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Estimation of genetic divergence analysis for yield and bacterial leaf blight (BLB) disease resistance in rice (*Oryza sativa* L.)

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Abstract

An investigation was carried out with hundred genotypes of indigenous collection of rice, were tested for the presence of diversity on the basis yield and bacterial leaf blight resistance using Mahalanobis D^2 statistics. ANOVA revealed the presence of considerable amount of variability among the genotypes. High estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were observed for area under disease progress curve followed by grain yield per plant and total spikelet per panicle. High heritability coupled with high genetic advance was recorded for total spikelets per panicle. On the basis of D^2 values the genotype were grouped into clusters. Eleven clusters were formed using Tocher's method. Cluster I, VII, V and IX were having 22, 17, 10 and 10 germplasm, respectively. The highest inter-cluster distance was found between cluster X and XI (458.41) whereas maximum distance intra-cluster distance was observed cluster IX (63.20) and cluster 1 sowing lowest intra-cluster distance (13.46) suggested a closer relationship and low degree of diversity among the genotypes. The maximum contribution towards divergence was accounted by plant 1000 grain weight (23.58%), grain yield per plant (22.9%) and effective tiller per plant (17.74%) followed by spikelets per panicle, plant height, grain weight per panicle and days to 50 percent flowering. On the basis of the divergence study the germplasm could be selected from the most divergent clusters for hybridization and further selection programme.

Keywords: GCV, PCV, diversity, D^2 analysis, rice

Introduction

Rice (*Oryza sativa* L.) ($2n=24$) is a monocotyledon angiosperm belongs to the family Poaceae and is widely cultivated in tropical and subtropical regions (Ezuka and Kaku, 2000) [6]. Rice is the prime food crop of the world for more than half of the global populations. The rising demand, saturation of cultivable field and low gross domestic production of rice are likely to cause a supply shortage of a crop in the near future (Dhanwani *et al.*, 2013) [4]. In India rice is grown over 43.19 mha, ranking first in the world and hold the second in production with 110.15 million tons in 2016-17 with the productivity of 2.50 t/ha. (Directorate of Economics and Statistics, Department of Agriculture and Cooperation, Government of India, 2016-17). To sustain self-sufficiency and to meet food grain requirement of future, India has to produce 135 to 140 million tons of rice by 2030.

However, rice production is constrained by considerable number of disease of fungal, bacterial and viral origin. Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *Oryzae*, is one of the most devastating disease of cultivated rice (*Oryza sativa* L.) in tropical and temperate regions worldwide (Nino-Liu Do *et al.*, 2006) [13]. This disease was first time noticed by farmers of Japan during 1884 (Tagami and Mizukami, 1962; Mizukami and Wakimoto, 1969; Devadath, 1992) [21, 11, 3]. The BLB usually occurs as lesions at the leaf tips. At the later stages, irregular yellow lesions are formed and margins become wavy. At extreme levels the entire leaf area is covered with white and greyish saprophytic growth (Ou, 1985; Tagami and Mizukami, 1962) [15, 21]. Result of severe disease occurrence will produce poor quality and sterile pollen grains. Disease incidence occurs at all growth stages of rice crop, causing drastic yield losses ranging between 20 and 30 per cent. The disease severity can cause a yield loss up to 80 per cent and is influenced by various crop stages, environmental conditions (28 to 34°C) and degree of susceptibility of the genotypes (Noh *et al.*, 2007) [14].

Genetic divergence studies between cultivars or accessions before any breeding programme would allow the breeder to concentrate efforts on those combinations which are more likely to be highly heterotic when different metric characters are available concerning a set of accessions. Mahalanobis's D^2 statistic based on multiple characters have been an efficient statistical tool for assessing genetic divergence among a set of genotypes which could be used

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for hybridization and thus the diverse genotypes incorporated in hybrids on selfing may be expected to throw desirable segregates with the accumulation of favorable genes into a single genetic background.

