

Bacterial Leaf Blight in Rice: Exploring Pathogen Life Cycle, Resistance Genes, and MAS Breeding Strategies

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Rice (*Oryza sativa* L.) is one of the most important food crops grown in various agro-climatic conditions and is the staple food for more than half of the world's population. More than 3.5 billion people depend on rice for more than 20 % of their daily calorie requirement (Maclean et al., 2013). Globally it is grown over 165.25 m ha with the production of 509.87 million tonnes milled rice (FAOSTAT, 2021). However, the production of rice is being adversely affected by various biotic and abiotic stresses. Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is a major disease affecting rice production worldwide, leading to significant yield losses. It is a serious problem in South-West monsoon season (Kharif season). It is a vascular disease and the symptoms can be visualized in terms of drying and yellowing of leaves which starts from the leaf tips and proceed downward. Understanding the pathogen's life cycle and its interaction with rice plants is crucial for developing effective management strategies. This review provides a comprehensive overview of the biology and epidemiology of Xoo, highlighting its infection mechanisms and life cycle. We explore the genetic basis of BLB resistance in rice, focusing on the identification and characterization of Resistance genes (R genes) that confer immunity against various Xoo strains. The integration of these R genes through conventional breeding and advanced molecular techniques such as Marker-Assisted Selection (MAS) has led to the development of resistant rice varieties.

Keywords: *Bacterial leaf blight; epidemiology; marker assisted selection (MAS); resistance GENES; Xanthomonas oryzae* pv. *oryzae*.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops grown in various agro-climatic conditions and is the staple food for more than half of the world's population. It is the source for more than 20 % of the total calorie intake. Rice is a rich source of carbohydrates, which are essential for providing energy to the body. It is also low in fat and contains a fair amount of protein, essential minerals (such as iron and zinc) and B-vitamins, making it a relatively balanced food choice. Rice holds significant cultural and culinary importance in numerous nations, frequently being a key element in traditional cuisines and ceremonial gatherings. This reflects the rich cultural identity and heritage of diverse communities. The knowledge of cultivating, harvesting, and cooking rice has been transmitted from one generation to another, playing a vital role in safeguarding and perpetuating these valuable cultural practices. Asia contributes to more than 90% production and consumption of rice. Globally it is grown over 165.25 m ha with the production of 509.87 million tonnes milled rice (FAOSTAT, 2021). In India, it is grown over 47.7 m ha with the production of 136 million tonnes in 2022 (usda.gov.in). It also provides employment for 30% of the 700 million people in absolute poverty living in rain fed rice cultivating areas of South Asia who either work directly on rice or in its related area (IRRI

Phillipines, 2010). The self sufficiency was achieved in this crop by early 1980s after the introduction of high yielding varieties in the mid 1960s. In order to sustain this self sufficiency, it is estimated that the global rice requirement will be 70% more than what we are producing now. However, the production of rice is being adversely affected by various biotic and abiotic stresses. These biotic stresses include damage caused by insect pests, bacterial, fungal, viral diseases and birds, whereas major abiotic stresses includes drought, salinity, high or low temperatures, flooding and heavy metal contamination. However, the production of rice is being adversely affected by various biotic and abiotic stresses. These biotic stresses include damage caused by insect pests, bacterial, fungal, viral diseases and birds, whereas major abiotic stresses includes drought, salinity, high or low temperatures, flooding and heavy metal contamination. So, to meet such huge demand, novel breeding strategies have to be employed.

1.1 Biotic Stress in Rice

"The production of rice is affected by wide range of fungi, bacteria, insects, nematodes and other pests. More than 70 diseases in rice have been reported which is due to the fungi, bacteria, viruses and nematodes" [1]. "The diseases affects the plant at various crop growth stages and almost all the parts of plant like leaves, stem,

inflorescence, developing grain etc. Approximately 52% of the global rice production is lost annually due to the biotic stress alone" [2]. "Among several biotic stresses, Bacterial leaf Blight, Blast, Sheath Blight, Brown spot, False smut and the insects such as Gall midge, Brown Plant Hopper, Stem Borer causes severe yield losses and also reduces the quality of grains" [3]. Approximately 52% of global rice production is lost each year due to various biotic stress factors, with around 21% attributed to insect pest attacks [2].

