

REVIEW ARTICLE

An insight into the etiology of *Magnaporthe oryzae* and the R genes in Rice for the inception of efficient breeding programmes

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. Resistance (R) genes are critical components of a plant's defense system, providing specific resistance to pathogens through a variety of mechanisms. R genes play a pivotal role in combating the rice blast disease caused by the fungus *Magnaporthe oryzae*. These genes are involved in two main forms of immunity: Pattern-Triggered Immunity (PTI) and Effector-Triggered Immunity (ETI). 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: Effector-Triggered Immunity, *Magnaporthe oryzae*, Marker Assisted Selection (MAS), Pattern-Triggered Immunity, Resistance genes

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. Origin and Distribution

Rice blast caused by fungal pathogen, *Magnaporthe oryzae*, is also known as rice fever disease, rice rotten neck, oval spot of gramineae, 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 of the pathogen

M. oryzae exhibits distinct morphological characteristics that are integral to its ability to infect and spread among rice plants. As a filamentous ascomycete fungus, it produces several types of structures throughout its life cycle, including conidia, appressoria, hyphae, and ascospores. The conidia of *M. oryzae* are the primary means of asexual reproduction and dissemination. These

conidia are typically pyriform (pear-shaped) and exhibit a light olive to pale brown color. They measure approximately 20-25 μm in length and 8-10 μm in width. Conidia are multicellular, usually consisting of three cells separated by septa, with the central cell being the largest. These spores are produced on specialized structures called conidiophores, which emerge from the surface of infected plant tissues, particularly during periods of high humidity or after rainfall (Valent *et al.*, 2013). Conidia are readily detached from conidiophores and are dispersed by wind, rain, or mechanical disturbances, facilitating the spread of the pathogen to new host plants. Upon landing on a suitable host surface, such as a rice leaf, the conidia germinate and produce a germ tube that soon develops into an appressorium. The appressorium is a specialized, dome-shaped structure that is essential for host invasion. It is highly melanized, which helps it withstand the high turgor pressure generated within. This pressure, crucial for penetration, is created by the accumulation of solutes like glycerol inside the appressorium, enabling it to physically breach the tough outer cuticle of the rice leaf (Ribot *et al.*, 2008). The appressorium typically measures around 10 μm in diameter and is darkly pigmented due to the presence of melanin, which strengthens its cell walls and protects it from environmental stress. After successful penetration of the host tissue, *M. oryzae* develops filamentous structures known as hyphae. These hyphae are typically 1-2 μm in diameter and can be either septate (having cross-walls) or aseptate (lacking cross-walls). Inside the host, the hyphae expand into a network that colonizes the plant tissues, spreading from cell to cell through plasmodesmata. The invasive hyphae initially remain within the living host cells during the biotrophic phase, extracting nutrients while avoiding triggering the plant's defense mechanisms (Fernandez and Orth, 2018). As the pathogen transitions to the necrotrophic phase, the hyphae continue to proliferate, breaking down plant cell walls and causing extensive tissue damage.

3.1. Perithecia and Ascospores

In addition to its asexual reproductive structures, *M. oryzae* can also undergo sexual reproduction, forming structures known as perithecia. These are flask-shaped fruiting bodies that develop under specific environmental conditions. Within the perithecia, sexual spores known as ascospores are produced. The ascospores are typically cylindrical and hyaline, and they are released into the environment to initiate new infections. The role of sexual reproduction in the disease cycle of *M. oryzae* is less prominent compared to asexual reproduction, but it contributes

to genetic diversity within the pathogen population, potentially aiding in the evolution of new virulent strains (Couch and Kohn, 2002).

4. Life cycle of the pathogen

4.1. Spore Germination and Appressorium Formation

The infection process of *Magnaporthe oryzae* starts when conidia, the asexual spores of the fungus, land on the surface of a rice leaf. These conidia, typically pear-shaped, adhere to the leaf surface and germinate, producing a germ tube that eventually forms an appressorium. The appressorium is a specialized structure essential for host invasion. It generates high turgor pressure, reaching up to 8 MPa, by accumulating solutes such as glycerol inside the cell. This immense pressure enables the appressorium to physically penetrate the tough outer layer of the rice leaf and invade the underlying cells (Talbot, 2003).