Material and Method

The present study was carried out to evaluate the hundred germplasm lines of rice obtained from National Bureau of Plant Genetic Resources, New Delhi through Department of Genetics and Plant breeding, Institute of Agricultural Sciences, Banaras Hindu University India with respect to yield attributes and AUDPC. All the genotypes were grown in an experiment in randomized complete block design in *Kharif* 2014 and *Kharif* 2015 at Agricultural Research Farm of Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (UP), India. Nursery was planted in second week of August and about 4 week old seedlings were transplanted during second week of September with row-to-row x plant-to-plant spacing maintained at 20 cm x 20 cm. Each plot consists of ten plants and represents a single entry in each replication. Recommended agronomic practices were followed to raise a good crop.

The culture of *Xanthomonas oryzae* pv. *oryzae* (strain BX043 wild type) was obtained from Department of Mycology and Plant pathology IAS, BHU, India and subcultured on peptone sucrose agar medium and maintained it at pH 7.2 - 7.4 (Fahy and Persley 1983) [7] and pathogenicity test, clipping method was used to inoculation the rice plants with *Xanthomonas oryzae* pv. *oryzae*. The artificial inoculation was conducted on fully developed leaves at 45-day-old-rice plants after transplanting. Following inoculation, the plants were observed after every 24 hrs time intervals to note the appearance of disease symptoms, and lesion length were recorded at 8, 16, 24 and 32 days after inoculation (DAI). (Anonymous, 1996) [1].

Table A: Scale for bacterial leaf blight.

Infection (%)	Score	Host response
0 %	0	Highly resistant (HR)
> 1-10%	1	Resistant (R)
> 10-30%	3	Moderately resistant (MR)
> 30-50%	5	Moderately susceptible (MS)
> 50-75%	7	Susceptible (S)
> 75-100%	9	Highly susceptible (HS)

Data were recorded on ten randomly selected. Five plants, excluding border plants, were randomly selected for recording of data from each replication on various yield traits such as days to 50% flowering (DF), days to maturity (DM), plant height (PH), number of effective tillers per plant (ET), panicle length (PL), number of spikelet per panicle (SP), Grain weight per panicle (GWP), Thousand grain weight, grain yield per plant (GYP), and Area under disease progress curve (AUDPC), and mean value were used for statistical analysis. AUDPC was calculated with the help of formula based on the per cent disease severity at different stages (Madden *et al.*, 2007) [9]:

$$\text{AUDPC} = \sum_{i=1}^n \left[\frac{(Y_i + Y_{(i+1)})}{2} \times (t_{(i+1)} - t_i) \right]$$

Where,

Y = disease level at time t_i

$[t_{(i+1)} - t_i]$ = time (days) between two disease scores

The genotypes were grouped into different clusters with the help of Tocher's method. Inter cluster and intra cluster distances and contribution of each trait towards total divergence among genotypes under investigation were estimated using Mahalanobis's D^2 analysis (Ranjith *et al.*, 2018) [19].

Result and Discussion

Analysis of variance showed significant differences for all the characters studied except for flag leaf width, suggesting the existence of high genetic variability among the genotypes. The presence of large amount of variability might be due to diverse source of materials as well as environmental influence affecting the phenotypes. Similar findings were reported by Mishra *et al.*, (2003). Both PCV and GCV estimates were highest for area under disease progress curve (35.43) followed by grain yield per plant (23.67) and total spikelet per panicle (23.17) (Table 1). Similar results were supported the earlier observations of Ponnaiah *et al.* (2018) [17]. High estimates of heritability (above 60%) in broad sense were recorded for all the ten characters under study (Ranjith *et al.*, 2018) [19], which ranged from 98.08% (grain yield per plant) to 68.21% (Panicle length). Johnson (1955) reported that high heritability should be accompanied by high genetic advance to arrive at more reliable conclusion.

Therefore, genetic advance was also computed. A perusal of genetic advance for all the quantitative characters under study ranged from 11.95% (panicle length) to 46.58 % (Total spikelets per panicle). High heritability coupled with high genetic advance was registered for total spikelets per panicle, suggesting predominance of additive gene action in the expression of these traits. Similar findings were reported by Govintharaj *et al.* (2016) [8].

