**REVIEW ARTICLE**

# Exploring Blast Pathogen and Causal Organism: The Significance of R Genes in Marker-Assisted Selection

## Abstract

Rice blast, caused by the fungal pathogen *Magnaporthe oryzae,* is one of the most severe diseases affecting rice production worldwide. It poses a significant threat to food security due to its potential to cause substantial yield losses under favorable conditions. The development of rice varieties with durable resistance to blast disease is a major focus of rice breeding programs.The deployment of these R genes through marker-assisted selection (MAS) has been instrumental in accelerating the development of blast-resistant rice varieties. MAS allows for the precise introgression of desirable resistance traits into elite cultivars, significantly reducing the time and resources required compared to conventional breeding methods. Furthermore, pyramiding multiple R genes into a single variety has proven to be an effective strategy to enhance the durability of resistance, as it reduces the likelihood of resistance breakdown due to pathogen evolution. This review provides a comprehensive overview of the progress made in understanding blast resistance genes and their application in breeding strategies.

Key words: *Magnoporthe oryzae,* R genes, Marker Assisted Selection (MAS)

## 1.Introduction

Rice (*Oryza sativa L.*) is one of the most important staple crops globally, providing a primary source of calories for more than half of the world's population. It is cultivated in a wide range of environments across Asia, Africa, and Latin America, with Asia alone accounting for about 90% of the world's rice production and consumption. The crop's adaptability to different agro-ecological zones has made it a critical component of global food security and economic stability, particularly in developing countries where rice farming is often the primary livelihood for millions of smallholder farmers (Khush, 2005; Mohanty, 2013). Rice cultivation faces several challenges, including the need for increased productivity to meet the demands of a growing global population and the pressures of climate change.Among these, rice blast disease, caused by the fungus *Magnaporthe oryzae* (formerly, *Pyricularia oryzae*), is considered one of the most devastating (Ou, 1985). Rice blast affects all aerial parts of the plant, including leaves, nodes, and panicles, leading to reduced photosynthetic capacity, lodging, and, ultimately, severe yield losses that can exceed 50% in epidemic conditions (Dean *et al*., 2012). The pathogen exhibits high genetic diversity and adaptability, which complicates the development of durable resistant rice varieties. Moreover, the emergence of new virulent races of *M. oryzae* continues to challenge rice breeding programs, necessitating ongoing research into the disease's epidemiology, resistance mechanisms, and management strategies (Skamnioti and Gurr, 2009).

## 2.Originand Distribution

Rice blast caused by fungal pathogen,*Magnoporthae oryzae*, is also known as rice fever disease, rice rotten neck, oval spot of graminae, pitting disease, rye grass blast etc., is one of the major disease in rice which can cause severe yield losses. It was first reported in China in 1637 by ‘Soon Ying Shin’ and later in Africa in 1922.

It has spread in about 85 countries of the world (Jia *et al.*,2009). During 1984-85, 40% of the rice growing area in China has been affected by this disease. In Japan, emergence of new races of pathogen has led to the 20-100% yield loss despite utilizing blast resistance genes in local cultivars (Khush and Jena, 2009). It was also reported by Miah *et al*.(1985) that blast is major disease in dry seed beds and sandy soils of Bangladesh.

In India, it was first recorded in 1913 and severe epidemic occurred in Tanjore delta of Tamil Nadu in 1919 (Padmanabhan,1965). It occurs in almost all parts of the country. It occurs normally during August due to the light drizzling for many days in most of the countries (Kim and Kim, 1993).

## 3. Morphology and Life cycle of pathogen

*M.oryzae* produces greyish coloured colonies with slight differences at dorsal surface. Conidia are small, 2 septate, 3 celled and pyriform in shape. It is an ascomycetefungi which produces sexual spores called ascospores. Asci are found within perithecia.

Blast fungus is a hemi-biotrophic pathogen which invades initially the living tissues and later it becomes necrotrophic. Infection cycle starts after initial landing of conidium on the leaf surface. The spore attaches to the cuticle and germinate, producing a germ tube (Koga and Nakayachi,2004) and forms appressorium, which adheres tightly to the plant surface using mucilage (Nnagbo,2021).Then, the penetration peg goes inside the host surface and peg differentiate into bulbous and lobed infectious hyphae that grow intracellularly and intercellularly and forms the lesions.