4.2. Invasive Growth and Biotrophic Phase

Once the host cell wall is breached, *M. oryzae* enters the biotrophic phase, where it proliferates within the host cells without killing them immediately. Inside the plant cells, the fungus forms invasive hyphae that move from cell to cell through plasmodesmata, the small channels that connect plant cells. During this phase, the pathogen manipulates the plant's cellular machinery to suppress immune responses and facilitate its growth. To do this, *M. oryzae* secretes a variety of effector proteins, some of which enter the host cell and modify its defense mechanisms. These effectors assist the fungus in evading detection and suppressing the plant's immune responses. Some of these effectors, known as avirulence (AVR) proteins, can be recognized by specific resistance (R) proteins in the rice plant, triggering a defense response called effector-triggered immunity (ETI). However, the fungus can evolve to overcome this resistance, rendering the host plant more susceptible to infection (Valent and Khang, 2010; Liu *et al.*, 2014).

4.3. Transition to Necrotrophy

After the biotrophic phase, *M. oryzae* transitions to a necrotrophic phase, during which it kills host cells and continues to feed on the dead tissue. This transition is characterized by the secretion of toxins and enzymes that break down host cell walls, leading to the development of necrotic lesions typical of rice blast disease. The necrotrophic phase is responsible for the visible damage to rice plants, including lesions on leaves, stems, and panicles. During this phase, the fungus produces large amounts of conidia on the surface of infected tissues. These conidia are then released into the environment, where they can infect new plants, perpetuating the disease.

cycle. The ability of *M. oryzae* to spread easily through airborne conidia makes it a particularly dangerous pathogen, capable of causing widespread epidemics under favorable environmental conditions (Skamniotiand Gurr, 2009).

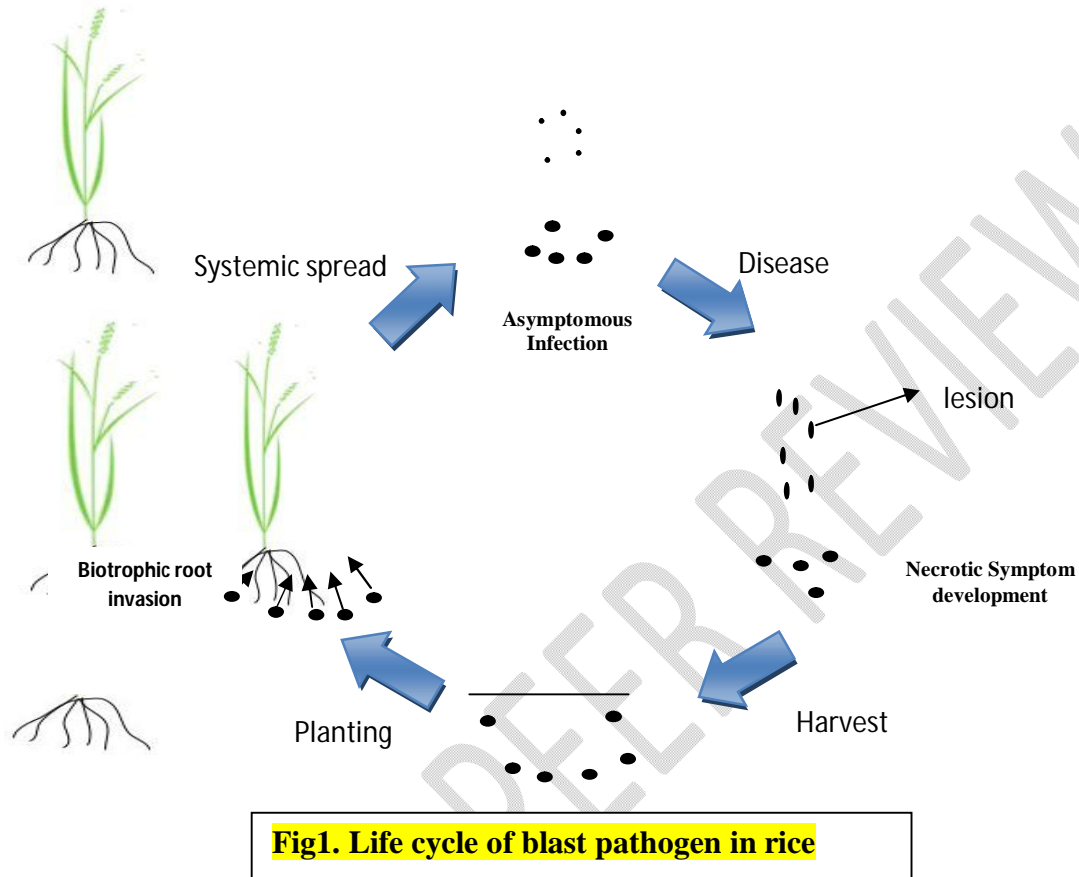


Fig1. Life cycle of blast pathogen in rice

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

Leaf blast is characterized by spindle-shaped lesions on rice leaves, which are wider at the center and taper towards the ends. Larger lesions often appear diamond-shaped, featuring a grayish center with a brown margin. Under optimal conditions, these lesions can merge and spread across the entire leaf, resulting in a burnt appearance across the field. This spread reduces the leaf's photosynthetic efficiency. As the disease advances, the lesions grow larger and may merge, covering significant portions of the leaf surface. In severe cases, the lesions can extend the full

