

Studies on Bacterial Blight of Rice in the below Mean Sea Level Tracts of Kuttanad

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Abstract: Studies on basic aspects of bacterial blight (BB) were conducted for three years from 2014-2018 in the Kuttanad tracts of Kerala state, India. Effects of concentration of pathogen, age of seedlings, position of leaves for artificial inoculation and wounding on disease severity were conducted. Screening trials with varieties released from the Kerala Agricultural University and survival of BB on weeds of rice ecosystem were also tried. Standardization of an inoculation method that required only less labor and time was attempted in comparison with other common methods. Relationship of disease severity with stomatal frequency and phenotypical characters like angle and width of leaf and internodal length were also tried. Bacterial concentration of 1×10^8 CFU/mL and method of leaf clipping at 35 days after sowing (DAS) were used for all artificial inoculation experiments. Disease severity was assessed based on International Rice Research Institute (IRRI) score chart. Results of the studies revealed the following facts. Inoculation of the lower most two leaves alone exhibited a disease severity of 48.25 per cent. Highest disease expression was observed with a bacterial concentration of 1×10^9 CFU/mL. In the newly developed artificial inoculation method of leaf clipping of 35 days old excised leaves gave good result with a disease severity as high as 90 per cent at 8 days after inoculation (DAI). It was observed that the disease severity showed an increasing trend up to 40 min after wounding and then declined afterwards up to 240 min. Kerala Agricultural University varieties Kayamkulam-2 and Pattambi-5 emerged as resistant varieties. Lowest internodal length and mean stomatal frequency were exhibited by the resistant variety Ptb-5. These resistant varieties could be suggested for breeding programs involving bacterial leaf blight (BB) resistance. The first report of new alternate hosts of BB like *Isachne miliacea* and *Eleocharis dulcis* will be helpful for the field control of disease.

Key words: Rice, BB, varieties disease severity, resistance.

1. Introduction

Bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* is found worldwide and is particularly destructive in Asia during the heavy rains of the monsoon season. In many Asian countries, the disease is becoming endemic consequent to repeated cultivation. Epidemics that began before panicle initiation significantly reduced grain yield, panicle fertility and kernel weight [1]. Reduction in yield might become as high as 50 per cent in fields where the crop was severely affected. Rice plants were found to be infected at maximum tillering stage and yields were reduced about 10-20 per cent [2]. In India, bacterial leaf blight (BB) of rice was first observed in

Maharashtra state during 1951 [3]. The disease is now prevalent in most of the rice growing regions of the country. Kerala, the southernmost state of India enjoys a tropical humid climate with an annual mean temperature of 27 °C and rainfall of 3,000 mm. Mean relative humidity is over 85 per cent. Rice is the major food crop cultivated in the state. Kuttanad, the rice bowl of Kerala, is situated towards the southern part spreading over an area of about 50,000 ha in Alappuzha, Kottayam and Pathanamthitta districts (Fig. 1). Annual flooding caused by southwest monsoon during the period of June-July is a unique feature of this area since it is located below mean sea level from 0.5 to 3 m. The climatic conditions prevailing especially heavy rains with strong winds are conducive for the occurrence of several diseases in rice [4]. In Kerala, BB was first observed in 1976 in Palakkad district.

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Fig. 1 Map of Kuttanad.

Later, in 1980 [5], it was reported that the disease was endemic in the major rice growing areas of Kuttanad and Palakkad. The disease remained as a major production constrain in the rice tracts of Kuttanad causing a grain loss of 6-60 per cent [6]. After 2010, the disease was found to occur regularly at an

alarming intensity during the additional crop season (June-July to September-October). Hence, the present study envisaged some basic aspects of the disease related to its occurrence, survival, alternate hosts and disease reaction of major rice varieties of Kerala.

2. Methodology

Bhadra, a highly susceptible variety was used for all artificial inoculation studies following leaf clipping method at 35 days after sowing (DAS). Degree of infection was noted as per the standard score chart of International Rice Research Institute (IRRI) (Fig. 2).

Among the 50 BB isolates collected, the most virulent Champakulam one (Fig. 3) that showed the highest degree of hypersensitive reaction towards *Nicotiana tabacum* variety *rustica* was used for all artificial inoculation studies.

Three replications with nine plants each were kept for each treatment. An observation on disease severity was taken at 15 days after inoculation (DAI) as per cent leaf area infected (Fig. 4).

2.1 To Determine the Most Effective Concentration of the Pathogen

A 24-h culture of the pathogen on Wakimoto Agar Medium was used. Different concentrations of Xoo, i.e. 1×10^6 CFU/mL to 1×10^{10} CFU/mL were tried in three replications with nine plants each per treatment. Concentrations were fixed using spectrophotometry.

