



RESEARCH ARTICLE

# Screening of recombinant inbred lines for resistance to bacterial leaf blight pathotypes in rice (*Oryza sativa* L.)

Duppala Manoj Kumar<sup>1\*</sup>, T. Srinivas<sup>1</sup>, L. V. Subba Rao<sup>2</sup>, Y. Suneetha<sup>1</sup>, R. M. Sundaram<sup>2</sup>, V. Prasanna Kumari<sup>1</sup>, V. Bhuvaneshwari<sup>1</sup> & B. Ganesh<sup>1</sup>

<sup>1</sup>Acharya N G. Ranga Agricultural University (ANGRAU), Andhra Pradesh, India

<sup>2</sup>ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

\*Email: manojgene7@gmail.com



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## Abstract

Sixteen recombinant inbred lines (RIL's) developed from the intra-specific cross between YH3 and AKDRMS 21-54 through Marker Assisted Pedigree Breeding Method were screened along with their parents and the checks, namely, BPT 5204, TN1 and Improved Samba Mahsuri (ISM) against the *IxoPt-20* pathotype at the ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, during *Rabi* season in 2021-22, and a new pathotype of *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight disease in rice at the Regional Agricultural Research Station (RARS), Maruteru during the *Kharif* season in 2022, to identify pathotype specific resistant sources. Morpho-Molecular screening was adopted to evaluate the recombinant inbred lines over two locations in the consecutive seasons of *Rabi* season in 2021-22 and *Kharif* season in 2022. Based on percent diseased leaf area, the genotypes were scored and categorised as per the Standard Evaluation System (SES) scale provided by the International Rice Research Institute (IRRI). The results revealed all 16 RIL's to be either resistant (11) or moderately resistant (6) to the *IxoPt-20* pathotype. However, only five RIL's were found to be resistant, while four RIL's were moderately resistant to the new virulent pathotype. Seven RIL's with resistant to moderately resistant reactions for the *IxoPt-20* pathotype, showed moderately susceptible reactions for the new virulent pathotype. Among the resistant RIL's identified for each pathotype, BPT-1901-72-10-6, BPT-1901-108-4-1, and BPT-1901-111-3-2 were found to be uniformly resistant, while BPT-1901-45-8-6 was uniformly moderately resistant to both *IxoPt-20* and the new virulent pathotype at Hyderabad and Maruteru, respectively, indicating their potential as genetic stocks for the development of new cultivars resistant to bacterial leaf blight disease.

## Keywords

Bacterial leaf blight, *IxoPt-20*, morphological screening, pathotype, resistant, Rice, *Xanthomonas oryzae* pv. *oryzae*

## Introduction

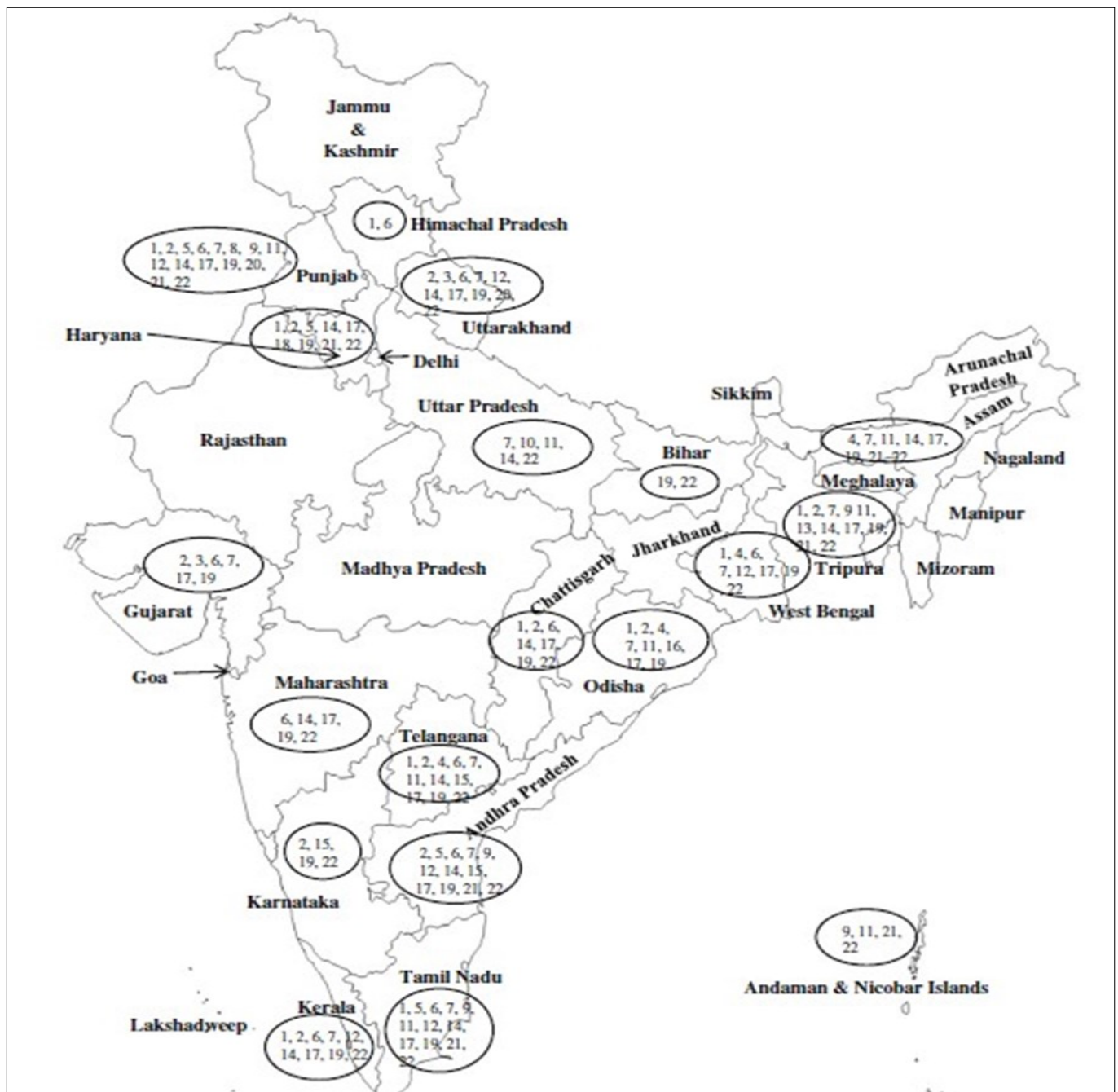
Rice is the staple food for more than 100 countries in the world and is the primary source of nourishment for almost 70% of the population. It is a crucial dietary and food security source for many Asian countries. India ranks second worldwide after China in the production of rice, with a share of 22% of the total world rice production (1). In spite of the significant improvement in rice production and productivity that was achieved through the green revolution, the productivity level is severely limited due to several

biotic and abiotic stresses. Among the primary ailments affecting rice, fungi are responsible for 45 of them (2), while bacteria account for 10 (3), viruses for 15, and insect-pests and nematodes for 75 (4).