Xanthomonas oryzae pv. *oryzae* (Xoo), a gram negative bacterium, which invades the xylem tissue either through wounds or stomata and leads to the systemic infection. It induces incomplete grain filling due to reduced photosynthetic activity, resulting in grain production losses of up to 50% under favorable conditions [4]. It may cause yield losses ranging from 70-80% in severe cases depending upon the cultivar's susceptibility, crop growth stage and the environmental conditions [5].

Hence there is need to understand about Bacterial leaf blight and its pathogenesis in order to breed for resistant cultivars.

2. BACTERIAL LEAF BLIGHT

2.1 Origin and Distribution

Bacterial leaf blight caused by *Xanthomonas oryzae* pv *oryzae* was first reported in 1884 by the Japanese farmers [6] where it was refereed as "white withering" disease. Later in 1908, Bacterial masses in dew drops of rice leaves were found by Taikashi [7]. In 1911, Bokura isolated the bacteria from the oozes and later named as *Bacillus oryzae*. The further study was carried out by Ishiyama in 1922 and renamed the bacteria as *Pseudomonas oryzae*. After several revision of name, the pathogen was elevated to a species status in 1990 and named as *Xanthomonas oryzae* pv *oryzae* (Xoo).

BLB has spread all over the world including several African countries, in Australia, North America, Central and South America, Taiwan, China, Cambodia, Malaysia, but it is of severe form and economic importance in Asia. It was reported in South East Asia in the early 1960s. In India, it was first reported in Maharashtra [8]. During 1980s, a major BLB epidemic occurred in Haryana, Punjab, Western Uttar Pradesh and plains of Uttarkhand which led to the severe crop

losses. It also occurs almost every year in Kerala. The BLB occurs mainly in South-West Monsoon Season in India. Application of high amount of Nitrogenous fertilizer also induce the disease.

2.2 Morphology and Life cycle of *Xanthomonas oryzae* pv *oryzae*

Xanthomonas oryzae pv *oryzae* is gram negative bacterium which is motile with polar monoflagella and slime producing. The length of individual cells vary from 0.6-2 μm and width varies from 0.4 μm to 0.7 μm . Colonies of *Xanthomonas* on glucose containing solid media are round, convex and yellow in colour due to the production of Xanthomonadin pigment. The capsular extracellular polysaccharides will be produced to prevent the bacteria from dessication and also it helps in dispersal through wind and rain [9]. Depending on the soil moisture and acidity, *Xanthomonas* can survive in the soil for 1-3 months whereas in tropical regions due to high temperature, humidity, *Xanthomonas* persists throughout the year. Primary inoculum of disease comes from the infected seed, stem and plant parts left out in the field after harvest. The wind, rain, splash disperse the pathogen from one place to the other place. The entry of pathogen is through hydathodes and wounds at the leaf tip and leaf margin of the rice leaves. Bacterium multiplies inside the vascular system and moves systematically to different parts of the plant. It leads to blockage of xylem vessels and lead to the wilting. Xoo pathogen can multiply at the temperature range of 26°C - 30°C and 20°C is ideal temperature for initial multiplication. It can tolerate pH range of 4 to 8.8 and it was observed that optimum pH is 6 - 6.5.

2.3 Symptoms of BLB Disease

The symptoms of the BLB can be divided into two distinct phases such as:

1. Kresek phase
2. Leaf Blight phase

Kresek phase: It is the most destructive manifestation of the disease and usually occurs from the seedling to the tillering stages. Leaves of the infected plants wilt and turns to grayish green by rolling up the leaves. Later as disease progress, the leaves turns yellow and wither and the entire plant dies. It may also leads to complete crop failure.