2.2 To Assess the Optimum Age of Seedlings

Seedlings were raised with three plants per pot. Nine plants each in three replications were kept for

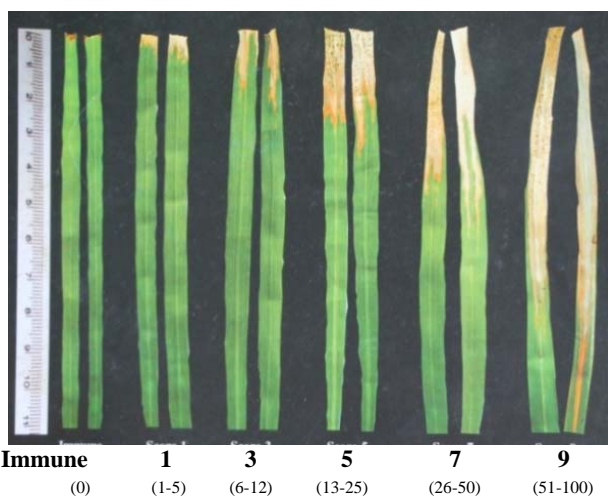


Fig. 2 Standard evaluation system of BB (in%) by International Rice Research Institute (IRRI).

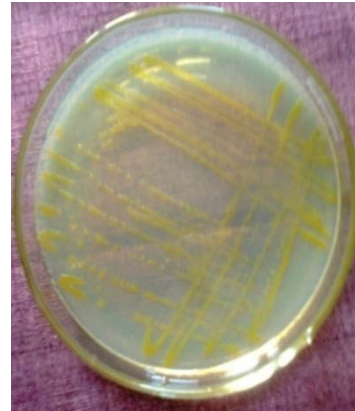


Fig. 3 Colonies of *Xanthomonas oryzae* on Wakimoto agar medium.



Fig. 4 Leaf symptom.

single treatment. Leaf clipping method was used for inoculation. Different stages of seedlings at 20, 35, 50 and 75 DAS were tried.

2.3 Effect of Position of Leaves on Disease Expression

Different treatments tried were lowest, middle and upper two leaves and whole leaves of rice plant. Number of plants per replication and the method of inoculation were the same as above.

2.4 Effect of Wounding on Disease Severity of Xoo

Treatments were inoculation at 0, 20, 40, 60, 80, 120 and 240 min after injury by leaf clipping. Standard procedure was followed for inoculation.

2.5 Effect of Storage on Survival of Xoo in Seeds of Different Varieties

Nine varieties were tested for the presence of Xoo

in seeds under storage from 0 to 75 days after harvest at 15 days interval. Seeds were stored in aluminum boxes after harvest. Dilution plating was the method followed. Moisture per cent of the seeds was also assessed in the same interval.

2.6 Standardization of a New Method of Inoculation

Four leaves each (35 DAS) with three replications were maintained. After cutting, lower ends of excised leaves were immediately covered with pressed cotton swab dipped in sterile water. The different methods of inoculation tried were clipping, clipping and swabbing, swabbing on upper side and swabbing on lower side and standard method. Leaves were kept in polypropylene bags of size 12×6 cm² equidistantly. They were incubated using moistened cotton swabs (Fig. 5). Observation on degree of infection was taken 8 DAI.



Fig. 5 Excised leaf technique.

2.7 Experiment on Varietal Resistance towards *Xoo*

Rice varieties released from the Kerala Agricultural University were tried in different groups like Pattambi, Moncompu, Kayamkulam and Vyttila using standard procedures. Standard method was followed for inoculation. Nine plants per replication were kept for each variety. Plants were incubated by covering with polythene bags and a relative humidity of about 85% was maintained up to 15 DAI. Observation on disease severity was taken at 15 DAI.

2.8 To Find out the Relationship between Disease Severity and Stomatal Frequency

Stomatal count was taken by microscopic method and stomatal frequency was calculated as total No. of stomata divided by 0.00895 cm². Means of five observations were taken and mean value was calculated.

2.9 Relation between Phenotypical Characters and *Xoo* Resistance with Respect to Three Rice Varieties Showing Differential Reactions

Varieties selected for study were MO-4 (susceptible) MO-16 (less susceptible) and Ptb-5 (resistant). Phenotypical characters studied were angle, width and mean stomatal frequency of leaf and inter nodal length of each variety.

2.10 Alternate Hosts of *Xoo*

Sixteen rice weeds commonly found in the rice ecosystem were raised in pots and were artificially inoculated as per the standard method. Weeds showing similar symptoms were selected. Koch's postulate was proved on rice also.