Among the bacterial diseases of rice, bacterial leaf blight (BLB) is considered to be the major disease, accounting for huge yield losses in rice. The disease is prevalent in all rice growing areas of India. It is characterised by initial lesions over the leaf, which gradually enlarge in length and width, resulting in a decrease in photosynthetic area, thereby effecting grain filling, culminating in a significant yield (5). Yield reductions of 10-12% for mild infection (6) and up to 81.3% for severe infection have been reported (7). The first report of bacterial leaf blight incidence was from the provinces of Maharashtra by (8). The disease epidemic was reported in the major rice cultivating states of India, namely, Andhra Pradesh, Punjab, Haryana, and Uttar Pradesh (9). Bacterial leaf blight is associated with

agronomic practices like closed spacing and high doses of nitrogen fertilizers, and hence, proper agronomic or cultural management has been reported to be effective in controlling the spread of the disease (10). However, chemical control using bactericides or antibiotics is not successful. Hence, the development and deployment of resistant cultivars have been reported to be the most economical and effective method of controlling the disease (11).

Several pathotypes of *Xanthomonas oryzae* pv. *oryzae* (Xoo) have been reported (Fig. 1) across the country (12). Owing to its dynamic virulence, new races of the pathogen have been reported to be co-evolving with the development and deployment of new cultivars at various geospatial locations across India (13). To combat the dynamic pathogen, 45 R genes showing bacterial leaf blight resistance have been identified and characterised (14). These R genes work in a gene-for-gene interaction mode and are the major sources for genetic enhancement for resistance



Distribution of *Xanthomonas oryzae* pv. *oryzae* pathotypes in different rice growing states of India. Source: Yugander et al. 2017

to the *Xoo* in rice crop (15). Among the resistance genes, *Xa13* and *Xa21* are considered to be major and broadly effective in India. These genes are used widely in gene pyramiding (16).

*Xa21* is a dominant resistance gene for bacterial leaf blight. It was initially introgressed from *Oryza longistaminata* and was consequently cloned and characterized (17). The gene confers broad-spectrum resistance owing to its stable protein against pathogen interaction in host plants, and pTA248 has been identified as a reliable simple sequence repeat (SSR) marker for plant breeders with respect to the gene for use in marker assisted selection for imparting *Xa21*-based bacterial leaf blight resistance (18). Several cultivars resistant to bacterial leaf blight disease have been identified and developed (16). However, a large number of research reports indicate the appearance of *Xoo* strains virulent on bacterial leaf blight resistance genes in India (19).

Breakdown of resistance has been reported in multi-

location trials due to the existence and evolution of new pathogenic strains (20) identified with the help of near isogenic lines (NIL'S) of the IRBB series used as differentials (Table 1). The appearance of a new pathotype of bacterial leaf blight during *Kharif* season in 2022 was observed at RARS, Maruteru, based on the disease response of the standard bacterial leaf blight differentials. In this context, the present investigation on screening of recombinant inbred lines possessing *Xa21* gene for bacterial leaf blight resistance derived from the AKDRMS 21-54 parent was taken up for their reaction to the bacterial leaf blight pathotypes, namely, *IxoPt-20* at ICAR-IIRR, Hyderabad, Telangana, and the new virulent pathotype at RARS, Maruteru, Andhra Pradesh.

## Materials and Methods

Morphological screening for bacterial leaf blight disease reaction following standard evaluation systems was car-

**Table 1.** Reaction of bacterial leaf blight differentials against *IxoPt-20* at Hyderabad, Telangana and new pathotype at Maruteru, Andhra Pradesh

S. No.	Differentials / Genes	Reaction	
		<i>IxoPt-20</i>	New Pathotype
1	IRBB -1( <i>Xa1</i> )	S	S
2	IRBB - 3( <i>Xa3</i> )	S	S
3	IRBB - 4 ( <i>Xa4</i> )	S	S
4	IRBB - 5( <i>xa5</i> )	MR	S
5	IRBB - 7 ( <i>Xa7</i> )	MR	S
6	IRBB - 8 ( <i>xa8</i> )	S	S
7	IRBB - 10( <i>Xa10</i> )	S	S
8	IRBB - 11( <i>Xa11</i> )	S	S
9	IRBB - 13( <i>xa13</i> )	S	S
10	IRBB - 14( <i>Xa14</i> )	S	S
11	IRBB - 21( <i>Xa21</i> )	S	S
12	IRBB - 50( <i>Xa4</i> + <i>xa5</i> )	R	S
13	IRBB - 51( <i>Xa4</i> + <i>xa13</i> )	R	S
14	IRBB - 52( <i>Xa4</i> + <i>Xa21</i> )	MR	S
15	IRBB - 53( <i>xa5</i> + <i>xa13</i> )	S	S
16	IRBB - 54 ( <i>xa5</i> + <i>Xa21</i> )	MR	S
17	IRBB - 55( <i>xa13</i> + <i>Xa21</i> )	MS	MS
18	IRBB - 56( <i>Xa4</i> + <i>xa5</i> + <i>xa13</i> )	MS	MR
19	IRBB - 57( <i>Xa4</i> + <i>xa5</i> + <i>Xa21</i> )	MR	R
20	IRBB - 58( <i>Xa4</i> + <i>xa13</i> + <i>Xa21</i> )	R	S
21	IRBB - 59( <i>xa5</i> + <i>xa13</i> + <i>Xa21</i> )	R	S
22	IRBB - 60( <i>Xa4</i> + <i>xa5</i> + <i>xa13</i> + <i>Xa21</i> )	R	S
23	IRBB - 61 ( <i>Xa4</i> + <i>xa5</i> + <i>Xa7</i> )	S	S
24	IRBB - 62( <i>Xa4</i> + <i>Xa7</i> + <i>Xa21</i> )	S	S
25	IRBB - 63( <i>xa5</i> + <i>Xa7</i> + <i>xa13</i> )	MR	S
26	IRBB - 64( <i>Xa4</i> + <i>xa5</i> + <i>Xa7</i> + <i>Xa21</i> )	MR	S
27	IRBB - 65( <i>Xa4</i> + <i>Xa7</i> + <i>xa13</i> + <i>Xa21</i> )	R	S
28	IRBB - 66 ( <i>Xa4</i> + <i>Xa5</i> + <i>Xa7</i> + <i>Xa13</i> + <i>Xa21</i> )	R	S
29	ISM (Resistant check)	R	S
30	TN1 (Susceptible check)	S	S