Leaf blight phase: It is the more common symptom which generally occurs from the maximum tillering stage. It usually appears four to six weeks after transplanting. Appearance of water soaked lesions and stripes on the leaf blades are common symptoms. Lesions get covered by the bacterial ooze. These bacterial ooze turns like yellow beads in dry weather and can be easily visualized. These lesions coalesce and increase in length and width, become yellow and later turns to white with wavy leaf margins [10]. The blight symptoms can extend to the leaf sheath and culm, killing the entire tiller. In severe cases, the glumes may get infected. The symptoms bacterial leaf blight can be distinguished from bacterial leaf streak whereas in latter, the lesions are light brown in colour. Ooze test is usually conducted to distinguish the Bacterial leaf blight from other pathogenic disease and physiological disorder. If the infected plant part is kept in the clear water, one can see the bacterial ooze coming out of the leaf [9].

Management strategies to control Bacterial leaf blight:

Preventive method: It includes seed treatment with Agrimycin @ concentration of 0.025%, hot water treatment for 30 min at 52-54°C, spraying of neem oil etc.

Cultural methods: Growing of tolerant varieties such as IR20, TKM6, Balanced fertilization, Destruction of weeds and collateral hosts

Biocontrol methods: Use of biological control agents (BCAs) like *Paenibacillus polymyxa* to suppress the incidence and severity of the disease.

Chemical methods: Application of bleaching powder @ 5 kg/ha in the irrigation water at kresek stage, Foliar spray with copper fungicides alternatively with Strepto-cyclin (250 ppm) to control secondary spread

Breeding approaches to mitigate the biotic stresses in rice: There are various measures to mitigate the biotic stress, namely, physical control, cultural control, biological control and chemical control. Use of chemicals for control of pathogens and pests is commonly practiced by farmers. Excessive use of chemicals is harmful for the environment and also leads to the resurgence of pests. Hence, use of resistant variety is an eco-friendly approach and also keeps the pest population below the threshold level. India has been involved in systematic research efforts in host plant resistance from more than 65 years. Based on the dynamic pest profile of the crop, various institutes have started biotic stress breeding programmes.

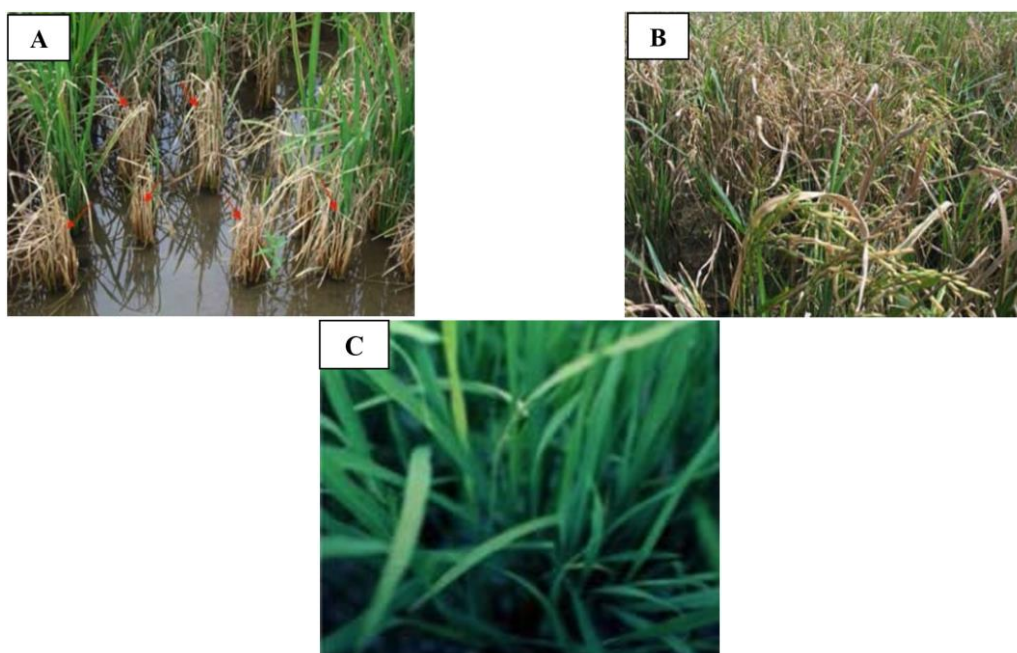


Fig. 1. Image Showing various stages of Bacterial Leaf Blight (A): Kresek stage, (B): Leaf Blight stage, (C): Pale Yellow stage

