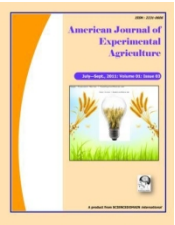

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Comparison of Different Parameters for Evaluation of Partial Resistance to Rice Blast Disease

N. K. Mohapatra[1], A. K. Mukherjee[2], A. V. Suriya Rao[2], N. N. Jambhulkar[2] and P. Nayak[3*]

[1]Christ College, Cuttack-753008, Odisha, India.
[2]Central Rice Research Institute, Cuttack-753006, Odisha, India.
[3]107/C, Goutam Nagar, Cuttack-753004, Odisha, India.

Authors' contributions

This work was carried out in collaboration between all authors. Authors NKM and AKM executed the experimental design, recorded all observations, tabulated data for statistical analysis and shared the entire process of interpretation, draft manuscript preparation under the supervision of the author PN. The mastermind behind all the statistical analyses was authors AVSR and NNJ. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: To evaluate and identify the most suitable parameter for easy and quick recognisation of rice genotypes possessing partial resistance to rice blast disease.
Experimental Design: The tested varieties were grown in one meter long single-row plots surrounded by the blast susceptible spreader rows of Karuna, with a spacing of 10 x 5 cm in a Uniform Blast Nursery pattern. The experiment was conducted in a randomized complete block design with three replications.
Place and Duration of Study: The experiments were conducted at the Central Rice Research Institute farm, Cuttack, continuously for nine seasons from 1998 to 2001.
Methodology: The disease severity was recorded at every alternate day intervals from disease initiation till end of epidemic. The disease scores were subjected to estimation of 12 parameters for evaluation of resistance. The data on 12 parameters for 42 rice genotypes tested across nine seasons were subjected to principal component analysis, in order to classify and ordinate the response of the genotypes and determine the relative

**Corresponding author: Email: nparsuramata@yahoo.co.in;*

importance of parameters.
Results: The cluster analysis classified the 42 genotypes into 4 groups of A&B as susceptible and C&D as partial resistant (PR) clusters, during each season of study. The PR genotype groups were characterized by lower estimates of Final disease severity(FDS), Mean disease severity(MDS), Area under disease progress curve(AUDPC), Relative AUDPC(RAUDPC), logistic infection rate(r), Gompertz infection rate(k), genotype score on first(PC-1) and second(PC-2) principal components, logit line intercept(logit-a), Gompit line intercept(gompit-a), and higher estimates of days to reach 50% severity in logistic model(T_{50r}) and Gompertz model(T_{50k}).Ordination of genotypes onto the PC-1 & PC-2 planes recognized 19 genotypes in group C and 12 genotypes in group D as PR, while rest of the 11 genotypes in cluster-A & B were susceptible. The relative importance of the parameters analyzed by factor analysis revealed that FDS, MDS, AUDPC, RAUDPC, r, k and PC-1 were the top ranking parameters with highly significant correlation among them.
Conclusion: One can choose any of the above seven top ranking parameters for easy identification of PR genotypes, depending upon the available resources for computation. Among the 42 rice genotypes, 12 in cluster-D and 19 in cluster-C were identified as possessing partial resistance. The technique of principal component analysis, the ordination and positioning of genotypes on the ordination figure emerged as a valuable tool in identification of rice genotypes possessing PR to blast disease.

Keywords: Magnaporthe grisea; multivariate analysis; ordination; principal components; partial resistance.

ABBREVIATIONS

***FDS-** Final disease severity; **MDS-** Mean disease severity; **AUDPC-** Area under disease progress curve; **RAUDPC-** Relative area under disease progress curve; **r-** Apparent infection rate in logistic model; **k-** Apparent infection rate in Gompertz model; **T_{50r} -** Time(days) required for the disease to reach 50% severity in logistic model; **T_{50k}-** Time(days) required for the disease to reach 50% severity in Gompertz model; **Logit-a-** logit line intercept; **Gompit-a-** gompit line intercept; **PCA -** Principal component analysis; **PC-1 -** Genotype score on first principal component axis; **PC-2 -** Genotype score on second principal component axis; **PR-** Partial resistance.*

1. INTRODUCTION

Frequent breakdown of vertical resistance in several plant-pathosystems, leading to serious yield loss, have developed renewed interest among the plant pathologists and breeders in exploitation of partial resistance, which is believed to be more stable and long lasting. The most devastating blast disease of rice (*Oryza sativa* L.), incited by *Magnaporthe grisea* (Hebert) Barr (*Pyricularia grisea* Sacc. = *Pyricularia oryzae* Cav.), is no exception to such types of serious yield losses due to breakdown of resistance [1,2]. The assessment of partial resistance in different plant pathosystems could be accomplished by analysis of different components of resistance [3,4], estimation of different parameters for evaluation of resistance [4,5,6,7,8] and analysis of the disease progress curves [7,9,10]. The second method has so far been commonly adopted worldwide by plant pathologist as well as breeders in different plant-pathosystems due to the ease in estimation of the parameters. The disease severity can be assessed either once at the peak of the epidemic or several times during the course of epidemics at certain intervals. The former method of assessment

measures the cumulative effect of the host-pathogen interactions operating during the course of an epidemic, while the later measures the path of epidemic progress by way of measuring the area under disease progress curves, the apparent infection rates and the time required for the disease to reach a specific level of severity.

The proportion of disease in the host plant, when plotted against time, gives a disease progress curve, which is usually sigmoid; although other types of curves are often encountered. The rate-reducing resistance can be assessed by quantification and comparison of such disease progress curves. Although it is difficult to classify disease resistance into discrete classes, the rate-reducing resistance can be recognized among several genotypes based on a sound knowledge on the host-pathogen system with the help of an efficient evaluation system. The disease resistance in different plant-pathosystems has so far been assessed through estimation of the parameters like the final disease severity (FDS), the means disease severity (MDS), the scoring by standard evaluation system (SES)[11], the area under disease progress curves (AUDPC) [8], the relative area under disease progress curve (RAUDPC) [6], the logistic apparent infection rates (r) [12], the Gompertz apparent infection rates (k)[5], the time required for the disease to reach a specific level of severity in logistic (T_{50r}) or Gompertz (T_{50k}) [8] models, the logit ($logit_a$) or gompit ($gompit_a$) line intercepts, the index-score values (IS) [13] or the genotype-scores on first (PC-1) and second (PC-2) principal components obtained through the principal component analysis (PCA) [14] of the disease scores. Each of the parameters has its own advantages and disadvantages as well. It also cannot be taken for granted that a single parameter will fit into all the plant-pathosystems.

The rate-reducing resistance to rice blast disease has been evaluated mostly through estimation of apparent infection rate [15]; AUDPC and r [16,17]; final disease severity and r [18]; diseased leaf area and AUDPC [17,19]; AUDPC, lesion number (LN) and lesion size [20]. Index score values using the single component index, 6.4 LN, was found to be highly effective in identification of slow-blasting rice genotypes with 93 % of relative selection efficiency [13]. Slow-blasting resistance was evaluated by adopting different parameters among which AUDPC, RAUDPC, r, k, T_{50r}, $T_{50k,}$ FDS, SES score, IS_{LN}, IS_{LN+NZA} and PC-1 were found to be superior expression of resistance [21]. The effect of nitrogen fertilization on the expression of slow-blasting resistance in rice was evaluated based on nine parameters, among which LN, AUDPC, RAUDPC, r, and k were found to be superior over T_{50r}, $T_{50k,}$ $logit_a$ and $gompit_a$ [7]. An attempt has been made in the present investigation to evaluate partial resistance through all these derived parameters and to identify the most suitable parameter for easy and quick identification of partial resistant rice genotypes.