R-Resistant; MR-Moderately Resistant; MS- Moderately Susceptible; S- Susceptible

ried out during *Rabi* 2021-22 and *Kharif* 2022, respectively, for 16 recombinant inbred lines (Table 2) developed (Fig. 2) from crossing between YH3, the improved breeding line with the genetic background of the Sri Dhruti (MTU 1121), possessing the major QTL *Pup1* conferring tolerance to low soil phosphorus, and AKDRMS 21-54, the breeding line of the variety, Akshayadhan, possessing the major bacterial blight resistance gene, *Xa21*, and the major blast re-

sistance gene, *Pi54*, following marker assisted pedigree method of breeding. These lines were screened together with their parental varieties, YH3 and AKDRMS 21-54, and susceptible checks (BPT 5204 and TN1), and a resistant check, Improved Samba Mahsuri (ISM), against the pathotypes *IXoPt-20* at ICAR-IIRR, Hyderabad, Telangana, and the new virulent pathotype at Regional Agricultural Research Station, Maruteru, Andhra Pradesh.

**Table 2.** Details of the experimental material screened for bacterial leaf blight reaction

S. No.	Genotype (s)
<b>Recombinant Inbred Line</b>	
1	BPT-1901-2-6-12
2	BPT-1901-11-1-3
3	BPT-1901-32-11-5
4	BPT-1901-38-5-10
5	BPT-1901-45-8-6
6	BPT-1901-53-16-10
7	BPT-1901-61-7-12
8	BPT-1901-72-10-6
9	BPT-1901-77-9-14
10	BPT-1901-84-2-9
11	BPT-1901-95-6-7
12	BPT-1901-108-4-1
13	BPT-1901-111-3-2
14	BPT-1901-128-12-4
15	BPT-1901-163-1-18
16	BPT-1901-177-17-1
<b>Parents</b>	
1	YH3
2	AKDRMS 21-54
<b>Checks</b>	
1	BPT5204 (Susceptible)
2	TN1(Susceptible)
3	ISM (Resistant)

### Artificial Inoculation and Disease Scoring

Bacterial culture of the pathogen was maintained on Hayward's agar media at 28 °C for 96 hours (Plate 1) and harvested after the incubation period. It was then diluted to get a final concentration of 10<sup>8</sup>cfu/ml (21). Later, inoculation was done at the 60 DAS (Days after sowing) stage of the crop following the leaf clip method described by (22 and 23). The leaf tip (1 to 2cm) of the uppermost leaf was clipped with scissors dipped into the inoculum for artificial inoculation of the experimental material (Plate 1). Symptoms were recorded 15 days after inoculation on the upper three leaves of the plant based on the Standard Evaluation Scale (24) developed for assessing diseased leaf area, as described in Table 3 and illustrated in Plate 1. The percentage of diseased leaf area (DLA) was obtained using the following formula:

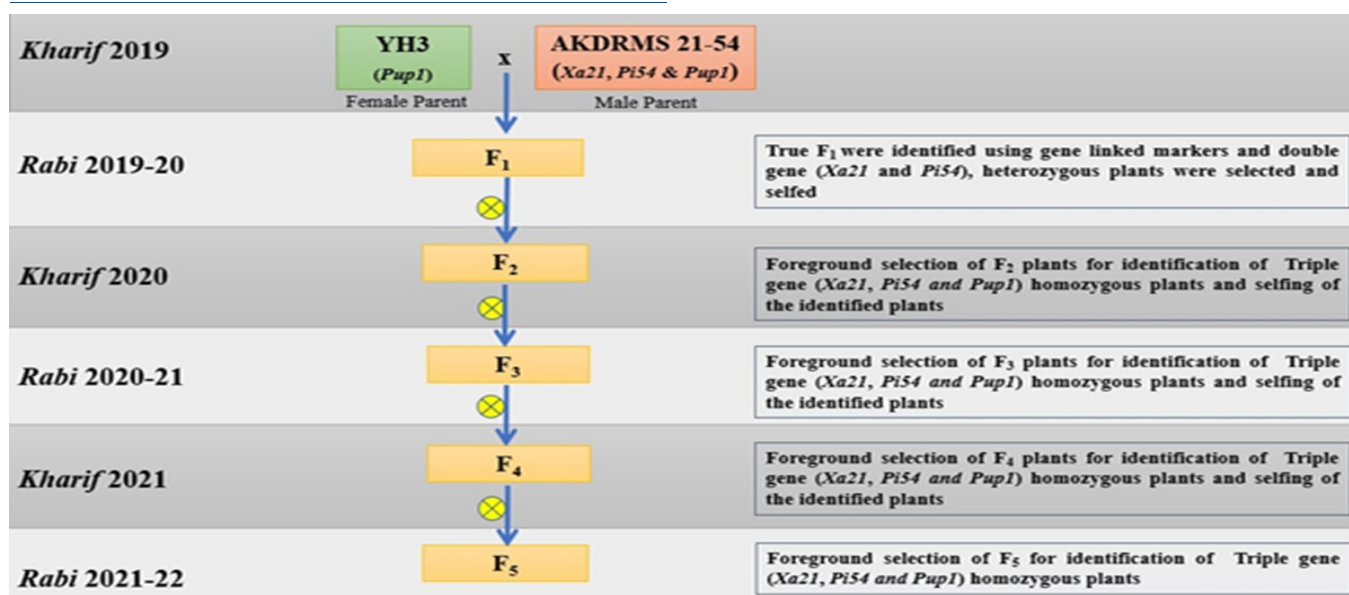
$$\text{Diseased Leaf Area (\%)} = \frac{\text{Lesion Length}}{\text{Leaf Length}} \times 100$$

