$	d	H^n
1. 52 isolates x 16 genotypes (this work)	Southern region	1.04	2.28	0.83	7.02	0.44	0.21	0.79	0.75
	Eastern region	1.21	2.90	1.06	6.24	0.35	0.22	0.79	0.75
	Northern region	0.93	2.30	0.83	3.90	0.43	0.21	0.71	0.85
	Western region	0.95	2.33	0.85	3.77	0.43	0.24	0.57	0.86
	India	1.31	2.95	1.08	9.48	0.34	0.21	0.79	0.73
2. 52 isolates x 15 differentials (Nayak et al., unpublished)	Southern region	0.85	2.06	0.72	4.31	0.49	0.18	0.64	0.61
	Eastern region	1.13	2.71	0.99	6.90	0.37	0.21	0.64	0.70
	Northern region	0.93	2.30	0.83	3.90	0.43	0.22	0.57	0.85
	Western region	0.95	2.33	0.85	3.77	0.42	0.25	0.43	0.86
	India	1.37	2.96	1.09	13.69	0.33	0.22	0.64	0.76
3. 18 isolates x 10 genotypes (Nayak, 1986a)	AP, BR, MN, OR, RJ, WB	0.99	2.44	0.89	3.64	0.41	0.22	0.36	-
4.11 pathotypes x14 genotypes (Gupta et al., 1986)	PB & Haryana	0.65	1.85	0.62	1.47	0.54	0.14	0.64	0.27
5. 10 strains x 11 NILs (Shanti et al., 2001)	OR, MP, UP	1.70	5.00	1.61	11.33	0.20	0.36	0.79	0.68
6. 10 isolates x 11 NILs (Shanti and Shenoy, 2005)	AP, OR, PB, TN	1.61	4.17	1.43	11.74	0.24	0.36	0.79	0.70

*AP – Andhra Pradesh, BR – Bihar, MN – Manipur, MP – Madhya Pradesh, OR – Orissa, PB – Punjab, RJ – Rajasthan, TN – Tamil Nadu, UP – Uttar Pradesh, WB – West Bengal, NILs – Near isogenic lines

Data of Mew (1987) and Vera Cruz and Mew (1989) indicated that Indian isolates of *Xoo* are most virulent compared with isolates from other Asian rice growing countries. The present findings of high diversity among the Indian isolates analyzed through host x pathogen interaction studies corroborate previous findings based on molecular analysis (Leach et al., 1992; Adhikari et al., 1995, 1999; and Shanti et al., 2001). This high diversity might be due to the diverse agro-ecological conditions under which rice is grown and the wide spectrum of host genotypes covering traditional, high yielding, hybrid varieties as well as wild rices of host plants, the pathogen encounters. Systematic sampling of pathogen population, covering widely different agro-ecological conditions in which rice is grown, and diversity analysis through host pathogen interactions, would help to provide a basic understanding of the population structure, especially where modern molecular techniques are not available.

These findings have practical implications in (i) giving a better understanding of the pathogen population structure over time and space, (ii) provide a preliminary basis for the design of strategies for the selection of resistant sources for regional breeding programs, (iii) the use of available resistant sources in disease control, (iv) understanding the impacts of host genotypes, climate and cropping intensity on pathogenic variability, and (v) tracking changes in population structure over time and space.

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