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Research Article

Genetic divergence analysis for bacterial leaf blight (BLB) disease resistance in rice (*Oryza sativa* L.)

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Abstract
The present study was undertaken to assess the genetic variability existed among 114 rice germplasm for BLB resistance. In field level screening, the genotypes PY5 and Kadaikannan showed immune against rice BLB. The rice germplasm were subjected to D^2 analysis for nine quantitative traits *viz.*, plant height, number of productive tillers per plant, days to fifty per cent flowering, panicle length, number of grains per panicle, thousand grain weight, single plant yield, grain length and grain breadth. On the basis of Mahalanobis D^2 statistics, the 114 genotypes were grouped into eighteen clusters.

Keywords
BLB, Mahalanobis D^2 statistics and PDI.

Introduction

The cultivated 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). More than 2.7 billion people in the world consume rice as staple food. Besides rice cultivation provides employment for more than one billion directly or in allied and supported activities (Das *et al.*, 2014). In India, rice crop is cultivated in an area of 433.88 lakh hectares with a production and productivity of 104.32 million tonnes and 2404 kg/ha respectively (Annual Report 2016-17, Dept. of Agriculture, Cooperation and Farmers Welfare). In Tamil Nadu, rice crop occupies an area of 21 lakh hectares with a production of 93 million tonnes (Policy Note 2015-16, Minister for Housing, Urban Development and Agriculture, Govt. of Tamil Nadu). To sustain self-sufficiency and to meet food grain requirement of future, India has to produce 135 to 140 million tonnes of rice by 2030.

Rice gets affected by more than 70 diseases by the infection of bacteria, fungi, and viruses. Among them, bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*) is the important disease around the rice cultivated areas (Khan, 1996). BLB is a γ-proteobacteria. The BLB usually occurs as lesions at the leaf tips. At 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). 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 (Ou, 1985; Shin *et al.*, 1992; Mew *et al.*, 1993; Noh *et al.*, 2007).

Mahalanobis' D^2analysis, serve as a potential tool for classification, a sort of multivariate analysis which helps the plant breeders in choosing suitable parents under stress situations for realizing superior segregants in breeding programmes.

Materials and Methods

The seeds of 114 rice germplasm were sown in a raised nursery beds and transplanted to the main field on 25 days after sowing with the spacing of 20 × 20 cm in two replications during Rabi 2017 - 18. All the crop management practices were followed throughout the crop period.

The screening was conducted in the paddy field at maximum tillering stage under natural environmental condition without using any BLB inoculum during Rabi season of 2017. Each germplasm is evaluated for the BLB resistance by calculating percentage disease index and by giving scales to the respective PDI reading. For one germplasm, twenty plants are taken for evaluating the BLB resistance. The Percentage Disease Index (PDI) and scales for evaluating the BLB resistance was analyzed based on the method suggested by Nagendran et al., (2013).

Percentage Disease Index (PDI) =

$$\frac{\text{Sum of all Numerical Ratings}}{\text{Total No of leaves graded}} \times \frac{100}{\text{Maximum Grade obtained}}$$

Scoring system used to evaluate breeding lines for BLB resistance in the field (IRRI, 2006 & Rafi et al., 2013)

Scale	Disease Leaf Area (%)	Description
0	0	Immune
1	1-10	Resistant
3	11-25	Moderate resistant
5	26-50	Moderate susceptible
7	51-75	Susceptible
9	76-100	Highly susceptible

The genetic divergence among one hundred and fourteen genotypes was estimated by Mahalanobis (1949) D^2 statistics for 9 quantitative characters. The D^2 values were calculated using the software Window Stat. The computed values were tested for significance. The average inter and intra cluster distance tables were obtained from the software output.

The grouping of the genotypes into cluster was done in Window Stat using Tocher's method (Rao *et al.,* 2002). The cluster diagram was drawn using the D^2 tables.

The cluster mean for the particular trait is the summation of mean values of the genotypes included in a cluster divided by number of genotypes in the cluster.