2. MATERIALS AND METHODS

2.1 Source of Material and Cultural Conditions

The seeds of 42 rice genotypes were collected from the list of donors for various biotic and abiotic stresses maintained at the International Rice Research Institute, Philippines and the national gene bank maintained at the Central Rice Research Institute, Cuttack, India. Seeds of these test varieties were grown in one meter long single-row plots surrounded by the blast susceptible spreader rows of Karuna, with a spacing of 10 x 5 cm in a Uniform Blast Nursery pattern, modified for screening for slow-blasting resistance as suggested by Marchetti(1983) [16]. The experiment was conducted in a randomized complete block design with three replications. Fertilizer in the form of ammonium sulphate was applied at the rate of 100 kg

Nha^{-1} in split doses. High relative humidity was maintained throughout the period of disease development by continuous overhead sprinkling with intermittent stoppages for 30 minutes after one hour of running of the sprinklers, during the hottest period of the day i.e. 10.00 AM to 3.30 P.M. The experiment was conducted continuously for nine seasons, spread over a period of five years from dry season, 1997 to dry season 2001 under upland conditions.

2.2 Recording Observation and Statistical Analyses

Critical observations were recorded at every alternate day intervals starting form the initiation of the disease till completion of the epidemic when the susceptible spreader rows completely succumbed to the disease. The disease severity was recorded beginning from the first day of disease initiation until the end of the epidemic in the spreader rows when it reached a severity level of 100% (completely succumbed), following the score chart developed by Padmanabhan and Ganguly [22]. The scoring was based on both number and type of spots, each assigned with a numerical value which is summarized below:

Type of spot	Numerical value
Type-A: Just brown specks	1
Type-B: Reddish brown circular discoloration without zonal differentiation	2
Type-C: Circular spots, 2–3 mm. diameters	4
Type-D: Broadly spindle shaped spots, slightly longer than broader with central ashy zone of 4–5 mm. diameters	8
Type-E: Elongated spindle shaped spots with central ashy zone of 3–5 mm. broad and up to 20–30 mm. long	16

The number of spots were also assigned numerical values as:

Number of spots	Score	Numerical value
Up to 5 spots	Score-1	1
6–15 spots	Score-2	5
>15 spots	Score-3	10

The products of the respective numerical values for the type and number of spots were considered as the numerical score value for a particular unit of observation (= a plant). The average of five such highest infected plants was considered as the disease score for a particular host genotype. These numerical score values were converted into severity scores of $0< x \leq 1$ by dividing each score by the highest score recorded in the susceptible check Karuna at the end of the epidemic and used for analysis of disease progress curves.

The per cent disease severity on a genotype on the last day of observation when the disease reached a level of 100% severity in the susceptible check Karuna was considered as the final disease severity (FDS). The final disease severity was divided by the number of days from the initiation of symptom appearance till the last day of observation in a particular genotype, in order to arrive at the mean disease severity (MDS) level and is expressed here as the per cent disease per day. The area under disease progress curve (AUDPC) was calculated as suggested by Shaner and Finney [8], which is given by:

$$AUDPC = \sum_{i=1}^{n} [(x_{i+1} + x_i)/2]\,[t_{i+1} - t_i]$$

where, x_i = the proportion of host tissue damaged at the i^{th} day; t_i = the time in days after appearance of the disease at the i^{th} day and n = the total number of observations. The values of AUDPC were normalized by dividing with the number of days from the first appearance of disease symptom until end of disease assessment [6]. The normalized AUDPC was referred to as RAUDPC. The apparent infection rates in logistic (r) model [12] as well as Gompertz (k) model [5], were estimated as the regression coefficients 'b' in the regression equations with logit (Y) or gompit (Y) as dependant variable and time in days as independent variable. The 'r' and 'k' are presented here as per unit per day and logit (Y) and gompit (Y) are $\log_e[Y/(1-Y)]$ and $-\log_e[-\log_e Y]$, respectively. The Y axis intercept 'a' for both logistic (logit-a) and Gompertz (gompit-a) models were also considered as two parameters in the present study. The number of days required for the disease to reach 50% severity was calculated both in logistic (T_{50r}) and Gompertz (T_{50k}) models [26] as:

T_{50r} = (logit 0.5 - a)/b and $T_{50}k$ = gompit (0.50 - a) /b, using the values of the point of intercept 'a' and the regression coefficient 'b' ,which was determined from the logit (r) or gompit (k) analyses for the respective disease progress curves. The genotype-scores on the first two principal components (PC-1 and PC-2); estimated from the principal component analysis (PCA) by using the genotypes as the entities and the disease severity at every alternate day intervals as the variables; were also considered as two independent parameters.

The data set on 12 parameters for evaluation of resistance of 42 genotypes over the period of nine seasons were subjected to multivariate analyses like the cluster analysis, principal component analysis and factor analysis, in order to classify and ordinate the genotypes on the basis of the parameters and also to find out the relative importance of the parameters in determining partial resistance [23]. The mean responses of the genotypes across nine seasons of study were considered for this purpose. The genotypes were classified on the basis of their estimates of 12 parameters by hierarchical agglomerative method of cluster analysis [24] by considering the genotypes as the entities and the parameters as the variables. The groups of rice genotypes, which responded similarly to the 12 parameters, could be delimited on a dendrogram showing distinct clusters of slow-blasting and fast-blasting genotypes.

The principal component analysis was carried out to extract the correlation matrix, eigen values and vectors, the per cent variation explained and finally the genotype-score on first few principal components accounting for more than 90% variation. The ordination of the genotype-scores on to the planes of PC-1 and PC-2 and super-imposition of the clustering pattern on the scattered diagram was done to display the position of each cluster of genotypes on the ordination figure. The field reactions of these clusters of genotypes were tested based on the average disease progress curves and the cluster means in respect of all 12 parameters.

The relative importance of the parameters was estimated for each season through factor analysis by considering the 12 parameters as the entities and the 42 genotypes as the variables. The analysis reduced the dimensionality and simplified the complexity by extracting the eigen values and vectors and rotated factor matrices for three factors, showing the inter-correlations among them. The question of which parameters are consistent in expressing disease response across nine seasons was answered by compilation and comparison of their inclusion into specific factors for each of the nine seasons. The entire set of multivariate analysis was carried out with the help of the INDOSTAT statistical package developed at Hyderabad, India [25].