### Evaluation of Agro-morphological Traits

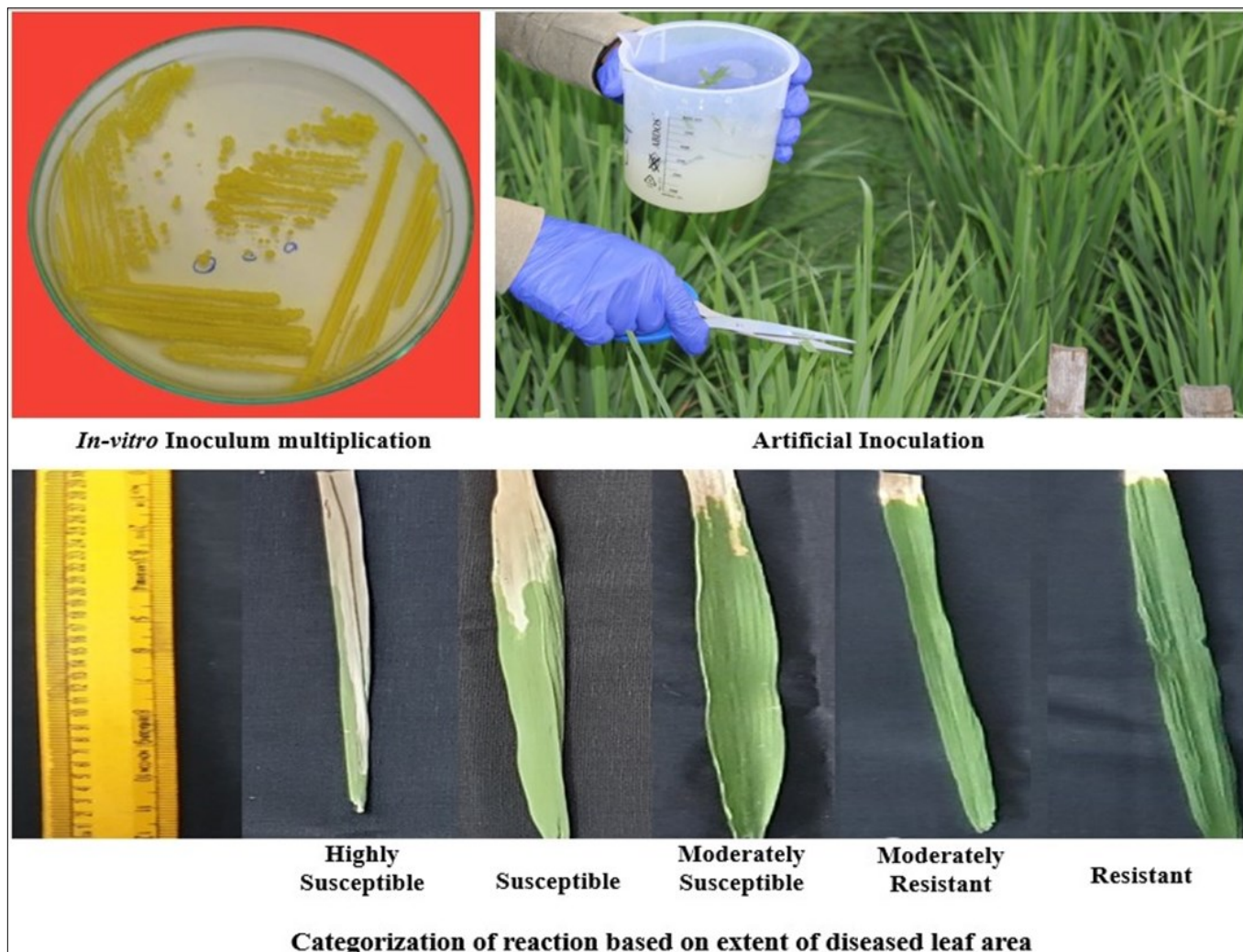
Quantitative data for six traits, namely, days to 50 percent flowering (DFF), plant height (cm), productive tillers per plant, panicle length (cm), filled grains per panicle, total grains per panicle, test weight (g), and grain yield per plant (g) were recorded using standard procedures for the recombinant inbred lines, parents, YH3 and AKDRMS 21-54, and the checks, BPT 5204, TN1, and Improved Samba Mahsuri.

### Molecular Screening for *Xa21* Gene

DNA was isolated from fresh leaves of parents (YH3 and



**Fig. 2.** Marker Assisted Pedigree Breeding strategy



**Plate 1.** Bacterial leaf blight inoculation and disease evaluation

**Table 3.** Standard Evaluation System (SES) scale for scoring bacterial leaf blight (IRRI, 2013)

Scale	Diseased Leaf Area (%)	Description
1	1 - 5	Resistant (R)
3	6 - 12	Moderately Resistant (MR)
5	13 - 25	Moderately Susceptible (MS)
7	26 - 50	Susceptible (S)
9	51 -100	Highly Susceptible (HS)

AKDRMS 2-154) and selected 16 recombinant inbred lines using the Cetyl trimethyl ammonium bromide (CTAB) method modified from the protocol of (25). The quality of genomic DNA was analysed in a 1% agarose gel, and the concentration was measured using the nanodrop method. Total genomic DNA samples were diluted to 100 ng/ml using sterilized distilled water and stored at 4 °C for PCR amplification. The DNA marker pTA248 with the forward se-

**Table 4.** Morphological screening for bacterial leaf blight reaction

S. No.	Genotypes	Rabi 2021-22 (IXoPt-20)			Kharif 2022 (New Pathotype)		
		Diseased Leaf Area (%)	BLB Score	Reaction	Diseased Leaf Area (%)	BLB Score	Reaction
1	BPT-1901-2-6-12	3.97	1	R	11.56	3	MR
2	BPT-1901-11-1-3	5.57	1	R	14.87	5	MS
3	BPT-1901-32-11-5	4.78	1	R	13.42	5	MS

quence, AGACGCGGGAAGGGTGGTCCCGGA and the reverse sequence, AGACGCGGTAATCGAAAGATGAAA at chromosome number 11 and amplification at 982 bp for resistance and 725 bp for susceptibility (26) was used to identify the plants homozygous for the *Xa21* gene.