Results and Discussion

The morphological screening of one hundred and fourteen rice varieties against the bacterial leaf blight pathogen under field condition was carried out. Here, there was no bacterial inoculum applied as well as there was no artificial favorable condition given to promote the pathogen growth. In order to test the host pathogen interaction at natural environment, the field screening of rice germplasm were carried out. Among the resistant genotypes PY 5 and Kadaikannan found to be immune with the lowest PDI value of 0.00 and 0.00 per cent respectively. The genotypes, Jai Shree Ram and Kurukot registered highest PDI value of 95.00 and 78.57 per cent respectively. The result for this screening of rice genotypes based on field screening were presented in the table 1 and figure 1.

Ramalingam et al., (2017) screened twenty five rice genotypes for bacterial leaf blight resistance and revealed that the improved rice lines in the genetic backgrounds of Samba Mahsuri, ASD 16, ADT 43 and IR 24 exhibited the higher level of resistance to most of the pathotypes studied, whereas the rice lines derived from ADT 47 background exhibited more susceptibility.

In the cluster diagram formed by the Tocher's method, eighteen major clusters were formed and their composition of D^2cluster was described in table 2 and figure 2. Cluster I included more genotypes with 38 individuals, followed by 30 genotypes in Cluster II, 20 genotypesin Cluster IV, 4 genotypes in XI Cluster and the remaining clusters contained single genotype each. The pattern of group constellation proved the existence of significant amount of variability. Similar findings also reported by Ramanjaneyulu *et al.,* (2014).

The highest intra cluster distance was recorded for cluster XI (29.02) followed by cluster V (27.28) and lowest intra cluster average distance was recorded by cluster I (20.17) and V (24.77). The intra and inter cluster average distance among eighteen clusters were variable and depicted in the table 3. Genotypes belonging to clusters separated by the higher genetic distance may be used in hybridization programme to obtain a wide spectrum of variation among the segregants. 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).

Cluster XI and XVII showed maximum inter cluster distance of 67.45 followed by cluster X and XVII (63.39). The lowest inter cluster distance was noticed between XIII and XIV (17.78), followed by the clusters VIII and XV (18.82). To realize much variability and high heterotic effect, Beevi and Venkatesan (2015) and Singh *et al.,* (2006) recommended that parents should be selected from two clusters having wider inter cluster distance. The cluster mean for all the biometric traits were studied character wise and are furnished below character wise and presented in Table 4 and Figure 3.Single plant yield exhibited cluster value ranging from 27.01 g (cluster XII) to 77.53 g cluster XI with an average of 46.54 g. The clusters, XI (77.53), VII (76.92), XVI (70.30) and X (70.24) exhibited higher cluster mean for single plant yield. Thus, the divergence of the one hundred and fourteen genotypes used in the study may be due to involvement of different ancestral pedigree or genetically diverse parentage.

The genotypes, PY 5, Kadaikannan,IR 12L 138, IR 11C 114, IR 12L 107, IR 12L 104, ACK 12001, ACK 13005, CR 1009, ADT 41, HHZ17-Y16-Y3-Y2, Mulampunchan and Veethiruppu were found to be resistant against rice bacterial leaf blight (BLB). Rice germplasm screened under field condition, one per cent were immune towards bacterial blight, nine per cent genotypes were resistant, thirty two per cent of them were found to be moderately resistant, thirty five per cent of rice genotypes were moderately susceptible, eighteen per cent were susceptible to bacterial blight and one per cent rice germplasm are highly susceptible to rice bacterial blight (BLB).On the basis of Mahalanobis D^2 statistics, the one hundred and fourteen genotypes were grouped into eighteen clusters. The BLB resistance in genotype dependent and not cluster based. Seven out of the thirteen resistant genotypes under field screening were positioned in I and II clusters. The cluster I had resistant genotypes IR 12L 104, IR 12L 107, ACK 12001, PY5 and IR 12L 138. Resistant genotypes in cluster II are Kadaikannan and Veethiruppu.

