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Screening of Rice Germplasm against blast disease for Identification of Resistant Sources

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ABSTRACT

Rice Leaf Blast disease is caused by means of *Magnaporthe oryzae* is one of the major biotic stresses of rice in India. To find the leaf blast resistance sources in rice accessions, an open field investigation was carried in natural and artificial epiphytotic form during *rabi* seasons in 2018 and 2019. A total of 97 rice genotypes including resistant check (Tetep) and susceptible check (NLR34242 and BPT5204) were grown, in uniform blast nursery (UBN). Rice Leaf blast disease severity assessment was scored according to 0-9 scale. Among rice genotypes,21.6 % were resistant, 29.8 % moderately resistant, 21.6 % moderately susceptible, 29.8 % susceptible and 16.4 % were highly susceptible during *rabi* 2018 whereas only 18.5 % resistant 29.8 % moderately resistant,15.4 % moderately susceptible and 23 % were susceptible and 12.37 % to rice leaf blast disease during *rabi* 2019. As per result, these resistant accessions with required agronomical traits can be used in leaf blast resistance breeding program as donor parent for the development of leaf blast resistant varieties in rice.

Keywords: Blast, Disease assessment, Genotypes, Rice, Resistant.

INTRODUCTION

Rice (*Oryza sativa* L.) is the primary nutrition food and accounts for over 20% of global calorie intake. Over 90% of the world's rice is produced and consumed in the Asian countries (China, India, Indonesia, Bangladesh, Vietnam and Japan) accounted for 80% in the world's production and consumption [1]. However, worldwide rice production is constrained by various biotic and abiotic stresses [2]. Among biotic stresses blast disease is very important constraints for rice production. An average yield loss of 25-30 per cent per annum commonly occurring due to diseases in India, among them most common and severe disease is leaf blast in rice [3]. Rice leaf blast is one of the most destructive diseases affecting rice production worldwide, is caused by the non-obligate filamentous *ascomycete Magnaporthe oryzae* (cyn. *Magnaporthe grisae*) (Anamorph = *Pyricularia grisea*) [4]. Rice Leaf blast disease incidence in Tanjavur delta of Tamil Nadu state in India during 1918 was recorded for the first time, the disease has occurred from time to time in the country causing severe losses in rice production [5, 6].

Blast disease is most common in all three types of cultivation *i.e* irrigated, rainfed uplands and lowlands regions. The occurrence and disease severity seems more at upland cultivation situation. The rice leaf blast disease is marked as most damaging diseases in worldwide and spread in approximately 85 countries in all areas wherever cultivation of rice crop. Alone in India, the total damage due to rice leaf blast disease during 1960–1961 was 2, 65,000 tons accounting 0.8% of total rice crop production. However, rice leaf blast disease under severe epiphytic conditions may result between 70–90% losses in isolated fields/localities condition [7]. Rice leaf blast disease results in yield failure as more as 70-80% when predisposition factors i.e., relative humidity higher than 85-89%, presence of dew, high temperature values, drought stress and excessive usage of nitrogen fertilizers ensures severe epidemic development [8]. The disease was under intensive study during the past four to five decades.

Usually, fungicides are used to control rice the blast disease incidence, however it creates extra costs in rice crop production, moreover causes pollution of environment and foods by using of chemicals. Hence by usage of highly resistant rice genotypes (host plant blast disease resistance) is one of the utmost well-organized method of crop protection both economically and environmentally. Till now about 100 rice leaf blast resistance genes have been recognized and among them 45%, 51%, 4% from *japonica* types, *Indica* lines and rest from *wild species* of rice respectively [9]. Blast resistance, tended to be unpredictable resistance is repeatedly breaking down, under field conditions. Even though several blast resistant varieties were developed every year, the leaf blast resistance is not stable as a result of severe pathogen plasticity in the field conditions which renders single leaf blast resistance gene crack down next 3 to 5 years after the rive cultivar release [10].

Hence the present study was carried out to find donors for resistant by screening 98 genotypes along with resistant check (Tetep) and local susceptible check cultivars NLR34242 and BPT5204 for blast resistance by artificial inoculation, following Uniform blast nursery method (UBN) and Standard evaluation system (SES) scale [11] for rice blast disease scoring.

