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IDENTIFICATION OF SLOW-BLASTING RICE GENOTYPES THROUGH MULTIVARIATE ANALYSIS OF DISEASE PROGRESS CURVES

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ABSTRACT

Forty two rice genotypes were tested in uniform blast nursery for a period of nine seasons, against blast disease caused by *Magnaporthe oryzae*. The highly susceptible check Karuna was used as the spreader row. Conducive atmosphere created by closer spacing, application of high nitrogen fertilizer and maintenance of high humidity through running of overhead sprinklers, resulted in 100% disease pressure in the spreader row as well as some of the susceptible test genotypes. The basic epidemiological data on per cent disease severity scores, recorded at every alternate day intervals were subjected to multivariate analysis. Cluster analysis classified the rice genotypes into clusters of slow-blasting and fast-blasting groups. Super-imposition of clustering pattern on to the planes of the ordination figures on the first two principal components (PC-1 and PC-2) clearly revealed the geometrical positioning of the slow-blasting genotype-clusters nearer to the intersection between the two ordinates and the fast-blasting genotype-clusters away from it along PC-1 axis. Thirty two stable slow-blasting genotypes were recognized by compilation of these data over a period of nine seasons. Application of the method of multivariate analysis to the basic epidemiological data on disease progress curve, facilitated in easy and quick identification of rice genotypes possessing slow-blasting resistance through clustering and ordination techniques.

Keywords: rice genotypes, multivariate analysis, ordination, *Magnaporthe oryzae*, slow-blasting, stable resistance.

INTRODUCTION

Disease resistance in any plant-pathosystem can not be classified unambiguously into distinct classes, nor is there an easy way to discern stable from unstable resistance, nor there is any simple characteristic by which one can always discern polygenic from monogenic resistance without fail. Only a sound knowledge on host-pathogen system is a good guarantee that one uses the resistance that is most appropriate for the situation concerned (Kranz, 2002). The disease severity can be assessed either once at the peak of epidemic development or several times from beginning till end of the epidemic. The former assessment measures the cumulative effects of all the factors operating during the course of an epidemic; while the later measures different parameters for evaluation and comparison among the disease progress curves. Rice genotypes possessing slow-blasting resistance have been recognized mostly through analysis of logistic apparent infection rates (r) and area under disease progress curves (AUDPC) (Mukherjee *et al*., 1995, 1998; Chiang and Huang, 2004). Among the 12 parameters; FDS, MDS, AUDPC, RAUDPC, r, k and the genotype-score on PC-1 were considered as the top ranking parameters in expression of consistent blast disease reactions (Mohapatra, 2002). Mukherjee *et al*. (2005) and Mohapatra *et al*. (2008) considered AUDPC, RAUDPC, r and k as the top ranking parameters for evaluation of slow-blasting resistance in rice. Linearization of disease progress curves help in determination of rate parameter for a better comparison of epidemics, prediction of future disease intensity and estimation of initial disease. Although the logistic model has been widely used for comparison of disease progress curves, of late Gompertz model proposed by Berger (1981) has proved more appropriate. Comparison between these two models resulted in fitting of 91% of disease progress curves into both the models (Mohapatra *et al*., 2008), while 78% of disease progress curves in a nitrogen experiment produced satisfactory fit into both logistic and Gompertz models (Mukherjee *et al*., 2005). However, each parameter has its own merit and demerits in expression of true host resistance.