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Table 1. Score for BLB resistance under Field Screening

S.No	Germplasm	Percentage Disease Index (PDI)	Scale	Description
1.	IR 20	48.00	5	MS
2.	IRRI 104	48.57	5	MS
3.	IR 12L 214	15.00	3	MR
4.	IR 11T 193	40.00	5	MS
5.	IRRI 163	50.00	5	MS
6.	IR 11C 465	40.00	5	MS
	IRRI C 134	40.00	5	MS
8.	IR 64	15.00	3	MR
9.	IR 12L 115	30.00	5	MS
10.	IR BL TAR – PI (Co)	71.43	7	S
11.	IR 10A 240	11.67	3	MR
12.	IR DL 25 – CA	15.00	3	MR
13.	IR 11C 114	10.00	1	R
14.	IR 11L 433	25.00	5	MS
15.	IR BL5 – M	72.00	7	S
16.	IR 12L 342	30.00	5	MS
17.	IR 12L 138	5.00	1	R
18.	IR 72	30.00	5	MS
19.	IR 50	33.33	5	MS
20.	IR 12L 107	5.00	1	R
21.	IR 12L 110	20.00	3	MR
22.	IR 12L 104	5.00	1	R
23.	BB 8	11.67	3	MR
24.	PY 2	20.00	3	MR
25.	PY 5	0.00	0	I
26.	ACK 12001	10.00	1	R
27.	ACK 13005	10.00	1	R
28.	ACK 14004	63.33	7	S
29.	CR 1009	10.00	1	R
30.	TN – 1	13.33	3	MR
31.	Co – 39	13.33	3	MR
32.	Co – 43	15.00	3	MR
33.	Co – 45	60.00	7	S
34.	Co - 49	18.33	3	MR
35.	Co – 50	26.67	5	MS
36.	MDU – 5	11.67	3	MR
37.	ADT – 37	40.00	5	MS
38.	ADT – 39	28.33	5	MS
39.	ADT – 41	5.00	1	R
40.	ADT – 42	23.33	5	MS
41.	ADT – 43	24.00	5	MS
42.	ADT – 46	20.00	3	MR
43.	ADT – 48	13.33	3	MR
44.	ASD – 16	25.00	3	MR
45.	ASD – 18	15.00	3	MR
46.	ASD – 19	25.00	3	MR
47.	TPS – 4	66.67	7	S
48.	HHZ17-Y16-Y3-Y2	10.00	1	R
49.	UMA	50.00	5	MS
50.	Mulampunchan	5.00	1	R
51.	Kadaikannan	0.00	0	I
52.	Kalakeri	13.33	3	MR
53.	Maranellu	34.29	5	MS
54.	Kayamma	24.00	3	MR
55.	Swarna	20.00	3	MR
56.	Thondi	42.00	5	MS
57.	Anjali	15.00	3	MR

58.	Virendra	40.00	5	MS
59.	Annada	31.67	5	MS
60.	Bharathi	25.00	3	MR
61.	Veethiruppu	10.00	1	R
62.	Namcheonbyeo	15.00	3	MR
63.	Sadabahar	35.00	5	MS
64.	Chinnapunjan	70.00	7	S
65.	Purple Puttu	25.00	3	MR
66.	Surakuruvai	75.00	7	S
67.	Kalyani	45.00	5	MS
68.	Adukan	20.00	3	MR
69.	White Sannam	30.00	5	MS
70.	Athira	45.00	5	MS
71.	Jai Shree Ram	95.00	9	HS
72.	Pusa Basmati	54.29	7	S
73.	Kalinga – 3	45.00	5	MS
74.	Kavya	40.00	5	MS
75.	Navarai	52.86	7	S
76.	Kerala Gandhakasala	46.67	5	MS
77.	Srilanka	60.00	7	S
78.	Krishna Hemavathi	66.67	7	S
79.	Poonkar	62.86	7	S
80.	Kottara Samba	40.00	5	MS
81.	Meikuruvai	38.33	5	MS
82.	Kurukot	78.57	9	HS
83.	Vanaprabha	51.00	7	S
84.	Sahbhagi Dhan	52.86	7	S
85.	Dhalaheera	66.00	7	S
86.	White Ponni	30.00	5	MS
87.	Karsamba	70.00	7	S
88.	Thooyamalli	28.33	5	MS
89.	Kullakar	31.00	5	MS
90.	Kitchadi Samba	22.00	3	MR
91.	Kuliyadichan	19.00	3	MR
92.	Kaivara Samba	65.00	7	S
93.	Mapillai Samba	35.00	5	MS
94.	Gowni	40.00	5	MS
95.	BPT 5204	25.71	3	MR
96.	LFR 293	25.00	3	MR
97.	Kattanur	24.00	3	MR
98.	Gowri	19.00	3	MR
99.	JGL 348	45.00	5	MS
100.	Seeraga Samba	25.00	3	MR
101.	AD-BIO- 09518	38.33	5	MS
102.	Abhya	28.33	5	MS
103.	Mattai	21.67	3	MR
104.	Varapukudaichan	50.00	5	MS
105.	SR – 22 B	15.00	3	MR
106.	Jaya	35.00	5	MS
107.	JGL 1798	65.00	7	S
108.	JGL 3855	35.00	5	MS
109.	CR. Dhan 70	23.00	3	MR
110.	Co – 51	25.00	3	MR
111.	Rajalakshmi	22.00	3	MR
112.	TP – 10008	60.00	7	S
113.	TP – 10106	35.00	5	MS
114.	Kuruvai Kalanjium	45.71	5	MS