3. RESULTS

3.1 Progress of Epidemic

The application of high nitrogen levels and maintenance of high relative humidity by overhead sprinkling resulted in 100% disease severity in the susceptible check Karuna during all the nine seasons of testing, as evidenced by early initiation, rapid rate of progress of the epidemic and completion of the epidemic within a short period ranging from 10 to 22 days. The disease was initiated in the resistant genotypes 2 to 4 days later, progressed very slowly and reached a maximum intensity of 4.6 to 33.3%, 26 days after initiation of disease in the susceptible check Karuna. Monitoring the disease development and progress through repeated assessment at every alternate day intervals, facilitated in describing the level, rate and shape of the resultant disease progress curves, through estimation of different parameters. Twelve such derived parameters averaged over nine seasons in respect of 42 rice genotypes arranged in ascending order of AUDP*C*, are presented in Table 1. The distribution of wide range of the FDS from 0.88 to 100% revealed that the set of genotypes possessed a broad spectrum of disease reactions. A similar wide spectrum of reactions was observed among the genotypes in respect of MDS, AUDPC, RAUDPC, r, k and the genotype score of PC-1.

3.2 Correlation among the Parameters

There was a highly significant correlation among the parameters (Table 2). The $T_{50r}$and T_{50k}were, however, not significantly correlated with logit-a, gompit-a and the genotype-score on PC-2 and also gompit-a was not correlated with PC-2. Although the arrangement of the genotypes in ascending order of AUDPC was not reflected in the parameters T_{50r}, $T_{50k,}$ logit-a, gompit-a and PC-2; the highest and lowest estimates were well within the range of resistance or susceptibility groups. The ranking of the genotypes based on FDS, MDS, AUDPC, RAUDPC, r, k and PC-1 were roughly continuous, each of them showing highly significant correlation with AUDPC.

3.3 Clustering Pattern

It is difficult to classify the genotypes into distinct groups of resistance or susceptibility by way of simple comparison of all the derived parameters. Classification of the 42 genotypes based on the 12 parameters using the hierarchical agglomerative cluster analysis, resulted in nine dendrograms, each depicting groups of genotypes with similarity in respect of their attributes of 12 parameters for each of the nine seasons. The cluster analysis invariably classified the 42 genotypes into two major groups at higher Euclidian distances, one of them consisting of highly susceptible genotypes and the second consisting of genotypes, which were further classified into three sub groups possessing both moderately susceptible and partial resistant reactions to the disease at lower Euclidian distances. The genotypes were thus grouped into four groups of A and B as susceptible and C and D as partial resistant clusters for each of the nine seasons of testing. This has been clearly depicted in the dendrogram for average of nine seasons of testing (Fig. 1).The cluster means in respect of all the 12 parameters exhibited distinct differences between the susceptible and resistant groups for each of the nine seasons.

Table 1. Average estimates of 12 parameters for comparison of blast disease progress curves of 42 rice genotypes tested over nine seasons, arranged in ascending order of AUDPC

Genotype	FDS	MDS	AUDPC	RAUDPC	r	k	T50r	T50k	Logit-a	Gompit-a	PC-1	PC-2
Milayeng-51	0.876	0.053	42.312	2.575	0.038	0.008	30.267	29.932	-2.349	-0.568	-1.975	-0.134
Chokoto-14	2.083	0.131	53.628	3.343	0.066	0.015	17.927	28.145	-2.506	-0.767	-1.813	-0.405
PTB-8	1.010	0.053	60.967	3.217	0.023	0.005	24.131	40.576	-1.666	-0.506	-2.011	0.012
Sechi aman	1.666	0.110	65.968	4.215	0.054	0.011	45.399	104.252	-3.024	-0.925	-1.496	-0.140
UCP-188	1.879	0.102	71.959	3.735	0.045	0.006	24.709	64.388	-2.456	-0.680	-1.703	-0.124
Salum pikit	1.667	0.107	73.204	4.570	0.063	0.012	43.920	84.695	-3.597	-1.101	-1.341	-0.256
Madhukar	2.667	0.155	74.489	4.169	0.076	0.018	27.193	87.452	-3.082	-0.947	-1.358	0.121
Tien-Phai	2.374	0.132	89.498	4.622	0.089	0.020	34.504	58.276	-3.877	-1.183	-1.472	-0.018
Sakai	2.322	0.146	92.625	5.384	0.079	0.018	48.305	77.981	-4.313	-1.317	-1.576	-0.015
DM-27	2.417	0.140	98.246	5.457	0.085	0.026	48.435	81.035	-4.395	-1.721	-1.426	0.039
Goda heenati	3.761	0.223	101.386	5.928	0.101	0.023	32.938	53.198	-4.371	-1.322	-1.432	0.021
Lien-tsan-50(B)	2.916	0.161	104.436	5.604	0.107	0.030	34.440	56.082	-3.517	-1.191	-1.249	0.192
IR-5533-PP-854-1	3.751	0.232	112.529	6.393	0.071	0.018	60.447	172.788	-3.366	-1.084	-1.188	0.188
Sam-houang	3.593	0.191	116.261	6.396	0.108	0.023	47.255	110.533	-4.662	-1.303	-1.160	0.111
Laurent-TC	3.404	0.212	117.094	6.814	0.128	0.026	56.157	106.963	-5.878	-1.737	-1.391	0.080
Raj bhawalta	3.377	0.187	118.059	6.483	0.084	0.022	69.474	117.868	-4.661	-1.474	-1.383	0.004
Chiang-Tsene-Tao	4.376	0.270	122.204	7.584	0.109	0.015	59.813	108.335	-5.391	-1.570	-1.345	-0.060
PTB-18	3.374	0.178	124.173	6.548	0.083	0.019	27.040	45.666	-3.408	-1.058	-1.292	-0.139
DNJ-155	3.583	0.201	127.847	7.045	0.084	0.019	47.406	83.773	-4.504	-1.409	-1.095	-0.188
DZ-192	3.228	0.164	129.054	7.073	0.104	0.021	45.081	79.708	-4.889	-1.481	-1.158	0.275
Dahanala-2014	6.541	1.224	133.941	8.025	0.140	0.036	46.854	78.316	-5.909	-1.829	-1.300	0.171
Seritus malam(B)	4.334	0.236	136.908	7.029	0.052	0.014	28.858	41.302	-2.499	-0.831	-0.682	-0.263
DJ-88	4.229	0.233	138.163	7.614	0.104	0.021	70.405	139.487	-5.276	-1.623	-1.072	-0.039
Prolific	5.601	0.446	146.525	7.599	0.137	0.037	43.676	70.213	-5.298	-1.646	-1.180	0.017
Lien-Tsan-50(A)	3.915	0.426	150.083	7.748	0.104	0.023	54.751	115.342	-5.345	-1.653	-0.841	0.105
CR-570	6.288	0.323	151.284	7.636	0.102	0.024	26.784	42.057	-3.511	-0.807	-0.628	0.378
Jumi-1	5.771	0.302	152.196	7.181	0.103	0.028	40.451	61.887	-4.356	-1.379	-1.000	0.254
Mak-thua	9.156	0.489	170.339	9.834	0.105	0.032	59.188	110.788	-4.825	-1.564	-0.954	-0.448
E-425	6.541	0.360	173.165	9.149	0.097	0.029	40.451	78.778	-4.693	-1.610	-0.401	-0.069

