Review Article

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

Abstract

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is a major disease affecting rice production worldwide, leading to significant yield losses. 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.

Key words: Bacterial Leaf Blight, *Xanthomonas oryzae pv. Oryzae*, Resistance genes, MAS

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.

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 (Ou, 1985). 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 annualy due to the biotic stress alone (Yarasi *et al.*, 2008). 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 (Singh *et al*., 2011). Approximately 52 % of global rice production is lost each year due to various biotic stress factors, with around 21 % attributed to insect pest attacks (Yarasi *et al*., 2008).

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 (Liu *et al.,* 2014). 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 (Baliyan *et al*., 2018).

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

BACTERIAL LEAF BLIGHT:

Origin and Distribution:

Bacterial leaf blight caused by *Xanthomonas oryzae* pv *oryzae* was first reported in 1884 by the Japanese farmers (Tagami and Mizukami, 1962) where it was refereed as “white whithering” disease. Later in 1908, Bacterial masses in dew drops of rice leaves were found by Taikashi (Mizukami and Wakimoto, 1969). 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 (Srinivasan *et al*., 1959). 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.

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 (Nino liu *et al*., 2006). 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.

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

1. 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.

2. 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 (Qudsia *et al*., 2017). 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 (Nino liu *et al*., 2006).

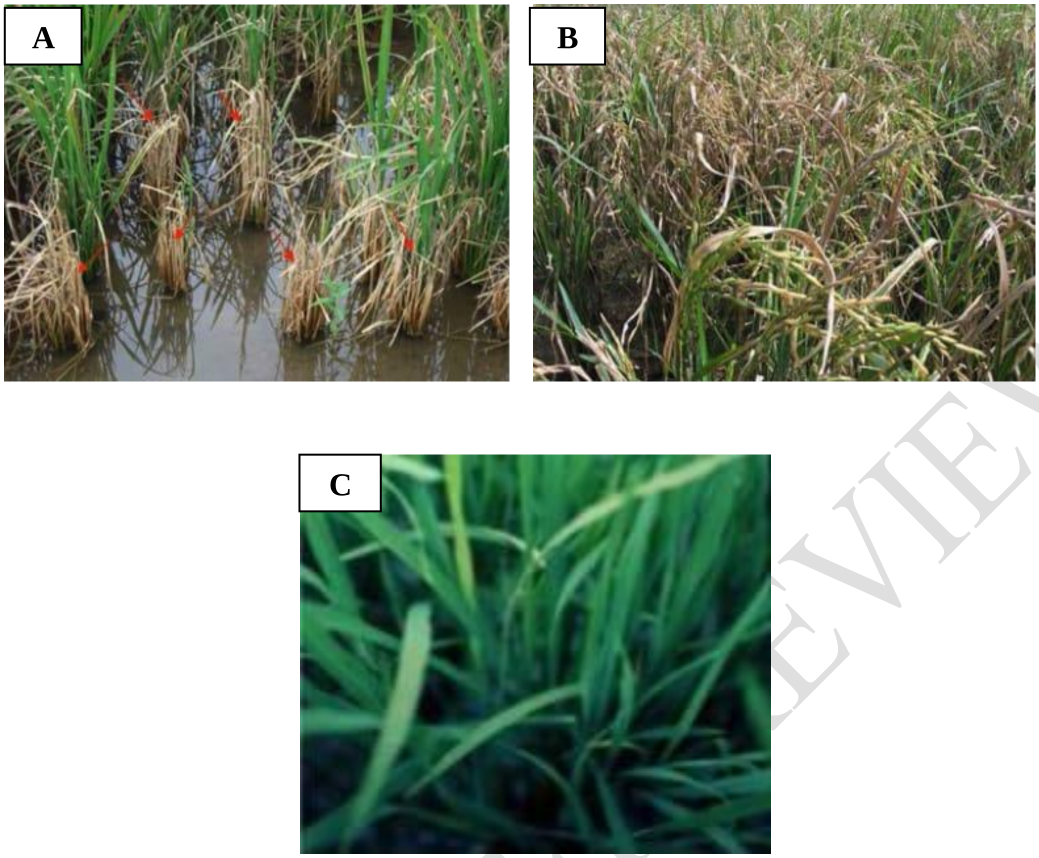

Fig 1 :- Image Showing various stages of Bacterial Leaf Blight (**A**): Kresek stage, (**B**): Leaf Blight stage, (**C**): Pale Yellow stage

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.

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 (Suh *et al*., 2013).

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 (Koizumi, 2007). 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 triat 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 seggregants efficiently as the markers linked to gene controlling the trait of interest co-seggregate with the trait phenotype across generations (Singh *et al.*, 2011)

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 (Chen *et al*., 2020). Many of these genes are tagged with markers (DNA based), while some have been cloned and characterized (Kumar *et al.*, 2020). 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 (Koide *et al.*, 2009). 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*. (2015), 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.

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	*O.longistaminata*
22	*Xa-22(t)*	11	China	Dominant	Zhachanglong
23	*Xa-23*	11L	China	Dominant	*O.rufipogon*
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	*O.minuta*
28	*xa-28(t)*	-	Bangladesh	Recessive	Lotasail
29	*Xa-29(t)*	1	-	Dominant	*O.officinalis(B5)*
30	*Xa-30(t)*	11L	India	Dominant	*O.rufipogon(*Y235)
31	*xa-31(t)*	4L	China	Recessive	Zhachanglong
32	*xa-32(t)*	11L	-	Recessive	*O.australiensis*
33	*Xa-33*	7	-	Dominant	*Oryza* wild species
34	*Xa-33(t)*	6	Thailand	Dominant	Ba7 *O.nivara*
35	*xa-34(t)*	1	Sri lanka	Recessive	BG1222
36	*Xa-35(t)*	11L	Philippines	Dominant	*O.minuta*
37	*Xa-36(t)*	11L	China	Dominant	C4059
38	*Xa-38*	4L	-	Dominant	*O.nivara*
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	*O.glabberina*
46	*Xa46*	11	Japan	Dominant	Mutant H120

Source : Chen *et al*. (2020)

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 (Naveed *et al*., 2010). To achieve more lasting disease resistance, researchers suggest combining major or minor resistance genes within a single genetic background (Koide *et al*., 2009). Extensively studying and incorporating the major BB resistance genes identified by various research groups have been 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 (Pandey *et al*., 2013). Pradhan *et al*. (2022) 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*. (2021) 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*. (2022) 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 BC_3F_3 lines. Kumar *et al*. (2023) 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*. (2019) 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. Developing rice varieties with combined resistance to BLB and tolerance to abiotic stresses will ensure productivity under changing climatic conditions.

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