DOI: https://doi.org/10.54083/978-81-980121-1-1-4

CHAPTER : 04

# Exploring Genetic Resistance for Sustainable Management of Blast Disease in Rice

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***Abstract***

The rice blast disease, caused by *Magnaporthe oryzae* or *Pyricularia oryzae*, poses a serious threat to rice production, affecting both yield and food security globally, highlights the devastating ability of the pathogen's to cause a reduction in yield that may go up as high as 70-80%. The host plant's genetic resistance is conferred mainly through two broad mechanisms. Resistant (*R*) gene in host plant conferring race specific, complete resistance in a 'gene-for-gene' relationship with *avirulent* (*AVR)* gene present in the pathogen and Quantitative Trait Loci (QTL) in the host plant conferring partial, race non specific, durable resistance against the pathogen. The integration of resistant genes into elite rice cultivars remains a primary goal in breeding programs, with advancements in molecular breeding offering new avenues for durable and broad-spectrum resistance. Dissecting the underlying genetic mechanism through novel molecular techniques helps better understanding the resistance for sustainable management of the disease. Various breeding approaches, right from conventional methods to genome editing were being increasingly employed for harnessing the potential genetic resistance in a concerted effort to breed for resistant cultivars.

***Keywords*** *avr* gene, Gene pyramiding, *Magnaporthe oryzae*, Multiline, *R* gene

## 1. Introduction

More than half of the world population, mostly in developing countries is largely depended on rice as their staple food. An estimated 20% of daily calorie need of these populations is met by rice alone. Apart from being Nutritional source of nearly 3.5 billion people globally, it is stated to be one of the primary income sources as well as employment for 200 million people (Asibi *et al.*, 2019). Growing global population demands 38% increased production by 2030 and an additional 116 million tons of rice is estimated to be required by 2035 to meet the food need (Seck *et al.*, 2012). However, this daunting task is further challenged by diminishing land and water resources, climate change induced stresses and emergence of new pest and diseases

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In: Current Trends in the Diagnosis and Management of Plant Diseases. (Eds.) Dutta, P., Upamanya, G.K., Pandey, A.K., 2024. Biotica Publications, Tripura, India.

to name a few. During the last few decades, innovations and advancements in technologies were successfully exploited to increase the yield, quality and other adaptive traits in most of the important crops plants including rice. In spite of these, various abiotic and biotic stresses still remain as a major threat in realizing the full potentiality of such advancement. It is reported that biotic stress can cause detrimental yield reduction in rice hindering in breaking the yield barrier.

It is estimated that, the farmers harvested on an average 5 tons $ha^{-1}$ of rice; while the mean potential yield of rice projected as 10 tons $ha^{-1}$ and this huge loss is attributed to various biotic factors like insect pest and diseases (Tanweer *et al.*, 2015). Among different biotic factors, diseases cause by fungus alone is estimated to cause a 14% overall annual yield reduction globally. Rice blast caused by *Magnaporthe oryzae* is regarded as one of the most devastating fungal disease capable of colossal yield loss that may go as high as 70-80% within a short time span (Sahu *et al.*, 2022). The pathogen infects rice plant in almost all stages of its growth from seedling to reproductive and in almost all plant parts from leaf, nodes, collar, panicles, panicle neck and roots *etc.*

Considering the detrimental impact of the pathogen on rice, carefully designed management practices are being adopted exploiting the latest innovations and techniques. Uses of suitable fungicide, nutrient optimization, appropriate timing of sowing, use of cultivars with durable resistance *etc.* are few approaches commonly adopted for management of the disease. Every management techniques come with its own benefits and challenges and proper combination of different techniques proven efficient in curbing the infestation. Deployment of cultivars with resistance genes against the pathogen is regarded as the most suitable, both economically and environmentally and advised throughout diverse ecological conditions (Yadav, 2019). In fact, genetic manipulation of disease reactions to the rice blast pathogen is regarded as one of the prime focus significantly influencing rice breeding programs.