Application of multivariate analysis directly to the epidemiological data by way of clustering and ordination results in easy and quick identification of resistant genotypes. Pattern analysis comprising of joint numerical classification and ordination of a set of entities on the basis of their attributes, has been widely applied in taxonomy (Sneath and Sokal, 1973; Hair *et al*., 1998), ecology (Orloci, 1975; James and McCulloch, 1990), social sciences (Stevens, 1992) and agricultural science (Williams, 1976; Sanogo and Yang, 2004), which could be used to more advantage in plant pathology. The application of multivariate analysis methods in plant pathology, although suggested during 1970s (Kranz, 1974a, 1974b, 1978; Williams, 1976), is still in its infancy. The numerical classification of the genotypes on the basis of their attributes of the disease assessment at different times has been made for slow-stem rusting in wheat (Rees *et al*., 1979a, 1979b; Thompson and Rees, 1979), slow-mildewing in lettuce (Lebeda and Jendrulek, 1988), disease resistance in vegetable crops (Lebeda and Jendrulek, 1987), early blight resistance in tomato (Madden and Pennypacker, 1979). The authors have earlier used this method for identification of slow-blasting rice genotypes, considering 14 components of resistance (Mukherjee *et al*., 2013) and 12 parameters for evaluation of resistance (Mohapatra *et al*., 2014, in Press), as the attributes. The objective of the present investigation was to classify rice genotypes on the basis of their attributes,

which are the actual blast disease scores at different assessment times, using multivariate analysis methods and to identify stable slow-blasting rice genotypes.

MATERIALS AND METHODS

Seeds of 42 rice genotypes were collected from the international list of donors for various stresses, maintained at international germplasm collections, IRRI, Philippines and the National Germplasm being maintained at the Central Rice Research Institute, Cuttack, India. Seeds were sown in Uniform Blast Nursery modified for screening of genotypes for slow-blasting resistance (Marchetti, 1983). The highly susceptible genotype Karuna was sown in alternate rows as well as all around the nursery as spreader rows. Each test genotype was sown in one meter long single-row plot surrounded by the spreader rows of Karuna. Seeds were sown with a spacing of 10cm between rows and 5cm between plants. The experiment was conducted in a randomized complete block design with three replications. Fertilizer was applied at 100kg N ha^{-1}, in the form of ammonium sulphate, in split doses. High relative humidity was maintained throughout the period of experimentation by running the sprinkler irrigation system during the hotter periods of the day (i.e., 10.00 A.M. to 3.30 P.M.) with an intermittent stoppage of half an hour after each hour of running. The experiment was conducted repeatedly for a period of nine seasons from dry season 1997 to dry season 2001 (four wet seasons + five dry seasons).

Critical observations on mean disease severity scores on percentage of total host tissue infected were recorded at every alternate day intervals, following the score chart developed by Padmanabhan and Ganguly (1959). The numerical score values of each assessment time were converted into per cent disease severity. These data were subjected to cluster analysis and principal component analysis to classify and ordinate the genotypes on the basis of similarity in their response to rice blast disease at different assessment times. For the purpose of analysis of any data set for any season, the genotypes were regarded as the entities possessing a number of attributes, which were the blast disease scores at various assessment times. The group of genotypes with similar response to the disease could be delimited on a dendrogram, thus classifying slow-blasting and fast-blasting genotypes in to distinct clusters.

This was followed by the principal component analysis, which extracted the correlation matrix, latent roots and vectors, percent variation explained by each root and finally the genotype-scores on the first few principal components, accounting for more than 90% variation in the communality. The ordination of the genotype-scores on to the planes of PC-1 and PC-2 resulted in a scattered diagram showing the position of individual genotypes on it. Super-imposition of the clustering pattern obtained from the dendrogram on to the scattered diagram depicted the positioning of the genotype-clusters on the ordination Figure.

The response of each genotype to rice blast disease was compiled over nine seasons indicating their inclusion in to a specific cluster. The genotype-response over nine seasons was further subjected to hierarchical method of cluster analysis (Sneath and Sokal, 1973) by considering the genotypes as entities and their response over each of the nine seasons as the variables, after assigning the numerical values of 1, 2, 3 and 4 to the clusters A, B, C and D, respectively. Thus the genotypes exhibiting similar response over nine seasons of testing could be clustered into distinct groups of stable resistance or susceptibility. The entire set of multivariate analysis, involving cluster analysis and principal component analysis, was carried out with the help of statistical package developed by M/s. Indostat Services, Hyderabad, India (INDOSTAT, 2004).