MATERIALS AND METHODS

Experimental Site and Materials

The experiment was carried out at Agricultural Research Station, Acharya NG Ranga Agricultural University, Nellore; Andhra Pradesh, India. A total of 97 rice genotypes comprising of *(landraces, wild species, japonica, mutants, aromatic varieties* and *modern indica cultivars*) are listed in (Table.1), were screened against rice leaf blast disease during *rabi s*eason 2018-19 & 2019-20 respectively, under natural epiphytotic field condition raising uniform blast nursery (UBN).

Table 1: List of genotypes used for blast screening during rabi 2018-19 & 2019-2020.

S. No	Genotypes
1.	ACARMATHI
2.	Aditya
3.	AMO
4.	ANJALI
5.	ARC 10955
6.	AUS 257
7.	B-370
8.	BHADRAKALI
9.	BINUHANGIM
10.	BPT 5204
11.	CHILARAI
12.	CHITTIMUTYALU
13.	CR401
14.	DALASHAITA
15.	DANGAR
16.	DHARIABOLIA
17.	DIKHOW
18.	DRR Dhan 38
19.	GULMURALI
20.	HIM 299
21.	HIM2216
22.	IC17020X
23.	IC454277X
24.	IC455374
25.	IC458459X
26.	JABORSAIL
27.	JAGABHANDHU
28.	JGL3844
29.	JUMA
30.	KALAMKATI
31.	KALIAUS
32.	Kasturi
33.	KAVYA
34.	KOLONG
35.	Krishna Hamsa
36.	LACHIT
37.	LUIT
38.	MIKHUDEB
39.	MOSHUR
40.	MTU1075
41.	MTU7029
42.	N 22
43.	NIDHI
44.	NL 148
45.	NL 16
46.	NL 22
47.	NL 24
48.	NL 32
49.	NL 34
50.	NL 380
51.	NL 42
52.	NL 44
53.	NL 46
54.	NL 50
55.	NL 60
56.	NLR 24-8
57.	NLR 3042
58.	NLR 3083
59.	NLR 3217
60.	NLR 3276
61.	NLR 33354
62.	NLR 33358
63.	NLR 3448
64.	NLR 4002
65.	NLR2422
66.	NLR3242
67.	NLR3247
68.	NLR3302
69.	NLR33057
70.	NLR34242
71.	NLR40024
72.	NLR4054
73.	NUMALI
74.	PANTSUGANDH15
75.	PSB68
76.	RAV1003
77.	RP BIO
78.	RP- Bio 150-7
79.	Sasyasree
80.	SHABAGHIDHAN
81.	SHOBHINI
82.	SIDDHI
83.	SM119
84.	SM277
85.	SM382
86.	SM385
87.	STBN-12-10
88.	TARAORI BASMATI
89.	TETEP
90.	THELAHAMSHA
91.	TKM 6
92.	Vardhan
93.	Vikramarya
94.	WAB4502432P18HB
95.	WARANGAL SAMBHA
96.	WGL11427
97.	wild rice 1

Methodology

Uniform Blast Nursery (UBN) pattern was followed. Each rice genotype was sown in a single row of 50 cm with row to row spacing of 10 cm. After every 10 genotypes, local susceptible checks (BPTS 5204 and NLR 34242) and resistant check (Tetep) were planted. The entire nursery was surrounded on all sides by two rows of susceptible check varieties. Inoculation was done at when the 6th leaf was half emerged with conidial suspension of @ 10^5/ ml containing 0.025% Tween 20 [13]. Disease reactions were scored by adopting following 0–9 scale [11].

Disease Assessment

Observations were recorded, 25 days after sowing and/or after 7th day post inoculation and plants were scored based on leaf blast severity by following Standard Evaluation System (SES) scale, International Rice Research Institute (IRRI), Philippines as given in Table.2.

Table 2: Description of SES Scale (IRRI, 2002) for blast disease scoring

0-9	Scale	Disease severity
0	No lesion observed.	Immune
1	Small brown specks of pin point size (smaller than 0.5 mm in diameter)	Resistant
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are mostly found on the lower leaves.	Moderately Resistant
3	Lesion types same as in 2 with 1-3 mm in diameter, but significant number of lesions on the upper leaves.	Moderately Resistant
4	Typical spindle shaped susceptible blast lesions, 3 mm or longer infecting less than 4% of leaf area.	Moderately Susceptible
5	Typical susceptible blast lesions of 3 mm or longer infecting 4- 10% of the leaf area.	Moderately Susceptible
6	Typical susceptible blast lesions of 3 mm or longer infecting 11-25% of the leaf area.	Susceptible
7	Typical susceptible blast lesions of 3 mm or longer infecting 26-50% of the leaf area.	Susceptible
8	Typical susceptible blast lesions of 3 mm or longer infecting 51-75% of the leaf area many leaves are dead.	Highly Susceptible
9	Typical susceptible blast lesions of 3 mm or longer infecting more than 75% leaf area affected	Highly Susceptible

