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Screening and management of rice germplasm against *Xanthomonas oryzae* pv. *oryzae*

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Abstract
The present studies on "Management of bacterial leaf blight of paddy (*Oryza sativa L*.) caused by *Xanthomonas oryzae* pv. *oryzae"* were undertaken in order to determine the status of the bacterial leaf blight of paddy in Jammu Division 2015. Maximum disease incidence was recorded in Kulian (45.33 per cent) of Jammu district and minimum in Jagatpur (15.21%) of Kathua district. Overall average disease incidence for all three districts was 33.53 per cent. Sixteen germplasm were screened under field condition against bacterial leaf blight, and no germplasm was found immune or resistant to the disease, four were moderately resistant, whereas eleven were moderately susceptible. However, Jaya was found to be susceptible. Fungicide and antibiotic for disease management revealed that minimum disease severity of 11.33% was recorded in streptomycin followed by copper oxychloride (16.00 % disease severity); second date of transplanting (15th July) and second irrigation schedule (water drained out after tillering stage) was found most effective among all the treatments of disease management with maximum 11.67 numbers of tillers per hill, plant height of 124.67cm, panicle length of 27.43 cm and yield of 41.34 q/ha while the first date of transplanting (5th July) with first irrigation schedule (stagnant water) was least effective treatment with 6.33 tillers per hill and plant height 105.00 cm and panicle length 18.23 cm along with 22.46 q/ha yield.

Keywords: BB, disease incidence, fungicides, antibiotic, transplanting and irrigation

Introduction
Rice is a primary staple food consumed by 60% of the world's population over 90% of the world's population in Asia, with China, India and Indonesia producing 30.85%, 20.12% and 8.21%, respectively of the total global rice production (USDA., 2012) [23]; (Kadu, *et al*., 2015) [13]. Although, In Jammu division paddy occupies 67 thousand hectares leading to production of 1500 thousand quintals (Statistical Digest J&K 2016-2017). Among these diseases, bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is considered as one of the most devastating disease that cause severe yield losses (Chukwu *et al*., 2019a) [7]. BLB caused severe yield losses of up to 50% depending upon variety, growth stage, the geographical location and environmental condition (Liu *et al*., 2014) [16]. BBL is a vascular disease which develops at various stages of development. The pathogen invades into the plants through the hydathode and wounds. Symptoms generally appears at the tillering stage along the veins of the leaf, forming grayish- white lesions. However, during the "Kresek" phase, the plant is systemically infected in between seedling to tillering stage. During this infection the leafs of plant turns white causing sudden withering of the plant.

Review
Bacterial leaf blight usually favors warm temperature, high humidity and is caused due to excessive nitrogen fertilization (Annonymous, 2010) [2] winds, irrigated water, trimming tools or even during the handling while transplanting (Mew, 1992). The gram negative bacteria consists of type III protein secretion system which directly inject the virulence factor into the host (Furutani *et al*., 2009) [10]. Conventionally, spraying of antibiotics such as copper oxychloride (Sulaiman and Ahmed, 1956), streptomycin solution (Seki and Mizukami, 1956) [19], techlofthalam (Nakagami *et al*., 1980) [20] were recommended for controlling disease. Resistant varieties is the economical, effective and eco- friendly strategy for controlling the endemic pathogen to minimize disease incident and yield losses (Chukwu *et al*., 2019b) [8]. Globally, 38 resistant genes are identified with tightly linked markers (Bhasin *et al*., 2012) [3]. Mandal *et al*. (2018) [17] reported that Quantitative trait loci mapping will facilitate isolation of novel bacterial blight resistant gene. Kim & Reinke (2019) [15] identified the novel BB resistance gene on chromosome 11 using QTL analysis.

Jiang (2020) investigated that for plant immunity, TAL effectors injected into plant cells have to be translocated into nucleus to bind to the target S or R broad spectrum gene. However, cultural and integrated plant management practices are also sensible practices for maintaining a sustainable ecosystem. As there is no single most effective control measure available against this disease, some culture practices can minimize the disease incident (Chaudhary *et al*., 2009) [6].