Table 1 continues

Seritus malam(A)	7.876	0.438	188.813	10.187	0.117	0.033	62.590	106.415	-4.917	-1.610	-0.353	-0.027
Surjamukhi	10.751	0.526	206.817	10.676	0.128	0.033	67.152	51.312	-5.135	-1.461	-0.283	0.088
N-22	15.667	0.835	260.503	12.622	0.143	0.063	38.204	55.199	-4.796	-1.607	0.688	0.113
Bakkabiasa	33.749	2.039	276.695	14.653	0.243	0.115	25.124	36.254	-5.986	-2.296	0.989	-0.507
Jaya	45.002	2.327	336.904	16.907	0.220	0.099	26.931	32.428	-4.907	-1.915	1.231	-0.254
Kalubalawee	26.417	1.466	349.566	16.678	0.181	0.081	47.565	73.086	-4.697	-1.779	1.241	-0.221
Ratna	40.832	2.407	402.725	19.726	0.297	0.128	17.840	38.916	-4.923	-1.855	2.951	-0.021
ARC-7046	57.083	3.065	443.438	21.179	0.303	0.136	15.424	124.762	-4.631	-1.714	3.835	0.219
Pusa-4-1-11	45.626	3.302	451.596	21.755	0.271	0.132	20.021	27.729	-5.210	-2.141	2.208	0.093
CR-289-1045-16	60.625	3.351	479.511	25.272	0.360	0.145	23.055	33.299	-6.321	-2.344	2.814	0.520
Tiace	77.917	4.888	652.775	31.234	0.446	0.247	11.249	11.024	-4.990	-2.213	6.532	-0.538
India dular	100.000	5.890	757.360	35.153	0.671	0.338	11.526	10.807	-7.368	-3.095	5.332	0.641
Karuna	100.000	8.343	1190.74	53.744	0.740	0.464	6.801	6.630	-5.076	-2.603	12.973	0.580
CD	14.748	1.151	133.93	6.154	0.136	0.101	25.546	54.277	2.405	1.211	1.359	0.975

Table 2. Association among 12 parameters for evaluation of partial resistance in rice

	MDS	**RAUDPC**	**r**	**k**	**T_{50r}**	**T_{50k}**	**Logit-a**	**Gompit-a**	**PC-1**	**PC-2**	**AUDPC**
FDS	0.977**	0.962**	0.971**	0.958**	0.617**	-0.502**	-0.517**	-0.795**	0.937**	0.313*	0.957**
MDS		0.985**	0.981**	0.988**	-0.603**	-0.504**	-0.488**	-0.779**	0.971**	0.343*	0.985**
RAUDPC			0.974**	0.980**	-0.527**	-0.461**	-0.523**	-0.797**	0.985**	0.356*	0.998**
r				0.987**	-0.552**	-0.475**	-0.579**	-0.828**	0.948**	0.411**	0.972**
k					-0.580**	-0.501**	-0.477**	-0.769**	0.972**	0.364*	0.984**
T_{50r}						0.763**	-0.131	0.184	-0.565**	-0.115	-0.539**
T_{50k}							-0.006	0.230	-0.455**	-0.026	-0.470**
Logit-a								0.899**	-0.421**	-0.310*	-0.493**
Gompit-a									-0.718**	-0.297	-0.776**
PC-1										0.317*	0.989**
PC-2											0.366*

** and ** Significant at P=0.05 and 0.01 levels, respectively.*

The susceptible cluster (cluster-A), consisting of three genotypes namely; Karuna, India Dular, CR-289-1045-16; and the moderately susceptible cluster (cluster-B) consisting of eight genotypes namely; Pusa 4-1-11, Jaya, Ratna, Prolific, ARC -7046, Tiace, Bakkabiasa and Kalubalawee were characterized by higher estimates of the parameters FDS, MDS, AUDPC, RAUDPC, r, k, PC -1 and PC-2 and lower estimates of T_{50r} , $T_{50k,}$ logit-a and gompit-a(Table 3). The reverse trend for the respective estimates was obtained for the rest of the genotypes under both the partial resistant clusters of C and D.

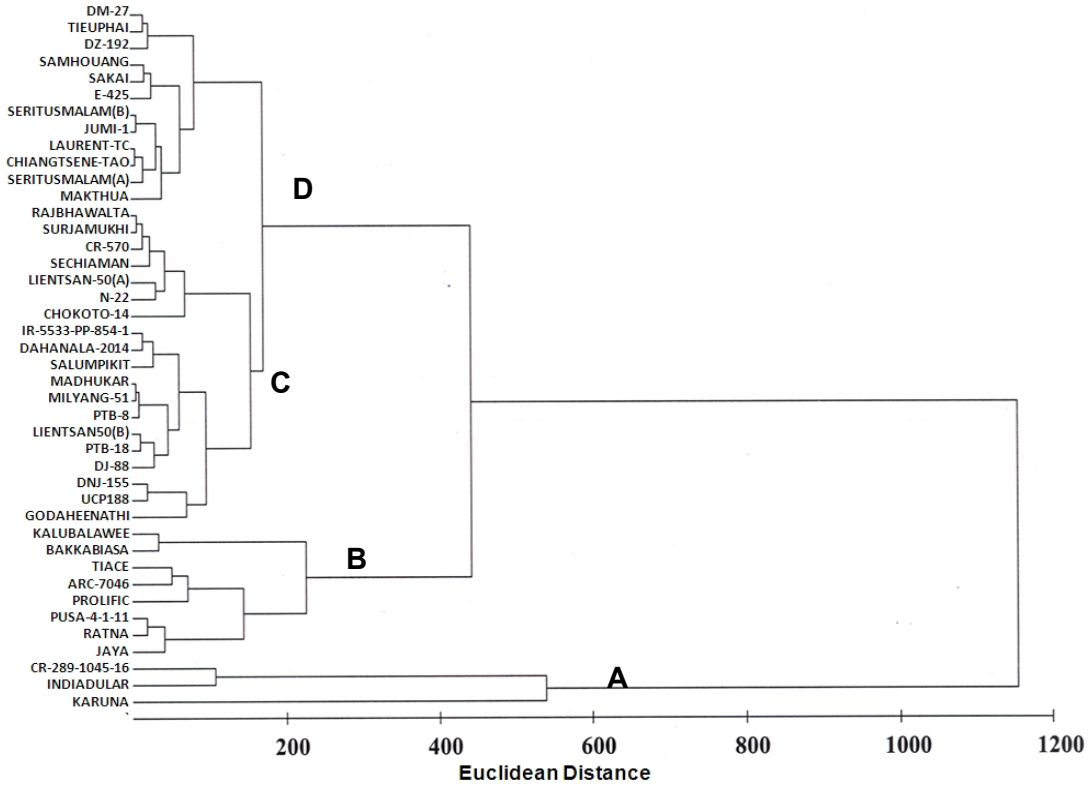

Fig. 1. A dendrogram showing the similarity and successive clustering of 42 rice genotypes based on 12 parameters for evaluation of resistance, averaged over nine seasons of testing

A critical insight into the inclusion of each genotype into different clusters across nine seasons of study revealed that the genotype Karuna (cluster-A) expressed high level of susceptibility consistently across all the nine seasons of testing. Eight genotypes, viz. DZ-192, DM-27, Tieu Phai, Sakai, Jumi-1, Laurent-TC, Chiang-Tsene-Tao and Chokoto-14 (cluster-D) expressed high levels of partial resistance, thus indicating high stability in their response across all nine seasons of testing (Table 4).