Statistical Analysis

Using Microsoft Excel package, the leaf blast disease data was analyzed statistically. Clustering of 97 rice genotypes was carried out with R-studio by r-package ape ver. 5.4-1 [14].

RESULTS AND DISCUSSION

The different blast disease pathogen isolates affect different parts of a rice plant during pathogenesis. One of the serious forms of rice blast is leaf blast. On the other hand, due to very complex nature of *M. oryzae*, the epidemiology of pathogen is not completely understood and the screening technique for leaf blast is precisely standardized. Therefore, in the present study we artificially created the suitable environment for phenotypic evaluation of leaf blast disease resistance.

Rice genotypes categorization on the basis of disease score

During *rabi* 2018-2019, 97 genotypes were screened for leaf blast disease in rice. Among the 97 genotypes, none of them showed highly resistant, while 21 lines scored resistant, 29 scored moderately resistant, 2 lines scored moderately susceptible, 29 lines were susceptible and 16 lines were highly susceptible (Figure 3, Table 3). Lowest leaf blast disease severity was observed in Amo (Scale 1) followed by other 20 genotypes, moderately resistant were seen in Dalashaita (Scale 2-3), followed by other 28 entries, HIM 299 and WAB4502432P18HB are seen under moderately susceptible (Scale 5), Bhadrakali (Scale 6-7) followed by 28 genotypes were susceptible and BPT5204 (Scale 9) followed by 15 lines are seen under highly susceptible. Similarly, in 2019-2020, out of 97 genotypes, none of them were found to be highly resistant, while 18 were resistant, 29 are moderately resistant, 15 were moderately susceptible, 23 lines are susceptible and 12 genotypes were highly susceptible.

The most effective way to manage rice leaf blast disease is use of resistant genotypes. Resistant to highly susceptible level of disease resistance range against leaf blast disease was observed in different rice cultivars. None of the genotype found to be under highly resistant category in both years. Accordingly, the 97 rice genotypes used in present study consists of diverse genetic background exhibited unlike interaction to leaf blast disease. These types of results are agreed by the work of [15,16,17].

Cluster analysis of 97 genotypes for leaf blast disease

The 97 rice genotypes were divided into 5 clusters viz. cluster 1 (resistant genotypes), cluster 2 (moderately resistant genotypes), cluster 3 (moderately susceptible genotypes), cluster4 (susceptible genotypes) and cluster 5 (highly susceptible genotypes) based on comparison in leaf blast disease reactions between 97 rice accessions in *rabi* 2018 and 2019. In cluster 1, 21 rice entries which contains 21.6 % of total genotypes were resistant types, 29 remained moderately resistant in cluster 2 representative of 29.8 % in total genotypes during *rabi* 2018. Equally, in cluster 3, 21 entries were moderately susceptible which represents 21.6 % and 29 entries were in cluster 4 (29.8 % in 97 rice genotypes) which denotes susceptible rice genotypes, where only 16 genotypes appear in cluster 5 representative of 16.4 % among 97 accessions were highly susceptible genotypes to leaf blast disease (5 clusters in 2018 is presented in Figure 2).

In 2019, cluster 1 has only 18 entries which signify 18.5 % of entire rice entries were resistant genotypes, 29 entries moderately resistant categorized in cluster II representative of 29.8 % in overall. In the same way, 15 entries scored moderately susceptible in cluster 3, which denotes 15.4 % then 29 genotypes observed in cluster IV which signifies susceptible rice genotypes containing 23.7 % of 97 rice genotypes, where 12 entries scored in cluster V representative of 12.37 % susceptible genotypes among 97 accessions to leaf blast disease (5 clusters in 2019 is presented in Figure 3).