## Results and discussion

Disease reactions of the lines developed by the marker assisted pedigree method of breeding are presented in Tables 4-6, Fig. 3-4 and Plates 2-4. The susceptible checks, BPT 5204 and TN1, planted along with the test materials exhibited 100 percent infection, indicating a good spread of the disease at both locations. Among the 16 lines evaluated for BLB with *IXoPt-20* during *Rabi* season in 2021-22, 72-10-6, BPT-1901-84-2-9, BPT-1901-95-6-7, BPT-1901-108-4-1, BPT-1901-111-3-2, BPT-1901-163-1-8 and BPT-1901-177-17-1) with score1 (1-5% diseased leaf area) and

4	BPT-1901-38-5-10	6.42	3	MR	4.92	1	R
5	BPT-1901-45-8-6	6.56	3	MR	10.85	3	MR
6	BPT-1901-53-16-10	4.82	1	R	17.54	5	MS
7	BPT-1901-61-7-12	7.13	3	MR	19.50	5	MS
8	BPT-1901-72-10-6	3.22	1	R	4.69	1	R
9	BPT-1901-77-9-14	7.19	3	MR	15.06	5	MS
10	BPT-1901-84-2-9	5.96	1	R	15.85	5	MS
11	BPT-1901-95-6-7	5.94	1	R	20.82	5	MS
12	BPT-1901-108-4-1	5.72	1	R	5.68	1	R
13	BPT-1901-111-3-2	4.03	1	R	5.04	1	R
14	BPT-1901-128-12-4	8.47	3	MR	4.63	1	R
15	BPT-1901-163-1-18	5.64	1	R	7.46	3	MR
16	BPT-1901-177-17-1	5.20	1	R	10.00	3	MR

**Parents**

	YH3 (Female Parent)	20.78	5	MS	15.77	5	MS
	AKDRMS 21-54 (Male Parent)	6.47	3	MR	6.72	3	MR

**Checks**

1	BPT-5204 (Susceptible)	32.17	7	S	26.13	7	S
2	Taichung Native-1 (Susceptible)	44.26	7	S	42.32	7	S
3	Improved Samba Mahsuri (Resistance)	4.9	1	R	1.53	1	R

R-Resistant; MR- Moderately Resistant; MS- Moderately Susceptible; S-Susceptible

**Table 5.** RIL'S of YH3 x AKDRMS 21-54 found to be uniformly resistant/ moderately resistant across pathotypes tested

Scale	Resistance reaction	Diseased Leaf Area (%)	Identified Recombinant Inbred lines (RIL's)
1	Resistant (R)	1-5 %	BPT-1901-72-10-6 BPT-1901-108-4-1 BPT-1901-111-3-2
3	Moderately Resistant (MR)	6-12 %	BPT-1901-45-8-6

**Table 6.** Morphological characters of the resistant and moderately resistant RIL's identified for IXoPt-20 and new pathotypes of BLB disease in Rice

Genotype	Days to 50 per cent flowering (DFF)	Plant height (cm)	Productive tillers per plant	Panicle length (cm)	Filled grains per panicle (g)	Test weight (g)	Grain yield per plant (g)	BLB Disease reaction
<b>Recombinant Inbred lines</b>								
BPT-1901-45-8-6	118.00	93.60	10.00	24.60	169.00	20.24	20.00	MR
BPT-1901-72-10-6	107.00	96.20	14.00	23.50	150.00	22.21	28.78	R
BPT-1901-108-4-1	110.00	102.00	13.00	29.80	175.00	20.65	26.43	R
BPT-1901-111-3-2	116.00	99.80	12.00	23.80	161.00	20.08	23.67	R
<b>Parents</b>								
YH3	105.00	82.50	10.00	24.00	190.00	26.50	34.00	MS
AKDRMS 21-54	118.00	92.00	12.00	22.00	111.00	19.50	23.50	MR
<b>Checks</b>								
BPT 5204	113.00	90.50	12.00	21.80	125.00	22.45	22.59	S
Improved Samba Mahsuri	115.00	87.80	11.00	22.50	121.00	21.87	23.23	R