The genetic resistance of the host plant is conferred mainly through two broad mechanisms. One is *R* gene mediated resistance that confers race specific complete resistance in a gene-for-gene relationship with the *AVR* gene present in the pathogen. Race-specific resistance is mediated through a large numbers of resistance genes (*R*-genes) characterized by race-specificity. Such R genes are identified in a wide range of the germplasms across the world. Another type of genetic resistance is conferred by minor genes or Quantitative Trait Loci (QTL) and provides a partial, race non specific, durable resistance against the pathogen. Both the types of resistance are increasingly being exploited in developing new resistant cultivars. Single gene conferred resistance is not often durable as new and new pathogen races rapidly evolved leading to complete breakdown of resistance. A study in Japan reported that the R-gene *Pik* introgressed resistant variety "Kusabue" witnessed breakdown of resistance only after 2 years of its release in 1963.

Similarly, there are several reports of complete breakdown of R gene conferred resistance available in rice (Fukuoka, 2019). Various factors like, relatively broad host range, continuous genetic variation, evolution and host shifts *etc.* considerably favors the evolution of newer and newer races of *M. oryzae*.

While breeding for blast resistance, some strategies like multiline varieties, varietal mixtures, gene pyramiding *etc* adopted the use of race specific, complete resistance genes (R genes);while several others are based on the accumulation of QTL conferring partial resistance through marker assisted selection (MAS). A wide range of breeding strategies starting with conventional breeding, mutation breeding, marker-assisted breeding, QTL mapping, transgenic approaches upto genome editing tools *etc.*, are being increasingly employed till date for the development of effective, durable cultivars with disease-resistant trait. (Yadav, 2019). Conventional breeding with various techniques like pedigree, backcross, multiline breeding, pureline selection, recurrent selection *etc.* are still in forefront of breeding resistant varieties, as evident from the release of numerous cultivars in diverse environment by different agencies across the globe. Current breeding approach primarily focuses on achieving partial and complete resistance against blast via combining genes taking help of molecular markers. Till now, nearly 350 quantitative resistance loci (QRLs) and 100 qualitative resistance (R) genes have been identified in rice and at least 32 of these R genes along with five QRL is reported to be fully cloned (Feng *et al.*, 2022). Recent innovations in molecular breeding open up new avenues in identification, validation and introgression of novel resistance at molecular level and their subsequent utilization in the genetic background of elite cultivars.

## 2. Economic Impacts of Rice Blast

Among different fungal diseases of rice, blast is believed to be one of the most devastating among all. Infestation causes enormous yield loss threatening global food security. Considering its impact on economic yield loss and its importance in scientific study, the fungal pathogen, *M. oryzae* has been rightly placed among the "top 10 plant fungal pathogens in the world" (Dean *et al.*, 2012).

Several factors like tolerance of the cultivar, the intensity of the infestation by the pathogen, fungicide application timing, relative humidity, onset of drought, heavy dew, high mean temperatures, high plant density and higher dose of nitrogenous fertilizer *etc* might influence the intensity of infestation to a varying degree and subsequent economic loss. The pathogen is capable of causing such devastating infestation due to its wide distribution as well as its ability to thrive in a wide range of environment

The first reported case of blast in rice came from China way back in 1637 where it was described as "rice fever". Subsequently, during the first decade of 1700 the incidence of the diseases was reported from Japan. The first incidence of blast infestation in India was reported from Tamil Nadu in 1913 and in 1919, Tanjore in South India witnessed the first ever blast epidemic.

However, the devastating effect of the disease was observed only after the introduction of semi dwarf HYV leading to green revolution during the 1960's. An estimated 85 countries around the globe, especially in South Asia and Africa, faced the outbreak of the disease to a varying degree incurring 10-80% total yield loss annually (Simkhada and Thapa, 2022). It is estimated that the annual cost incurred in managing the blast and the yield loss due to the disease collectively cost around 204 USD, sufficient to feed nearly 60 million people throughout the globe. Chemical fungicide alone cost more than 70USD/ha annually in managing the disease (Nalley *et al.*, 2016). As per the estimation, a total of around 157 million tonnes of rice accounting for nearly 30% of global production were lost due to the outbreak of the blast disease during 1975 to 1990. Crop losses ranging from 20 to 100% due to various races of blast pathogens were reported in Japan (Khush, 2005).