Screening of 97 rice accessions exhibited different level of rice leaf blast disease resistance in the screened accessions during *rabi* 2018 and 2019. Rice genotypes was classified into five clusters viz. cluster 1 (resistant genotypes), cluster 2 (moderately resistant genotypes), cluster 3 (moderately susceptible genotypes), cluster4 (susceptible genotypes) and cluster 5 (highly susceptible genotypes) based on resemblance in leaf blast disease responses between 97 rice accessions in *rabi* 2018 and 2019, similar cluster pattern of results was agreed with [12]. Maximum genotypes of 97 were resistant, moderately resistant type and susceptible category towards leaf blast disease in both years indicating good sources of disease resistance among genotypes screened during *rabi* 2018 showed more entries resistant type than in *rabi* 2019 due to host plant specificity of pathogen, climatic conditions and genomic status of genotypes. Environment impacts the different expressions of genotypes grow from horizontal resistance and so results in durable resistance of genotypes [18]. Besides, further factors like moisture stress and unnecessary level usage of nitrogenous fertilizer increases leaf blast disease severity incidence in rice [19]. In rice blast management genotypes with highly resistance for both leaf and neck blast disease have been most widely used [20]. Pyramiding of resistance genes for leaf blast is the most important challenge to rice scientists against continuously developing and geographically diverse races of *M. oryzae* [9]. Therefore, these types of studies essential to conduct continuously to screen virulence of the different leaf blast pathogen and to detect novel sources of resistant genotypes and useful in different National and International plant breeding program for the development of leaf blast resistant rice varieties in future.

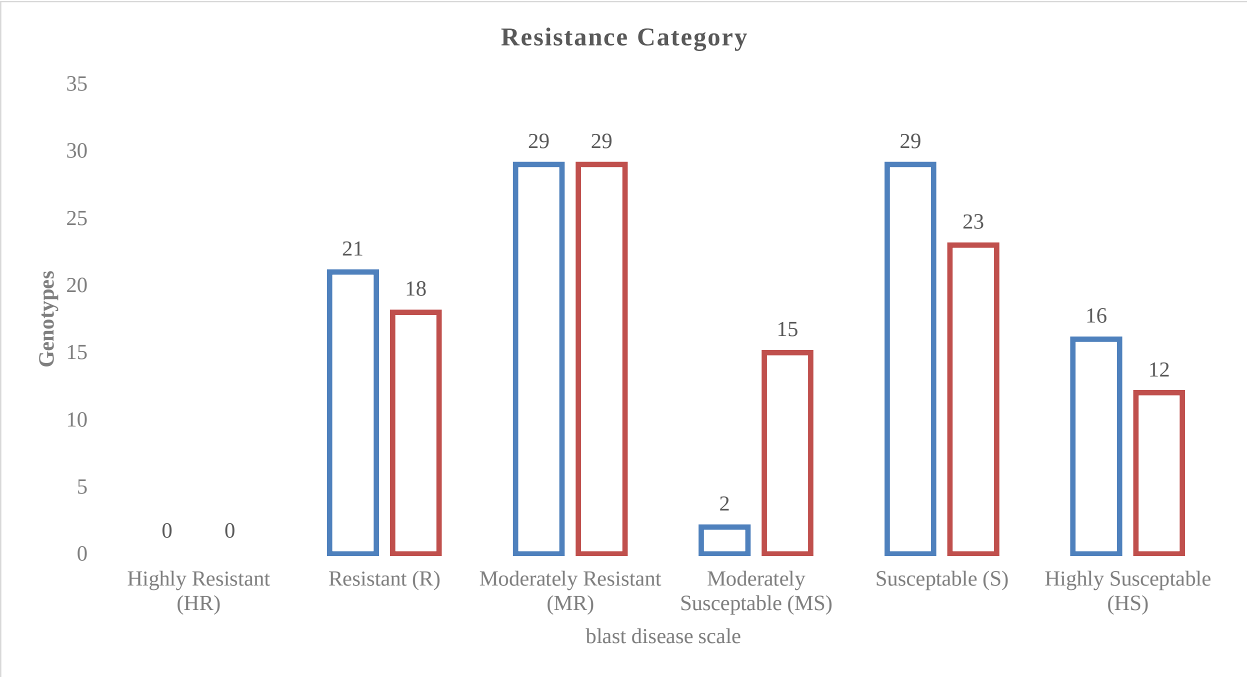

Figure 1: Frequency of 97 rice genotypes with different scale of resistance to leaf blast disease during rabi 2018 and 2019 at Agricultural Research Station, Nellore; Andhra Pradesh