R-Resistant; MR- Moderately Resistant; MS- Moderately Susceptible; S-Susceptible



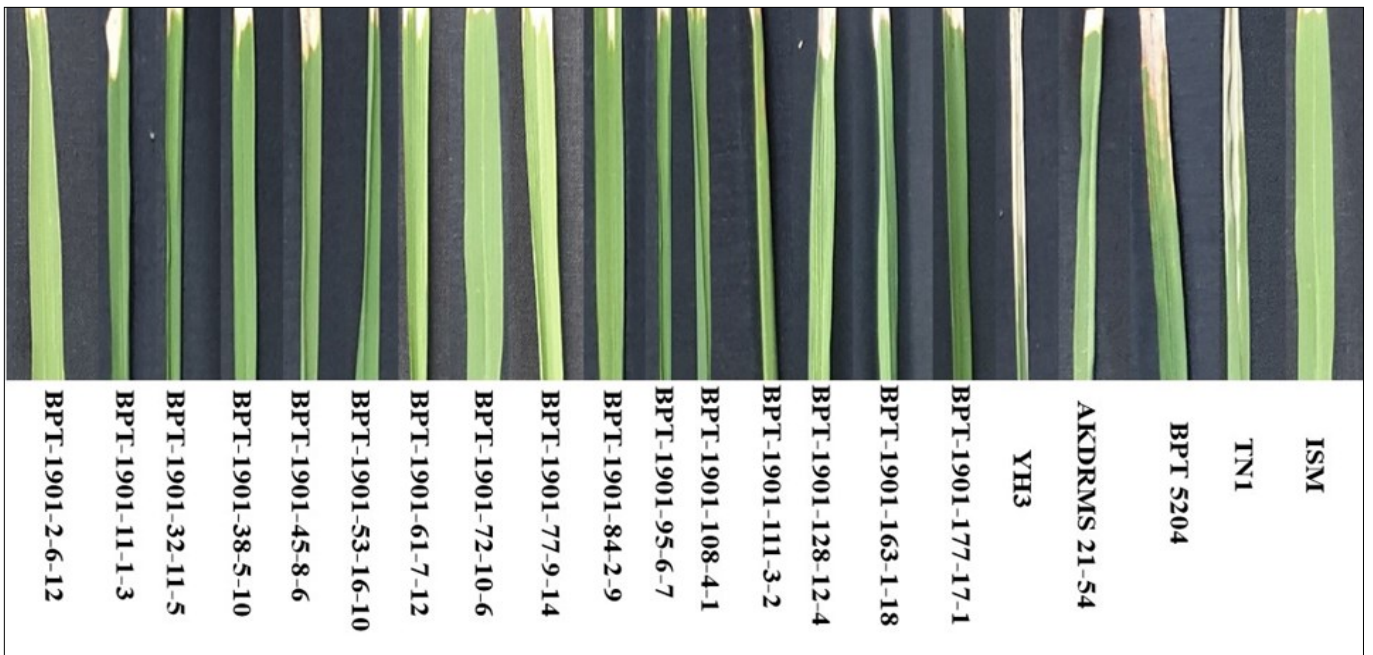


Plate 2. Morphological screening for *Ixopt-20* of BLB disease in rice



Plate 3. Morphological screening for new pathotype of BLB disease in rice

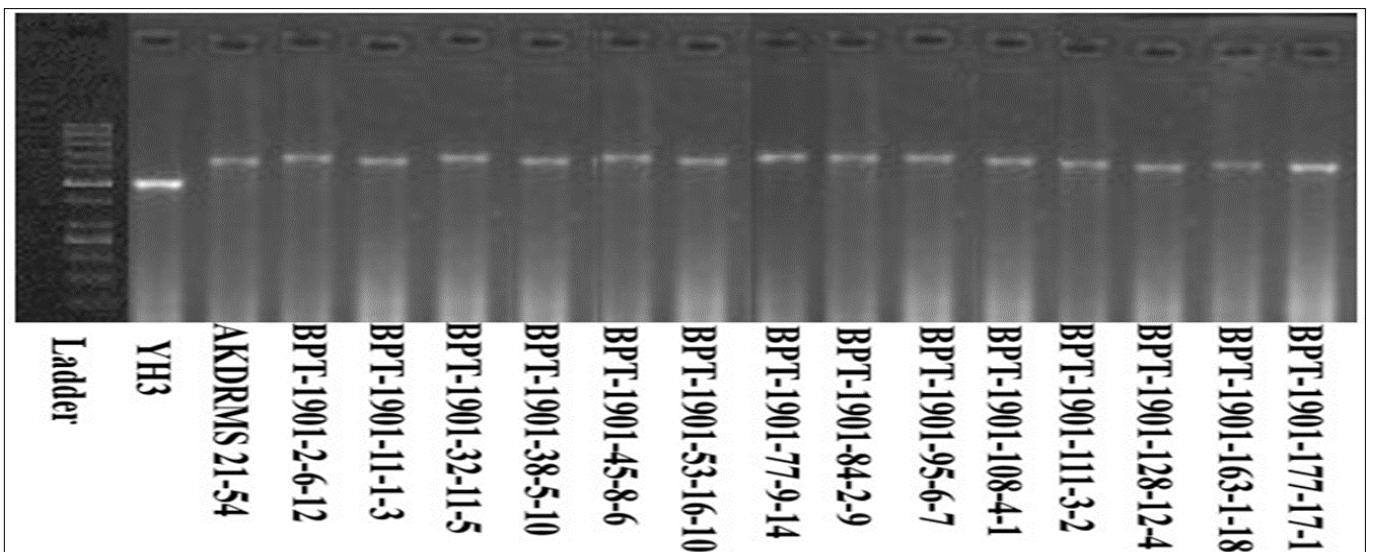


Plate 4. Molecular screening for *Xa21* gene in Recombinant Inbred lines.

ease reactions of the checks are in agreement with the reports of (27) for the pathotype, *Ixopt-20*.

The diseased leaf area percentage of the experimental material for the new virulent pathotype at Marute-



ru during *Kharif* season in 2022 ranged from 1.53 (ISM) to 42.32 (TN1). Among the recombinant inbred lines studied, five improved lines were noticed to be resistant (BPT-1901-38-5-10, BPT-1901-72-10-6, BPT-1901-108-4-1, BPT-1901-111-3-2, and BPT-1901-128-12-4) with score of 1 (1-5 % diseased leaf area), and four lines were moderately resistant (BPT-1901-2-6-12, BPT-1901-45-8-6, BPT-1901-163-1-18 and BPT-1901-177-17-1), with score of 3 (6-12 % diseased leaf area), whereas seven lines (BPT-1901-11-1-3, BPT-1901-32-11-5, BPT-1901-53-16-10, BPT-1901-61-7-12, BPT-1901-77-9-14, BPT-1901-84-2-9, and BPT-1901-95-6-7) exhibited moderately susceptible reaction (13-25 % diseased leaf area). The female parent, YH3, also exhibited a moderately susceptible reaction (15.77% diseased leaf area), and the male parent, AKDRMS 21-54 exhibited a moderately resistant reaction (6.72 % diseased leaf area), while the checks, TN1 (42.32%) and BPT 5204 (26.13%), showed a susceptible reaction with a score of 7 (26-50 % diseased leaf area), indicating the validity of the screening trial. The positive and resistant check, Improved Samba Mahsuri (1.53%), also showed a highly resistant reaction to the new virulent pathotype of BLB at RARS, Maruteru, with a score of 1.

The recombinant inbred lines BPT-1901-11-1-3 (5.57% vs 14.87%), BPT-1901-32-11-5 (4.78% vs 13.42%), BPT-1901-53-16-10 (4.82% vs 17.54%), BPT-1901-84-2-9 (5.96% vs 15.85%), and BPT-1901-95-6-7 (5.94% vs 20.82%) showed significant increase in percent disease leaf area for the new pathotype of Maruteru compared to the *IXo-Pt-20* pathotype, and their reaction changed from a resistant to *IXo-Pt-20* to a moderately susceptible reaction for the new virulent pathotype at Maruteru. Breakdown of resistance of lines possessing *Xa21* by new races of *Xoo* has also been reported in Japan, Korea, and India (28).

The recombinant inbred lines BPT-1901-2-6-12 (3.97% vs 11.56%), BPT-1901-163-1-18 (5.64% vs 7.46%), and BPT-1901-177-17-1 (5.20% vs 10.00%) also showed a significant increase in percent disease leaf area for the new pathotype of Maruteru, Andhra Pradesh, compared to the *IXo-Pt-20* pathotype, and their reaction changed from a resistant to *IXo-Pt-20* to moderately resistant reaction for the new virulent pathotype at Maruteru, Andhra Pradesh. A change in disease reaction from moderately resistant for *IXo-Pt-20* to moderately susceptible reaction for the new virulent pathotype at Maruteru, Andhra Pradesh, was noticed for the recombinant inbred lines, BPT-1901-61-7-12 (7.13% vs 19.50%) and BPT-1901-77-9-14 (7.19% vs 15.06%). A similar breakdown of resistance with the development of a new pathotype was reported earlier studies (29,30).

In contrast, the recombinant inbred lines BPT-1901-38-5-10 (6.42% vs 4.92%), and BPT-1901-128-12-4 (8.47% vs 4.63%) showed a decrease in percent disease leaf area, and the disease reaction changed from moderately resistant for *IXo-Pt-20* to resistance for the new pathotype. However, no significant change was observed in the diseased leaf area and disease reaction of the parents, YH3 and AKDRMS 21-54; and the checks, namely, BPT 5204, TN1 and Improved Samba Mahsuri (ISM). The female par-

ent, YH3 (20.78% vs 15.77%); male parent, AKDRMS 21-54 (6.47% vs 6.72%); susceptible checks, BPT 5204 (32.17% vs 26.13%) and TN1 (44.26% vs 42.32%); and resistant check, Improved Samba Mahsuri (4.9% vs 1.53%) showed uniform responses to both the pathotypes studied.