RESULTS

Progress of epidemics

The application of high nitrogen and maintenance of high humidity levels through the sprinkler irrigation system created congenial atmosphere for disease development during all the seasons of study. The disease symptoms were initiated first in the susceptible check Karuna and a few other genotypes grouped under cluster-A, increased sharply to reach a level of 100% severity within a short period of 10 days during wet season 1998 and 22 days during dry season 2000 (Figure-1). The group of genotypes under cluster-B, exhibited disease symptoms almost at the same time or two days later progressed at a comparatively slower rate to reach a maximum severity level of 50% after 22 days during wet season 1999 to 100% after 24 days during dry season 1997 and 1998. The disease was initiated in group of genotypes in cluster-C either at the same time or 2 to 6 days later than those in cluster-A, progressed slowly and reached a maximum level ranging from 4.6% during dry season 2000 after 24 days to 33.3% after 26 days of initiation in susceptible check Karuna during dry season 1998. The group of genotypes under cluster-D exhibited disease symptoms 2 to 6 days later than Karuna, progressed very slowly and reached a maximum level of 1.0% after 26 days during dry season 1997 to 4.8% during dry season 1999 after 24 days of disease initiation in the susceptible check Karuna.

Clustering pattern

The hierarchical classification of the genotypes based on the blast assessment scores at alternate day intervals for each of the nine seasons was done separately. Due to lack of space, the resulting dendrogram in respect of one representative season has been presented in Figure-2. The disease progress curves having similarity in disease proportions, could be clustered into distinct group of cluster-A, B, C and D arrayed in a resistance spectrum of highly resistant or slow-blasting resistant groups at the top (clusters-D and C) to moderately susceptible or highly susceptible fast-blasting groups (clusters-B and A) at the bottom of the dendrogram. The groups of genotypes in cluster A and B were characterized by early initiation of the disease, rapid rate of disease development and attainment of 50 to 100% severity within a short period of

18 to 22 days. On the other hand, the groups of genotypes under clusters C and D were characterized by late initiation of the disease, slow rate of disease progress and attainment of maximum severity levels of 1.7 to 5.7% by end of the epidemic.

Ordination of genotypes

Principal component analysis extracted data Tables on the correlation matrix, latent roots and vectors, percent variation explained by each root and finally, the genotype-scores on the first few principal components accounting for more than 90% variation in the communality. The PC-1 and PC-2 together accounted for a maximum variation of 90.3% during wet season 1998 to 97.4% during wet season 1997. The ordination of the genotype-scores on PC-1 and PC-2 distinctly displayed the positioning of the genotypes on the planes of the ordination figure (Figure-3). Super-imposition of the clustering pattern obtained from the dendrogram on to the respective ordination Figure displayed the positioning of the fast-blasting genotype-cluster (A and B) away from the point of intersection between the two ordinates along PC-1 in positive direction and the slow-blasting resistant genotype-clusters (C and D) nearer to the point of intersection, either in positive or negative direction or both. The ordination was in general agreement with the hierarchical classification over all the nine seasons of testing.

Stable resistance

The responses of each of the 42 genotypes over a period of nine seasons of testing were compiled (Table-1) in terms of the inclusion of each genotype into specific cluster in each season. Classification of genotypes was again performed through hierarchical cluster analysis resulting in a dendrogram (not presented), which distinctly classified 42 genotypes into four clusters (Table-2). Cluster-A constituted four genotypes namely; Karuna, India dular, Tiace and ARC-7046 which were grouped under cluster-A and B consistently for eight or nine seasons and thus exhibited stable susceptibility. Cluster-B consisting of six genotypes namely; Kalubalawee, Jaya, Bakka biasa, Ratna, Pusa 4-1-11 and CR 289-1045-6, which were grouped irregularly under one of the four clusters and hence were considered as most unstable susceptible genotypes. Rest of the 32 genotypes were grouped consistently in clusters-C and D. Eight of them namely; DZ-192, DM-27, Tieu-phai, Sakai, Jumi-1, Laurent-TC, Chiang-Tsene-Tao and Chokoto; exhibited consistently high degree of resistance and were grouped under cluster-D in all the seasons. The response of the rest of 24 genotypes were expressed by their inclusion in to the clusters-C and D during different seasons of testing. Thus 32 genotypes in clusters-C and D exhibited consistently resistant reaction and hence were identified as possessing stable slow-blasting resistance.