In India, blast related yield loss in rice is reported to be gone as high as 50%. During the 1980 and 1987, wide spread blast epidemics in states like Himachal Pradesh, Andhra Pradesh, Tamil Nadu and Haryana incurred substantial yield loss in rice resulting negative impact on overall economy. During natural epidemics of blasts in the *Kharif* season, incidence of blast infestation went upto 14 to 27% above the economic threshold level, leading to 27 to 35% loss in economic yield (Annegowda *et al.*, 2022). Numbers of countries in Europe *viz.* Italy, Spain, Portugal, Greece and France *etc.* also witness the severity of the blast, reporting 20% to 50% yield reduction.

## 3. Pathogenesis of Blast Disease

Controlling the blast pathogen effectively through various curative measures including continuous use of fungicides and other are proved to be futile. A durable control is possible only when the infection mechanism of the pathogen is thoroughly studied and measures taken against such mechanism. The pathogen *M. oryzae* conidia formed against the factors of the crop and climate act as disease propagules in a congenial field condition. This mechanism of infection in the host crop by the pathogen is termed as pathogenesis. It is the series of events that occur step by step that leads to the development of the disease.

In many plant species, the resistance mechanism against a certain pathogen is seen in a variety- or accession-specific type. The defense mechanism mediated by specific interactions involve rapid area specific cell death, called as the hypersensitive reaction (HR), the evolution of phytoalexins and different antimicrobial secondary metabolic substances and appearance of "pathogenesis-related" (PR) proteins. The blast fungus utilizes a hemibiotrophic infection – a process characterized by initial extension inside living host cells prior to turning into vicious necrotrophic mode. This infection process of paddy by *M. oryzae* depicts a developmental course which has been seen in numerous other leaf fungal pathogens. The infection starts with *P. oryzae* spore sticking to the hydrophobic exterior of leaf. The fungus identifies the cuticle substances and then, triggers spore germination

with creation of specialized appendages for diffusion (Martin-Urdiroz *et al.,* 2016). The pathogen generates high turgor pressure within the melanized appressorium, to pierce the host surface by a thin penetration peg exerting pressure to enter the leaf surface cell. After entry, the peg proliferates into globular and lobed infectious hyphae growing both intra- and inter-cellularly (Heath *et al.*, 1990), finally developing into blast lesions (Tucker *et al.*, 2001).

The symptoms are observed on whole body of the plant that include leaves, collars of leaf, necks, pedicels, panicles and seeds. As reported, roots of the plant also can be infected by the disease. The diamond shaped lesions, most common and characteristic symptom of rice blast mainly observed on leaves, while symptomatic lesion marks on the leaf sheaths are seen relatively rare.

A few factors like age of the plant, environmental conditions and resistance level of the host influence the occurrence of the symptoms on the leaves. The lesions initially appear as water soaked gray-green on susceptible cultivars, with a border of dark green colour. These expand rapidly in length and with time they become low tan colour with peripheral necrotic areas. On the other hand, lesions generally stay small in size with a colour of brown to dark brown in resistant cultivars. In collars, necrostic patch is observed at the juncture of the leaf and sheath tissues. These infections may results in total death of the whole leaf, consequently extending into and around the sheath. Spores can be formed in such lesions. Neck blast is reported to be attributed with the maximum grain yield loss in rice (Filippi *et al.*, 2009).

The fungus infects the neck of the panicle at the node that called rotten neck or neck blast. This may as destructive as completely unfilled grains (blanking) or fall over of the whole panicle as it got rotted. This may attack while the seeds form and lesions found on branches of panicles, sipke and spikelets which lead to discolorations and break down of the branches at the lesions. The main end result of the severity of blast is the decrease in grain yield effected by the blocking of the way of the nutrients, affecting grain development, that may lead to infertility of panicle (Prabhu *et al.,* 1995).When pedicels got infected seeds are not produced at all, a condition commonly called as blanking. Infections in the florets ultimately lead to the seed infection as they mature into the seeds.