Table 3: List of rice genotypes showing resistant response against leaf blast disease in 2018 and 2019 at Agricultural Research Station, Nellore, Andhra Pradesh

Experimental year	**Resistant (R) Genotype (Score 1).**
2018	AMO, KALAMKATI, NLR3302, NLR3247, NLR 3083, NLR 3217, NL 32, NL 148, N 22, RP- BIO 150-7, SM119, TETEP, VIKRAMARYA, WGL11427, MTU7029, NLR 3276, NLR2422, NLR 3042, NLR 33354, NLR33057 and WILD RICE 1.
2019	ADITYA, KALAMKATI, NLR3302, NLR3247, NLR 24-8, NLR 3083, NLR 3217, NL 16, SM119, SM277, TETEP, VIKRAMARYA, ANJALI, NLR 3276, NLR 4002, NLR 33354, NLR33057 and WILD RICE 1.
	Moderately Resistant (R) Genotypes (Score 2).
2018	DHARIABOLIA, NLR3242, NLR 24-8, NL 16, NL 22, NL 24, NL 34, NL 46, NL 50, NL 60, PANTSUGANDH15, RP BIO and NLR 4002
2019	AMO, DHARIABOLIA, NLR3242, NL 22, NL 24, NL 32, NL 34, NL 46, NL 50, NL 60, N 22, PANTSUGANDH15, RP- Bio 150-7, SHABAGHIDHAN, RP BIO, NLR2422 and NLR 3042.
	Moderately Resistant (R) Genotypes (Score 3).
2018	Aditya, B-370, GULMURALI, HIM2216, MOSHUR, NL 42, NL 44, NL 380, RAV1003, SM277, ANJALI, MIKHUDEB, NLR40024, and MTU1075.
2019	B-370, DANGAR, MOSHUR, NL 42, NL 44, NL 148, NL 380, MTU7029, NLR40024 and MTU1075.
	Moderately Resistant (R) Genotypes (Score 4).
2018	PSB68
2019	JAGABHANDHU & PSB68.

	Moderately Susceptible (MS) Genotypes (Score 5).
2018	HIM 299 &WAB4502432P18HB.
2019	DALASHAITA, DIKHOW, GULMURALI, HIM 299, HIM2216, IC454277X, IC17020X, LUIT, RAV1003, SM382, Sasyasree, Vardhan, WGL11427 and JGL3844.
	Susceptible (S) Genotypes (Score 6).
2018	BHADRAKALI, CHITTIMUTYALU, DANGAR, JAGABHANDHU, NIDHI, SM382, SHABAGHIDHAN, KALIAUS, JGL3844 and WARANGAL SAMBHA.
2019	BHADRAKALI, CHITTIMUTYALU, CR401, IC458459X, NIDHI, KALIAUS and WARANGAL SAMBHA.
	Susceptible (S) Genotypes (Score 7).
2018	BINUHANGIM, CHILARAI, CR401, DIKHOW, IC454277X, JABORSAIL, KASTURI, KRISHNA HAMSA, KOLONG, LACHIT, LUIT, NUMALI, NLR 3448, STBN-12-10, SM385, SASYASREE, THELAHAMSHA, TARAORI BASMATI and VARDHAN.
2019	BINUHANGIM, DRR DHAN 38, IC455374, JABORSAIL, KASTURI, KRISHNA HAMSA, LACHIT, NLR 3448, SM385, SHOBHINI, THELAHAMSHA, TKM 6, TARAORI BASMATI, WAB4502432P18HB, NLR4054 and MIKHUDEB.
	Highly Susceptible (HS) Genotypes (Score 8).
2018	AUS 257, IC458459X, IC17020X, JUMA, SHOBHINI, TKM 6, NLR4054 and NLR 33358.
2019	AUS 257, JUMA, NUMALI and NLR 33358.
	Highly Susceptible (HS) Genotypes (Score 9).
2018	ACARMATHI, ARC 10955, BPT 5204, DRR Dhan 38, IC455374, KAVYA, SIDDHI and NLR34242.
2019	ACARMATHI, ARC 10955, BPT 5204, KAVYA, KOLONG, STBN-12-10, SIDDHI and NLR34242.