Material and method

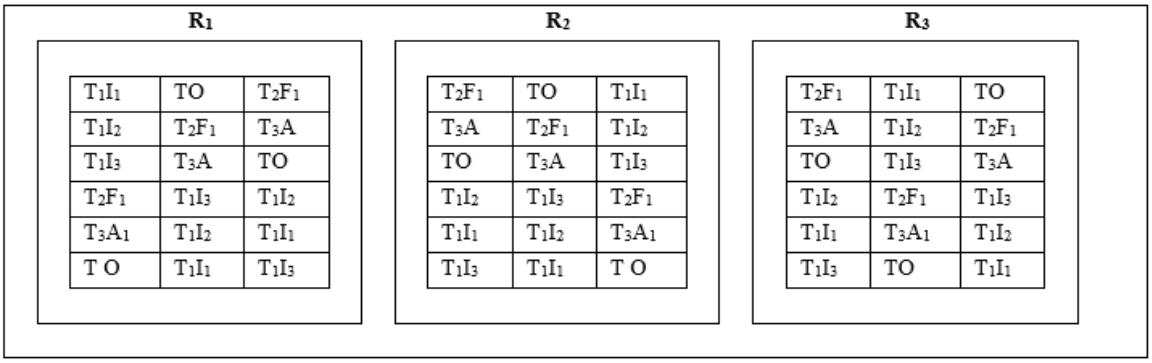
1. Survey of Disease

The disease samples were randomly from three rice growing area of Jammu, Kathua and Samba, here five fields locations were selected for estimation of disease incidence and collection of samples. At each place four diseased plants were examined and upper three leafs of each plant were collected. The disease incidence was calculated by the formula of (IRRI, 1996) [11].

$$\text{Percent disease incidence} = \frac{\text{Number of bacterial blight infected plants}}{\text{Total number of plants examined}} \times 100$$

2. Design, Layout and Treatments

The experiment was laid out in a split- plot design with three replications. The rice cultivar Basmati- 370 was tested. Three dates of transplanting (5th , 15th ,and 25th July) were done in the main plots and three irrigation schedule (I1- Stagnant water, I2- Water drained out after tillering stage, I3- Water drained out two days after transplanting and tillering stage) and chemical treatments (streptomycin and copper oxychloride) were tested in sub plots of size 0.76 * 0.76 m^2 each. The treatment were randomly assigned to experimental units.



Experimental Design: Split-plot (variety in main plot and treatments in subplot) with 3 replications. Factor A: Varieties: V = Basmati-370. Factor B: Treatments: T_1 = 5th July 2015, T_2 = 15th July, T_3 =25th July, T_4 =Stagnant water level, T_5 = Water drained out after tillering stage, T_6 = Water drained out two days after transplanting and after tillering stage, T_7 = Spray of copper oxycloride @ 3gm/litre of water at the time of disease appearance, T_8 = Spray of streptomycin @ 100 ppm at the time of disease appearance, T_9 = Control. Plot size: 0.76 m x 0.76 m; R_1, R_2, R_3 = Replications.

3. Seed bed preparation and Raising of Seedlings

The land was well ploughed, marshy and rich in organic matter, therefore no fertilizers were applied to the seed bed. Seeds of Basmati-370 was collected from the Plant pathology division, SKUAST-J and later, 30 days old seedlings were transplanted in well- puddle field.

4. Land Preparation and Fertilizer Application

The land was ploughed with tractor driven disc plough followed by harrowing. The field prepared stubble and weed free for transplanting of rice seedlings. Eventually, fertilizers for N P K were applied in the plots at the rate of 30, 20 and 10 kg per hectare. Half quantity of N and whole quantity of P and K was applied as basal dose and half in two split doses as top dressing.

5. Screening

Sixteen rice genotypes viz. PUSA-1121, B-370, PR-113, RR-8585, PC-19, IET-1410 SJR-5, Jaya,PB-1, IARI-1460, Ranbir basanti, Saanwal basmati, Basmati-564, IR-10 Basmati-387 and Ratna collected from sources were grown under field conditions. Data of severity was recorded as percentage of tissue area infected out of total leaf area examined. Percentage average lesion area of 15 leaves collected was measured for disease severity in each plot using the following scale (Chaudhary, 1996) [5].

Disease Rating	Lesion size (% of leaf length)	Disease Reaction
0	0	Immune
1	1-10	Resistant
3	11-30	Moderately Resistant
5	31-50	Moderately Susceptible
7	51-75	Susceptible
9	76-100	Highly Susceptible

$$\text{Disease Index} = \frac{n(1) + n(3) + n(5) + n(7) + n(9)}{tn}$$

Where: n (1), n (3), n (5), n (7) and n (9) = Number of leaves showing severity score of 1, 3, 5, 7 and 9; tn = Total number of leaves scored

6. Management

For management of disease two chemicals viz. streptomycin and copper oxychloride 100 ppm and 0.3 per cent respectively were applied along with three irrigation schedules (I_1- Stagnant water, I_2- Water drained out after tillering stage, I_3- Water drained out two days after transplanting and after

tillering stage) on three dates of transplanting viz 5th July,15th July and 25th July. The lesion length was measured after 2 and 3 weeks and for maintaining optimal humidity for infection development adequate water was supplied as per schedule.