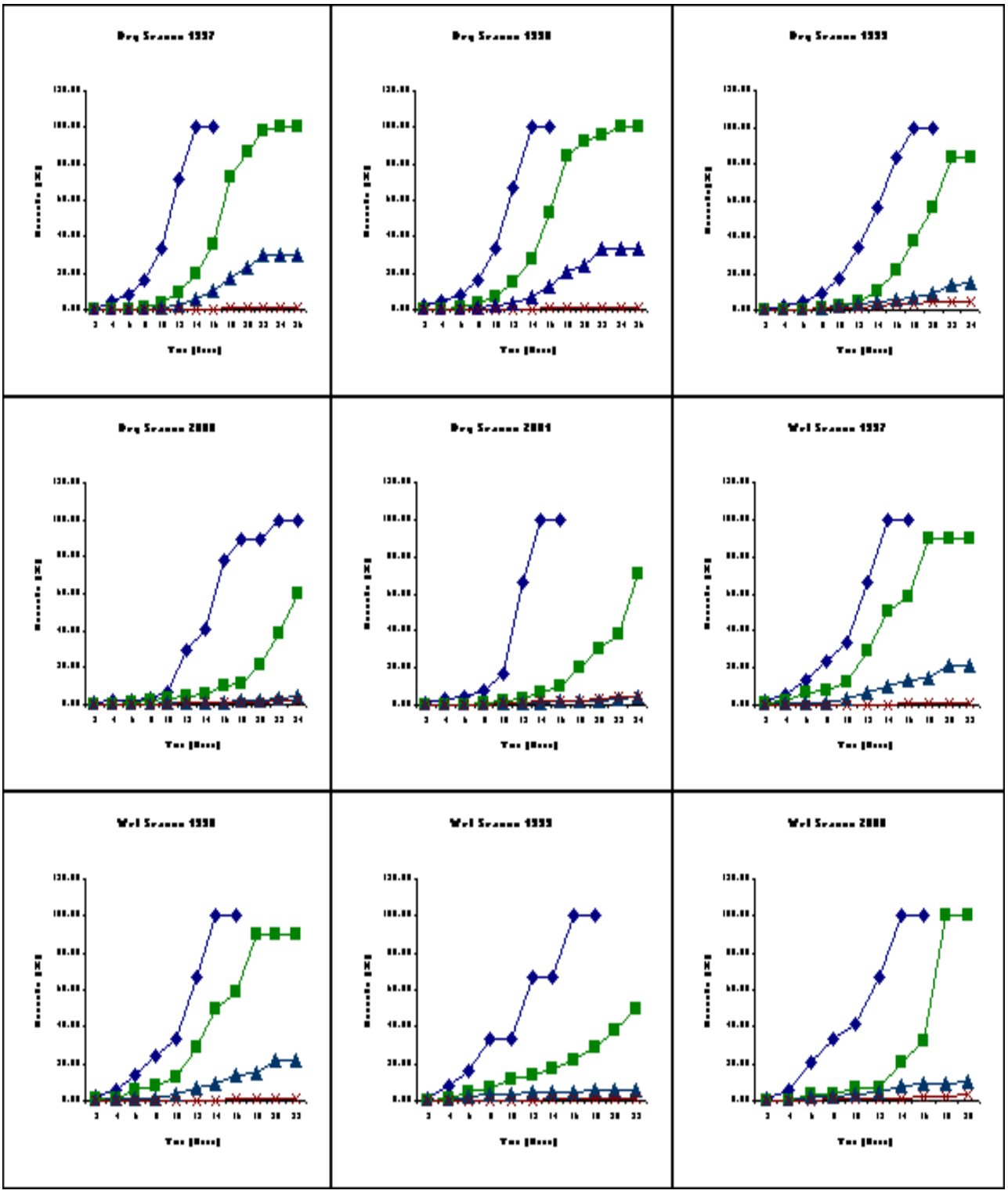

Figure-1. Average disease progress curves for each of the four clusters of rice genotypes in respect of nine seasons of testing. Cluster-A (-♦-), Cluster-B (-■-), Cluster-C (-▲-) and Cluster-D (-×-).