length of the leaf blade, leading to a blighted appearance where substantial areas of the leaf become necrotic. The affected leaves may appear scorched, curl, or wither, substantially diminishing the plant's photosynthetic capability. One distinctive feature of leaf blast lesions is their "eye-shaped" or "diamond-shaped" morphology, with a gray or whitish center and a dark brown or reddish-brown edge. This specific pattern aids in field identification of the disease. Under favorable conditions such as high humidity and moderate temperatures (20-28°C), the lesions can develop abundant conidia, giving the leaf a velvety texture due to the presence of fungal spores.

b. Nodal Blast

Nodal blast initially manifests on the nodes of the rice plant—where leaves, stems, and panicles intersect. The infection starts with dark brown to black lesions on these nodes, which are often water-soaked and irregularly shaped. These initial lesions can be small and localized but expand rapidly under conducive conditions. As the disease progresses, the lesions deepen and may encircle the entire node, severely compromising the plant's structural integrity. When a node is fully encircled by a lesion, it disrupts the transport of water and nutrients between the roots and the upper plant parts. This disruption leads to wilting and yellowing of the leaves above the infected node, as the plant struggles to maintain its physiological functions. A notable feature of nodal blast is the discoloration and increased brittleness of the affected nodes. The nodes can turn dark brown or black and become fragile, making them susceptible to breaking. In severe cases, the collapse of infected nodes can cause the stem to break, resulting in the plant toppling over. This is especially detrimental during the flowering stage, potentially leading to a complete loss of developing grains.

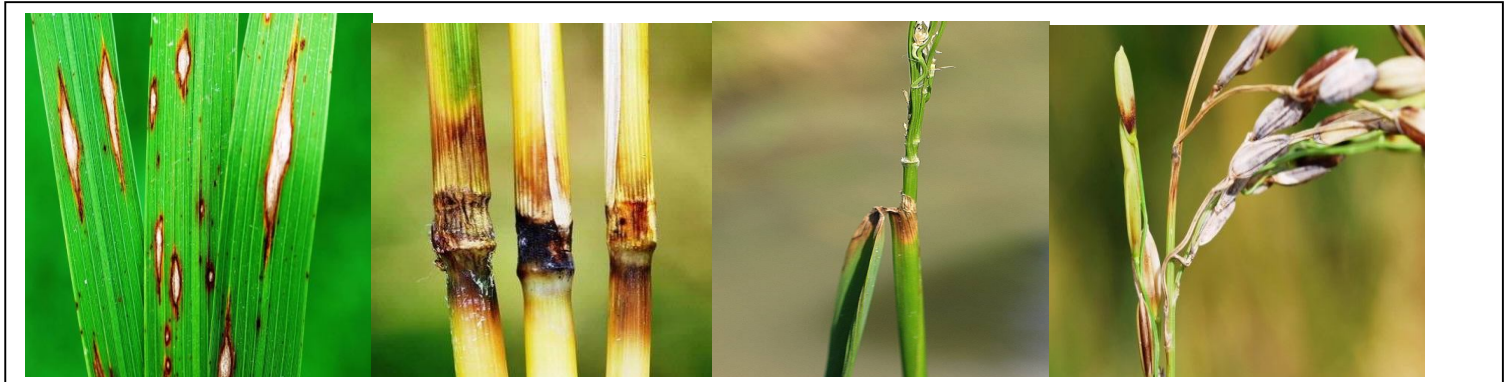
c. Collar blast

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 (Pinnschimdtet *al.*, 1994).

d. Panicle blast

Panicle blast starts with small, water-soaked spots on the panicle branches and spikelets. Initially, these spots are grayish or light brown and may appear slightly depressed. As the disease advances, the lesions grow larger and more pronounced, causing substantial damage to the panicle. This results in significant necrosis of the branches and spikelets. Spikelets that become

infected often turn brown and shrink, leading to poor development or complete abortion of the grains.