Host plant resistance: The Hypersensitive Response (HR), which is the genetically programmed death of infected cells, as well as tissue reinforcement and antibiotic production at the infection site, are all inducible defence responses that plants may activate very effectively. As a result of these local reactions, the plant may develop systemic acquired resistance (SAR), a long-lasting response that can protect it from a variety of infections. This multifaceted reaction necessitates a significant investment of cellular resources, including major metabolic re-allocation and genetic reprogramming. Hence, defences are kept under strict genetic control and only become active when the plant recognises a potential attacker. Plant cells autonomously maintain constant vigilance against infections because they produce wide arrays of "R genes" (R, resistance) because plants lack the benefit of a circulating antibody system. R genes encode putative receptors that are thought to react to the avirulence-producing byproducts of 'Avr genes' produced by the pathogen during infection. When added to a plant of the same species that has previously been vulnerable to one or more strains of a specific disease, a single R gene can frequently provide total resistance. R genes have been utilised in traditional resistance breeding programmes for many years because of this [11].

Conventional breeding approaches such as pedigree method, back crossing, recurrent selection and mutation breeding were used for the incorporation of resistance genes. Through conventional breeding programme, major genes of blast and BLB resistance genes such as *Pib*, *Pita*, *Pikh*, *Pi2*, *Pi4*, *Pi1*, *Pia* and *Xa21* have been introduced into the rice varieties [12]. However, the conventional breeding approaches which target major genes have been not fruitful for many years due to the evolution of new pathotypes thus leading to the breakdown of resistance.

With the invent of molecular markers, the resistance breeding programme got a new avenue. Marker Assisted Selection (MAS) which refers to the selection of trait of interest through the use of molecular markers tightly linked to trait of interest. MAS is an efficient, effective and more reliable approach. Since the beginning of crop improvement, the selection of segregants carrying the desired gene has been a part of the breeding programme. Plant breeders generally relied on phenotype, morphological markers and statistical methods to find out the segregants. But

this selection criteria is not reliable due to the less abundance of morphological markers, effect of the environment on the expression of phenotype and stage specific expression of characters. With the help of MAS, one can easily find out the segregants efficiently as the markers linked to gene controlling the trait of interest co-segregate with the trait phenotype across generations [3].

Molecular Genetics of R genes conferring resistance to Bacterial leaf blight: "A total of 46 R genes conferring resistance to bacterial leaf blight have been identified till date" [13]. "Many of these genes are tagged with markers (DNA based), while some have been cloned and characterized" [14]. "Marker-assisted introgression of disease resistance genes into a single genetic background might be expected to give more durable disease resistance, as more resistance genes are incorporated into single genotype" [15]. Out of the 46 R genes identified, 17 [*xa5*, *xa8*, *xa13*, *xa15*, *xa19*, *xa20*, *xa24*, *xa25(t)*, *xa26(t)*, *xa28(t)*, *xa31(t)*, *xa32(t)*, *xa34(t)*, *xa41(t)*, *xa42*, *xa44(t)* and *xa45(t)*] are recessive, while 29 are dominant and 11 of these have been cloned and characterized [*Xa1*, *Xa3/Xa26*, *Xa4*, *xa5*, *Xa10*, *xa13*, *Xa21*, *Xa23*, *xa25(t)*, *Xa27(t)* and *Xa41(t)*]. Different types of proteins were found to be encoded by the cloned genes, pointing to multiple mechanisms of R gene-mediated Xoo resistance. R genes involved in disease resistance fall into two main categories. According to Sekhwal et al. [16], "one is the receptor kinase (RLK) class and the other is the nucleotide-binding site leucine-rich repeat (NBS-LRR). First R gene of the RLK class, *Xa21* is widely employed in rice breeding efforts and has a broad spectrum of resistance. The largest R gene class that confers resistance against many bacteria, fungi, and viruses is the NBS-LRR class".