A perusal of the results presented in Table 5 and Fig. 4 revealed that the recombinant inbred lines, BPT-1901-72-10-6 (3.22% vs 4.69%), BPT-1901-108-4-1 (5.72% vs 5.68%) and BPT-1901-111-3-2 (4.03% vs 5.04%) were found uniformly resistant for both pathotypes, while BPT-1901-45-8-6 (6.56% vs 10.85%) was found to be uniformly moderately resistant for both the pathotypes namely, *IXoPt-20* and the new virulent pathotype at RARS Maruteru. Similar uniform resistance for the existing and new pathotypes was earlier reported by (31) and (32) in their studies.

An analysis of the molecular screening of the recombinant inbred lines and the parents with pTA248, gene specific SSR marker for the *Xa21* gene (Plate 4), revealed amplification of the resistance specific fragment of size 982 bp with respect to the gene, *Xa21*, for the resistant parent, AKDRMS 21-54, and the recombinant inbred lines, while the susceptibility-specific fragment of size 725 bp was amplified in the female parent, YH3. All 16 of the selected recombinant inbred lines were found homozygous for the *Xa21* gene.

Agro-morphological characteristics of the uniformly resistant and moderately resistant recombinant inbred lines, along with parents and checks, are presented in Table 6. A perusal of these results revealed the RIL's, namely, BPT-1901-72-10-6, BPT-1901-108-4-1, and RIL BPT-1901-111-3-2 recorded relatively higher yields (>23.50 g/plant), compared to the checks and male parent, AKDRMS 21-54. These lines were also observed to be uniformly resistant to both *IXoPt-20* and the new virulent pathotype of *Xoo*. Further, these RIL's recorded semi-dwarf plant height with a greater panicle length and number of filled grains per panicle compared to the checks. Among these, BPT-1901-72-10-6 and BPT-1901-108-4-1 were of mid-late duration (105-115 DFF), while BPT-1901-111-3-2 was of late duration (116DFF). The moderately resistant line BPT-1901-45-8-6 was of late duration. These lines may therefore be registered as potential genetic stocks with resistance/moderate resistance to the pathotypes, *IXoPt-20* and the new virulent pathotype for bacterial leaf blight, for use in rice crop improvement programmes aimed at the development of cultivars with broad spectrum resistance for different bacterial leaf blight pathotypes.

## Conclusion

The study identified lines resistant to the pathotypes, *IXoPt-20* and the new virulent pathotype of bacterial leaf blight, in addition to specific resistance sources for the pathotypes. A thorough screening of all the available rice genotypes for their reaction to different pathotypes of bacterial leaf blight is therefore inferred to be essential for the identification of resistant and tolerant sources for use in various rice breeding programs aimed at the development of resistant cultivars and the broadening of the genetic base

of the existing resistant cultivars towards effective management of bacterial leaf blight disease, since there is no effective chemical control.

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## Authors contributions

DMK generated the material and evaluated; TS guided all the research work; LVS coordinated research work at ICAR-IIRR; YS involved in coordinated the crossing work at ARS Bapatla; RMS formulated the research work; VPK guided evaluation of material for BLB; VB carried out the differential tests for BLB and BG evaluated material for BLB at RARS-Maruteru.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None.

## References

- Kumar MD, Srinivas T, Rao LS, Suneetha Y, Sundaram RM, Kumari VP. Study of genetic variability and trait associations in F<sub>2</sub> Population of YH3 x AKDRMS 21-54 intra-specific cross of rice. The Pharma Innovation Journal. 2022;11(9):1735-42. <https://www.thepharmajournal.com/archives/2022/vol11issue9/PartV/11-9-53-527.pdf>
- Wei CT. Manual of rice pathogens (in Chinese). Revised. Science Press, Beijing. 1975;300.
- Goto M. Dissemination of *Erwinia chrysanthemi*, the causal organism of bacterial foot rot of rice. Plant Disease Reports. 1979;63:100-03. <https://doi.org/10.1094/Phyto-69-213>
- Ou SH. Rice diseases, Commonwealth Mycological Institute Publication (II Edn), Kew, Surrey, UK.1985;280-82.
- Tandon AL, Chaliganjewar, SD. An overview of bacterial blight disease of rice caused by *Xanthomonas oryzae* pv. *oryzae*. International Journal of Advanced Research. 2016; 4 (5):1129-36. <http://dx.doi.org/10.21474/IJAR01/346>
- Khan M, Rafi A, Abbas A, Ali T, Hassan A. Assessment of yield losses caused by bacterial blight of rice in upper dir, Khyber pakhtunkhwa province. Asian Journal Agricultural Biology. 2015;3(2):74-78. <https://www.asianjab.com/wp-content/uploads/2015/07/3-MS-15-151.pdf>
- Prasad D, Singh R, Deep S. *In-vitro* and *in-vivo* efficacy of anti-bacterial compounds against *Xanthomonas oryzae* pv. *oryzae*, A cause of bacterial leaf blight of rice. International Journal of Current Microbiology and Applied Sciences. 2018;7(5):2960-69. <https://doi.org/10.20546/ijcmas.2018.705.344>
- Srinivasan MC, Thirumalachar MJ, Patel MK. Bacterial blight of rice. Current Science. 1959;28:469-70.
- Shekhar S, Sinha D, Kumari A. An overview of bacterial leaf blight disease of rice and different strategies for its management. International Journal of Current Microbiology and Applied Sciences. 2020;9(04):2250-65. <https://doi.org/10.20546/ijcmas.2020.904.270>
- Mandal D, Pal R, Mohanty AK. Management of bacterial leaf blight of rice in an integrated way. Journal of Mycopathological Research. 2017;54(4):539-41. <https://www.cabdirect.org/cabdirect/abstract/20173083326>
- Laha GS, Reddy CS, Krishnaveni D, Sundaram RM, Srinivas Prasad M, Ram T, Muralidharan K, Viraktamath BC. Bacterial blight of rice and its management. DRR Technical Bulletin, No. 41. 2009;1-37.
- Yugander A, Sundaram RM, Ladhakshmi D, Hajira SK, Prakasam V, Prasad MS, Sheshu Madhav M, Ravindra Babu V, Laha GS. Virulence profiling of *Xanthomonas oryzae* pv. *oryzae* isolates, causing bacterial blight of rice in India. European Journal of Plant Pathology. 2017;149(1):171-91. <https://doi.org/10.1007/s10658-017-1176-y>
- Sharma P, Bora LC, Puzari KC, Baruah AM, Baruah R, Talukdar K, Katakya L, Phukan A. Review on bacterial blight of rice caused by *Xanthomonas oryzae* pv.*oryzae*: different management approaches and role of *Pseudomonas fluorescens* as a potential biocontrol agent. International Journal of Current Microbiology and Applied Sciences. 2017;6:982-1005. <https://doi.org/10.20546/ijcmas.2017.603.117>
- Joshi JB, Arul L, Ramalingam J, Uthandi S. Advances in the *Xoo*-rice pathosystem interaction and its exploitation in disease management. Journal of Biosciences. 2020;45(1):1-17. <https://doi.org/10.1007/s12038-020-00085-8>
- McDowell JM, Woffenden BJ. Plant disease resistance genes: recent insights and potential applications. Trends in Biotechnology. 2003;21:178-83. [https://doi.org/10.1016/s0167-7799\(03\)00053-2](https://doi.org/10.1016/s0167-7799(03)00053-2)
- Sundaram RM, Laha GS, Viraktamath BC, Sujatha K, Natarajkumar P, Hari Y, Srinivasa Rao K, Reddy CS, Balachandran SM, Madhav MS, Hajira SK, Rani NS, Vishnupriya MR, Sonti RV. Marker assisted breeding for development of bacterial blight resistant rice. In: K Muralidharan and EA. Siddiq (Eds.). Genomics and crop improvement: Relevance and Reservations, Institute of Biotechnology, Acharya NG Ranga Agricultural University, Hyderabad. 2011;154-82.
- Khush GS. "Breeding rice for resistance to insects." Protection Ecology. 1984;7:147-65.<http://repository.ias.ac.in/83779>
- Nanayakkara D, Edirisingha I, Dissanayake L, Weerasinghe D, Suriyagoda L, Herath V, Perera C, Jayatilake D. A novel intragenic marker targeting the ectodomain of bacterial blight-resistance gene *Xa21* for marker-assisted selection in rice. Journal of Crop Improvement. 2020;34(6):824-41. <https://doi.org/10.1080/15427528.2020.1771643>
- Mishra D, Vishnupriya MR, Anil MG, Kotilingam K, Raj Y and Sonti RV. Pathotype and genetic diversity amongst Indian isolates of *Xanthomonas oryzae* pv. *oryzae*. Plos One. 2013;8(11):1-11. <https://doi.org/10.1371/journal.pone.0081996>
- Preece TF, Rhodes ME, Skinner FA. Progression of bacterial disease within plants. Bacteria and plants, Academic Press, London. 1982; Pp: 71-83.
- Fiyaz RA, Shivani D, Chaithanya K, Mounika K, Chiranjeevi M, Laha GS, Viraktamath BC, Rao LS, Sundaram RM. Genetic improvement of rice for bacterial blight resistance: Present status