7. Yield and yield parameters

The crop was harvested at maturity stage and plants of each sub plots were harvested separately to obtain yields. All harvested plants of individual sub plots were threshed and grains were dried (12 per cent moisture content) before recording the weight of the grains. The data for the important agronomic parameters such as number of tillers/hill; plant height, panicle length, and yield kg per quintal were recorded.

8. Analysis of data

The data were subjected to statistical analysis. Analysis of variance was done following split-plot design for the concerned field experiment with the help of computer package O.P Stat followed by calculator

Result

1. Survey for Disease Incident Analysis

In order to determine the prevalence of bacterial leaf blight of paddy in Jammu ranged from 34.10 to 45.33 per cent with the mean of 34.59 per cent, Kathua ranged from 15.21 to 40.13 per cent with mean of 29.67 per cent and Samba districts ranged from 15.23 to 40.21 per cent with mean of 36.35 per cent of Jammu division of Jammu and Kashmir, an exhaustive survey was conducted during *kharif* 2015 at boot leaf stage of the crop and data presented in Table 1. Maximum disease incidence was recorded in Kulian (45.33 per cent) of Jammu district and minimum at Jagatpur (15.21 per cent) of Kathua district. Overall disease incidence for all three districts was 33.53 per cent. Three varieties viz. Basmati-370, Pusa-1121and IET-1410 were grown at different locations of Jammu district. Maximum disease incidence (45.33 per cent) was recorded IET-1410 whereas minimum incidence (15.21 per cent) was recorded in Pusa-1121 and Neha.

Table 1: Disease incidence in Jammu, Kathua and Samba district

S. No.	District	Location	Variety	PDI (%)
I	Jammu			
1		Udheywala	B-370	40.21
2		B.Bramhana	B-370	38.11
3		Bhor Camp	B-370	34.10
4		Gagian	Pusa-1121	15.21
5		Kulian	IET-1410	45.33
6	Mean			34.59
7	Range			34.10-45.33
II	Kathua			
1		Nagari	IET-1410	40.13
2		Kalibari	Pusa-1121	17.15
3		Jagatpur	Neha	15.21
4		Rathwal	PHB-71	39.33
5		Bhujwal	B-370	36.56
6	Mean			29.67
7	Range			15.21-40.13
III	Samba			
		Ramgarh	B-370	37.11
1		Koh Bramhana	Neha	15.55
2		Raiper	Ratna	40.21
3		Sordi	Ratna	35.34
4		Bainglarh	Neha	15.23
5		ChakSalarian	B-370	38.32
6	Mean			36.35
7	Range			15.23-40.21
	Overall mean			33.53

2. Screening

Sixteen varieties were grown in order to determine the severity for bacterial leaf blight of paddy. The data presented in Table 2 revealed that maximum severity was observed on Jaya (60.22 per cent) whereas, minimum severity was observed on Pusa-1121 (25.21 per cent). Four varieties i.e. PUSA-1121, PR-113, PB-1 and IR-10 were found as moderately resistant, eleven varieties viz. B-370, RR-8585, PC-19, IET-1410, SJR-5, IARI-1460, Ranbir basmati, Saanwal basmati, Basmati-564, Basmati-385, Ratna as moderately susceptible and one Jaya as susceptible. Out of twenty varieties none of the variety was found immune.

Table 2: Screening of Variety

S. No.	Variety	Disease severity (%)	Score	Disease reaction
1	PUSA-1121	25.21	3	MR
2	B-370	38.11	5	MS
3	PR-113	27.45	3	MR
4	RR-8585	39.34	5	MS
5	PC-19	42.77	5	MS
6	IET-1410	42.21	5	MS
7	SJR-5	45.33	5	MS
8	Jaya	60.22	7	S
9	PB-1	30.55	3	MR

10	IARI-1460	40.65	5	MS
11	Ranbir basmati	35.22	5	MS
12	Saanwal basmati	38.33	5	MS
13	Basmati-564	40.72	5	MS
14	IR-10	27.22	3	MR
15	Basmati-385	38.33	5	MS
16	Ratna	45.21	5	MS

