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Occurrence of Bacterial Blight of Rice in Kerala State and Variability Among the Isolates of the Pathogen, *Xanthomonas oryzae* pv.*oryzae*

Research Article

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Abstract

Bacterial blight of rice caused by *Xanthomonas oryzae* pv.*oryzae* is becoming a major production constraint worldwide. In India, the disease has been reported to be major threat causing yield loss. In the state of Kerala disease is appearing in an epidemic nature during kharif season in recent years. A survey has been carried out to assess the intensity of the disease in major rice growing districts of the state and to isolate pathogen from different locations. The predominantly cultivated rice varieties in farmers field such as Uma and Jyothi were affected in all the districts surveyed *viz*., Palakkad, Malappuram and Thrissur. Both *kresek* as well as leaf blight symptoms were observed. The severity of the leaf blight upto 95.10 per cent was recorded in variety Uma and 90.5 per cent in variety Jyothi. *Kresek* symptom was observed in Palakkad, Malappuram and Thrissur districts in varieties Jyothi, Uma and Ponmani. In Palakkad district the disease severity ranged from 5.28 to 50.80 per cent. In Malappuram district *kresek* symptom was observed in Ponmani and the severity ranged from 10.13 to 15.05 per cent. In Thrissur district *kresek* symptom was recorded in variety Jyothi and Uma, the disease severity ranged from 10.35 to 50.15 per cent. The 168 *Xanthomonas oryzae* pv.*oryzae* isolates from different locations of major rice growing tracts varied in their virulence upon inoculation to the susceptible variety Jyothi. The disease severity caused by the isolates ranged from score 5 to score 9. The disease reaction caused by the Xoo isolates varied widely indicating the variability.The genetic variability was further tested by DNA fingerprinting of representative isolates from different districts, which showed high variability among the isolates. Further studies on pathotyping of the Xoo isolates are needed to understand the genes/ gene combinations effective for imparting broad spectrum resistance to rice varieties against prevailing pathogen population of the state.

Keywords: Bacterial Blight; Rice; Genetic Variability; *Xanthomonas oryzae* pv.*oryzae*

Introduction

Bacterial blight caused by *Xanthomonas oryzae* pv.*oryzae* is a devastating disease of rice causing considerable yield loss worldwide. The losses due to this disease depends on the crop stage, variety of the crop and climatic condition. The yield loss ranging from 20 to 80 per cent has been reported by various researchers. [1-3]. Understanding the pathogen population prevailing in a geographical area is important for the development of management strategies. Periodic survey to assess the disease occurrence andstudy of pathogen population is essential for understanding evolution new pathotypes of the pathogen. Many of the resistance genes have been used and transformed to several cultivars [4,5]. However large scale and long-term cultivation of the varieties would result in its breakdown of resistance. Survey and study of the pathogen population have helped to report resistance status of predominant varieties as well as the structure of pathogen population [6,7]. In Kerala, bacterial blight of rice is causing yield loss recently in every year particularly during

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kharif season. After the floods experienced during the years 2018 and 2019, the incidence of bacterial blight has increased in all the major rice growing areas of the state. Understanding the extent of severity of the disease in predominantly cultivated varieties of the state and the structure of the pathogen population prevailing in the state is essential for the development of control measures of bacterial blight of rice in Kerala. The present study was taken up to assess the severity of bacterial blight in major rice growing districts of the Kerala *viz*., Palakkad, Thrissur, Malappuram, Alappuzha and Kottayam and to collect pathogen for studying the virulence pattern as well as the variability.

Materials and Methods

Survey

A purposive sampling survey was conducted to assess the occurrence and severity of bacterial blight of ricein major rice growing districts of Kerala state *viz*., Alappuzha, Kottayam, Thrissur, Palakkad and Malappuram during the years 2018 and 2019. During the survey 52 panchayats coming under these five districts were covered. In Palakkad 24 panchayats *viz*., Kuthannur, Kuzhalmannam, Peringottukurissi, Kottayi, Kollengode, Pirayiri,Chittur, Pattanchery, Perumatty, Muthalamada, Vallappuzha, Alathur, Thenkurissi, Kannambra, Anakkara, Kappur, Pattithara, Chalisseri, Nagalasseri, Koppam, Vilayur, Pattambi, Ongallurand Kumaramputhur. 82 fields were surveyed from 31 locations coming under these 24 Panchayats. In Malappuram district, the survey was conducted in five panchayats*viz.,* Angadippuram, Perumpadappa, Alamkode, Vettattur and Thazhekode. 20 fields were covered during the survey. In Thrissur district, 13 panchayts *viz*., Nadathara, Thrissur, Tholur, Kodakara, Varandarapally, Venkitangu, Elavally, Mundathikode, Thekkumkara, Wadakkanchery, Pazhayannur, Thiruvilwamala and Chelakkara, were surveyed.Four panchayats *viz*., Vaikom. Vechoor, Thalayazham and Arpookkara were surveyed in Kottayam district. Five panchayats in Alappuzha district, *viz*., Thakazhi, Karuvatta, Chambakkulam, Edathua and Mannar were covered during the survey. During the symptoms observed, disease severity, stage of the crop and variety were recorded.