- and future prospects. *Rice Science*. 2022;29(2):118-32. <https://doi.org/10.1016/j.rsci.2021.08.002>
22. Kauffman HE, Reddy APK, Hsieh SPY, Merca SD. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. *Plant Diseases Report*. 1973;56:537-40. <https://www.scienceopen.com/document?vid=80dbc4e6-0f0e-4949-b4df-676d8a822bd2>
  23. Jabeen R, Rahman SU, Rais A. Evaluating BLB resistance/aggressiveness in rice through best inoculum concentration, inoculation and application methods. *Pakistan Journal of Botany*. 2011;43(5):635-38. [http://mail.pakbs.org/pjbot/PDFs/43\(5\)/PJB43\(5\)2635.pdf](http://mail.pakbs.org/pjbot/PDFs/43(5)/PJB43(5)2635.pdf)
  24. International Rice Research Institute (IRRI). Standard Evaluation System of Rice. 2013; 13-17. <http://www.knowledgebank.irri.org/images/docs/rice-standard-evaluation-system.pdf>
  25. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small amount of fresh leaf tissue. *Phytochemistry Bulletin*. 1987;19:11-15.
  26. Ronald PC, Albano B, Tabien R, Abenes L, Wu K, Mc Couch SR and Tanksley SD. Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. *Molecular Genetics and Genomics*. 1992; 236:113-20. <https://doi.org/10.1007/BF00279649>
  27. Prasanna BL, Dangi KS, Raju CHD, Jagadeeshwar R, Rekha G, Sinha P, Aleena D, Harika G, Mastanbee SK, Ravindra KS, Kousik MBVN, Mahadevaswamy HK, Anila M, Kale RR, Kumar TD, Punniakotti E, Hajira SK, Bhadana VP, Sundaram RM. Marker assisted pedigree breeding based improvement of the Indian mega variety of rice MTU1010 for resistance against bacterial blight and blast and tolerance to low soil phosphorus. *Plos One*. 2022;17(1):260-535. <https://doi.org/10.1371/journal.pone.0260535>
  28. Acharya B, Sujata KC. Field screening of rice (*Oryza sativa* L.) genotypes for resistance to bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) disease. *Journal of Agriculture and Natural Resources*. 2021;4(1):42-49. <https://doi.org/10.3126/janr.v4i1.33201>
  29. Suryadi Y, Samudra IM, Priyatno TP, Susilowati DN, Lestari P, Fatima Kadir TS. Determination of pathotypes from Indonesian *Xanthomonas oryzae* pv. *oryzae* population causing bacterial leaf blight and their reactions on differential rice. *Makara Journal of Science*. 2016;20(3):109-18. <https://doi.org/10.7454/mss.v20i3.6241>
  30. Tekete C, Cunnac S, Doucoure H, Dembele M, Keita I, Sarra S, Dagno K, Koita O, Verdier V. Characterization of new races of *Xanthomonas oryzae* pv. *oryzae* in Mali informs resistance gene deployment. *Phytopathology*. 2020;110(2):267-77. <https://doi.org/10.1094/PHYTO-02-19-0070-R>
  31. Srinivas T, Reddy MV, Jain KC, Reddy MSS. Screening for resistance to two isolates of sterility mosaic pathogen in Pigeon pea (*Cajanus cajan* (L.) Millsp.). *Crop Research*. 1977;13(1):169-75.
  32. Chumpol A, Monkham T, Saepaisan S, Sanitchon J, Falab S, Chankaew S. Phenotypic broad spectrum of bacterial blight disease resistance from Thai indigenous upland rice germplasms implies novel genetic resource for breeding program. *Agronomy*. 2022;12(8):1930-48. <https://doi.org/10.3390/agronomy12081930>