## 4. Protocols for Screening of Blast Resistance

Resistance breeding needs continuous efforts of increasing the reservoir pool of resistance genes/alleles for efficient management of a disease. Effective screening protocol for disease resistance is required for fast and reliable screening of germplasm. In a crop, resistant or susceptible varieties might be identified based on the correctness of the screening techniques. A few screening methods used by researchers are briefly discussed hereunder.

*a) Abscissic Acid Assay Method*: Elevation of abscissic acid (ABA) is one resistance response of plants (Flors *et al.*, 2009) that may be used to identify resistance and susceptible interactions.

*b) Inoculation of Plants using Blast Inoculum*: In this method prepared spore suspension of blast is sprayed onto 18-day-old paddy seedlings in plastic trays with a motor sprayer. The motor sprayer is washed with alcohol and then rinsed with distilled water for individual isolate inoculated to prevent contamination of spore suspension. The inoculated plants are incubated in a moist chamber covered by jute sacks for 24 hours with an air temperature of 28 °C to promote symptom development (Bonman *et al.* 1996). The plants are then transferred to humidified room (mist room) with temperature of 25-30 °C and sprinkled with water for lesion development. For disease assessment, a measured quantitative scoring is done at seven days of inoculation by calculating Percent Diseased Leaf Area (0-82). Plants with 0-4% DLA are considered resistant when plants with 8-82% DLA are considered susceptible.

*c) Screening technique at field condition* : To screen for rice blast in the field, identification of defiant germplasm/varieties is important for further breeding activities. In field, screening of leaf blast is done in Uniform Blast Nursery (UBN) (Vasudevan *et al.,* 2014). The nursery has a size of 10 m and 1 m in length and width. Thirty plants for one entry to be tested are planted in a bed at a spacing of 10 X 50 cm plant to plant and row to row respectively. The vulnerable check entries are planted after each 10 lines of the entries tested as spreader lines. The susceptible checks are also grown in the border of UBN for uniform spread of the infection. Any one or more than one entries of susceptible checks are used as border or spreader lines. Concurrently, isolation, maintenance, and multiplication of blast cultures (fungal conidia) is performed as per the standard method. Furthermore, artificial inoculation is done with a local and vastly race of the pathogen (conidial suspension of the fungus at a concentration of 1X105 spores $mL^{-1}$) spray in 25-30 days after sowing in the UBN. In later period, the beds are sprayed with water 3–4 times in a day and covered by polythene sheets in night to keep a high humid condition until border and spreader lines develop and progress the disease. The observations are recorded in each entry in 10–15 days of inoculation with 5 days intervals, 2–3 times a day following Standard Evaluation System 2002 of IRRI..

d) Technique for Screening in Greenhouse/Polyhouse /Controlled Conditions: In the screening procedure for greenhouse/polyhouse, 15 plants of test entries are sown in plastic trays in 4–5 batches for inoculation with different individual blast isolates (The number depends on the availability of individual isolates of the pathogen for screening). Plants are grown in normal conditions for 10–15 days. The pathogen cultured on culture medium is used for preparation of conidial suspension All the plants are then inoculated with 50 mL of spore suspension and incubate in a moist chamber for 24 h at 26–28 $^0$C to maintain humidity and temperature. Plants are transferred to the incubation chamber at 25 $^0$C for 7 days, and water is sprayed three to four times during day time to maintain high humidity (humidity is near 100% for the initial 72 h to favor disease initiation). Then the disease reaction is assessed after nine days of inoculation and scored on a zero to nine rating

scale as per the Standard Evaluation System 2002 (SES) of the IRRI.