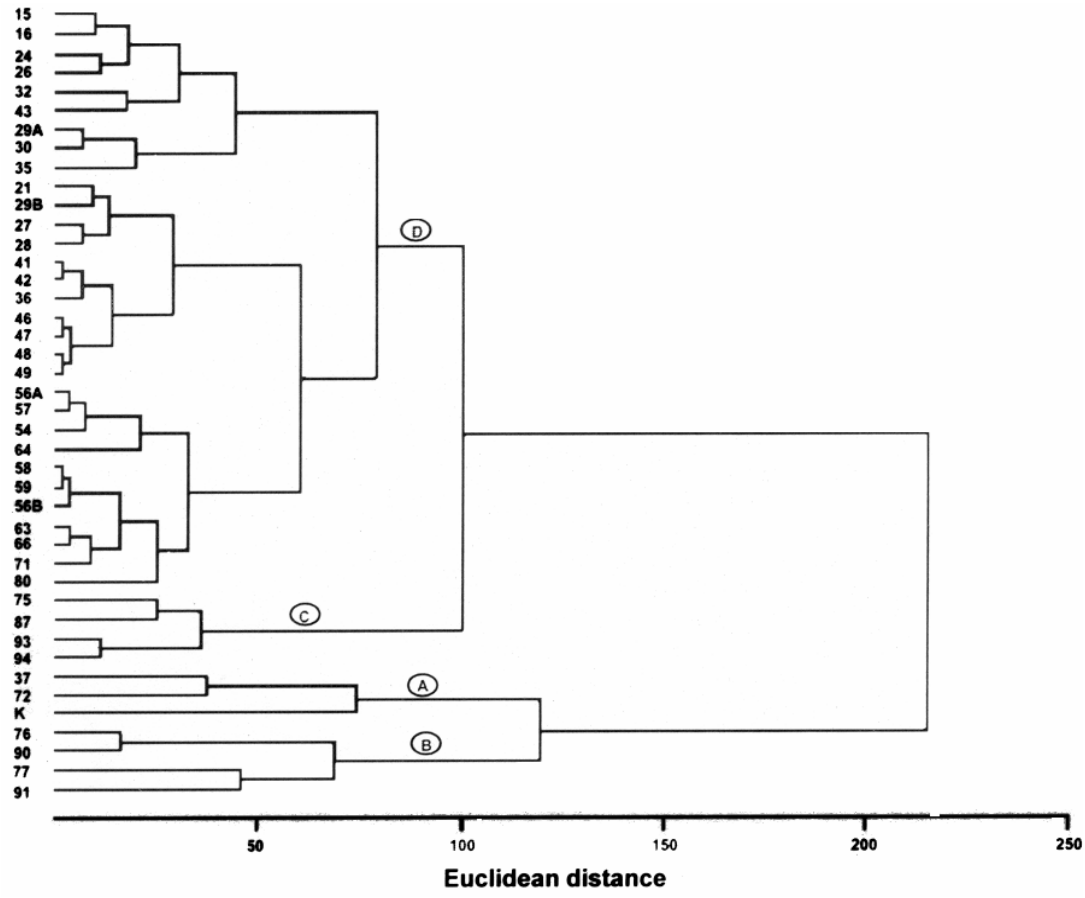

Figure-2. A dendrogram showing the similarity and successive clustering of 42 rice genotypes for one representative season of testing. The numerals indicate the respective genotypes listed in Table-1.