Table 2. Composition of D^2 cluster for rice genotypes

Cluster	Number of genotypes	Name of the genotypes
I.	**38**	TN 1, Co 50, IR 12L 104, IRRI 104, Co 39, IR DL 25 CA, ADT 37, IR 12L 115, IR BL 5M, BB 8, ASD 18,CR 1009, IR 12L 107, Uma, ASD 16, AD BIO 09518, IR 12L 342, Co 43, IR 72, TPS 4, ACK 12001, ADT 43, IR 12L 214, BPT 5204, PY 5, ACK 14004, LFR 293,IR 64, ADT 42, IR 11L 433, IR 50, IRRI C 134, IR 12L 138, ADT 48, White Sannam, Adukan, IR 11C 114 and IRRI 163.
II.	**30**	Kadaikannan, Kalakeri, Kitchadi samba, Karsamba, Veethiruppu, Kalakeri, Annada, Chinnapunjan, Jaya, Kuliyadichan, Thondi, Surakuruvai, Maranellu, Navarai, Anjali, Purple Puttu, Virendra, Sahbhagi Dhan, Rajalakshmi, Athira, Kurukot, Gowri, Kaivara Samba, Gowni, Swarna, Bharathi, Kayamma, White Ponni, Meikuruvai and Abhya.
III.	**1**	SR22B
IV.	**20**	ACK 13005, ADT 39, Kullakar, Srilanka, Kalyani, Kavya, Jai Shree Ram, Kalyani, Sadabahar, ADT 46, IR 11C 465, CR Dhan 70, JGL 1798, IR 11T 193, Co 49, HH-Z17-Y16-Y3-Y2, JGL 348, Kuruvai Kalanjium, TP10008 and Namcheonbyeo.
V.	**9**	IR 12L 110, MDU 5, Co 45, ADT 41, PY 2, Co 51, Kalinga 3, Pusa Basmati and TP 10106.
VI.	**1**	IR 10A 240
VII.	**1**	Kottara Samba
VIII.	**1**	IR 20
IX.	**1**	Vanaprabha
X.	**1**	Thooyamalli
XI.	**4**	Mapillai Samba, Varapukudaichan, Mattai and Dhalaheera.
XII.	**1**	Poonkar
XIII.	**1**	Kerala Gandhakasala
XIV.	**1**	Kattanur
XV.	**1**	Seeraga Samba
XVI.	**1**	JGL 3855
XVII.	**1**	IR-BL-TAR-PI (Co)
XVIII.	**1**	Krishna Hemavathi