**5. Mechanism of Resistance at Genetic level**

In rice, R genes present in host plant interact with corresponding avirulence (*AVR*) genes present in the pathogen in a gene-for-gene manner (Flor, 1956). The specific R protein encoded by the R gene in host interacting with the effector molecule encoded by *AVR* gene in the pathogen resulted in the resistance reaction. This interaction either directly or indirectly, detected the invasion of the pathogen, thereby triggering a series of reactions inducing resistance. R genes were first reported way back in 1960s, where three independent R genes *viz.* '*Pia*', '*Pii'* and '*Pik'* were discovered (Yamasaki and Kiyosawa, 1966). Till now, nearly 100 resistance genes or loci have been identified in rice conferring resistance to blast pathogen (Korinsang *et al.*, 2020). Among the identified 100 resistance (R) genes, *indica* genotypes alone harbor 51 R genes whereas, 45 and 4 were identified in *japonica* genotypes and wild species respectively (Sharma *et al.*, 2012). Adopting map-based cloning, *Pib* was the first R gene to be isolated and characterized. The protein encoded by this gene contains a "nucleotide binding site" (NBS) and "leucine-rich repeats" (LRRs); making the gene a member of 'NBS-LRR' family of genes (Wang *et al.,* 1999). Till date 37 more R genes have been molecularly cloned and characterized. A few of these are: "*Pib, Pb1, Pita, Pi9, Pi2, Pizt, Pid2, Pi33, Pii,Pi36, Pi37, Pikm, Pit, Pi5, Pi3, Pid3-A4, Pikh, Pish, Pik, Pikp, Pia, PiCo39, Pi25, Pi1,Pi21, Pi50* and *Pi65"* (Zhu *et al.*, 2016).

R genes are characteristically clustered across the genome of rice, with special hot spots spread over chromosomes 6, 11 and 12. A minimum of 11 R genes *viz* "*Pi2*, *Pi9*, *Pi22*, *Pi25*, *Pi26*, *Pi40*, *Pi42*, *Pigm*, *Piz*, *Pizt* and *Pi50"*, are documented to be located as gene cluster in and around the centromeric region of short arm in the chromosome 6 and 7. Few R genes like *Pik*, *Pi-kg(t)*, *Pikm*, *Pik-h*, *Pik-p*, *Pi54* and *Pi1*are located in chromosome 11 at the long-arm region. More than 20 R genes including '*Pi-ta, Pi-ta2, Pi-tan, Pi19, Pi20, Pi30, Pi31* and *Ptr'* were documented on the chromosome 12 (Jiang *et al.*, 2020). Some of these genes like '*Pi-1 (t), Pi2, Pi9, Pi20 (t), Pi27 (t), Pi39 (t), Pi40 (t)* and *Pikh'* were reported to provide non race specific, broad spectrum of resistance, whereas some other including '*Pia, Pib, Pii, Pi- km, Pi-t, Pi12 (t)* and *Pi19 (t)'* reportedly provide resistance to specific races of the pathogen (Koide *et al.*, 2009).

Most of the R genes are dominant in nature and exhibit complete resistance. However, few recessive R genes are also been reported. Few of the R genes are constitutively expressed, but mostly they are induced by pathogen infection. Blast disease affect rice plant from vegetative stage till reproductive stage and the loss is more prominent if it affects the panicle. Therefore, R genes that exhibit panicle blast resistance such as Pb1, Pi25 and Pi64 are more desirable. However, only a few such genes have been cloned till date. On the other hand, majority of the R genes that have been cloned confer resistance to leaf blast affecting seedling stage. Involvement of both major and minor

resistance genes in regulating the resistance reactions makes the genetics of blast resistance more complex in nature. This complexity is even exaggerated by the interaction of complementary or additive effects, and environmental effects (Wu *et al.*, 2005).

Usually, two strategies are deployed for attaining resistance- use of broad-spectrum resistance genes and gene pyramiding. Due to the presence of a high numbers and different types of R genes in addition to the complex gene interaction of the disease, gene pyramiding remain as challenge for the plant breeders. This complexity becomes even more intense because of rapid emergence of new virulent races that lead to sufficient variability in *M. oryzae* population. Therefore, achieving broad spectrum and durable resistance against rice blast is one of the important challenges for the breeders. Considering the continuous evolution of *M. oryzae*, there is an urgent need to identify new *R* genes/alleles and also minor resistance genes.