3. Results

Results of various experiments are presented below.

3.1 To Determine the Most Effective Concentration of the Pathogen

It was found that disease severity of BB increased with increase in concentration up to 1×10^9 CFU/mL and then decreased. Highest disease expression was observed with a bacterial concentration of 1×10^9 CFU/mL (Table 1).

3.2 To Assess the Optimum Age of Seedlings

For all concentrations of bacteria, the highest disease severity was expressed for leaf clipping at 35 DAS (Table 1). Optimum age of seedlings obtained was 35 DAS which was similar to the results of previous workers [7].

Table 1 Effect of age and concentration on disease severity of bacterial blight (BB).

Sl No.	Concentration (CFU/mL)	Disease severity at 110 days after sowing (DAS) (per cent leaf area infected)			
		Root dipping at 20 DAS	Leaf clipping at 35 DAS	Leaf clipping at 50 DAS	Leaf clipping at 75 DAS
1.	1×10^6	40.60	55.60	38.40	28.00
2.	1×10^7	50.20	60.00	51.60	38.00
3.	1×10^8	53.00	76.85	58.60	44.00
4.	1×10^9	57.00	80.05	59.00	49.00
5.	1×10^{10}	39.00	65.25	42.40	35.05

Table 2 Effect of position of leaves on disease severity of Xoo.

Sl. No.	Position	Disease severity at 15 days after inoculation (DAI) (% leaf area infected)
1	Upper	0.00
2	Middle	37.85
3	Lower	48.50
4	All leaves	50.75

3.3 Effect of Position of Leaves Used for Inoculation

Among the different positions of leaves tried, inoculation on lower most leaves alone gave the disease severity of 48.25 per cent and was very near the result of that in inoculation of all leaves as for the standard procedure while inoculation of upper most leaves alone gave no symptom of Xoo (Table 2).

3.4 Developing a New Method of Artificial Inoculation

Clipping of excised leaves gave the highest disease severity of 90 per cent even at 8 DAI. This was found to be a better method for getting maximum severity of infection within a minimum time (Table 3). Disease severity was the least for swabbing of lower side of leaves (41.6 per cent).

3.5 Effect of Wounding on Xoo Infection

It was observed that disease severity showed an increasing trend to 40 min after wounding and then declined afterwards up to 240 min (Table 4).

Similar result was obtained with other research workers [8].

3.6 Effect of Storage on Survival of Xoo in Seeds of Different Varieties

Studies showed that no survival of the pathogen was noted in any variety after 45 days of storage under a moisture percent of 8.5. MO-4, where the pathogen survival was maximum, was found to be a susceptible variety with a score of 9 (Table 5). A decreasing trend in the survival of BB pathogen was observed with respect to the period of storage of infected seed material [9].

3.7 Disease Reaction

Among the different varieties tested, Kayamkulam-2 and Ptb-5 showed resistance towards BB (score 3) and PTB-4, PTB-8, A1, K1, MO-10, MO-19 and Vytilla-1 exhibited tolerance with a score of 5 (Fig. 6, Tables 6 and 7).

Table 3 Standardization of inoculation methods.

Sl. No.	Method of inoculation	Disease severity at 15 DAI (per cent leaf area infected)
1	Clipping of excised leaves	90.0
2	Pricking and swabbing	73.5
3	Swabbing of upper side	62.0
4	All leaves of live plant	50.8

Table 4 Effect of wounding on bacterial leaf blight (BB) infection.

Sl. No.	Time (min)	Percent infection	Lesion length (cm)
1	0	35	5.0
2	20	35	5.3
3	40	50	6.0
4	60	75	8.0
5	80	75	7.9
6	120	50	6.2
7	240	25	4.9

Table 5 Survival of BB pathogen in seeds.

Sl. No.	Days after harvest	Moisture percentage	Varieties that showed the presence of pathogen
1	0	13.0	Kalanamak, Sabita, NDR-8002 RP Bio226, Ajaya, Vikramarya, MO-4, MO-7, MO-16
2	15	12.5	Kalanamak, Sabita, MO-4, MO-7
3	30	9.5	Kalanamak, MO-4
4	45	8.5	-
5	60	7.1	-
6	75	6.9	-

3.8 Studies on Stomatal Frequency and Disease Severity of BB of Rice

Among the Vytilla varieties, Vytilla-3 with a stomatal frequency of 4,245.81 showed the highest disease

severity of 58.8 and score 9, while Vytilla-1 having the lowest stomatal frequency of 2,905.02 exhibited the

lowest reaction of 25.0 with a score of 5. Similar trend was observed in Kayamkulam and Moncompu

Table 6 Evaluation of KAU varieties for disease resistance based on artificial inoculation.