Collection of Diseased Plants

Rice plants showing the typical symptoms of bacterial blight, *kresek* as well as leaf blight were collected from the fields surveyed for the isolation of the pathogen. The samples were collected in polythene bags and brought to the laboratory for isolation of the pathogen. For long term storage the freshly collected infected leaf samples were cut into small pieces and kept in small plastic tubes on cotton in which calcium chloride wasadded and stored at 4^0C for long term preservation.

Isolation of Bacterial Blight Pathogen *Xanthomonas oryzae* pv.*oryzae*

Freshly collected samples showing typical bacterial blight symptoms were used for isolation of the pathogen. Isolation of *Xanthomonas oryzae* pv.*oryzae* was carried out on Peptone Sucrose Agar medium (PSA). Diseased samples were washed in tap water for removing the external materials. The leaf samples were cut into small pieces of size one cm along with some healthy tissue. The leaf bits surface sterilized using 0.1 per cent mercuric chloride for 30 seconds, then washed thrice thoroughly with sterilized distilled water. The leaf bits were transferred to a sterilized Petri plate and added 0.5 ml of sterile water. The leaf bits were crushed using forceps until the water turns turbid. By using a heat sterilized loop, a loopful of the water containing the bacterial ooze was streaked on peptone sucrose agar (PSA) media in plates. The plates were incubated at 28^0C for 3 - 4 days. After the development of bacterial colonies, based on morphological and cultural characters single yellow colonies were picked up and sub culturedinto peptone sucrose agar slants. Bacterial cultures were also stored in 15 per cent glycerol at $^-20^o$C for long term preservation. A total of 168 isolates were purified and maintained. The *Xanthomonas oryzae* pv.*oryzae* cultures were serially numbered starting from Xoo1 onwards.

Pathogenicity Studies

To prove the pathogenicity of *Xanthomonas oryzae* pv.*oryzae* isolates suspension of 48 hour old culture was clip inoculated on local variety Jyothi. Seeds were sown in pots and grown in glass house. 20 days old seedlings were transplanted to pots filled with potting mixture. Three plants per pot were transplanted. Other cultural practices as per recommendations were followed. Plants were inoculated 40 days after transplanting with individual isolates of Xoo separately adopting clip inoculation [8]. The leaf tips were cut off by using sterilized scissors dipped in bacterial suspension containing 10^8 CFU/ml. The observations were recorded 15 days after inoculation as per Standard Evaluation System scale of IRRI (2013) [9].

DNA fingerprinting of representative isolates of bacterial blight pathogen

1. *Xanthomonas oryzae* pv.*oryzae* cultures were grown in 1.5 ml of LB broth for 15 hours at 28^oC on a rotary shaker (200 rpm). Genomic DNA was extracted by the procedure of Leach *et al* (1990) [10]. PCR amplification of the DNA was performed as described by George *et al* (1995) [11] with slight modifications. Reaction was performed in 25µl volume, containing 50 picomoles each of the two opposing primers, pJEL1 (5'CTCAGGTCAGGTCGCC3') and pJEL2 (5'GCTCTACAATCGTCCGC3') complementary to each end of IS1112, 20 ng of genomic DNA, 185µM each of four dNTPs, 2.5 units of Taq polymerase in 1x taq polymerase buffer containing 1.5 % $Mgcl_2$ supplemented with 10% dimethysulfoxide (vol/vol), and 7.5 µl of Tris-HCl (pH-9.5). The reaction mixture was overlaid with one drop of mineral oil, initially denatured for one minute at 94^0C, and then subjected to 35 cycles of PCR (10 s denaturation, at 94^0C, one minute annealing at 62^0C and 8 minuteextension at 65^0C) and a final extension for 8 minutes at 65^0C using Master cycler Eppendorf. Electrophoresis was carried out using gel containing agarose 0.5% + Synergel 0.75% in 0.5X tris borate buffer for 4 hours ata voltage of 100 V, stained with ethidium bromide and documented in gel documentation system (Bio-Rad, USA).