Table 3. Average inter and intra cluster D^2 values for rice genotypes

Cluster	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.	XIII.	XIV.	XV.	XVI.	XVII.	XVIII.
I.	20.19	37.28	22.32	25.85	32.42	24.99	36.48	24.85	32.85	44.28	48.44	37.32	38.58	39.88	36.15	30.26	30.19	46.16
II.		20.35	33.91	33	42.07	45.31	26.52	34.53	43.1	26.4	31.22	28.51	25.73	30.02	33.06	44.47	52.81	34.25
III.			0	19.49	28.92	33.33	33.78	31.12	29.05	33.21	40.95	39.88	41.48	43.59	40.38	25.19	41.98	46.99
IV.				24.77	31.39	34.71	32.99	31.11	30.73	35.3	40.22	38.47	38.8	41.67	38.81	29.4	42.21	44.81
V.					27.28	44.58	45.17	35.8	34.61	46.42	45.89	52.46	45.95	50.62	41.9	34.56	45.23	43.04
VI.						0	39.66	29.23	36.15	53.48	56.69	33	46.85	45.82	44.85	39.09	28.84	58.43
VII.							0	33.55	40.18	26.36	32.99	28.77	35.64	31.5	38.15	38.57	50.56	47.65
VIII.								0	45.65	47.97	48.57	34.55	32.75	28.53	18.82	41.52	33.11	34.96
IX.									0	40.66	42.6	46.11	50.92	57.44	55.63	25.91	46.32	59.19
X.										0	25.47	38.42	39.99	43.86	48.69	42.2	63.39	49.04
XI.											29.02	43.11	44.08	49.09	49.06	49.34	67.45	46.21
XII.												0	31.56	31.55	39.18	51.13	49.15	47.42
XIII.													0	17.78	23.3	46.76	44.88	26.62
XIV.														0	20.58	49.9	44.35	33.15
XV.															0	49.32	42.6	21.6
XVI.																0	38.19	57.43
XVII.																	0	56.25
XVIII.																		0

Intra cluster – Diagonal values, Inter cluster – Off diagonal values

Table 4. Cluster mean among rice genotypes for various biometric traits

S.No.	Cluster	PH	NPT	PL	SPY	DFF	NGP	TGW	GL	GB
1.	I.	112.34	16.2	22.18	36.22	75.63	146.85	13.65	8.32	2.92
2.	II.	150.36	14.76	24.15	46.89	94.44	131.94	17.63	8.17	3.09
3.	III.	106.04	19.4	23.99	41.06	75.5	138.75	19.95	7.5	2.35
4.	IV.	121.93	19.81	23.34	46.76	78.32	142.49	17.7	8.13	2.84
5.	V.	132.65	16.41	22.52	53.26	73.39	229.12	16.13	8.16	2.99
6.	VI.	112.29	14.94	22.3	31.63	73	87.55	9.8	9.65	2.9
7.	VII.	112.79	20.43	27.88	76.92	91.5	79.8	16.52	8.8	3.05
8.	VIII.	99.61	17.97	22.86	35.55	84.5	198.72	11.1	9.25	2.95
9.	IX.	162.59	17.94	21.59	69.81	68.5	96.02	16.33	8.5	3.1
10.	X.	141.45	15.78	22.15	70.24	93.5	76.01	24.42	7.5	2.85
11.	XI.	168.09	16.33	22.93	77.53	92.75	132.57	21.69	8.7	2.89
12.	XII.	150.37	11.8	25.91	27.01	93	58.89	13.56	9.45	3.1
13.	XIII.	154.45	20.82	21.38	30.29	97.5	149.74	12.48	6.75	3.1
14.	XIV.	111.18	20	28.16	33.7	99	144.61	11.61	7.45	3.2
15.	XV.	112.58	21.82	21.35	32.1	94.5	227.16	10.98	7.2	2.2
16.	XVI.	107.43	33.22	21.89	70.3	70.5	105.63	17.14	7.4	3.55
17.	XVII.	98.53	14.59	20.52	30.29	72	146.55	5.5	7.85	3.6
18.	XVIII.	157.86	15.19	21.94	28.22	97.5	279.91	13.36	6.85	2.35
Grand mean		**128.47**	**18.18**	**23.16**	**46.54**	**84.72**	**142.90**	**14.97**	**8.09**	**2.94**