3. Effect of disease management on yield and yield components

The effects of the different treatments on yield and yield attributes such as number of tillers, plant height, and panicle length are presented in Table 3, 4 and 5. The effects of the treatments were recorded as follows:

Table 3: Effect of agronomic inputs on the severity of BLB and yield components of paddy

DOT	I_1				I_2				I_3				Mean			
	NT	PH	PL	Y	NT	PH	PL	Y	NT	PH	PL	Y	NT	PH	PL	Y
D1	7.33	106.00	19.33	25.20	8.67	109.66	20.83	25.22	9.00	106.67	19.56	25.23	8.33	107.44	19.90	25.21
D2	8.00	121.67	25.50	36.11	11.33	123.33	26.43	39.21	10.00	121.67	25.77	34.44	9.77	122.22	25.90	36.58
D3	6.33	91.33	20.60	30.30	8.33	94.33	21.43	31.25	7.00	92.33	20.80	32.45	7.22	92.66	20.94	31.32
Mean	7.22	106.33	21.82	30.53	9.44	109.11	22.90	31.88	8.67	106.88	22.04	30.70	-	-	-	-
CD (p=0.05)	0.53	0.60	0.32	0.11	-	-	-	-	-	-	-	-	0.94	0.59	0.60	0.20

NT = No. of tillers, PH = plant height (cm), PL = panicle length (cm), Y = Yield (q/ha), DOT = Date of transplanting, I_1, I_2, I_3 = irrigation schedule, D1 = 5th July, D2 = 15th July, D3 = 25th July

I_1- Stagnant water, I_2- Water drained out after tillering stage, I_3- Water drained out two days after transplanting and after tillering stage

Effects on effective number of tillers

Maximum number of effective tillers (11.33) were recorded during second date of transplanting (15th July) and second irrigation schedule(Water drained out after tillering stage) while the minimum 6.33 tillers were recorded in plants grown on third date of transplanting (25th July) with first irrigation schedule (Stagnant water) whereas numbers of tillers were significantly affected in all treatments. Mean number of tillers ranged from 7.22 to 9.44 in different dates of transplanting.

Effects on plant height

Plant height ranged from 106.33 to 109.11 cm in different irrigation schedules. The maximum plant height of 123.33 cm was recorded in plants grown on second date of transplanting and second irrigation schedule while minimum plant height 91.33 cm was recorded in plants grown on third date of transplanting (25th July) and first irrigation schedule stagnant water in sub plot. Mean plant height ranged from 92.66 to 122.22 cm in different dates of transplanting.

Effects on panicle length

Panicle lengths were significantly affected in all treatments. Mean of panicle lengths ranged from 21.82 to 22.90 cm in different irrigation schedule. The maximum panicle length 26.43 cm was recorded in plants grown on second date of transplanting (15th July) and second irrigation schedule (water drain out after tillering stage) while the minimum panicle length 19.33 cm was recorded in plants grown on first date of transplanting (5th July) and first irrigation schedule.

Effects on yield

Yield was significantly affected by different treatments (Table 3). Mean yield ranged from 30.53 to 31.88 q/ha in irrigation schedule. The maximum 39.21 q/ha yield was recorded in plants grown on second date of transplanting and second irrigation schedule while the lowest yield of 25.20 q/ha was recorded plants grown on first date of transplanting (5th July) and first irrigation schedule. Mean yield ranged from 31.32 to 36.58 q/ha in dates of transplanting in different dates of sowings.

Table 4: Effect of cultural and chemical management on the severity (%) of bacterial leaf blight of paddy

DOT	Irrigation schedule				Chemical			
	I_1	I_2	I_3	Mean	Streptomycin 100ppm	Copper oxychloride 0.3%	Control	Mean
D1	24.33	19.33	20.67	21.44	13.33	16.00	27.67	19.00
D2	17.33	14.33	14.33	15.33	11.33	17.33	19.33	16.00
D3	17.33	14.33	14.33	15.33	21.67	28.33	43.33	34.25
Mean	19.67	16.00	16.44	-	15.44	20.56	30.11	-
CD P=0.05	1.19	-	-	1.52	2.03	-	-	1.73

I_1-Stagnant water, I_2-Water drained out after tillering stage, I_3-Water drained out two days after transplanting and after tillering stage, D1 = 5th July, D2 = 15th July, D3 = 25th July

Results from Table 4 revealed that there was significant effect due to transplanting dates, irrigation schedules and chemicals on the severity of bacterial leaf blight of paddy. Mean severity due to transplanting dates ranged from 15.33 to 21.44 per cent. Maximum disease severity 24.33 percent was recorded from first date of transplanting (5th July) and in irrigation schedule first (stagnant water) whereas all other dates of transplanting and irrigations schedule were at par (14.33% disease severity).