Results

A purposives ampling survey was conducted to study the occurrence and intensity of bacterial blight disease of rice in major rice growing tracts of Kerala and to isolate pathogen form these places. Survey was conducted in five districts *viz,* Palakkad, Malappuram, Thrissur, Kottayam and Alappuzha covering 52 panchayats. The details of survey locations, the varieties grown in different locations, stage of the crop, symptoms noticed and disease severity are given in Tables 1 to 5. In Palakkad district among the 24 locations surveyed,

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Table 1: Crop details and severity of bacterial blight in different locations of Palakkad district

Panchayat	Location/field	Variety	Stage of crop (days old)	Symptom	Disease Severity %	Xoo isolate code
Kuthannur	L1 F1	ASD	100	Leaf blight	40.88	Xoo 1
	L1 F2	Uma	100	Leaf blight	40.95	Xoo 2
	L1F3	Jyothi	100	Leaf blight	40.10	Xoo 3
Kuzhalmannam	L1 F1	Jyothi	100	Leaf blight	40.15	Xoo 4
	L2F1	Jyothi	90	Leaf blight	75.48	Xoo 5
	L3F1	Kanchana	100	Leaf blight	30.18	Xoo 6
Peringottukurissy	L1F1	ASD	100	Leaf blight	10.08	Xoo 7
Kottayi	L1F1	Jyothi	110	Leaf blight	10.83	Xoo 8
	L2F1	Uma	90	Leaf blight	10.85	Xoo 73
Kollengode	L1F1	Jyothi	110	Leaf blight	5.30	Xoo 9
Pirayiri	L2F1	Uma	100	Leaf blight	10.70	Xoo 10
	L2F2	Uma	100	Leaf blight	15.33	Xoo 11
	L2F3	Uma	100	Leaf blight	10.08	Xoo 12
	L2F4	Uma	100	Leaf blight	90.50	Xoo13
Chittur	L1F1	Jyothi	100	Leaf blight	50.90	Xoo 39
	L2F1	Jyothi	100	Leaf blight	90.28	Xoo 74
Pattanchery	L1F1	Jyothi	90	Leaf blight	95.10	Xoo 76
	L1F2	Uma	100	Leaf blight	80.38	Xoo 77
	L1F3	Jyothi	100	Leaf blight	10.20	Xoo 88
	L1F4	Jyothi	90	Leaf blight	40.10	Xoo 89
	L1F5	Uma	90	Leaf blight	25.03	Xoo 164
Perumatty	L1F1	Uma	100	Leaf blight	20.08	Xoo 163
Muthalamada	L1F1	Jyothi	95	Leaf blight	40.18	Xoo 165
Vallappuzha	L1F1	Uma	75	Leaf blight	50.10	Xoo 168
Alathur	L1F1	Uma	95	Leaf blight	30.03	Xoo 58
	L1F2	Jyothi	100	Leaf blight	10.55	Xoo 59
	L2F1	Uma	100	Leaf blight	10.25	Xoo 60
Thenkurissi	L1F1	Jyothi	90	Leaf blight	30.80	Xoo 75
Kannambra	L1F2	Jyothi	30	Kresek	25.03	Xoo 103
	L1F3	Uma	30	Kresek	50.80	Xoo 104
Anakkara	L1F1	Ponmani	90	Leaf blight	30.80	Xoo 101
	L1F2	Uma	90	Leaf blight	50.15	Xoo 102
Kappur	L1F1	Uma	20	Kresek	40.75	Xoo 80
Pattithara	L1F2	Uma	90	Leaf blight	10.20	Xoo 44
	L1F3	Jyothi	90	Leaf blight	30.88	Xoo 45
Chalissery	L1F1	Ponmani	60	Leaf blight	15.33	Xoo 71
Nagalassery	L1F1	Jyothi	80	Leaf blight	10.13	Xoo 147
	L1F2	Jyothi	90	Leaf blight	25.85	Xoo 161
Koppam	L1F1	Uma	90	Leaf blight	10.00	Xoo 25
Vilayur	L1F1	Supriya	100	Leaf blight	30.98	Xoo 24
Pattambi	L1F1	Uma	90	Leaf blight	5.78	Xoo 20
	L1F2	Jyothi	90	Leaf blight	50.25	Xoo 21
	L1F1	Jyothi	20	Kresek	60.18	Xoo 50
	L1F2	Uma	80	Leaf blight	15.58	Xoo 160
	L1F3	Ponmani	75	Leaf blight	20.28	X00 162
	L2F1	Ponmani	75	Leaf blight	50.85	Xoo 127
	L2F2	Uma	75	Leaf blight	20.08	Xoo 128
	L2F3	Uma	80	Leaf blight	10.10	Xoo 129
	L2F4	Uma	80	Leaf blight	30.00	Xoo 130
	L2F5	Uma	80	Leaf blight	50.30	Xoo 131
	L2F6	Uma	80	Leaf blight	15.33	Xoo 132
	L2F7	Jyothi	80	Leaf blight	10.58	Xoo 133
	L2F8	Uma	80	Leaf blight	10.10	Xoo 134