The genetic divergence existing in hundred rice genotypes was studied by employing D^2 analysis for ten quantitative characters. The hundred genotypes were grouped into eleven distinct clusters. The distribution of these genotypes in eleven clusters has been given in Table 2 & Fig 1. Among the clusters the highest twenty two genotypes were appeared in cluster I and followed by cluster VII had seventeen genotypes and cluster V & IX had ten genotypes in each. Clusters VIII had nine genotypes, Cluster II had eight genotypes and cluster VI had six genotypes. Remaining cluster IV, X, XI and III had 5 and 3 respectively. The pattern of group constellation proved the existence of significant amount of variability.

The estimates of intra and inter cluster distances for hundred rice genotypes for eleven clusters are presented in Table 2. The intra cluster distance ranged from 13.46 (cluster I) to 63.20 (cluster IX). The inter cluster distance was maximum between cluster X and XI (458.41) and minimum inter cluster distance was observed between cluster V and cluster VII (18.40). An examination of average intra and inter cluster distance indicated that genotypes of within cluster had little divergence from each other with respect to aggregate of effects of ten characters under study. Similar findings were also reported by Nayak *et al.*, (2004) [12], Chaturvedi and Maurya (2005) [2] and Yadav *et al.* (2011) [24]. Therefore, the chance of obtaining recombinants in segregating generations by crossing the members of same are very low. It is therefore, suggested that crosses should be attempted between the genotypes belonging to cluster separated by large inter cluster distances. To realize much variability and high heterotic effect, recommended that parents should be selected from two clusters having wider inter cluster distance (Thomas and Lal, 2012) [22].

The cluster mean values showed a wide range of variations for all the characters undertaken in the study (Table 3). Cluster XI exhibited highest mean value for grain yield per plant (29.75), Grain weight per panicle (4.07), Total spikelets per panicle (182.12), lowest mean value of Area under disease progress curve (190.77), while cluster X contained genotypes with highest mean value for panicle length (28.61). Cluster VIII recorded highest value for effective tillers per plant (9.39) and plant height (140.77) while highest mean value for days to 50% flowering (133) and days to maturity (163) was recorded by cluster VII. In Hybridization programme involving genetically diverse parents belonging to different distant clusters would provide the opportunity for bringing together gene constellations of diverse nature (Ramanjaneyulu *et al.*, 2014; Singh *et al.*, 2008) [18, 20].

The contribution of different characters towards the expression of genetic divergence has been given in Table 4. The highest contribution in manifestation of genetic divergence was exhibited by thousand grain weight (23.58%), grain yield per plant (22.91%), Effective tiller per plant (17.74%), total spikelets per panicle (17.33%), plant height (12.97%), grain yield per plant (3.54%), days to maturity and

days to 50 percent flowering (less than 1%). The contribution of no of spikelets per panicle and yield in divergence has been also observed by (Nayak *et al.*, 2004, Eswaran R., 2012 and Ovung *et al.*, 2012) [12, 5, 16]. The contribution of various characters towards the expression of genetic divergence should be taken into account as criterion for choosing parents for crossing programme for the improvement in such characters (Nayak *et al.*, 2004 and Tripathi *et al.*, 2013) [12, 23]. In present study cluster X and cluster XI were most diverse to each other. These clusters are suggested to provide a broad spectrum of variability in segregating generations and the genotypes present in them may be used as parents for future hybridization programme to develop desirable types. The mode of distribution of genotypes from different eco-regions into various clusters was at random indicating that geographical and genetic diversity were not related. Thus, the crossing of genotypes belonging to cluster separated by high inter cluster distances and differing markedly for characters having high contribution towards total genetic divergence would be more fruitful for isolating superior segregates in segregating generations.