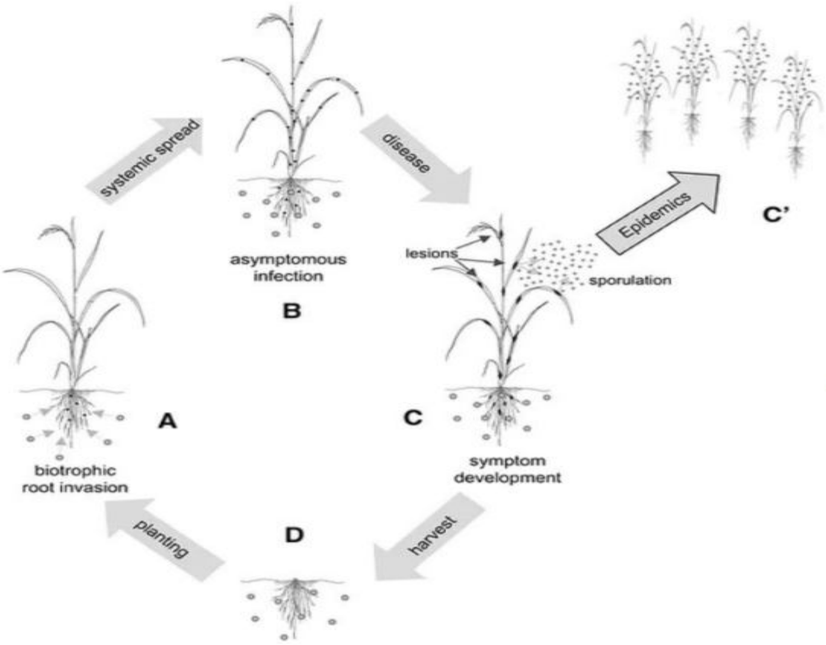


Fig. 1. Life cycle of blast pathogen in rice

## 4. Symptoms of blast diseases:

The disease infects the various parts of the plant including leaf, panicles, nodes at various growth stages of the plant. Based on the part of the plant getting infected, the symptoms can be classified into four distinct types:

### a. Leaf blast

Spindle shaped lesions appears on the leaves which wide at the centre and tapering towards the end. Large lesions are diamond shaped with greyish centre and brown margin. Under favourable conditions, lesions coalesce and spreads over the entire leaf which results in burnt appearance of the field. These lesions reduce the net photosynthetic rate of leaves.

### b. Nodal blast

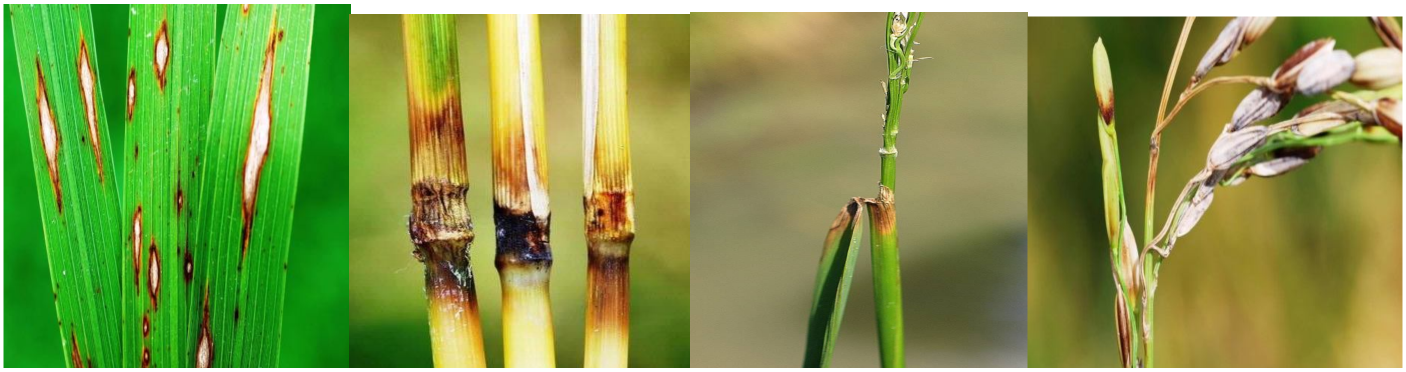
Pathogen affects the node and it turns black and the culms can be easily pulled out. Glumes and rachis can also get affected and brown to black spots can be seen (Padmanabhan, 1965).