Table 3. Cluster means of 12 parameters for evaluation of partial resistance, based on average of nine seasons of testing

Cluster*	Parameters											
	FDS	**MDS**	**AUDPC**	**RAUDPC**	**r**	**k**	**T_{50r}**	**T_{50k}**	**Logit-a**	**Gompit-a**	**PC-1**	**PC-2**
A (3)	92.639	6.374	866.956	40.043	0.619	0.350	9.859	9.487	-5.811	-2.637	8.279	0.228
B (8)	40.625	2.349	375.117	18.599	0.252	0.113	26.770	52.709	-5.184	-1.956	1.994	-0.007
C (19)	5.247	0.349	142.919	7.737	0.103	0.025	50.202	90.607	-4.659	-1.428	-0.984	0.023
D (12)	2.136	0.126	77.393	4.401	0.069	0.016	34.347	63.834	-3.263	-1.019	-1.571	-0.059

** Figures in parentheses indicate the number of genotypes under corresponding clusters.*

Name of the genotypes in each cluster:

Cluster-A Karuna, India dular, CR-289-1045-16.

Cluster-B Pusa-4-1-11, Jaya, Ratna, Prolifc, ARC-7046,Tiace, Bakka-biasa, Kalubalawee.

Cluster-C Goda heenati, UCP-188, DJ-88, DNJ-155, PTB-8, PTB-18, Lien-Tsan-50(B), Milayeng-51, Madhukar, Salum Pikit, Dahanala-2014, IR-5583-pp-85-1, Chokoto-14, N-22, Lien-Tsan-50(A), Sechi aman, CR-570, Surjamukhi, Raj Bhawalta.

Cluster-D Mak-Thua, Seritus Malam(A), Chiang-Tsene-Tao, Laurent-TC, Jumi-1, *Seritus Malam(B), E-425, Sakai, Sam-Houang, DZ-192, Teu-Phai, DM-27.*

Twenty four genotypes, viz. E-425, Mak-thua, Sam-houang, Seritus malam(A), Seritus malam(B), Raj bhawalta, Sechi aman, Surjamukhi, IR-5533-PP-854-1, Madhukar, Milayeng-51, PTB-8, Dahanala-2014, Lien Tsan-50(A), Lien tsan-50(B), N-22, Salum-Pikit, PTB-18, DNJ-155, DJ-88, UCP-188, Goda Heenati, Prolific and CR-570 were consistently included into the partial resistant clusters-C & D during all the seasons of testing, thus indicating high stability in their response to the disease across nine seasons of testing. The genotypes India Dular and Tiace were consistently included into the susceptible clusters-A & B during all the nine seasons. Rest of the genotypes exhibited variable reactions by way of their inclusion into Clusters-ACD or ABCD or BC or BCD, which might be due to the variability in host response or pathogen population or host x pathogen x environment interactions.

Table 4. Compilation of data on the response of genotypes across nine seasons of testing

Genotypes	Number of genotypes	Clusters
Karuna	1	A
India Dular, Tiace	2	AB
Kalubalawee	1	ACD
CR-289-1045-16	1	ABCD
ARC-7046, Pusa-4-1-11, Ratna	3	BC
Bakka-Biasa, Jaya	2	BCD
E-425, Mak-Thua, Sam-Houang, Seritus Malam(A), Seritus Malam(B), Raj Bhawalta, Sechi Aman, Surjamukhi, IR-5533-PP-854-1, Madhukar, Milayeng-51, PTB-8, Dahanala-2014, Lien Tsan-50(A), Lien Tsan-50(B), N-22, Salum-Pikit, PTB-18, DNJ-155, DJ-88, UCP-188, Goda Heenati, Prolific, CR-570	24	CD
DZ-192, DM-27, Tieu-Phai, Sakai, Jumi-1, Laurent-TC, Chiang-Tsene-Tao, Chokoto-14	8	D

The results of possible seasonal variations in assessment of disease reactions of the 42 genotypes for each of the 12 parameters are presented in Table 5. There was no significant difference among the seasons based on PC-1 and PC-2 while other parameters showed significant differences.

3.4 Ordination of Genotypes

The principal component analysis of 42 rice genotypes, carried out by considering the genotypes as entities and 12 parameters as the variables, extracted data tables on correlation matrix, latent roots and vectors, per cent variation explained by each root and finally the genotype scores on the first two principal components. The per cent variation explained by the first two principal components was 71.05 and 14.73, respectively.

Table 5. Seasonal variations analysed through different parameters

Season	FDS (%)	MDS (%)	AUDPC	RAUDPC	*r*	*k*	T_{50r}	T_{50k}	Logit '*a*'	Gompit '*a*'	PC-1	PC-2
1	24.682a	1.784a	282.60a	13.87a	0.206a	0.109a	21.09c	27.38d	-3.985ab	-1.584abc	0.262a	-0.028a
2	20.704ab	1.166bcd	223.05abcd	11.10abc	0.179ab	0.082ab	19.21c	24.12d	-3.687a	-1.240a	-0.054a	0.000a
3	24.047a	1.487ab	247.25abc	11.54abc	0.209a	0.095ab	22.23c	28.70cd	-4.195ab	-1.534ab	0.158a	-0.010a
4	9.802e	0.643d	212.23bcd	9.18c	0.106c	0.043c	64.98a	100.07a	-3.666a	-1.245a	-0.000a	0.000a
5	19.959abc	1.246bc	262.80ab	13.46ab	0.198ab	0.077abc	38.95b	49.48bc	-5.700cd	-1.948cde	0.075a	-0.006a
6	12.817de	0.823cd	193.79cd	10.55c	0.137bc	0.062bc	47.98b	65.87b	-4.062ab	-1.410ab	0.127a	0.103a
7	15.873bcde	0.829cd	184.67d	9.92c	0.178ab	0.064bc	45.66b	56.65b	-6.292d	-2.125e	0.063a	0.017a
8	13.655cde	1.079bcd	180.25d	10.79bc	0.199ab	0.084ab	45.09b	63.69b	-4.918bc	-1.671bcd	0.084a	0.007a
9	18.321abcd	0.956bcd	209.59bcd	10.80bc	0.194ab	0.071bc	42.17b	51.37b	-6.179d	-2.026de	-0.000a	0.000a
CD	6.827	0.533	61.99	2.85	0.063	0.035	32.886	21.1	0.98	0.38	0.63	0.45

Figures in a column followed by the same letter do not differ significantly at P=0.05 level.