Alternatively, quantitative trait loci (QTL), with smaller individual effects can be effectively utilized for attaining broad-spectrum, non-race-specific and durable disease resistance (Kou and Wang, 2010). Such race non specific and partial resistance is durable because of low selection pressure on the pathogen. Hence, the identification and utilization of novel QTLs and their deployment for achieving broad-spectrum resistant varieties are becoming important breeding objective for rice blast resistance. Though, till date more than 350 QTLs are reported from different genetic background using different mapping populations (RIL, $F_2$, Back cross population, double haploid), their deployment are limited due to associated uncertainty. However, there is probability that these QTLs may contain few potential candidate genes with major effect. Identification and map based cloning of these genes may open up future prospective in blast resistance. In a recent meta QTL (MQTL) analysis, 71 MQTLs were projected from analyzing a total of 435 QTLs across all the chromosomes of rice and identified a total of 199 putative rice blast resistant genes within 53 MQTL regions (Devanna *et al.*, 2024). These regions contained 48 characterized resistant gene analogs and related proteins like "NBS–LRR type, LRR receptor-like kinase, NB-ARC domain, pathogenesis-related TF/ERF domain, elicitor-induced defense and proteins involved in defense signaling".

However, there is large interval among QTLs as well as recombination rate is low that results in undesirable linkage drag and ultimately reduces the effectiveness of QTLs in marker assisted selection (MAS). Fine-mapping the QTLs may narrow down the QTL interval delimiting them to one or two genes. Such genes may be then characterized and utilized in future breeding programme. Meta-QTL analysis is one of the recent approaches that could be helpful for dissecting QTL intervals and subsequently validating their association with rice blast resistance.

## 6. Breeding Blast Resistance in Rice

Available reports suggested that nearly 350 quantitative resistance loci

(QRLs) and 100 qualitative resistance (R) genes conferring resistance to the pathogen *M. oryzae* is being discovered till date. Among the identified genes and loci, not less than 32 R genes and five QRL genes had been reportedly cloned (Li *et al.*, 2017; Yin *et al.*, 2021). QRL genes, *viz Pi21* and *bsr-d1*, generally confers broad-spectrum, durable resistance. This is attributed to the absence of 'gene-for-gene' relationship for resistance reaction and consequently absence of a strong selection pressure on pathogens. Most of these resistant genes and QRLs are located on certain chromosomal regions forming a "gene clusters". Such clusters are found to be spread over all the rice chromosomes except for the chromosome 3. Reports suggested that 3 loci *viz.* Piz locus, Pik locus and Pita locus, found in rice chromosome number 6, 11 and 12, respectively, served as the hotspots of resistance gene clusters (Wang *et al.*, 2014). Introgression of R gene as well as QRL conferring resistance to the blast pathogen into the genetic background of elite cultivars is the one of the primary challenge in breeding resistant varieties. Nevertheless, numerous rice varieties with varying degrees of resistance towards blast have been reported from throughout the world. Different breeding strategies were being efficiently employed considering the importance of the disease.

### *6.1. Conventional Breeding*

In conventional rice breeding programme, especially for breeding resistance to disease, hybridization based methods like pedigree and backcrossing; population improvement based recurrent selection and mutation breeding are extensively used for developing resistant cultivars (Srivastava *et al.*, 2017). Recurrent selection method exploiting the male sterility in rice, were employed for development of a resistant cultivar *viz.* CG-91 for upland condition in Brazil (Miah, 2013). Mutation breeding, using different chemical mutagens, successfully developed several mutant lines with durable resistance to blast in Malaysia, China, Thailand and India. Linkage drag, the phenomenon of co-transfer of undesired characters tighly linked with the desired resistance gene, is a major challenge in conventional breeding. However, successful introgression of several major blast resistance genes *viz.* 'Pib', 'Pita', 'Pia', 'Pi1', 'Pikh', 'Pi2' and 'Pi4' achieved through this strategy (Miah *et al.*, 2013). Many notable rice varieties conferring resistance to blast were being developed through conventional approach. The success of conventional breeding is often challenged by some inherent weakness. These include comparatively long cycle of breeding especially, while breeding for quantitative traits, low selection efficiency, involvement of crossing, difficulty in distant crossing *etc.* These may usually hinders the progress in developing new resistant cultivars causing a lag between varietal development and emergence of new pathotypes. Advent of the molecular biology techniques has opened new vistas in reinforcing the efforts of conventional breeding the developing and release of varieties conferring resistance to not only blast but also other biotic and abiotic stress.