Leaf blast

Nodal blast

Collar blast

Panicle blast

Fig 2: Microphotographs of different plant disease

5. Management of paddy blast

➤ **Nursery/Seedling Stage to Transplanting Stage**

Field Management: Maintaining of field cleanliness by removing infected seeds, panicles, and plant debris after harvest to minimize disease sources.

Crop Rotation: Implementation of crop rotation with non-host crops to help break the cycle of pathogens by separating viable spores in crop residue from new seedlings.

Seed Quality: Use of high-quality, disease-free certified seeds to prevent the introduction of inoculum that could spread and develop into new infections.

Seed Treatment: Treatment of seeds with *Pseudomonas fluorescens* at 10 g/L of water for 30 minutes or *Trichoderma viride* at 5-10 g/kg of seeds. These treatments offer protection against soil-borne and seed-borne diseases.

➤ **Vegetative to Panicle Initiation Stage**

Fertilizer and Nutrient Management: Application of fertilizers and micronutrients according to local recommendations. Split nitrogen application into three doses: 50% at planting, 25% during the tillering stage, and 25% at panicle initiation.

Disease Management: Removal and destruction of (burn) any diseased plant parts to prevent disease spread.

Targeted Fungicide Use: Application of targeted fungicides based on observed disease presence

➤ **Flowering to Harvest Stage**

Ongoing Monitoring: Regular monitoring of disease incidence throughout this stage.

Targeted Fungicide Use: Application of fungicides based on observed disease presence and choose those with a shorter waiting period before harvest.

6. Mechanism of Disease Resistance

Plants have developed a complex system for detecting and responding to pathogen attacks, which is mediated by specialized receptors known as pattern-recognition receptors (PRRs). The initial defense mechanism, termed pattern-triggered immunity (PTI), plays a crucial role in preventing pathogen invasion. This early response activates a distinct class of intracellular receptors called nucleotide-binding domain leucine-rich repeat-containing receptors (NLRs), leading to a more advanced defense response known as effector-triggered immunity (ETI). PTI not only limits pathogen entry but also maintains a beneficial microbial community on the plant surface, supporting overall plant health. Key components involved in PTI include PRRs such as PRR1 and PRR2, calcium signaling through CDPK, the transcription factor WRKY, defense-related genes like PR1 to PR3, and secondary metabolism genes such as PAL and CHS. In the ETI pathway, components include R proteins R1 and R2, effector proteins AVR1 and AVR2, and the signal transduction gene SGT1, which interact via shared MAPK cascades, common transcription factors, overlapping gene transcription, or modifications to the cell wall (Malik *et al.*, 2020; Chen *et al.*, 2020; Singh *et al.*, 2019; Yuan *et al.*, 2021; Younas *et al.*, 2024)

7. 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*, *Pi-ta*, *Pi-ta2*, *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 *Pi-ta* are explained below.

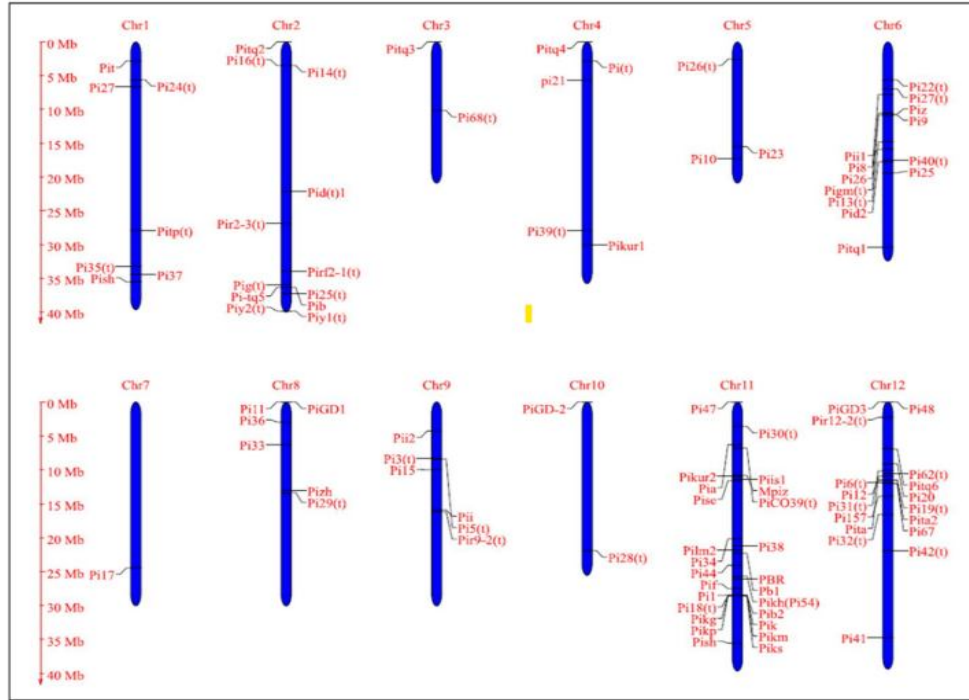


Fig 3.Rice blast resistance genes identified and mapped on to different Rice chromosomes (Devanna *et al.*,2022)

7.1. 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.