Ordination of the genotype-scores on to PC-1 and PC-2 clearly displayed the response of the genotypes to the rice blast disease. Super-imposition of the clustering pattern obtained from the dendrogram (Fig. 1) onto the ordination figure distinguished positioning of the four clusters of genotypes on the planes of PC-1 and PC-2 ordinates (Fig. 2). A critical insight into the ordination figure revealed that 3 genotypes in cluster-A and 8 genotypes in cluster-B, positioned away from the point of intersection in positive direction, exhibited higher degree of susceptibility. The 19 genotypes in clusters-C and 12 genotypes in cluster-D, positioned along the PC-2 ordinate, exhibited higher degree of resistance (= partial resistance). This fact was further confirmed through the field reaction of these clusters of genotypes presented as cluster means (Table 3) and also the average disease progress curves for the respective clusters of genotypes (Fig. 3).

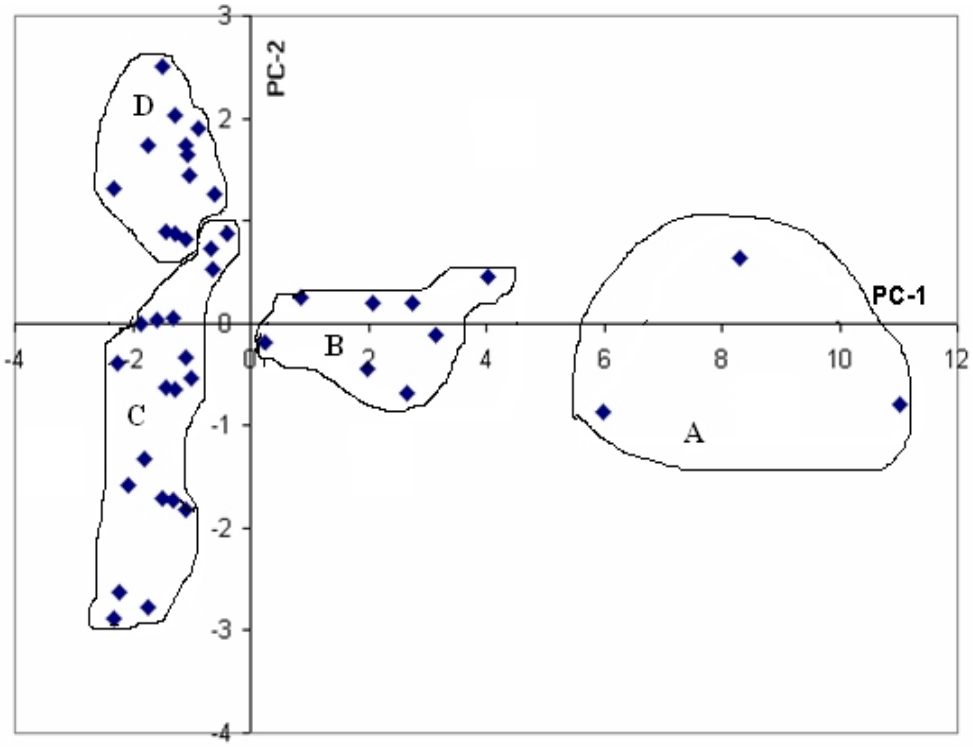

Fig. 2. Ordination of 42 rice genotypes onto the planes of vector-1 and 2 from principal component analysis. The groups of genotypes encircled are the main clusters obtained from Fig. 1

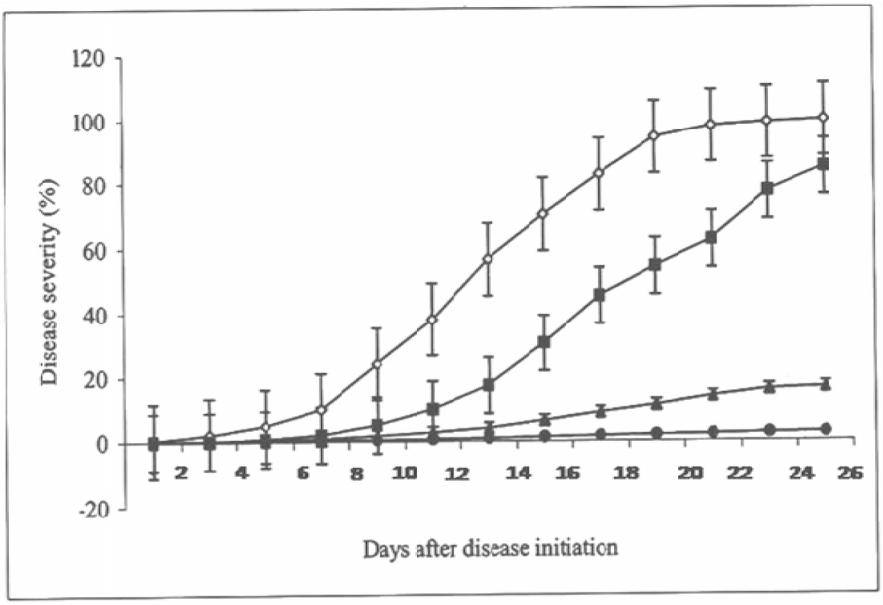

Fig. 3. Average disease progress curves for each of the four clusters of genotypes based on the clustering pattern obtained from the dendrogram (Fig. 1)

3.5 Relative Importance of the Parameters

The relative importance of the 12 parameters in characterization of disease progress curves could be determined by the degree of variability and inter-correlations, analyzed through factor analysis by considering the parameters as the entities and the 42 genotypes as the variables, over a period of nine seasons of testing individually. Three factors were extracted from the rotated correlation matrix for each of the nine seasons of testing through factor analysis. The most interesting inter-correlations were in the first and second factors. The first factor proves a high positive inter-correlations for FDS, MDS, AUDPC, RAUDPC, r, k and PC-1 for each of the nine seasons of testing, thus signifying high relative importance of these parameters for evaluation of slow-blasting resistance in rice. The second factor proves high positive inter-correlation for T_{50r} and T_{50k} and high negative inter-correlation for the logit-a as well as gompit-a. The third factor proves high positive inter-correlation recorded for PC-2 during each of the nine seasons of testing. The first two factors together explained 80.10% to 91.18% of the variation. The authors are restricted to present these data for all nine seasons in nine tables for economizing the printing space in the journal.

The compilation of data on the consistency in disease assessment through each parameter over nine seasons of testing, revealed maximum inter-correlations among the genotypes expressed through the parameters FDS, MDS, AUDPC, RAUDPC, r, k and PC-1 as evidenced by their consistent incorporation in to factor-1 during the all the periods of testing (Table 6). Hence these were considered as the top ranking parameters for assessment of partial resistance. The parameters $T_{50r}$and T_{50k}were recognized as the second ranking parameters due to their inclusion into factor-2. The two parameters on logit-a as well as gompit-a were inconsistent in expression of the disease reaction of the genotypes as evidenced by their inclusion, sometimes into factor-1 and sometimes into factor-2. The parameter PC-2 emerged as the third ranking parameter by way of its constant inclusion into

factor-3 during each of the nine seasons of testing. These findings based on the analysis and compilation of each of the nine seasons data were also confirmed through analysis of the data based on the average of all the nine seasons with a few exceptions.

Table 6. Relative importance of each of the 12 parameters based on their inclusion into different factors estimated over nine seasons of testing.