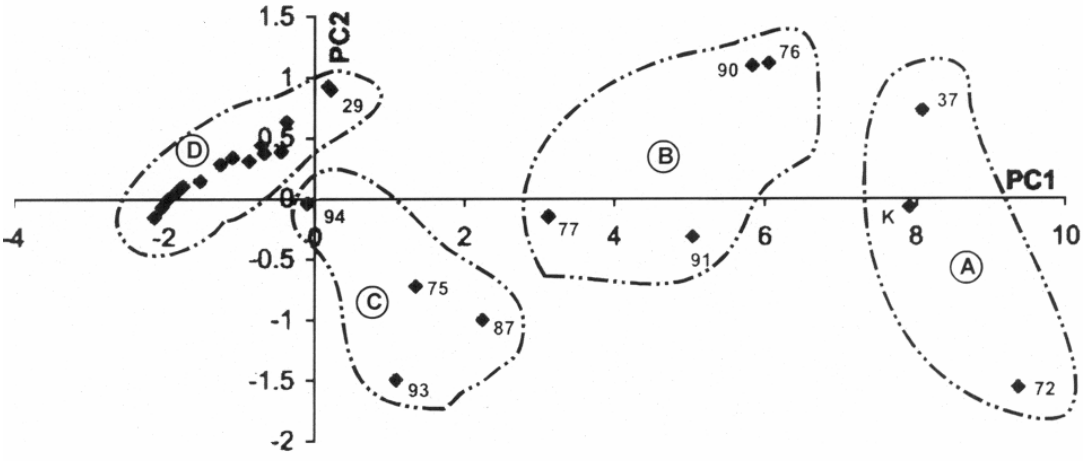

Figure-3. Ordination of 42 rice genotypes on to the planes of vector 1 and 2 from principal component analysis. The groups of genotypes encircled are the main clusters obtained from the dendrogram (Figure-2).

Table-1. Summary results of the blast disease reaction of 42 rice genotypes tested over nine seasons for identification of stable resistance.

Genotypes		DS	WS	DS	WS	DS	WS	DS	WS	DS	**Total			
		1997	1997	1998	1998	1999	1999	2000	2000	2001	A	B	C	D
15	DZ-192	*D	D	D	D	D	D	D	D	D	-	-	-	9
16	DM-27	D	D	D	D	D	D	D	D	D	-	-	-	9
21	Tieu-phai	D	D	D	D	D	D	D	D	D	-	-	-	9
24	E-425	D	D	D	D	D	C	D	D	D	-	-	1	8
26	Mak-thua	C	D	C	D	D	C	D	D	D	-	-	3	6
27	Sam houang	D	D	D	D	D	C	D	D	D	-	-	1	8
28	Sakai	D	D	D	D	D	D	D	D	D	-	-	-	9
29A	Seritus malam-A	D	D	D	D	D	C	D	C	D	-	-	2	7
29B	Seritus malam-B	D	D	D	D	D	C	D	C	D	-	-	2	7
30	Jumi-1	D	D	D	D	D	D	D	D	D		-	-	9
32	Laurent-TC	D	D	D	D	D	D	D	D	D	-	-	-	9
35	Chiang-tsene-tao	D	D	D	D	D	D	D	D	D	-	-	-	9
36	Chokoto	D	D	D	D	D	D	D	D	D	-	-	-	9
37	India dular	A	A	B	B	A	B	A	B	B	4	5	-	-
41	Raj bhawalta	D	D	D	D	D	D	D	D	C	-	-	1	8
42	Sechi aman	D	D	D	D	D	C	D	D	C	-	-	2	7
43	Surjamukhi	C	D	D	C	D	C	D	D	C	-	-	4	5
46	IR-5533-PP-854	D	D	D	D	D	C	D	D	C	-	-	2	7
47	Madhukar	D	D	D	D	D	C	D	D	C	-	-	2	7
48	Milayeng-51	D	D	D	D	D	C	D	D	C	-	-	2	7
49	PTB-8	D	D	D	D	D	D	D	D	C	-	-	1	8
54	Dahanala-2014	C	D	D	D	D	D	C	D	C	-	-	3	6
56A	Lien-tsan-50-A	D	D	D	D	D	C	C	D	C	-	-	3	6
56B	Lien-tsan-50-B	D	D	D	D	D	D	C	D	C	-	-	2	7
57	N-22	C	D	C	C	C	C	C	C	C	-	-	8	1
58	Salum pikit	D	D	D	D	D	D	C	D	C	-	-	2	7
59	PTB-18	D	D	D	D	D	D	C	D	C	-	-	2	7
63	DNJ-155	D	D	D	D	D	C	C	D	C	-	-	3	6
64	DJ-88	D	D	D	D	D	D	C	D	C	-	-	2	7
66	UCP-188	D	D	D	D	D	D	C	D	C	-	-	2	7
71	Goda heenati	D	D	D	D	C	D	C	D	C	-	-	3	6
72	Kalubalawee	C	A	C	D	C	C	C	D	C	1	-	6	2
75	Bakka-biasa	B	C	B	B	C	D	C	D	B	-	4	3	2
76	Tiace	B	B	B	B	B	B	B	A	B	1	8	-	-
77	ARC-7046	B	B	B	B	C	B	B	B	B	-	8	1	-
80	Prolific	C	D	C	D	C	D	C	D	C	-	-	5	4
87	Pusa-4-1-11	B	C	B	C	B	B	B	C	B	-	6	3	-
90	Ratna	B	B	B	B	C	C	B	C	B	-	6	3	-
91	Jaya	D	B	D	C	B	C	B	C	B	-	4	3	2
93	CR-289-1045-16	B	C	B	C	A	B	B	D	B	1	5	2	1
94	CR-570	D	C	D	D	C	D	C	D	C	-	-	4	5
K	Karuna	A	A	A	A	A	A	A	A	A	9	-	-	-