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	L3F1	Uma	75	Leaf blight	40.18	Xoo 116
	L3F2	Jyothi	75	Leaf blight	50.23	Xoo 117
	L3F3	Uma	75	Leaf blight	50.03	Xoo 118
	L3F4	Uma	75	Leaf blight	50.63	Xoo 119
	L3F5	Jyothi	90	Leaf blight	20.15	Xoo 145
	L3F6	Uma	80	Leaf blight	15.00	Xoo 146
	L4F1	Jyothi	100	Leaf blight	15.45	Xoo 86
	L4F2	Uma	100	Leaf blight	10.83	Xoo 87
	L4F3	Uma	75	Leaf blight	50.10	Xoo 120
	L5F1	Ponmani	40	Kresek	15.80	Xoo 78
	L5F2	Uma	40	Kresek	5.28	Xoo 79
	L5F3	Kattamodan	90	Leaf blight	10.20	Xoo 81
	L5F4	Karuthamodan	90	Leaf blight	10.00	Xoo 82
	L5F5	Swarnaprabha	90	Leaf blight	15.25	Xoo 83
	L5F6	Vysakh	90	Leaf blight	35.78	Xoo 84
	L5F7	Mattatriveni	90	Leaf blight	20.95	Xoo 85
	L5F8	Jyothi	20	Kresek	30.83	Xoo 100
	L5F9	Jyothi	100	Leaf blight	40.18	Xoo 101
	L5F10	Uma	100	Leaf blight	30.40	Xoo 102
	L5F11	Annapoorna	90	Leaf blight	10.85	Xoo 148
Ongallur	L1F1	Uma	100	Leaf blight	40.10	Xoo 166
	L1F2	Jyothi	75	Leaf blight	40.18	Xoo 167
Kuamaramputhur	L1F1	Uma	100	Leaf blight	20.30	Xoo 135
	L1F2	Uma	100	Leaf blight	20.38	Xoo 136
	L1F3	Uma	100	Leaf blight	5.30	Xoo 137
	L1F1	Uma	60	Leaf blight	5.45	Xoo 138
	L1F2	Uma	75	Leaf blight	20.08	Xoo 139
	L1F3	Jyothi	75	Leaf blight	35.70	Xoo 140
	L1F1	Jyothi	75	Leaf blight	40.45	Xoo 141

Table 2: Crop details and severity of bacterial blight in different locations of Malappuram district.

Panchayat	Location /Field	Variety	Stage of crop (Days old)	Symptom	Disease Severity %	Xoo isolate code
Angadippuram	L1F1	Ponmani	50	Kresek	5.30	Xoo 14
	L2F1	Ponmani	60	Kresek	10.20	Xoo 15
	L2F2	Ponmani	60	Kresek	10.00	Xoo 16
	L3F3	Ponmani	20	Kresek	15.05	Xoo 17
	L3F2	Jyothii	90	Leaf blight	10.13	Xoo 18
	L4F1	Ponmani	45	Kresek	10.33	Xoo 19
Perumpadappa	L1F1	Uma	65	Leaf blight	10.38	Xoo51
	L1F2	Uma	65	Leaf blight	20.08	Xoo 52
	L1F3	Uma	65	Leaf blight	10.00	Xoo 53
	L1F4	Uma	65	Leaf blight	10.70	Xoo 54
	L2F1	Uma	65	Leaf blight	5.28	Xoo 55
Alamkode	L1F1	Uma	60	Leaf blight	5.45	Xoo 56
	L1F2	Uma	60	Leaf blight	5.78	Xoo 57
Vettathur	L1F1	Uma	90	Leaf blight	70.20	Xoo 121
	L1F2	Uma	90	Leaf blight	70.60	Xoo 122
	L2F1	Uma	75	Leaf blight	50.80	Xoo 123
	L3F1	Uma	75	Leaf blight	75.75	Xoo 124
Thazhekode	L1F1	Uma	40	Leaf blight	50.15	Xoo 125
	L1F2	Jyothi	40	Kresek	50.10	Xoo 126

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Table 3: Crop details and severity of bacterial blight in different locations ofThrissur district.