Results for chemical management of the disease showed that the chemicals tested were significantly superior in comparison to control. Maximum disease severity 43.33 per cent was recorded in control sub-plot whereas minimum severity 11.33 per cent was recorded in streptomycin treated plots 16.00 per cent in copper oxychloride treated plots.

Table 5: Effect of cultural and chemical management on the yield and yield related parameters of paddy

DOT	Streptomycin 100ppm				Copper oxychloride 0.3%				Control				Mean			
	NT	PH	PL	Y	NT	PH	PL	Y	NT	PH	PL	Y	NT	PH	PL	Y
D1	9.67	114.00	22.67	29.13	9.00	111.33	20.33	27.25	6.33	105.33	18.23	22.46	8.33	110.22	20.41	26.28
D2	11.67	124.67	27.43	41.34	10.67	122.67	26.30	37.14	8.67	120.33	24.50	27.54	10.33	122.56	26.07	35.34
D3	8.67	99.00	22.80	35.46	8.00	96.00	22.40	35.23	6.33	91.00	20.80	28.72	7.67	95.33	22.00	33.13
Mean	10.00	112.56	24.30	35.31	9.22	110.00	23.01	33.20	7.11	105.56	21.17	26.24	-	-	-	-
CD (P=0.05)	0.42	0.92	0.57	0.13	-	-	-	-	-	-	-	-	1.15	0.85	0.94	0.50

NT= No. of tillers, PH = plant height (cm), PL = panicle length (cm), Y = Yield (q/ha), DOT = Date of Transplanting, D1 = 5th July, D2 = 15th July, D3 = 25th July

Application of chemicals had significant effects on number of tillers, plant height, panicle length, and yield. Mean no. of tillers ranged from 7.11 to 10.00. Maximum 11.67 tillers were recorded when streptomycin was sprayed and sub plot was transplanted on 15th of July and minimum number of tillers 6.33 were recorded in control sub plot, except sub plot which was transplanted on 15th of July. Plant height mean ranged from 105.56 cm to 112.56cm in all treatments. Maximum, minimum plant height plant height and mean panicle length of 124.67 cm, 91.00 cm and from 27.43 cm to 20.80 cm was recorded when streptomycin was sprayed on 15th of July and 25th of July respectively. Maximum panicle length 27.43 cm was observed in sub plots sprayed with streptomycin and transplanted on 15th July and minimum panicle length 18.23 cm was observed in control sub plot transplanted on 5th July. However, maximum yield of 41.34 q/ha was recorded in sub plots sprayed with streptomycin and transplanted on 15th of July and minimum yield 22.46 q/ha was recorded in control sub plot which was transplanted on 5th July.

With regards to different dates of transplanting maximum number of tillers, plant height, panicle length, and yield were observed in second date of transplanting D2 (15th July) and minimum number of tillers and plant height were recorded in third date of transplanting D3 (25th July) while panicle length and yield were observed in first date of transplanting D1(5th July).

Discussion

It was reported that from the three districts (Jammu, Kathua and Samba) Kulian village of Jammu had maximum 45.33% disease incidence and Jagatpur of Kathua had minimum 15.21% disease incidence our finding are consonance with Khan *et al*. (2009) [14] reported maximum disease incidence of 80% in Sheikpura. Out of sixteen Germplasm which were screened against *Xanthomonas oryzae pv oryzae*, none was found immune. Out of 16 varieties 4 were moderately resistant, whereas 11were moderately susceptible and Jaya was found to be susceptibleour results were similar with finding of Bhat *et al*. (2015) [4] reported Basmati -370 as a susceptible variety while developing potential bacterial blight of rice resistant donor. While in the chemical fungicide and antibiotic evaluation minimum 11.33% disease severity was recorded in streptomycin followed by Copper Oxychloride 16.00% and our results are conformity with Patil *et al*. 2017 indicated that, Streptocycline plus copper Oxychloride treatment showed lowest percentage disease incidence of 22.33%. During second transplanting (15th July) along with second irrigation schedule (water drained out of tillering stage) turned to be the most effective management. Murad Ali (2013) also reported that sowing dates × varieties interaction indicated that 1000 grain weight was enhanced till 15th June in every variety tested but further delay in sowing resulted in decline in 100 gram weight of all varieties at both location and year.

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