7.2. Molecular genetics of *Pi-ta*

The *Pi-ta* 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, *Pi-ta* was introduced into the rice cultivar Katy from a Vietnamese cultivar called 'Tetep'. Subsequently, Jia *et al.* (2005) used Katy as the donor of *Pi-ta* for cultivars Madison, Drew, Kaybonnet, Cybonnet, and Ahrent. The region carrying *Pi-ta* has been observed to remain stable in the rice genome. Chen *et al.* (2002) identified *Pi-ta* as a single copy gene located near the centromere of chromosome 12, a region often associated with recombination suppression. The specificity of *Pi-ta*'s resistance is determined by a single amino acid, alanine, at position 918 of the *Pi-ta* protein. In the cultivar Apura, *Pi-ta* 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).

8. 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. Recent research has focused on pyramiding multiple R genes to achieve enhanced resistance. For example, the combination of the *Pi-ta*, *Pi-b*, and *Pi-kh* genes in rice has been shown to provide robust and broad-spectrum resistance to various strains of *M. oryzae*. The pyramiding of these genes into a single rice variety results in a plant that can resist multiple pathogenic races, reducing the likelihood of resistance breakdown due to pathogen adaptation (Zhao *et al.*, 2021; Fujita and Yoshida, 2022). Advances in genomic selection and CRISPR/Cas9 technology have further enhanced gene pyramiding efforts. Genomic selection allows for the prediction of breeding values based on the entire genome, improving the efficiency of selecting desirable traits, including disease resistance. Additionally, CRISPR/Cas9 technology enables precise editing of the rice genome to introduce or enhance R genes, facilitating the creation of pyramided lines with multiple resistance traits (Yang *et al.*, 2023; Chen and Zhou, 2022). One of the key benefits of gene pyramiding is the potential for long-term durability of resistance. By combining multiple R genes with different mechanisms of action, pyramided varieties are less likely to be overcome by new pathogen races. Studies have also explored the sustainability of gene pyramiding, emphasizing the importance of continuous monitoring and management practices to maintain resistance over time (Ghosh and Sharma, 2021; Singh and Sharma, 2022).

9. 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 (Ashkaniet *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*, *Pi-ta*, and *Pik*) in a Italian rice variety which is highly

susceptible to blast. Molecular analysis of Backcross lines showed the presence of *Pi-ta2* gene which is linked to *Pi-ta* and hence number of blast genes introgressed increased to five. Phenotypic evaluation also confirmed the effectiveness of introgressed lines against 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 *Pi54MAS*, 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 BC₂F₁ 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 were 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. Rathoure *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. Rice varieties with pyramided R genes such as *Pi-a*, *Pi-2*, and *Pi-33* have shown high levels of resistance to rice blast in diverse environments. These varieties have been tested in multiple locations and under varying disease pressures, confirming their robustness and potential for commercial release (Kumar and Kumar, 2022; Liu and Xu, 2023). The introgressed line displayed high level of resistance and also shown superior agronomic performance compared to recurrent parent.

10. 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.

11. Conclusion

Rice blast disease, caused by *Magnaporthe oryzae*, poses a significant threat to global rice production. The pathogen's complex infection cycle involves spore germination, appressorium formation, invasive growth, and transition to a necrotrophic phase. The disease manifests in various forms, including leaf, nodal, collar, and panicle blast, each presenting distinct symptoms and impacts on rice plants. To manage rice blast, integrating strategies such as gene pyramiding, marker-assisted selection (MAS), and genomic tools has shown promise. Gene pyramiding involves combining multiple resistance (R) genes to provide broad-spectrum and durable resistance. Recent studies highlight the effectiveness of pyramiding genes like *Pi-ta*, *Pi-b*, and *Pi-33*, supported by advances in MAS and CRISPR/Cas9 technology. These methods enhance the efficiency of developing resistant varieties and ensure robust performance in diverse environments.

Disclaimer (Artificial intelligence)

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