Panchayat	Location/field	Variety	Stage of crop (days old)	Symptom	Diseases Severity %	Xoo isolate code
Nadathara	L1F1	Uma	60	Leaf blight	20.23	Xoo 42
	L1F2	Uma	60	Leaf blight	5.20	Xoo 43
Thrissur	L2F1	Jyothi	75	Leaf blight	25.00	Xoo 142
Tholur	L1F1	Uma	49	Leaf blight	10.13	Xoo 32
	L2F1	Uma	50	Leaf blight	20.40	Xoo 33
	L3F1	Uma	65	Leaf blight	5.10	Xoo 34
	L4F1	Uma	65	Leaf blight	10.78	Xoo 35
	L5F1	Jyothi	90	Leaf blight	80.18	Xoo 36
Kodakara	L1F1	Uma	75	Leaf blight	85.83	Xoo 22
	L1F2	Jyothi	75	Leaf blight	30.75	Xoo 23
	L1F3	Jyothi	80	Leaf blight	20.53	Xoo 97
	L1F4	Jyothi	80	Leaf blight	50.28	Xoo 98
Varandarappally	L1F1	Uma	75	Leaf blight	25.35	Xoo 40
	L2F1	Uma	75	Leaf blight	20.03	Xoo 41
Vengittangu	L1F1	Mattatriveni	90	Leaf blight	10.43	Xoo 90
	L1F2	Mattatriveni	90	Leaf blight	5.65	Xoo 91
	L1F3	Mattatriveni	90	Leaf blight	5.40	Xoo 92
	L2F1	Uma	90	Leaf blight	10.08	Xoo 93
	L2F2	Uma	90	Leaf blight	10.23	Xoo 94
	L2F3	Uma	90	Leaf blight	5.25	Xoo 95
	L2F4	Uma	90	Leaf blight	5.05	Xoo 96
Elavally	L1F1	Jyothi	15	Kresek	35.05	Xoo 46
	L1F2	Jyothi	20	Kresek	50.15	Xoo 47
	L2F1	Uma	40	Kresek	25.20	Xoo 48
	L2F2	Uma	40	Kresek	10.35	Xoo 49
Mundathikkode	L1F1	Uma	50	Leaf blight	50.43	Xoo 26
	L1F2	Uma	50	Leaf blight	30.08	Xoo 27
	L2F1	Uma	60	Leaf blight	50.15	Xoo 28
	L2F2	Shreyas	50	Leaf blight	50.25	Xoo 29
	L3F1	Uma	60	Leaf blight	35.05	Xoo 30
Thekkumkara	L1F1	Uma	75	Leaf blight	50.43	Xoo 149
	L1F2	Uma	75	Leaf blight	45.53	Xoo 150
	L1F1	Uma	75	Leaf blight	30.03	Xoo 151
	L1F2	Uma	75	Leaf blight	30.83	Xoo 152
	L1F3	Uma	75	Leaf blight	20.23	Xoo 153
Wadakkanchery	L1F1	Uma	70	Leaf blight	30.55	Xoo 154
	L1F2	Uma	80	Leaf blight	60.90	Xoo 155
	L2F1	Uma	57	Leaf blight	60.18	Xoo 31
Pazhayannur	L1F1	Jyothi	90	Leaf blight	80.18	Xoo 72
Thiruvillamala	L1F1	Uma	90	Leaf blight	40.98	Xoo 143
Chelakkara	L1F1	Uma	110	Leaf blight	50.38	Xoo 156
	L1F2	Uma	110	Leaf blight	40.08	Xoo 157
	L1F3	Uma	110	Leaf blight	40.80	Xoo158
	L1F3	Uma	110	Leaf blight	30.78	Xoo 159

Table 4: Crop details and severity of bacterial blight in different locations of Alappuzha district

Panchayat	Location/field	Variety	Stage of crop (days old)	Symptom	Disaese Severity %	Xoo isolate code
Thakazhy	L1F1	Jyothi	90	Leaf blight	10.78	Xoo 61
Karuvatta	L1F1	Uma	90	Leaf blight	20.03	Xoo 62
	L2F1	Uma	60	Leaf blight	50.05	Xoo 63
	L3F1	Uma	90	Leaf blight	30.55	Xoo 64
Chambakkulam	L1F1	Jyothi	100	Leaf blight	50.70	Xoo 65
Edathua	L1F1	Uma	90	Leaf blight	10.63	Xoo 66
	L1F2	Uma	90	Leaf blight	50.70	Xoo 67
Mannar	L1F1	Uma	60	Leaf blight	70.68	Xoo 68
	L1F2	Uma	60	Leaf blight	30.58	Xoo 69
	L2F1	Jyothi	85	Leaf blight	5.38	Xoo 70
	L2F2	Jyothi	85	Leaf blight	5.30	Xoo 71

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Table 5: Crop details and severity of bacterial blight in different locations ofKottayam district

Panchayat	Location/Field	Stage of crop (days old)	Variety	Symptom	Disease Severity %	Xoo isolate code
Vaikom	L1F1	100	Uma	Leaf blight	30.18	Xoo 105
	L1F2	120	Uma	Leaf blight	25.85	Xoo 106
Vechoor	L1F1	135	Uma	Leaf blight	50.15	Xoo 107
	L1F2	135	Uma	Leaf blight	50.63	Xoo 108
Thalayazham	L1F1	125	Uma	Leaf blight	25.03	Xoo 109
	L1F2	125	Uma	Leaf blight	50.30	Xoo 110
	L1F3	125	Uma	Leaf blight	10.33	Xoo 111
	L1F1	100	IR 5	Leaf blight	5.80	Xoo 112
Arpookkara	L1F1	120	Uma	Leaf blight	10.55	Xoo 113
	L1F2	130	Uma	Leaf blight	10.23	Xoo 114
	L1F3	130	Uma	Leaf blight	5.45	Xoo 115

Table 6: Disease severity on rice variety Jyothi under artificial inoculation of Xoo isolates.