Sl. No.	Source	Varieties showing (based on disease score)		
		Resistance (0-3)	Tolerance (> 3-5)	Susceptibility (> 7)
1	Pattambi	Ptb-5	Ptb-4, Ptb-8	Ptb-2
2	Ambalavayal	-	A1	-
3	Kayamkulam	K2	K1	-
4	Vytilla	-	V1	V6, V2, V4
5	Moncompu	-	MO-5, MO-10, MO-11, MO-21	MO-4, MO-20

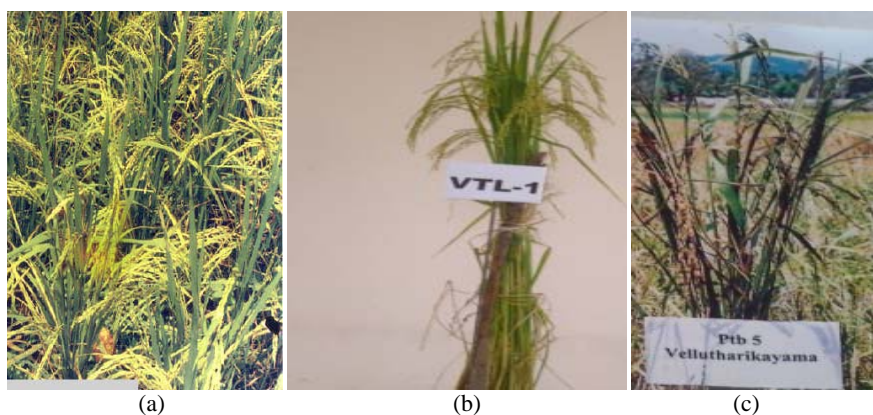
**Fig. 6** (a) Kayamkulam-2, (b) Vytilla-1, (c) Ptb-5.

Table 7 Evaluation of Pattambi varieties for disease resistance.

Sl. No.	Varieties	Disease reaction (%)	Score
Pattambi varieties			
1	Ptb-1	37.0	7
2	Ptb-2	57.3	9
3	Ptb-3	37.5	7
4	Ptb-4	25.0	5
5	Ptb-5	10.0	3
6	Ptb-6	36.1	7
7	Ptb-7	50.0	7
8	Ptb-8	25.0	5
9	Ptb-9	41.6	7
10	Ptb-10	36.5	7
11	Ptb-11	17.5	5
12	Ptb-12	28.3	7
13	Ptb-13	25.0	5
14	Ptb-14	37.5	7
15	Ptb-15	37.1	7
16	Ptb-16	37.5	7
17	Ptb-17	70.0	9
18	Ptb-18	65.6	9
19	Ptb-19	56.9	9
20	Ptb-20	50.5	9
21	Ptb-21	61.5	9
22	Ptb-22	55.8	9
23	Ptb-23	35.8	7
24	Ptb-24	52.5	9
25	Ptb-25	56.5	9
26	Ptb-26	58.5	9
27	Ptb-27	13.5	3
28	PTB28	72.8	9
29	Ptb-29	45.7	7
30	Ptb-30	47.5	7
31	Ptb-31	61.8	9
32	Ptb-32	45.8	7

varieties also (Table 8). Disease severity was found to be directly related with stomatal frequency for the above rice groups.

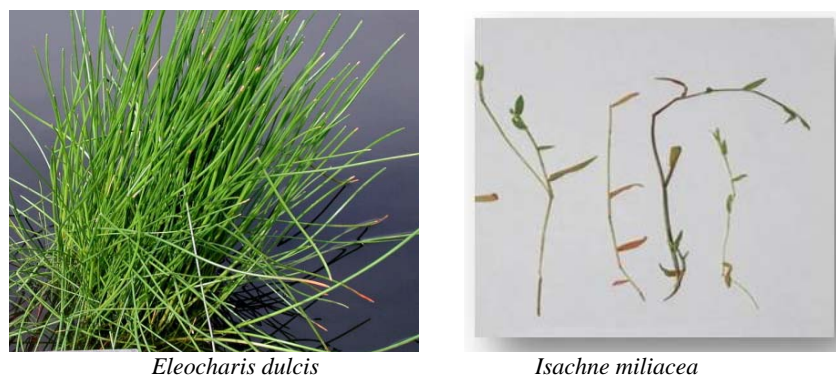
3.9 Phenotypical Characters of Three Rice Varieties and Disease Severity

The most susceptible variety Mo-4 had the highest leaf angle of 35.3°. The resistant variety Ptb-5 exhibited the largest mean leaf width of 0.68 cm along with the lowest stomatal frequency of 3,918.35 with that of MO-16 (less susceptible) and Mo-4 (highly susceptible) varieties (Table 9).