### *6.2. Multiline Varieties*

Multiline varieties are another strategy effectively utilized in blast management in rice. Multilines are the mixture of several isogenic lines carrying different resistant genes for different races of the pathogen. Efficacy of multiline variety in producing durable resistance is primarily depended on numbers of isogenic lines comprising the variety, the rate at which new blast race develops and the coverage of the cultivated area under the variety (Miah *et al.*, 2013). This strategy of using Multiline had been found to be successful in managing the blast severity in many studies. It was suggested that mixing genotypes consisting 80-90% resistant plants and 10-20% susceptible plants with similar genetic background effectively limits the emergence of new virulent pathotype of blast in rice (Zhu *et al.*, 2000). Japan developed and released the multiline variety "Sasanishiki" comprising seven lines with seven different resistant genes in 1997 through a series of backcrossing "Sasanishiki" as recurrent parent with several landraces of rice having resistance towards the pathogen as a donor parents. (Abe, 2004). In another study, the multiline "Koshihikari BL" was developed from a popular nonglutinous cultivar, "Koshihikari", in 2005. It was found to be reduced the infestation by 79.4% in leaves and 81.8% in panicles , from a period spanning 2005 to 2019 in comparison to 2004 (Ishikawa *et al.*, 2022).

### *6.3. Marker Assisted Backcross Breeding (MABC)*

This method **i**s not only cost effective and time saving, but also it minimizes the linkage drag. Marker-assisted backcross breeding (MABC), involves two basic steps: (i) MAS for the gene of interest-known as "foreground selection" and (ii) MAS for recovery of the recurrent parent genome- known as "background selection". MABC based gene pyramiding of several blast resistant genes in to single elite cultivar is an attractive strategy to create durable and broad spectrum resistance. This approach is also efficient in recovering recurrent genome thus reducing the genome from donor parent in the genetic background of developed cultivars. In a recent study, four R genes *viz.* 'Piz', 'Pib', 'Pita' and 'Pik' were introgressed into a highly susceptible japonica Italian rice cultivar through MABC using "Kompetitive allele specific PCR" (KASP) marker (Zampieri *et al.* 2023). In another experiment, blast resistance genes *viz.*, *Piz-5* and *Pi54*, from the donor lines "C101A51" and "Tetep" were transferred to PRR78 to develop "Pusa1602" (PRR78 + *Piz5*) and "Pusa1603" (PRR78 + *Pi54*) PRR78, a highly susceptible line, was the restorer parent in the popular basmati hybrid Pusa RH10, subsequently making the hybrid susceptible. Inclusion of improved PRR78 lines was successful in producing hybrid at par with the original "Pusa RH 10" for all agronomic traits with benefit of resistance to blast. The strategy was successfully used for pyramiding two major genes for blight (*'Xa21'* and *'xa13'*) and blast (*'Pi54'* and *'Pi1'*) resistance into "Tellahamsa", a popular cold tolerant variety in Telengana. Similarly, National Rice Research Institute (NRRI) in India introgressed two major R genes *viz. Pi2(t)* and *Pi9(t)* along-with another novel gene from wild rice *O. minuta* into popular variety "Kalinga III".