MAS for pyramiding of Bacterial leaf blight resistance genes in rice cultivars: Enhancing rice varieties to withstand prevalent and destructive diseases is crucial for maintaining sustainable rice production. Previous efforts to create varieties resistant to bacterial blight (BB) have been unsatisfactory due to the high variability of disease populations in cultivation regions [17]. To achieve more lasting disease resistance, researchers suggest combining major or minor resistance genes within a single genetic background [15]. Extensively studying and incorporating the major BB resistance genes identified by various research groups have been

Table 1. List of Resistant Genes against Bacterial leaf Blight

Sl. No	Resistant gene (R)	Location on chromosome	Source	Gene action	Donor cultivar/ lines
1	Xa-1	4L	Japan	Dominant	Kogyoku,IRBB 1
2	Xa-2	4L	Vietnam	Dominant	IRBB2
3	Xa-3	11	Japan	Dominant	IRBB3, Minghui 63
4	Xa-4	11	India	Dominant	TKM6, IRBB4
5	xa-5	5S	Bangladesh	Recessive	IRBB5
6	Xa-6	11	USA	Dominant	Zenith
7	Xa-7	6	Bangladesh	Dominant	DZ78
8	xa-8	7	-	Recessive	P1231128
9	Xa-9	11	Laos	Dominant	Sateng
10	Xa-10	11L	-	Dominant	Cas 209
11	Xa-11	3L	Philippines	Dominant	IRS
12	Xa-12	4	Japan	Dominant	Kogyoku, Java14
13	xa-13	8L	India	Recessive	BJ1, IRBB13
14	Xa-14	4L	Taiwan	Dominant	TN1
15	xa-15	-	-	Dominant	M41 Mutant
16	Xa-16	-	Vietnam	Dominant	Tetep
17	Xa-17	-	South korea	Dominant	Asominori
18	Xa-18	-	Philippines,	Dominant	IR24, Miayang 23
19	xa-19	-	-	Recessive	XM5
20	xa-20	-	-	Recessive	XM6
21	Xa-21	11L	Africa, Mali	Dominant	<i>O. longistaminata</i>
22	Xa-22(t)	11	China	Dominant	Zhachanglong
23	Xa-23	11L	China	Dominant	<i>O. rufipogon</i>
24	xa-24	2L	Bangladesh	Recessive	DV86
25	xa-25(t)	12	China	Recessive	Minghui 63, HX-3
26	xa26	11L	China	Recessive	Nep Bha Bong
27	Xa-27	6	Philippines	Dominant	<i>O. minuta</i>
28	xa-28(t)	-	Bangladesh	Recessive	Lotasail
29	Xa-29(t)	1	-	Dominant	<i>O. officinalis(B5)</i>
30	Xa-30(t)	11L	India	Dominant	<i>O. rufipogon(Y235)</i>
31	xa-31(t)	4L	China	Recessive	Zhachanglong
32	xa-32(t)	11L	-	Recessive	<i>O. australiensis</i>
33	Xa-33	7	-	Dominant	<i>Oryza</i> wild species
34	Xa-33(t)	6	Thailand	Dominant	Ba7 <i>O. nivara</i>
35	xa-34(t)	1	Sri lanka	Recessive	BG1222
36	Xa-35(t)	11L	Philippines	Dominant	<i>O. minuta</i>
37	Xa-36(t)	11L	China	Dominant	C4059
38	Xa-38	4L	-	Dominant	<i>O. nivara</i>
39	Xa39	11	Chinese	Dominant	FF329
40	Xa40(t)	11	Korea	Dominant	IR65482-7-216-1-2
41	xa41(t)	11	-	Recessive	-
42	xa42	3	Japan	Recessive	XM14
43	Xa43	11	Japan	Dominant	P8 and lipum
44	xa44	11	Japan	Recessive	IR73571 and lipum
45	xa45(t)	8	Phillipines	Recessive	<i>O.glabberina</i>
46	Xa46	11	Japan	Dominant	Mutant H120