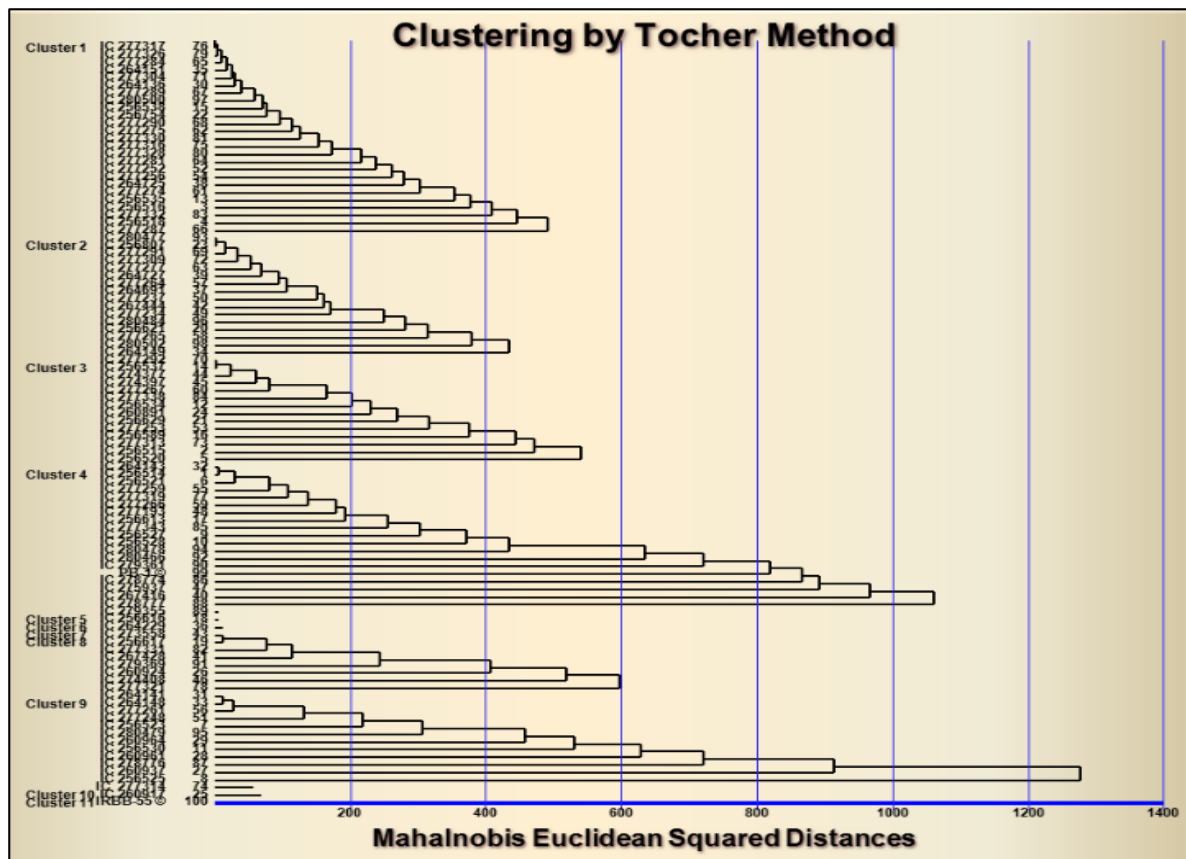


Fig 1: Distribution of hundred genotypes of rice into different clusters.

Table 1: Genetic Parameter of ten traits in selected rice germplasm.