### c. Collar blast

Collar region is the junction between the leaf and sheath of the stem. Necrotic lesions will be appeared on the collar which may spread to the leaves. Spores appears on these lesions also (Pinnschimdt *et al*., 1994).

### d. Panicle blast

It is the most destructive stage of the disease which directly reduces the economic value of theproduce. Fungus attacks the neck portion of the plant and get rotten. As a result, grain filling would not occur properly and leads to sterility and panicle turns white.



Leaf blast Nodal blast Collar blast Panicle blast

## 5. Molecular Genetics of R genes conferring resistance to Blast

Sasaki studied inheritance of resistance in blast for the first time in 1923. Later in 1965, systematic studies were conducted by Goto *et al*. (1964) and established the differential system for blast fungus races in Japan. The first blast resistant gene ‘*Pia*’ was isolated from japonica variety Aichi Asahi by Kiyosowa in 1967 and also he and his colleagues identified 13 genes for resistance by using seven Japanese strains of blast fungus (Kiyosawa,1981). These were named as *Pia, Pii*, *Piks*, *Pik*, *Piz*, *Pita*, *Pita2*, *Pizt*, *Pikp*, *Pikm, Pikn*, *Pib,* and *Pit.* About more than 100 R genes have been identified for Blast Resistance (Sahu *et al*., 2022). The details of molecular genetics of *Pi54* and *Pita* are explained below.

### 5.1. Molecular genetics of *Pi54/Pi-kh*

The *Pi-kh* gene, originating from the Tetep indica rice line, is a prominent dominant gene that imparts resistance to the PLP-1 isolate of *M. grisea*. It demonstrates high efficacy against the pathogen population in the Northwestern Himalayan region of India (Sharma *et al.*, 2002). Previous research has located this gene on the long arm of rice chromosome 11 using the RAPD marker S129700. Through linkage analysis of 208 individual F2 plants in the mapping population, using four rice microsatellite markers (RM202, RM536, RM206, and RM224) and a cleaved amplified polymorphic sequence (CAPS) marker derived from RAPD marker 129700 (Sharma *et al.*, 2005), it was determined that S129700 and RM206 were closely associated with the *Pi-kh* locus at distances of 4.5 and 0.7 cM, respectively.

Like *Pi-b* and *Pi-ta* genes, the *Pi-kh* gene was also isolated using map-based cloning (Sharma *et al.*, 2005). In contrast to *Pi-ta*, the *Pi-kh* gene does not demonstrate constitutive expression, as evidenced by transcriptional studies. Instead, it is activated in response to a pathogen attack, similar to *Xa1* and *Pi-b* (Yoshimura *et al.*, 1998). Following pathogen injection into both resistant and susceptible lines, the candidate gene was activated, with the susceptible genotype showing lower expression levels compared to the resistant genotype. By examining the different motifs present in the Pi-kh protein, it is possible to predict the mechanism of resistance conferred by the *Pi-kh* gene. In combination with one or more of the alleles (*Pi-k, Pi-ks, Pi-km* and *Pikp*) reported in this region of rice chromosome 11 (McCouch *et al.*, 1994), *Pi-kh* may trigger resistance in Tetep.

### 5.2. Molecular genetics of *Pita*

The *Pita* locus in rice has been widely utilized for rice blast management globally (Lee, 2011) and in India (Ramkumar *et al.*, 2011). Initially, in the southern United States, *Pita* was introduced into the rice cultivar Katy from a Vietnamese cultivar called ‘Tetep’. Subsequently, Jia *et al*. (2005) used Katy as the donor of *Pita* for cultivars Madison, Drew, Kaybonnet, Cybonnet, and Ahrent. The region carrying *Pita* has been observed to remain stable in the rice genome. Chen *et al*. (2002) identified *Pita* as a single copy gene located near the centromere of chromosome 12, a region often associated with recombination suppression. The specificity of *Pita*'s resistance is determined by a single amino acid, alanine, at position 918 of the *Pita* protein. In the cultivar Apura, *Pita* was mapped using a DH population flanked by markers RG457 and RG869, at distances of 13.5±4.3 cM and 17.7±4.5 cM, respectively. The gene *Pi-ta* was suggested to be the same as *Pi-4(t)* (Inukai *et al*., 1996), which has been mapped at 15.3 cM from the restriction fragment length polymorphism (RFLP) marker RG869 (Yu *et al*., 1996).