Source: Chen et al. [13]

instrumental in the development of rice varieties with improved resistance to the disease. Genes like *Xa1*, *Xa4*, *xa5*, *Xa21*, *Xa26*, and *Xa27* have been widely employed in the process of pyramiding into numerous susceptible rice

varieties and traditional cultivated varieties. This is because these genes offer a broad spectrum of resistance against various pathogens [18]. Pradhan et al. [19] introgressed Bacterial blight resistant genes into the background of Ranidhan,

a late maturing popular variety but susceptible to bacterial leaf blight. In BC3F2 they obtained seven lines obtaining different combination of three resistance genes. The lines carrying two resistance gene combinations (*Xa21* + *xa13* and *Xa21* + *xa5*) exhibited enhanced resistance against the BB pathogens. Biswas et al. [20] pyramided four BB resistant genes into the population derived from the cross between Ciherang and IRBB60 promising recombinant introgressed lines in F2 were evaluated against three virulent bacterial strains of Xoo. The RIL's which had two or three resistance genes along with *Xa4* showed broad spectrum resistance and higher agronomic performance than the donor and recipient parents. Chandrasekar et al. [21] carried out research to pyramid *xa13*, a major BB resistance gene, into the background of the improved CO43 carrying two major genes for Gall Midge resistance *Gm1* and *Gm4*. Marker Assisted Backcrossing (MAB) was employed to develop three gene pyramided homozygous BC₃F₃ lines. Kumar et al. [22] introgressed three BB resistant genes (*Xa21* + *xa13* + *xa5*) through marker assisted selection into the background of popular aromatic short grain rice cultivar HUR 917. The NIL's obtained from this showed broad spectrum resistance against Bacterial Leaf Blight. Yugander et al. [23] introgressed *Xa38*, a dominant resistance gene in the genetic background of APMS 6B from the donor parent PR 114. At BC2F6, these introgressed lines exhibited high degree of resistance against Bacterial leaf Blight.

Future prospects: Breeding for bacterial leaf blight (BLB) resistance in rice is evolving rapidly with advances in genomics, biotechnology, and precision breeding techniques. Wild rice species offer a valuable reservoir of novel resistance genes that can be incorporated into cultivated varieties to increase genetic diversity and resistance durability. Combining multiple resistance genes (R genes) into a single variety can enhance resistance durability and broaden the spectrum against various strains of *Xanthomonas oryzae* pv. *oryzae* (Xoo). Advances in transcriptomics and functional genomics help identify key regulatory genes involved in resistance, providing new targets for breeding and genetic engineering. CRISPR-Cas9 and other genome editing tools enable precise modifications, such as inserting resistance genes or knocking out susceptibility genes, creating highly resistant varieties efficiently. Recent study by Jiang et al. [24] highlighted the importance of use of phages in controlling BLB. Developing rice

varieties with combined resistance to BLB and tolerance to abiotic stresses will ensure productivity under changing climatic conditions.

3. CONCLUSION

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), remains one of the most devastating diseases affecting rice crops globally. Understanding the complex interplay between the rice plant, the pathogen, and the disease's life cycle is crucial for developing effective control strategies. The pathogen's ability to rapidly evolve and overcome host resistance necessitates continuous research and breeding efforts. Breeding approaches, including traditional methods and Marker-Assisted Selection (MAS), have been instrumental in developing BLB-resistant rice varieties. The identification and utilization of resistance (R) genes have provided a strong foundation for breeding programs. These R genes, when effectively integrated into rice varieties, can offer durable resistance against multiple strains of the pathogen. MAS has further accelerated the development of BLB-resistant rice by enabling the precise introduction of R genes into elite rice varieties. This strategy not only saves time but also enhances the accuracy of breeding programs, ensuring that new rice cultivars possess the desired resistance traits without compromising other agronomic qualities.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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