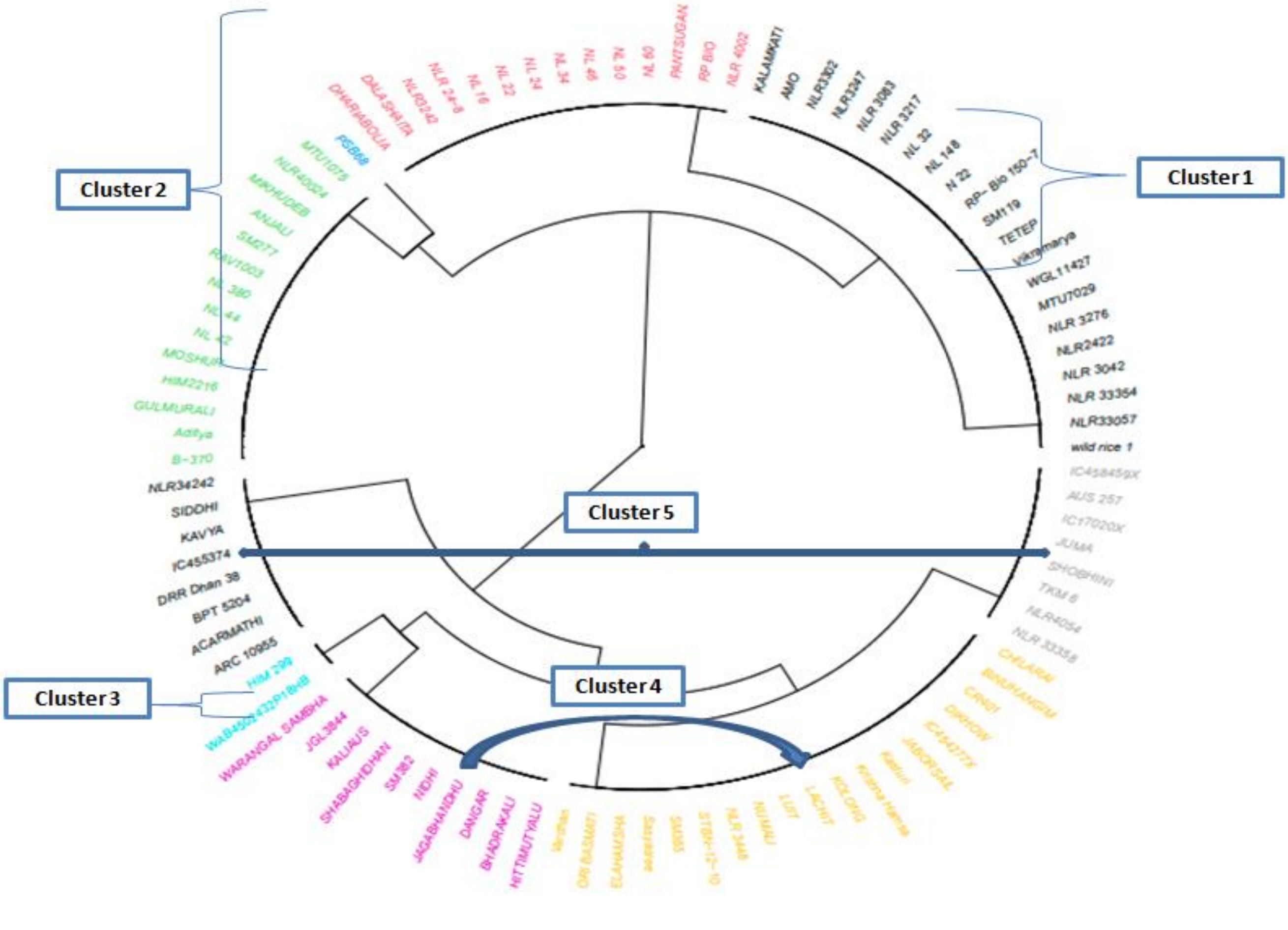

Figure 2: UPGMA cluster analysis of 97 rice genotypes based on final scoring for leaf blast disease in 2018