## 6.Gene pyramiding

The concept of gene pyramiding was proposed by Nelson (1978) to develop crop varieties with durable resistance to diseases by bringing together few to several different oligogenes for resistance to the given disease. This hypothesis was based on the idea that a host variety with two or more distinct oligogenes for pathogen resistance could be attacked by a pathogen race or pathotype that is virulent to all the resistance genes. As a result, this variety's resistance would be much more robust than that of types with just one resistance gene. It was also hoped that even if the pathogen was able to overcome all of the resistance genes, residual effects of these genes could still offer some defence against the infection; this appears to be the case, at least in some host-pathogen systems (Melchinger, 1990). Gene pyramiding is a generic phrase that refers to combining two or more genes that govern the same feature in a single line or variety.

## 7.MAS for pyramiding of Blast resistance genes in rice cultivars

Plant breeders have successfully created numerous blast-resistant cultivars using traditional plant breeding methods (Miah *et al*., 2013). However, the focus of breeders has now shifted towards Marker-Assisted Selection (MAS) to develop resistant cultivars (Ashkani *et al*., 2013). This shift is primarily due to the lengthy breeding cycle and limited selection efficiency associated with conventional breeding. Marker-assisted backcross breeding has emerged as a crucial tool in transferring novel genes to desirable rice varieties or hybrid rice parental lines, effectively incorporating resistance genes against the blast pathogen *Magnaporthe oryzae*.Zampieri *et al*. (2023) carried out research on MABC based on KASP Marker assays to introgress four *Pi* genes (*Piz, Pib, Pita*, and *Pik)* in a Italian rice variety which is highly susceptible to blast. Molecular analysis of Backcross lines showed the presence of *Pita2* gene which is linked to *Pita* and hence number of blast genes introgressed increased to five. Phenotypic evaluation also confirmed the effectiveness of introgressed lines agains many strains of blast pathogen.Thulasinathan *et al*. (2023) successfully introgressed the blast resistance gene *Pi9* into elite rice cultivar CO 51 which already contains *Pi54* gene. Through foreground selection using functional markers such as NBS4 and *Pi54*MAS, they confirmed the presence of *Pi9* and *Pi54* genes in Advance backcross breeding lines. They noticed that the Advance Backcross Breeding Lines containing two resistance genes were more effective than those containing single resistance gene.Samal *et al.* (2019) carried out study to genetically improve Ranbir Basmati variety for semi dwarf stature and blast resistance by introgressing *sd1* and *Pi9* genes respectively. The donor line, Pusa Basmati 1637 was crossed with the Ranbir Basmati and $BC_2F_1$ line having maximum genome recovery of recurrent parent was selected. The selected line was forwarded through anther culture to produce homozygous doubled haploid lines. The lines derived from anther culture was short statured and resistant to blast. Thus, the combination of Double Haploidy along with Marker Assisted Backcross Breeding (MABB) schemes speed up the process of obtaining superior recombinant lines.Rathour *et al.* (2022) used MABB to employ blast resistance in the temperate variety of rice 'Himalaya741' by introgressing *Pi9* gene from the basmati donor PB1637. The introgressed line displayed high level of resistance and also shown superior agronomic performance compared to recurrent parent.

## 8.Future prospects

The future prospects of breeding for blast resistance in rice are promising, driven by advancements in genomics, biotechnology, and breeding techniques. As rice blast disease remains a persistent threat to global rice production, the focus on developing durable blast-resistant varieties is intensifying. A deeper understanding of the genetic basis of blast resistance, coupled with new technologies, is paving the way for more effective breeding strategies. One of the key areas of progress lies in the identification and characterization of new resistance (R) genes. As the genome sequences of various rice cultivars and *Magnaporthe oryzae* isolates become increasingly available, researchers are better equipped to uncover novel R genes that can provide resistance against a broad spectrum of pathogen races. The use of genomic tools, such as genome-wide association studies (GWAS) and high-throughput sequencing, is accelerating the discovery of these genes and their integration into breeding programs.Moreover, the advent of genome editing technologies, particularly CRISPR-Cas9, has revolutionized the field of plant breeding. These tools allow for precise modifications in the rice genome, enabling the direct manipulation of genes to either enhance resistance or knock out susceptibility genes. This approach can create rice varieties with tailored resistance profiles, potentially reducing the time required to develop new varieties compared to traditional methods.

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