Xoo code	Disease severity (%)	Score	Reaction
1	41.18	7	S
2	41.32	7	S
3	24.13	5	MS
4	30.64	7	S
5	31.87	7	S
6	47.68	7	S
7	42.69	7	S
8	31.91	5	MS
9	54.31	9	HS
10	59.70	9	HS
11	30.98	7	S
12	38.70	7	S
13	51.17	9	HS
14	44.48	7	S
15	49.64	7	S
16	44.97	7	S
17	43.59	7	S
18	49.82	7	S
19	51.63	9	HS
20	41.86	7	S
21	43.13	7	S
22	37.15	7	S
23	40.02	7	S
24	48.86	7	S
25	30.49	7	S
26	34.74	7	S
27	39.61	7	S
28	43.41	7	S
29	43.47	7	S
30	28.41	7	S
31	39.12	7	S
32	35.18	7	S
33	42.12	7	S
34	40.02	7	S
35	24.18	5	MS
36	43.96	7	S
37	31.24	7	S
38	25.52	7	S
39	24.63	5	MS
40	30.13	7	S
41	37.29	7	S
42	34.64	7	S
43	40.49	7	S
44	35.69	7	S
45	43.91	7	S
46	15.15	5	MS
47	44.46	7	S
48	50.06	7	S
49	60.93	9	HS
50	40.54	7	S
51	38.39	7	S
52	60.07	9	HS
53	47.66	7	S
54	39.21	7	S
55	55.09	9	HS
56	41.59	7	S
57	51.27	9	HS
58	41.36	7	S
59	28.66	7	S
60	36.95	7	S
61	42.97	7	S
62	47.15	7	S
63	57.81	9	HS
64	32.82	7	S
65	49.16	7	S
66	34.10	7	S
67	36.94	7	S
68	57.55	9	HS
69	39.38	7	S
70	25.00	5	MS
71	37.08	7	S
72	21.05	5	MS
73	45.84	7	S
74	35.04	7	S
75	45.80	7	S
76	51.56	9	HS
77	40.69	7	S
78	43.11	7	S
79	36.55	7	S
80	46.87	7	S
81	48.25	7	S

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82	43.67	7	S
83	35.51	7	S
84	30.94	7	S
85	25.71	7	S
86	23.44	5	MS
87	30.94	7	S
88	37.58	7	S
89	33.24	7	S
90	32.41	7	S
91	44.09	7	S
92	32.57	7	S
93	38.60	7	S
94	55.47	9	HS
95	31.21	7	S
96	34.68	7	S
97	36.07	7	S
98	43.52	7	S
99	27.44	7	S
100	52.98	9	HS
101	36.57	7	S
102	24.32	5	S
103	23.19	5	S
104	24.10	5	S
105	40.03	7	S
106	27.03	7	S
107	31.79	7	S
108	44.93	7	S
109	28.02	7	S
110	32.56	7	S
111	34.02	7	S
112	37.14	7	S
113	46.89	7	S
114	33.70	7	S
115	26.54	7	S
116	26.37	7	S
117	21.51	5	MS
118	27.26	7	S
119	41.06	7	S
120	30.22	7	S
121	17.96	5	MS
122	27.17	7	S
123	27.86	7	S
124	27.48	7	S
125	27.79	7	S
126	23.60	5	MS
127	33.01	7	S
128	35.31	7	S
129	35.71	7	S
130	42.53	7	S
131	33.36	7	S
132	45.95	7	S
133	24.45	5	MS
134	22.26	5	MS
135	18.39	5	MS
136	29.47	7	S
137	26.27	7	S
138	29.01	7	S
139	44.74	7	S
140	31.92	7	S
141	20.61	7	S
142	22.64	7	S
143	32.79	7	S
144	37.90	7	S
145	14.37	5	MS
146	25.52	7	S
147	22.05	5	MS
148	29.05	7	S
149	26.44	7	S
150	21.76	5	MS
151	21.84	5	MS
152	33.21	7	S
153	48.11	7	S
154	26.64	7	S
155	51.59	9	HS
156	47.01	7	S
157	31.14	7	S
158	31.47	7	S
159	29.13	7	S
160	33.46	7	S
161	31.51	7	S
162	31.95	7	S
163	33.11	7	S
164	19.69	5	MS
165	22.76	5	MS
166	17.51	5	MS
167	36.27	7	S
168	17.95	5	MS

the leaf blight severity ranging from 5.30 to 95.10 per cent was recorded (Table 1). The *kresek* symptom was noticed in six locations and the severity ranged from 5.28 to 60.18 per cent. The highest severity was recorded in eastern parts of the district Chittur (90.28 %) and Pattancheri (95.10 %) in variety Jyothi. During the period of survey, in most of the fields the crop was in reproductive phase which is highly susceptible to the disease. Among the 82 fields surveyed, 24 fields were under cultivation of variety Jyothy and in 35 fields the variety cultivatedwas Uma. The other varieties grown in the district affected with disease were ASD (10.08 -40.88 %), Kanchana (30.88%), Ponmani (15.33 to 50.85%), Mattatriveni (20.95%), Annapoorna (10.85%). The upland varieties Kattamodan (10.20%), Karuthamodan (10%), Swarnaprabha (15.25%) and Vaisakh (35.78%) showed relatively less severity in upland condition. *Kresek* symptom was observed in varieties Jyothi, Uma and Ponmani in earlytransplanted stage in six fields surveyed.