PH - Plant height
NPT - No of productive tillers
PL - Panicle length
SPY - Single plant yield
DFF - Days to 50% flowering
NGP - Number of filled grains per panicle
TGW - 1000 grain weight
GL - Grain Length
GB - Grain breadth

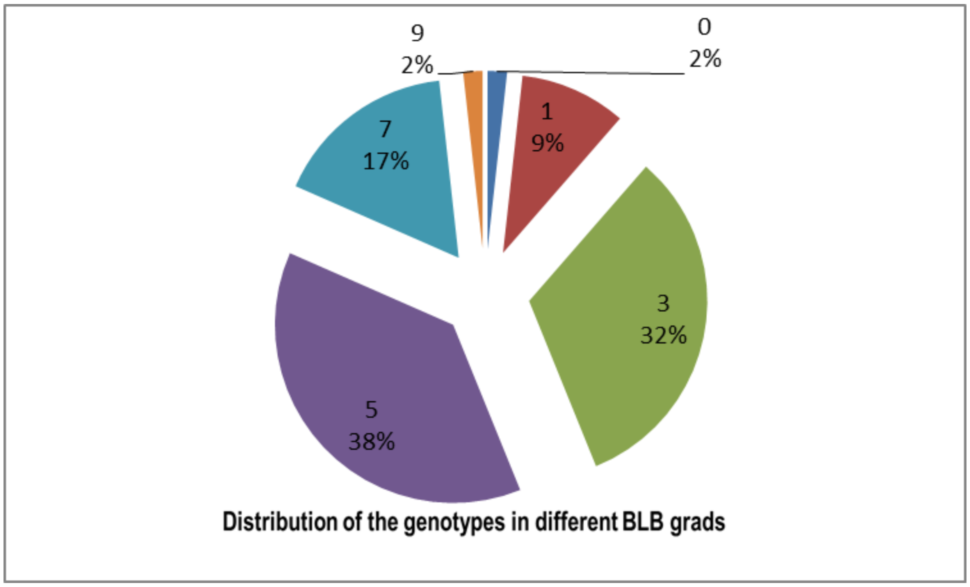

Fig. 1. Frequencies distribution of the genotype in different BLB grads

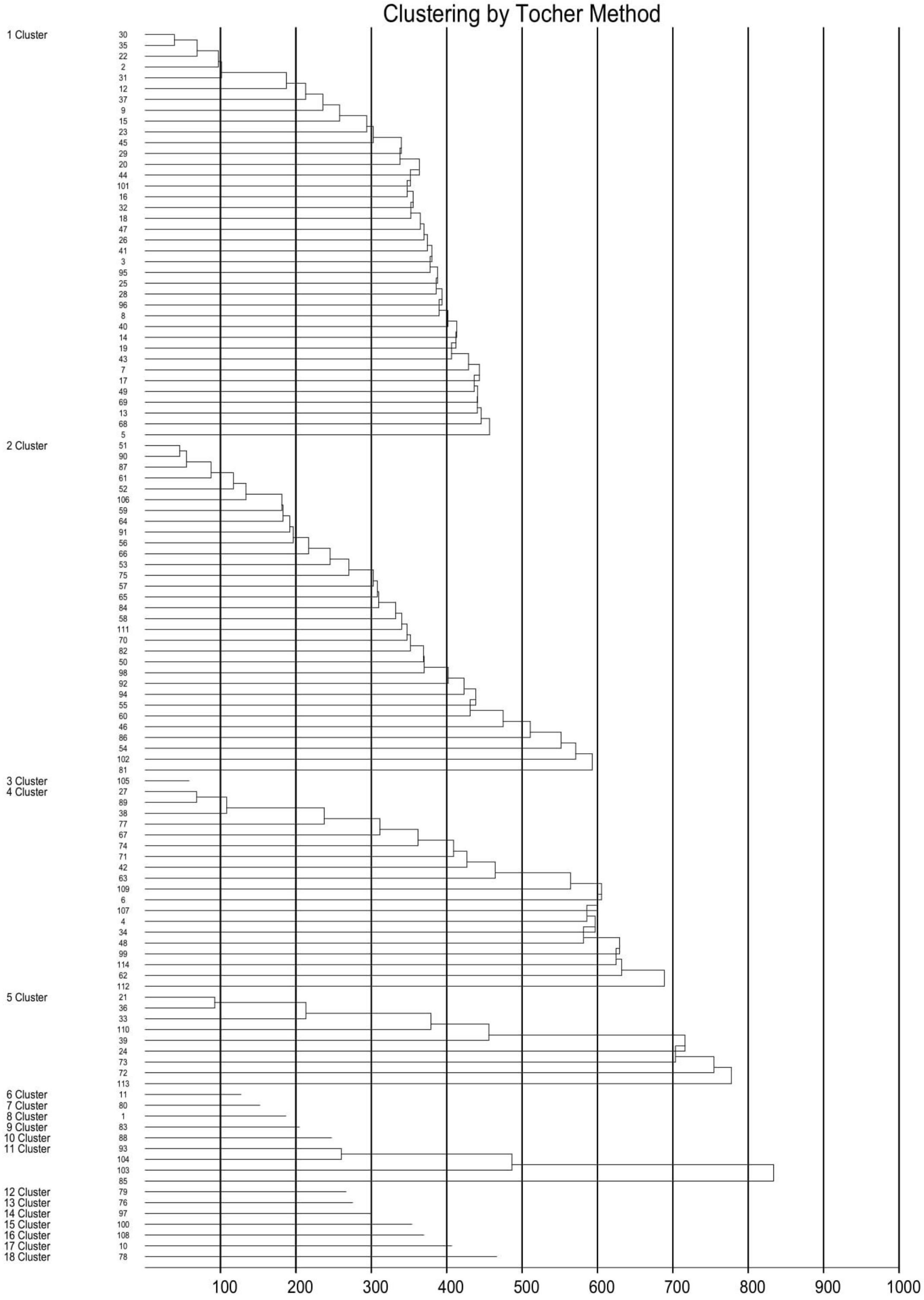

Fig. 2. Dendrogram constructed based on Morphological Observations

1-TN 1, 2-Co 50, 3-IR 12L 104, 4-IRRI 104, 5-Co 39, 6-IR DL 25 CA, 7-ADT 37, 8-IR 12L 115, 9-IR BL 5M, 10-BB 8,11- ASD 18,12-CR 1009, 13-IR 12L 107, 14-Uma, 15- ASD 16,16- AD BIO 09518, 17-IR12L 342,18- Co 43, 19-IR 72, 20- TPS 4, 21-ACK 12001, 22-ADT 43, 23-IR 12L 214, 24-BBT 4204, 25-PY 5, 26-ACK 14004, 27-LFR 293, 28-IR 64, 29-ADT 42, 30-IR 11L 433, 31-IR 50, 32-IRRI C 134, 33-IR 12L 138, 34-ADT 48, 35-White Sannam, 36-Adukan, 37-IR 11C 114, 38-IRRI 163, 39-Kadaikannan, 40-Kalakeri, 41-Kitchadi samba, 42-Karsamba, 43-Veethiruppu, 44-Kalakeri, 