*A, B, C and D are extracted from the Tables of clustering pattern for each genotype during respective season of testing.
** The total value relates to the total number of cases (out of nine seasons), a genotype exhibited a specific reaction.
DS = Dry Season; WS = Wet Season

Table-2. Clustering pattern of 42 rice genotypes obtained from the dendrogram based on the compiled data (presented in Table-1) for nine seasons of study.

Clusters	Number of genotypes	Name of the genotypes
A	4	Karuna, India dular, Tiace, ARC-7046
B	6	Kalubalawee, Jaya, Bakka-biasa, Ratna, Pusa-4-1-11, CR-289-1045-16
C	10	Lien Tsan-50-B, Salum-pikit, PTB-18, DJ-88, UCP-188, Dahanala-2014, Goda heenati, CR-570, Prolific, N-22
D	22	DJ-192, DM-27, Tieu-phai, Jumi-1, Laurent-TC, Chian-tsene-tao, Chokoto, E-425, Sam houang, Seritus malam-A, Seritus malam-B, Mak-thua, Raj bhawalta, PTB-8, Sechi aman, IR-55-33-PP-854-1, Madhukar, Milayeng-51, Lien-tsan-50-A, DNJ-155, Surjamukhi, Sakai

DISCUSSIONS

Disease assessment scores at frequent intervals when plotted against time, results in a disease progress curve, which are usually compared by means of estimation of different parameters. The senior author made an attempt to recognize slow-blasting resistant rice genotypes by means of estimation and comparison of host response through 12 parameters for evaluation of resistance and also their relative importance (Mohapatra, 2002). Among them FDS, MDS, AUDPC, RAUDPC, r, k and PC-1 were recognized as the first ranking parameters due to consistent expression of true resistance over a period of nine seasons of testing. Significant differences in treatment effects due to nitrogen fertilization on slow-blasting attributes in rice have been reported by comparison of disease progress curves (Long *et al*., 2000) and by estimation of nine parameters for evaluation of resistance (Mukherjee *et al.*, 2005). Recently, Mukherjee et al. (2010), Jeger and Viljanen (2001), and Haynes and Weingartner (2004) suggested estimation of AUDPC from two data points that gives similar results to those estimated from all data points without any loss of information.

One can use any of these parameters from amongst various options to represent the level, rate and shape of disease progress curves. However, each of these parameters has its own merit and demerits as well. Although these parameters may describe the level, rate and shape of the curves; the two main associated problems are: (i) the parameters are inter-correlated, thereby interfering with univariate statistical testing and (ii) it may not be possible to determine as to which curve elements contribute to the epidemic structure or their relative importance. In view of these reasons, it was suggested to use multivariate analyses like, cluster analysis and principal component analysis for comparison of disease progress curves (Kranz, 1974a; Williams, 1976; Thompson and Rees, 1979; Rees *et al*., 1979a, 1979b; Madden and Pennypacker, 1979; Lebeda and Jendrulek, 1988), since components are uncorrelated and it is possible to evaluate the relative importance of curve elements by determining the per cent variability explained by each of them.