Parameters	Factor 1	Factor 2	Factor 3
MDS	9	0	0
FDS	9	0	0
AUDPC	9	0	0
RAUDPC	9	0	0
r	9	0	0
k	9	0	0
T_{50r}	0	9	0
T_{50k}	0	9	0
logit-a	4	9	0
Gompit-a	6	9	0
PC-1	9	0	0
PC-2	0	0	9

Table 7. Eigen values and vectors; varimax factor matrix of 12 parameters for evaluation of blast disease progress curves in respect of 42 rice genotypes averaged over nine seasons of testing

Parameters	Eigen values and vectors			Varimax factor matrices	
	Vector-1	Vector-2	Vector-3	Factor-1	Factor-2
MDS	0.335	-0.030	-0.060	**0.742**	-0.641
FDS	0.339	-0.036	-0.016	**0.744**	-0.654
AUDPC	0.337	-0.003	0.006	**0.767**	-0.616
RAUDPC	0.338	0.016	-0.020	**0.784**	-0.598
r	0.340	0.035	0.021	**0.805**	-0.582
k	0.337	-0.030	0.012	**0.745**	-0.645
T_{50r}	-0.202	0.543	-0.100	-0.011	**0.932**
T_{50k}	-0.180	0.494	0.087	-0.002	**0.841**
logit 'a'	-0.192	-0.537	0.210	**-0.884**	-0.209
Gompit 'a'	-0.283	-0.346	0.237	**-0.932**	0.155
PC-1	0.330	-0.046	-0.009	**0.715**	-0.647
PC-2	0.133	0.216	0.937	0.483	-0.018
Root	8.526	1.768	0.845	5.903	4.391
σ^2 % explained	71.050	14.732	7.044	57.344	42.656
$\Sigma\sigma^2$ % explained	71.050	85.782	92.826	57.346	100.000

Bold figures indicate high inter-correlations.

The factor analysis based on the average of nine seasons data on 12 parameters with regard to 42 genotypes, extracted two factors accounting for 57.35% and 42.66% variations present in the communality (Table 7). The most interesting inter-correlations were in factor-1. The first factor proves high positive inter-correlations for FDS, MDS, AUDPC, RAUDPC, r, k and genotype-score on PC-1. This was in conformity with similar findings obtained from the analysis in respect of each of nine season's data. The second factor proves high positive inter-correlations for T_{50r} and T_{50k}, which is similar to the observations on individual nine season data. The only deviation was high negative inter-correlations for logit-a and gompit-a,

and low positive inter-correlation for genotype-score on PC-2, all of which were incorporated into factor-1.

4. DISCUSSION

The disease progress curve encompasses within it various elements of the host, the pathogen and the environment, active at different stages during the course of the epidemic development and thus can be considered as a complete expression of the anatomy of the disease. One can dissect out these elements, analyze, compare and classify the disease progress curves. Estimation, compilation and comparison of 12 parameters in respect of 42 genotypes over a period of nine seasons of testing revealed FDS and MDS to be more variable. Moreover, these parameters cannot be used for assessment of partial resistance since they do not express certain important elements of disease progress curve like the delay in initiation of the disease; the rate of disease progress, prediction of the time at which the disease would reach specific level of severity etc. On the other hand, the estimates of the parameters AUDPC, RAUDPC, logistic as well as Gompertz apparent infection rates, the $T_{50r}$and T_{50k}incorporate all these elements effective during the process of epidemic development. The logit and gompit line intercepts express the probable day of initiation of the epidemic and the genotype-score on PC-1 and PC-2 express the overall score of the genotypes to place them in their respective geometric positions on the ordination figure.

The relative importance of 12 parameters in characterization of disease progress curves, determined by the degree of variability and inter-correlations among them estimated through factor analysis, revealed maximum inter-correlations among FDS, MDS, AUDPC, RAUDPC, r, k and PC-1 consistently for all the nine seasons by way of their inclusion into factor-1 which accounted for 44.98 to 62.16% of variation with a mean of 54.54%. Besides the inter-correlation among themselves, each of them was significantly associated with rest of the parameters. Among them, the logistic apparent infection rate has been widely used for analysis of epidemics as a very useful parameter in several plant-pathosystems including rice-blast [7,26]. However, serious drawback in the logistic apparent infection rate as a statistic for studying the rate-reducing resistance has been pointed out [5,8,27,28]. According to Berger (1981), some of the information in the disease progress curve are lost in the calculation of r due to the errors introduced by lack of linearity[5], since it is strongly influenced by minor differences in low disease severities early in the season, which becomes much larger when transformed to logit x/(1-x). On the other hand, Gompertz model avoids the curvilinearity associated with the logistically transformed values resulting in accurate estimation of the epidemic rate, projection of future disease severity and determination of initial disease in nine plant-pathosystems [5]. The Gompertz transformation was also reported to be more consistent in detecting the degree of slow-rusting in oats [27], late blight of potato, leaf spot of celery and rust of beans [29] and several other plant-pathosystems. On the other hand, a better fit of the logistic model was claimed with wheat powdery mildew-pathosystem [30]. Both logistic as well as Gompertz models have been reported fitting well for linearization of disease progress curves in rice blast-pathosystem [7, 26]. The inclusion of both the parameters r and k into factor-1 consistently for all the nine seasons, leads to the conclusion that both the models fit well into rice blast-pathosystem, thus confirming the previous findings [26] reported by Mohapatra et al. [26].

The two parameter, logit-line intercept (logit-a) as well as gompit-line intercept (gompit-a) were found to be of some value for comparing the disease progress curves, as an indicator of initial start of the epidemics, but were highly inconsistent in expression of the true nature of the disease progress curve, as evidenced by their poor association with other parameters

over nine seasons of testing. The lower '*a'* values obtained for more resistant genotypes could be interpreted as an indicator of initial date of start of epidemic and greater delay in onset of epidemic. On the other hand, the reverse should have happened for the susceptible genotypes, which was not always true. This is probably the reason why these parameters were inconsistently included into either factor-1 or 2 across different seasons of testing. The two parameters, $T_{50r}$and T_{50k} had maximum inter-correlations and were consistently included in factor-2, accounting for 24.72 to 36.17% of the variation with mean of 31.03% for all the nine seasons of testing and thus were considered as the second ranking parameters, even though they embodied both the position and the slope of the transformed disease progress curves.

The estimates of the parameter AUDPC in the present experiment resulted in a better visual comparison among the genotypes, correctly reflected the disease development using all the available data, did not obscure the variation in rate of disease development, exhibited distinct differences among the genotypes, proved most convenient for summation without involving complicated data transformations and was least influenced by minor differences in disease severity early in the season, and hence was considered superior to other parameters. Kranz [31] analyzed different elements of disease progress curves in various pathosystems through factor analysis and reported AUDPC as one of the important elements in addition to the logistic apparent infection rate. Similar conclusions were also drawn for stem rust resistance in wheat [28], slow-mildewing in Knox wheat [8], late blight resistance in potato [6], slow-blasting resistance in rice [9,21,26]. The only disadvantage that, it has to be calculated from a common time base, since it is a product of time and severity, could be avoided by estimating the RAUDPC for easy comparison between genotypes over different seasons of study.