45-Annada, 46-Chinnapunjan, 47-Jaya, 48-Kuliyadichan, 49-Thondi, 50-Surakuruvai, 51-Maranellu, 52-Navarai, 53-Anjali, 54-Purple Puttu, 55-Virendra, 56-Sahbhagi Dhan, 57-Rajalakshmi, 58-Athira, 59-Kurukot, 60-Gowri, 61-Kaivara Samba, 62-Gowni, 63-Swarna, 64-Bharathi, 65-Kayamma, 66-White Ponni, 67-Meikuruvai, 68-Abhya- 69-SR22B, 70-ACK 13005, 71-ADT 39, 72-Kullakar, 73-Srilanka, 74-Kalyani, 75-Kavya, 76-Jai Shree Ram, 77-Kalyani, 78-Sadabahar, 79-ADT 46, 80-IR 11C 465, 81-CR Dhan 70, 82-JGL 1798, 83-IR 11T 193, 84-Co 49, 85-HH-Z17-Y16-Y3-Y2, 86- JGL 348, 87-Kuruvai Kalajium, 88-TP10008, 89-Namcheonbyeo, 90-IR 12L 110, 91-MDU 5, 92-Co 45, 93-ADT 41, 94-PY 2, 95-Co 51, 96-Kalinga 3, 97-Pusa Basmati, 98-TP 10106, 99-IR 10A 240, 100-Kottara Samba, 101-IR 20, 102-Vanaprabha, 103-Thooyamalli, 104-Mapillai Samba, 105-Varapukudaichan, 106-Mattai, 107-Dhalaheera, 108-Poonkar, 109-Kerala Gandhakasala, 110-Kattanur, 111-Seeraga Samba, 112-JGL 3855, 113-IR-BL-TAR-PI (Co) and 114-Krishna Hemavathi.