Pattern analysis consisting of joint numerical classification and ordination of a set of entities on the basis of their attributes, are tools for revealing similarities and differences among the entities (Thompson and Rees, 1979). The numerical classification produces discrete groups of like-entities such that the similarities within groups are greater than between groups. The ordination simply displays the geometric positions of these entities within a multidimensional space defined by the attributes. Application of such a method of hierarchical agglomerative cluster analysis to classify 42 entities (rice genotypes), based on their attributes of blast disease severity scores at each assessment date over a period of nine seasons of repeated testing, resulted in invariable consistent grouping of the genotypes into four clusters of A = highly susceptible; B = moderately susceptible; C and D = moderately resistant to resistant (= slow-blasting resistant) nature. The change in genotype-placements into specific groups over seasons was rare, with shifts within susceptible or resistant clusters. Ordination of the genotypes on to the planes of PC-1 and PC-2, both together accounting for 90 to 98% variability in the communality, clearly displayed the geometric position of the genotypes within the multidimensional space defined by the attributes. The distribution of genotype-scores are linear on the plane of PC-1, with the resistant groups of genotypes positioned nearer to the point of intersection between both the axes and the susceptible groups away from it, in the positive direction on PC-1, in contrast with those reported to be curved for wheat stem rust (Rees *et al*., 1979a; Thompson and Rees, 1979) and leaf rust epidemic progress curves (Rees *et al.*, 1979b). The present findings corroborate with those reported by Lebeda and Jendrulek (1988), who reported positioning of the clusters of cultivars along the horizontal axis both for the dynamics of lettuce downy mildew disease proportion as well as the number of infected leaves, with the extreme susceptible groups being located at the right hand side and an increased field resistance groups towards the left hand side.

Genotypes expressing consistent disease reactions over repeated testing at the same location over a period of time or at different locations over the same period of time; are considered to have possessed stable resistance. In the present experiment, repeated testing of the 42 rice genotypes at the same location over a period of nine seasons and compilation of consistency in their response revealed that a group of 32 genotypes possessed

stable slow-blasting attributes of late disease initiation, slow disease development.

The application of the methods of multivariate analyses to rice blast-pathosystem involving (i) the cluster analysis used for natural data clustering to simplify the description of vast multivariate data set and to generate hypothesis concerning the nature of the data and (ii) the principal component analysis which displays the relative geometric position of the genotypes on the plane of the first two ordinates; has opened up a new dimension for easy and quick identification of sources of stable field resistance. The application of the methods of multivariate analyses for the purpose of comparing and classifying genotypes appears simpler to classify and ordinate on the basis of their attributes of the actual disease assessment scores at different assessment dates, rather than the derived parameters, which involve complicated data transformation and estimation. The only difficulty in the tedious, labor-intensive and time consuming process of recording the disease severity at frequent intervals can be reduced by restricting the observations to the lesion number (LN) on the third leaf at seedling stage and fourth leaf at tillering stage of the plants (Mohapatra *et al*., 2001), since the single-component index based on LN alone has been identified as the best and most efficient with 93% relative selection efficiency, compared with other single and multiple-component indices (Mukherjee *et al*., 1996).

The multivariate analysis methods will have wider application in future, for analysis and simplification of vast multivariate data sets obtained from large scale screening trials like UBN or IRBN or even testing of the segregating population for understanding the genetic basis of host-pathogen interaction, to delimit more objectively grouping of individuals, strains, races, varieties, treatments, reactions etc., which are more similar in epidemiological behavior. The method would provide a valuable complement to the other methods of identification of stable resistant genotypes and create avenues as a prospective test for identification of resistant genotypes for use in long term disease control strategy not only in rice blast-pathosystem, but also in other plant-pathosystems in future.

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