Parameters		Days to 50% flowering	Days to Maturity	Plant Height (cm)	Effective Tillers / plant	Panicle Length (cm)	Total Spikelets/ Panicle	Grain Weight/ Panicle	1000 Grain Weight (g)	Grain Yield/ Plant (g)	AUDPC
Range	Min	57.00	86.75	69.43	4.00	19.28	47.21	1.41	12.42	8.34	190.77
	Max	146.50	172.12	174.65	11.00	28.67	182.12	4.12	32.19	29.75	1095.26
Mean		117.58	147.49	129.37	7.54	24.51	91.34	2.87	22.63	16.86	622.11
SEm(±)		2.20	2.16	1.30	0.07	0.41	1.15	0.06	0.20	0.18	58.00
Variability	PCV	17.73	14.32	15.95	17.12	8.50	23.17	23.67	17.27	23.01	35.43
	GCV	16.91	13.70	15.69	16.90	7.02	22.89	22.81	17.07	22.79	23.50
Heritability (%)		90.99	91.50	96.79	97.54	68.21	97.61	92.88	97.76	98.08	44.02
GA as % of mean		33.23	26.99	31.81	34.39	11.95	46.58	45.28	34.77	46.49	32.13

Table 2: Average Intra & Inter cluster distances: Tocher Method

	1Cluster	2Cluster	3Cluster	4Cluster	5Cluster	6Cluster	7Cluster	8Cluster	9Cluster	10 Cluster	11 Cluster
1 Cluster	13.460	28.116	35.652	37.651	46.689	63.659	61.544	67.183	76.235	162.306	266.098
2 Cluster		20.946	41.925	55.986	55.794	54.990	46.771	63.051	65.355	171.520	209.818
3 Cluster			34.969	66.740	43.903	45.351	62.287	100.820	71.868	132.815	300.176
4 Cluster				37.119	84.429	80.692	99.852	104.558	101.348	156.987	301.078
5 Cluster					0.000	79.166	18.409	103.963	73.136	187.260	318.529
6 Cluster						0.000	84.774	155.895	66.264	58.007	324.544
7 Cluster							0.000	95.689	73.892	232.714	265.529
8 Cluster								60.357	123.077	290.674	181.847
9 Cluster									63.208	143.443	234.004
10 Cluster										0.000	458.410
11 Cluster											00.000

Table 3: Mean value of eleven clusters for ten morphological characters in rice genotypes.

	Days to 50 % Flowering	Days to Maturity	Plant Height (cm)	Effective Tillers Per Plant	Panicle Length (cm)	Spikelets Per Panicle	Grain Weight Per Panicle (g)	1000 Grain Weight (g)	Grain Yield Per Plant (g)	Area Under Disease Progress curve
1 Cluster	127.582	158.058	131.899	8.181	24.293	84.232	2.721	22.053	15.557	652.374
2 Cluster	124.336	153.750	139.572	7.227	24.340	104.131	2.784	21.803	18.505	563.341
3 Cluster	124.500	154.625	141.708	7.152	24.835	81.092	3.496	25.568	15.820	692.116
4 Cluster	105.842	135.171	116.766	7.478	24.247	86.442	2.464	19.689	13.622	699.737
5 Cluster	125.375	156.875	130.742	7.000	28.405	47.211	3.419	24.030	21.234	536.744
6 Cluster	130.250	160.375	128.346	5.000	24.175	101.187	3.057	26.536	14.023	683.301
7 Cluster	133.125	163.125	139.326	6.000	24.708	68.100	2.835	21.072	24.917	426.556
8 Cluster	110.656	141.219	140.779	9.391	25.340	107.029	2.606	21.256	21.175	524.556
9 Cluster	105.906	135.135	114.287	6.417	24.011	95.562	3.281	25.982	19.859	517.512
10 Cluster	65.875	94.500	95.300	5.000	28.621	99.256	2.652	32.194	8.343	1007.546
11 Cluster	87.250	120.625	80.650	9.125	25.016	182.125	4.072	23.503	29.755	190.775

Table 4: Relative contribution of different characters to genetic divergence.

Source	Contribution %
1 Days to 50 % Flowering	1.84
2 Days to Maturity	0.02
3 Plant Height (cm)	12.97
4 Effective Tillers Per Panicle	17.74
5 Panicle Length (cm)	0.08
6 Spikelets Per Panicle	17.33
7 Grain Weight Per Panicle	3.54
8 1000 Grain Weight (g)	23.58
9 Grain Yield Per Plant (g)	22.91
10 Area Under Disease Progress Curve	0.01

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