### *6.4. Genome Editing*

Susceptibility factor-encoding genes (S-genes) present in host plant either helps infection by the pathogen or enhances compatibility between the host and pathogen. Thus efficient manipulation such genes in host plant are considered an effective alternate strategy in plant disease management over breeding for race specific R genes. Employing genome editing techniques to knockout a single gene was found to be considerably increasing the plant tolerance towards disease. A key player in plants inherent stress tolerant mechanism is ethylene responsive factors (ERF). RNAi silencing of ERF922 in rice was reported to be associated with the increased tolerance of rice to blast indicating a negative regulation of resistance (Liu *et al.*, 2012). Similarly CRISPR/Cas9 edited ERF transcription factor mutation was found to be improving the resistance as evident from the lesser numbers of lesion in blast infected mutant in comparison to the wild type. For other agronomic traits, both mutant and wild types lines were comparable (Wang *et al.*, 2016). Disruption of 'OsSEC3A', an important subunit component of exocyst complex, through CRISPR/Cas9 exhibited increased blast resistance in rice (Ma *et al.*, 2018).

CRISPR/Cas9 mediated knockout of two rice genes 'OsDjA2' and 'OsERF104', regulating production of a chaperone protein and an 'APETELA2/ ethylene-responsive factor', respectively, leads to substantial increase in resistance to blast in rice (Távora *et al.*, 2022).

CRISPR/Cas9 is being effectively used for functional validation of resistance gene for blast in rice. Several reports indicated that this technique is effectively used for functional validity of R gene like *Pi-d2*, *Ptr etc.* in rice against blast disease.

Hence, different genome editing tools like CRISPR/Cas has proved their potentiality not only in developing resistant varieties but also in functional validation of putative defense response genes (Devanna *et al.*, 2022).

### *6.5. Transgenic*

It is emerging as an important approach of blast management in rice. R gene cloning and characterization was carried out by transgenic expression of the genes in susceptible lines. Till date 38 R genes in rice blast are being transformed into different rice lines with diverse background (Devanna *et al.*, 2022). *Pi54* and its orthologs are the most extensively studied blast R gene among these 38 cloned genes through transgenic approach. Although, reports of large scale commercial cultivation of such transgenic lines are not available due to regulatory approval, nevertheless they serve as a invaluable genetic resources harboring resistance.

### *6.6. QTL Mapping*

As QTLs confers partial resistance characterized by a susceptible infection that is non-race specific and durable, mapping QTLs against blast resistance becoming increasingly important. This enhanced durability might be due to low selection pressure against the pathogen. First report of QTLs association

with blast resistance came from an African cultivar *viz*. Moroberekan in 1994. Till now more than 350 QTLs are being mapped in rice dispersed across different chromosomes. Numerous reports are available on mapping QTLs on different mapping population involving mostly indica japonica crosses (Tanwar *et al.*, 2015). A plethora of molecular markers including SSRs, RFLP and ISSR are currently being used in detecting QTLs in diverse population across a wide range of environment. Subsequent to its cloning of *'Pi21'* the first cloned gene for resistance to rice blast disease, a large numbers of blast resistance loci were detected in QTLs. However, small individual effects make identification of QTLs a daunting task. Linkage drag is another challenge in introducing QTLs to elite lines owing to low resolution in genetic map (Fukuoka *et al.*, 2014).

## 7. Conclusion

Blast disease in rice caused by the fungus *Magnaporthe oryzae,* is one of the most devastating biotic stresses causing great economic loss challenging food security across the rice growing countries, especially in developing nations. Resistant cultivars are recognized as one of the best strategies in sustainable management of the disease. The pathogen's ability to evolve and adapt to varying environment demands a concerted efforts in research and management in spite of humongous advancements in breeding and molecular techniques aimed at enhancing resistance. While deploying resistant varieties, particularly those with broad-spectrum resistance, has proven effective, combining various approaches such as genetic breeding, molecular markers and genome editing will be essential for long-term control. Given the pathogen's complex interaction with rice, future strategies must focus on durable resistance, utilizing innovations like QTL mapping and CRISPR/Cas9 to sustain productivity and mitigate the economic losses caused by this devastating disease.

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