The difficulty in the tedious, labor-intensive and time consuming process of recording 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 the fourth leaf at tillering stage of the plant [32], since the single component index score (IS), 'LN(IS_{LN})' alone has been identified as the best and most efficient , with 93% of relative selection efficiency, compared with other single and multiple component indices [13]. AUDPC can also be estimated from two data points, *i.e.* initial and final disease scores of the disease progress curves, since it provides the information similar to that from all data points, thereby saving valuable time, labor and economic resources [33,34].

It is of interest to note here that the genotype-score on PC-1 emerged as one of the first ranking parameters due to the fact that the ranking of the genotypes on PC-1 was consistent during all the nine seasons of testing and PC-1 alone accounted for more than 90% of the variation in the communality, with a strong association with all the parameters. The parameter PC-2 was consistently included into factor-3 during all the nine seasons of testing, which accounted for only 8.60 to 18.89% of the variation with a mean of 11.43% and was not associated with the parameters under factor-2 i.e. $T_{50\,r}$ and $T_{50\,k}$ and also gompit-a. Both PC-1 and PC-2 estimated consistently similar types of disease reactions across all nine seasons of study without exhibiting significant differences among seasons (Table 5).In view of these findings, PC-1 appears to have emerged as the most important parameter for evaluation of partial resistance to blast disease. The seasonal variation in disease reactions might be due to environmental fluctuations or variation in pathogen population. The rice genotypes identified as possessing partial resistant reactions in the present study, were also tested in multi-location blast testing trials conducted by the International Rice Research Institute, Manila, and identified as possessing resistant reactions across several locations all over the

world. Thus the genotypes have been exposed to a wide spectrum of environments and pathogen population and still exhibiting resistant reactions. Hence, they might be possessing partial resistance to the disease, which has been proved from the present experiment.

5. CONCLUSION

The seven parameters FDS, MDS, AUDPC, RAUDPC, r, k & PC-1 were identified as the top ranking parameters from among the 12 parameters. One can choose any of these parameters depending upon the available resources for computation. Among the 42 rice genotypes, 12 in cluster-D and 19 in cluster-C were identified as possessing partial resistance. The technique of principal component analysis, involving cluster analysis, factor analysis, and ordination and positioning of genotypes on the ordination figure emerged as a valuable tool in identification of rice genotype clusters possessing PR to blast disease.

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RECOMMENDATION

It can be recommended from the present study that (***i***) any one of the seven parameters FDS, MDS, AUDPC, RAUDPC, r, k & PC-1 can be chosen by the researcher for identification of partial resistant rice genotypes, (***ii***) the principal component analysis could be used as a valuable tool for easy identification of genotypes possessing partial resistance, and (***iii***) the genotypes possessing partial resistance could be used as donors in breeding for resistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

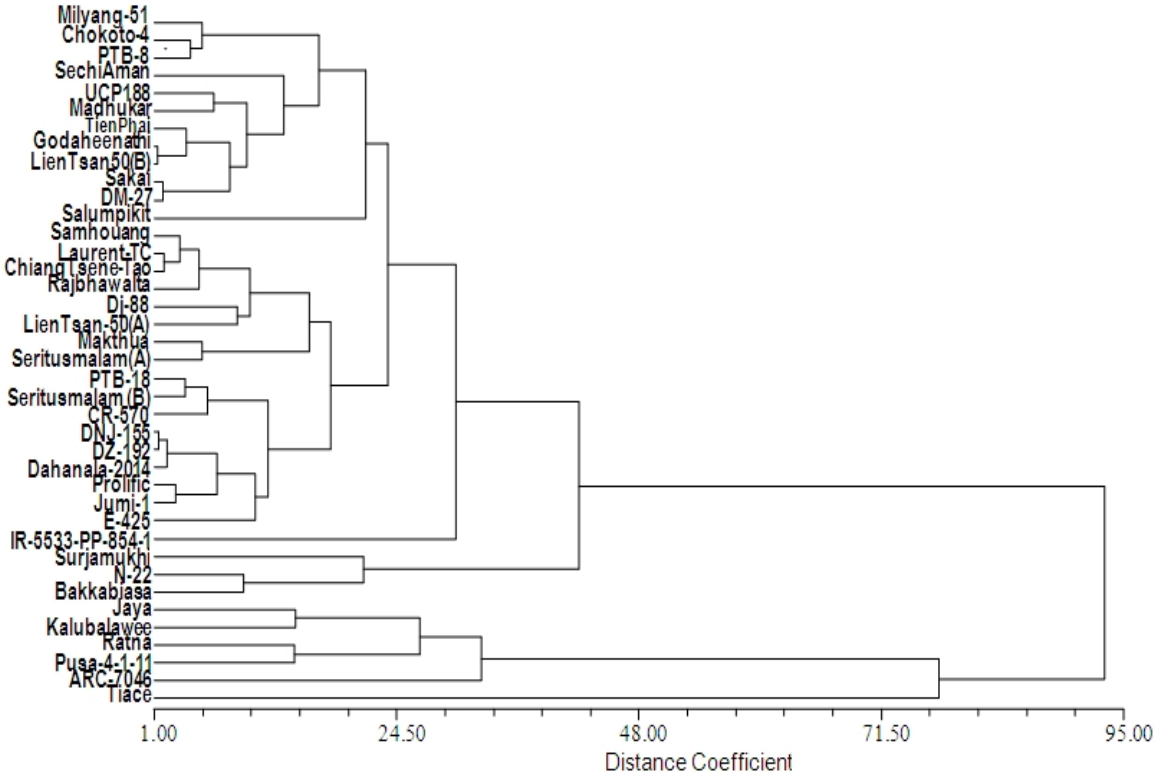

Fig. 1. Dendrogram (without Karuna, CR-289-1045 and Indiadular)

Table 1: Correlation between parameters for evaluation of partial resistance to rice blast disease, calculated from all data points

Parameter	FDS	MDS	AUDPC	RAUDPC	r	k	T50r	T50k	Logit-a	Gompit-a	PC1	PC2
FDS	1.000	0.935**	0.911**	0.924**	0.909**	0.919**	-0.301**	-0.297**	-0.393**	-0.630**	0.833**	0.107*
MDS		1.000	0.896**	0.916**	0.911**	0.940**	-0.289**	-0.280**	-0.356**	-0.596**	0.824**	0.125*
AUDPC			1.000	0.986**	0.875**	0.911**	-0.181**	-0.185**	-0.393**	-0.628**	0.914**	0.093
RAUDPC				1.000	0.897**	0.926**	-0.173**	-0.180**	-0.437**	-0.667**	0.905**	0.113*
r					1.000	0.952**	-0.259**	-0.257**	-0.580**	-0.747**	0.769**	0.186**
k						1.000	-0.279**	-0.275**	-0.397**	-0.651**	0.836**	0.157**
T50r							1.000	0.912**	-0.267**	-0.144**	-0.237**	0.014*
T50k								1.000	-0.178**	-0.062	-0.230**	0.041
Logit-a									1.000	0.902**	-0.221**	-0.119*
Gompit-a										1.000	-0.455**	-0.147**
PC1											1.000	0.011
PC2												1.000

** and ** Significant at P=0.05 and 0.01 levels, respectively.*

Peer-review history:
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