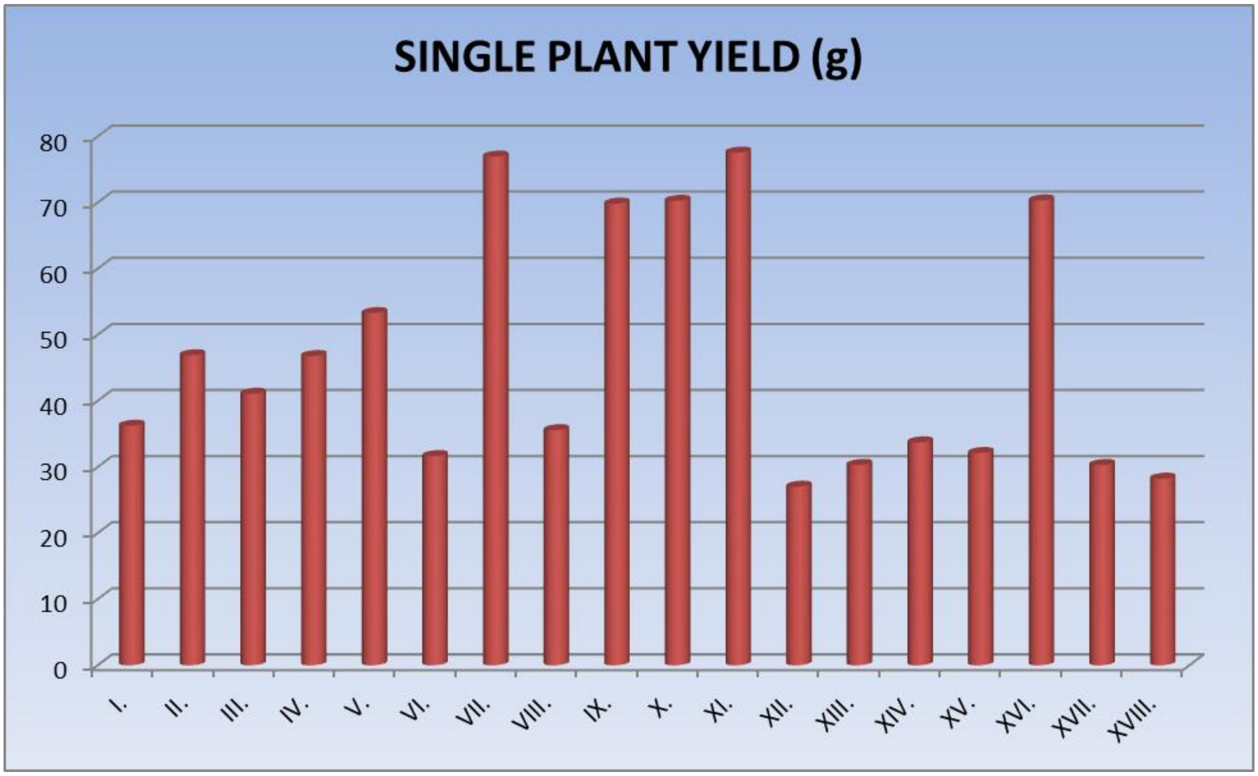

Fig. 3. Cluster mean variability for single plant yield (g)