In Malappuram district, fivePanchayats covering 19 fields were surveyed (Table2). In one panchayat, Angadippuram, in five locations the variety Ponmani and in one location Jyothi was grown. The crop was in early transplanted stage and was affected by *kresek* phase of bacterial blight.In other fields surveyed covering three panchayats, Uma variety was in flowering stage. The leaf blight symptom ranging from 5.28 to 75.75 per cent severity was recorded. In one panchayat,

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Thazhekode, in two fields, the varieties Uma and Jyothi were in early transplanted condition where *kresek* symptom was recorded (5.33 - 15.05% severity).

In Thrissur district 13 panchayatscovering 29 locations in which 44 different fields were surveyed (Table3). In 32 fields the variety cultivated was Uma and the disease severity ranged from 5.25 per cent (Nadathara and Venkitangu) to 85.83 per cent (Kodakara). In two fields *kresek* symptom was noticed in 10.35-25.25 per cent severity. In 8 fields surveyed variety Jyothi was cultivated. Disease severity ranged from 25-80.18 per cent. In two fields where direct sowing was practiced, *kresek* symptom was recorded at 35-50.15 per cent severity level in variety Jyothi and 10 to 25 per cent in variety Uma. The other varieties affected with leaf blight wereMattatriveni (5.40-10.43%) in three fields of other panchayats and Shreyas in one location (50.25%).

In Alappuzha district, survey was carried out in five panchayats, eightlocations covering11 fields (Table 4). Variety Uma was cultivated in seven fields and Jyothi was cultivated in three fields. The crop was in flowering to maturity stage. Bacterial leaf blight was noticed 10.63-70.68 per cent severity in variety Uma. In Jyothi 5.38- 50.70 per cent severity was noticed.

In Kottayam district, among the four panchayats surveyed the variety Uma was grown in all the 11 fields except one where IR5 was grown (Table 5). The crop was in maturity stage. In all the surveyed locations leaf blight symptom was recorded. The disease severity ranged from 5.45 to 50.63 per cent.

Isolation of the Pathogen

The pathogen was isolated from the all the diseased samples collected from different locations five districts surveyed, in peptone sucrose agar medium. Yellow pigmented bacterial colonies typical to that of *Xanthomonas oryzae* pv.*oryzae* were picked up and sub cultured on to PSA slants and maintained at -20 ^{0}C. 168 isolates were obtained from different locations.

Pathogenicity and Virulence Studies

Pathogenicity of the all the isolates were proved by inoculation to susceptible variety Jyothi. The bacterial isolates produced symptoms on the test variety upon inoculation. The pathogen was reisolated from the symptomatic plants and upon reisolation the same bacterial isolate wereobtained. The severity of symptoms obtained on the susceptible variety Jyothi varied with the isolate, showing the variability in virulence among the *Xanthomonas oryzae* pv.*oryzae* isolates. The data is given in table 6. The disease severity varied from 14.37 per cent (Score 5) to 60.93% (Score 9). Among the 168 isolates, 14 isolates (8.33% produced highly susceptible reaction with score 9, 128 isolates (76.19 %) produced susceptible reaction with score 7 and 26 isolates (15.48%) produced moderately susceptible reaction with score 5 upon artificial inoculation on susceptible variety Jyothi.

Genetic Variability Studies

To understand whether there is variability exists among the isolates of Xoo, DNA fingerprinting analysis using IS112 and IS113 repetitive element-based primers JEL1 and JEL2 was carried out for 24 isolates representing different locations from five districts. The genetic fingerprinting revealed high genetic variability among the isolates even within a small geographical area (Figure 1). Fingerprinting showed distinct banding patterns. Fingerprints comprising to 15 bands were generated with JEL1 and JEL2 primers. The number and position of bands varied for different isolates showing the difference among the isolates.

Discussion

The survey conducted in major rice growing tracts of Kerala revealed the wide spread occurrence of bacterial blight of rice. The predominantly cultivated varieties Uma and Jyothi were affected in moderate to severe intensities. Among the districts surveyed, the disease severity was high in Palakkad district. In variety Jyothi the highest severity recorded was 90.5 per cent and in Uma it was 95.10 per cent. This was followed by Thrissur district, where the highestdisease severity of 85.35 per cent and 80.18 per cent was recorded in varieties Uma and Jyothi respectively. In Malappuram district, highest disease severity recorded in variety Uma was 75.75 per cent. Jyothi was cultivated in only one field surveyed where the disease severity was low (10.13%).In Alappuzha district, the highest disease severity recoded in variety Uma was 70.68 and in variety Jyothi was 50.70 per cent. In Kottayam district, in all the locations surveyed except one, the variety cultivated was Uma and the highest severity recorded was 50.63 per cent. The incidence of bacterial blight as major disease of rice has been reported from the states of Punjab, Haryana, Uttaranchal, Bihar, West Bengal, Tripura, Assam. Tamil Nadu, Uttar Pradesh, Andhra Pradesh, Andaman and Nicobar Islands, Maharashtra, Chhattisgarh, Gujarat, Himachal Pradesh, Karnataka and Kerala [12]. The bacterial blight epidemics causing yield loss due to bacterial blight ranging from 30 to 90 per cent have been reported by several researchers depending on the variety cultivated, stage of the crop and climatic conditions [2,13-15]. The yield loss 10-20 percent is common. In the state of Kerala, after the floods experienced during the year 2018 and 2019, the incidence and spread of bacterial blight has increased considerably. The systematic collection of diseased samples, isolation of pathogen and study of its virulence is important to develop effective management strategies