3.10 Hosts of Xoo

Among the 16 rice weeds studied, 12 were found to be alternate hosts of BB pathogen. *Isachne miliacea* and *Eleocharis dulcis* were found to be new hosts of Xoo (Fig. 7).

Xoo survived in several grasses and sedges belonging to Poaceae and Cyperaceae families. The weeds mainly included were *Leptochloa chinensis*, *L. panicea*, *Leersia oryzoides*, *Zizania latifolia*, *Cyperus rotundis* and wild *Oryza* species (*O. rufipogon* and *O. australiensis*) [10].

*Eleocharis dulcis**Isachne miliacea***Fig. 7** New hosts of Xoo.**Table 8** Stomatal frequency and disease severity of BB of rice.

(1) Vytilla varieties

Sl. No.	Varieties used	Stomatal frequency (No./cm ²)	Disease severity at 15 DAI (% of leaf area)	Disease score
1	Vytilla 3	4,245.81	58.8	9
2	Vytilla 8	3,764.70	58.0	9
3	Vytilla 2	3,687.15	55.0	9
4	Vytilla 5	3,575.41	51.3	9
5	Vytilla 6	3,463.68	50.8	9
6	Vytilla 4	3,128.49	50.0	9
7	Vytilla 7	3,128.49	40.0	7
8	Vytilla 1	2,905.02	25.0	5

(2) Kayamkulam varieties

Sl. No.	Varieties used	Stomatal frequency (No./cm ²)	Disease severity at 15 DAI (% leaf area)	Disease score
1	Kayamkulam 7	3,394.38	50.1	9
2	Kayamkulam 4	3,073.06	50.0	9
3	Kayamkulam 6	2,901.46	30.0	7
4	Kayamkulam 5	2,120.08	50.0	7
5	Kayamkulam 3	2,745.11	25.0	5
6	Kayamkulam 1	2,801.75	25.0	5
7	Kayamkulam 2	2,210.05	10.0	3

(3) Moncompu varieties

Sl. No.	Varieties used	Stomatal frequency (No./cm ²)	Disease severity at 15 DAI (% leaf area)	Disease score
1	MO-4	5,228.35	61.3	9
2	MO-12	5,205.15	59.1	9
3	MO-7	5,108.17	54.1	9
4	MO-20	5,015.82	51.1	9
5	MO-14	5,006.15	49.4	7
6	MO-19	4,501.85	47.2	7
7	MO-16	4,025.18	45.2	7
8	MO-9	3,502.13	42.6	7
9	MO-8	3,135.82	41.9	7
10	MO-17	3,015.21	40.2	7
11	MO-18	2,895.12	28.1	7
12	MO-15	2,587.12	28.1	7
13	MO-6	2,682.17	25.2	7

14	MO-13	2,598.13	25.0	7
15	MO-5	2,502.18	23.1	5
16	MO-10	2,138.05	23.2	5
17	MO-21	2,013.12	22.2	5
18	MO-11	2,031.15	20.0	5

Table 9 Phenotypical characters of three rice varieties.

Sl. No.	Parameters/rice varieties (mean of 5 values)	MO-4 (susceptible)	MO-16 (tolerant)	Ptb-5 (resistant)
1	Angle (°)	35.3	24.0	26.6
2	Width (cm)	0.5	0.5	0.68
3	Inter nodal length (cm)	2.0	1.8	1.50
4	Stomatal frequency	5,228.35	4,025.18	3,918.35

4. Conclusions

From the results it could be concluded that inoculation on lower and middle leaves of 35 days old seedlings by leaf clipping method with a bacterial concentration of 1×10^9 CFU/mL gave the maximum disease severity of BB and can be recommended for all artificial screening trials. Among the different positions of leaves tried, inoculation on lower most leaves alone gave the highest disease severity and this can be followed for artificial inoculation trials in order to save time and energy. The new method of inoculation, the excised leaf technique can be adopted for all artificial screening trials for getting the highest disease severity within a limited time of eight days after pathogen application. Time and energy of researchers can be saved by following this method. Result of experiment on wounding revealed that fresh wounds acted as better site of infection. KAU varieties like Kayamkulam-2 and Pattambi-5 that emerged as resistant varieties can be suggested for breeding programs of BB disease resistance. The first reports on identification of new alternate hosts of BB like *Isachne miliacea* and *Eleocharis dulcis* will be helpful for the control of disease in the field.

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