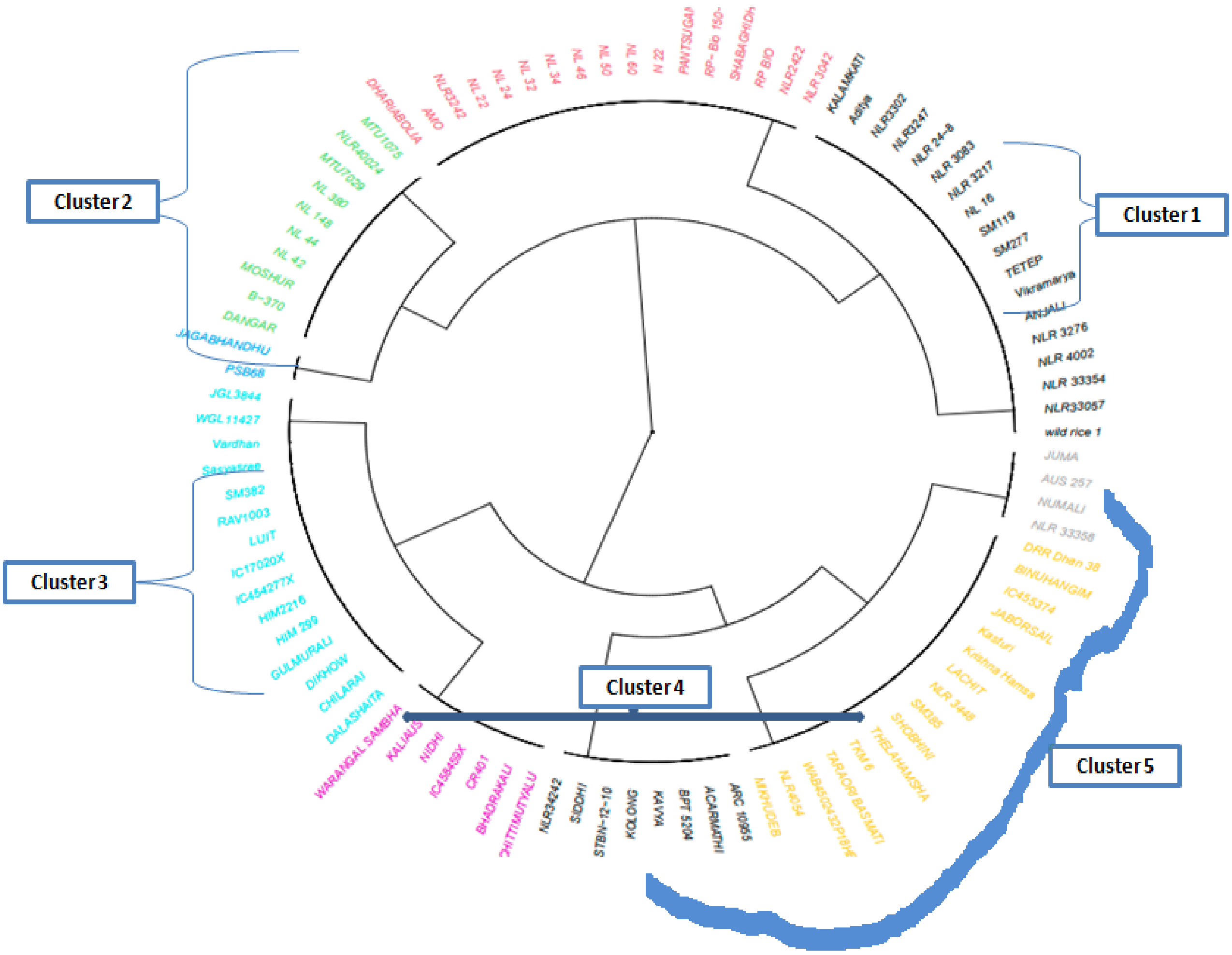

Figure 3: UPGMA cluster analysis of 97 rice genotypes based on final scoring for leaf blast disease resistance in 2019

CONCLUSION

Rice leaf blast disease caused by *M. oryzae* is one of the major damaging biotic stresses in rice crop of India. Among rice genotypes, 21.6 % were resistant, 29.8 % moderately resistant, 21.6 % moderately susceptible, 29.8 % susceptible and 16.4 % were highly susceptible in 2018 whereas only 18.5 % resistant 29.8 % moderately resistant, 15.4 % moderately susceptible and 23 % were susceptible and 12.37 % to leaf blast disease resistance in *rabi* 2019. Consequently, these resistant and moderate resistance genotypes along with desirable agronomical traits can be used as donor parent in leaf blast resistance breeding program for the development of leaf blast resistant varieties in rice.

Conflict of interest

None declared.

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