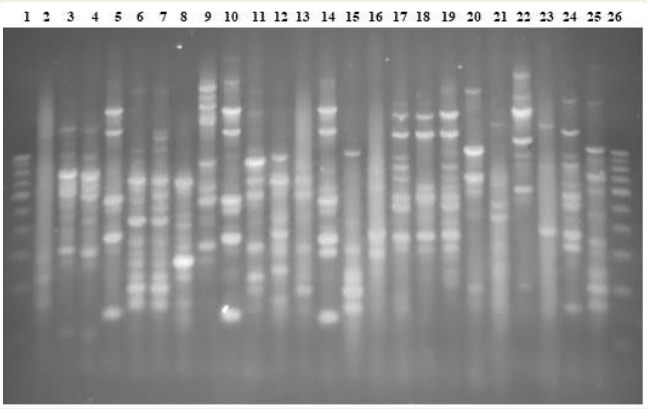

Figure 1: DNA fingerprints of *Xanthomonas oryzae* pv.*oryzae* strains using PCR with IS primers pJEL1 and pJEL2 Lane 1and 26:100 bp ladder; Lane 2-25 Xoo isolates from different parts of Palakkad, Malappuram and Thrissur, Aleppy and Kottayam districts.

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including development of resistant varieties. Similar studies were conducted in the states of India such as Punjab and Andhra Pradesh. Rajarajeswari and Muralidharan (2008) [16] conducted a study to assess the occurrence of bacterial blight of rice in four rice growing districts of Nellore (Andra Pradesh), Rangareddy (Andra Pradesh), West Godavari (Andra Pradesh), Karnal (Hariyana). Singh *et al* (2003) [6] reported the prevalence and intensity of bacterial blight in 10 districts of Punjab. Rafi *et al* (2013) [17] conducted a survey and reported the disease incidence and severity of bacterial blight in all rice growing zones of Pakistan.

The inoculation of the isolates in susceptible variety Jyothi revealed that the isolates from different locations are highly virulent capable of producing moderately susceptible to highly susceptible disease response. This indicate the chances of epidemics in predominant varieties like Jyothi is very high under favourable climatic conditions due to the prevailing pathogen population in different rice growing tracts of the state. Among the isolates 168 isolates collected, all the isolates are virulent on susceptible variety Jyothi causing a reaction of moderately susceptible (score 5) and above up to maximum score of 9 (highly susceptible). The disease response varies with the isolates indicating the variability among the isolates. Bakade *et al* (2020) [18] studied the virulence of Xoo isolates collected from different geographical regions of South India by inoculation of one susceptible variety TN-1. They reported high variability among the isolates in their virulence.

To confirm the existing variability of the pathogen as evidenced from the pathogenicity studies, genetic variability of representative Xoo isolates from various locations were carried out. It revealed high genetic variability among the isolates.The existence of high genetic variability of bacterial blight pathogen of rice *Xanthomonas oryzae* pv.*oryzae* isolates from different parts of Punjab was reported by Singh *et al* 2003 [6] and Lore *et al* 2011 [7]. All these suggests the need for detailed study on pathogen population prevailing in the state particularly pathotyping of the pathogenson differentials / NILs carrying different resistance genes so as to identify the resistance genes/gene combinations effective against the prevailing pathotypes of the state.

Conclusion

A survey has been carried out in five major rice growing districts of Kerala to assess the severity of disease in predominant varieties of the state and collect the pathogen isolates. The predominant rice varieties Jyothi and Uma grown in these districts were affected severely by bacterial blight even up to 90-95 per cent. This suggests an urgent need to replace these mega varieties of the state with new varieties with bacterial blight resistance. Among the districts surveyed bacterial blight was more widely seen with high intensity in Palakkad district, the rice bowl of Kerala. 168 isolates collected from different locations of the state varied in their virulence on susceptible variety Jyothi. The DNA fingerprinting of representative isolates from different locations exhibited high variability. Further studies on pathotypic variability and identification of genes/gene combination effective against major pathotypes prevailing in the state is essential for development of bacterial blight resistant varieties suitable for the state of Kerala.

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