INCORPORATION OF BLAST RESISTANCE INTO JYOTHI AND KANCHANA RICE (*Oryza sativa* L.) VARIETIES THROUGH MARKER ASSISTED BREEDING

By

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(2018-11-143)

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THESIS

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DEPARTMENT OF PLANT BREEDING AND GENETICS

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DECLARATION

I, hereby declare that this thesis entitled 'Incorporation of blast resistance into Jyothi and Kanchana rice (*Oryza sativa* L.) varieties through marker assisted breeding' is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of other University or Society.

Vellanikkara

Date : 20/7/2022

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CERTIFICATE

Certified that this thesis, entitled **'Incorporation of blast resistance into Jyothi and Kanchana rice (*Oryza sativa* L.) varieties through marker assisted breeding'** is a bonafide record of research work done independently by **Ms. T. Anusha** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship, or associateship to her.

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T. ANUSHA

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LIST OF ABBREVIATIONS

%	Per cent
BB	Bacterial Blight
bp	Base pairs
cm	Centimeter
cM	Centimorgan
CTAB	CetylTrimethyl Ammonium Bromide
COH	College of Horticulture
DBT	Department of Bio Technoilogy
DNA	DeoxyRibo Nucleic acid
EDV	Essentially Derived Variety
ISM	Improved Samba Mahsuri
MAB	Marker Assisted Backcrossing
MAS	Marker Assisted Selection
µg	Microgram
µl	Microliter
ml	Milliliter
OD	Optical Density
PCR	Polymerase Chain Reaction
PDS	Percent Disease Severity
RFLP	Restriction Fragment Length Polymorphism
RLK	Receptor Like Kinase
RM	Rice Microsatellite
SAP	Specific Amplicon Polymorphism
STS	Sequence Tagged Site
TBE	Tris Boric acid EDTA

Introduction

INTRODUCTION

Most of the world's population relies on rice (*Oryza sativa* L.) as a staple meal, with Asia and Africa being the two continents with the highest consumption rates (Muthayya *et al*., 2014). Around 154 million hectares of land are used to grow rice, producing 744.9 million tonnes annually (FAOSTAT, 2013). India alone contributes 26 per cent of the world's rice production, having the largest cultivable area (44.62 million hectares) and the second largest production (159.3 million tonnes) (FAOSTAT, 2014). More than 3.5 billion people eat rice as a source of energy (Spence *et al*., 2014). In terms of total food grain output, rice share roughly 52 per cent of the total while cereals make up 55 per cent. In India, 65 per cent of the population relies on rice (Bishwajit *et al*., 2013).

Due to rising population, there is an increase in demand for rice, which is expected to result in a 26 per cent increase in rice output over the next 25 years (Khush, 2013). The only way to meet these needs is to increase rice production and productivity with the limited amount of resources and arable land that are available (Ronald, 2014).

However, there are a number of limitations in rice cultivation, including an adverse environment, biotic and abiotic stresses that result in huge losses with respect to yield. The primary factors affecting yield loss are diseases (Divya *et al*., 2014). Productivity in rice is endangered with diseases such as bacterial, fungal and viral. Due to infestation of these diseases 20-70 per cent loss may occur in overall yield in rice (Fahad *et al*., 2014). *Magnaporthe oryzae*, a filamentous ascomycete that causes rice blast disease, is regarded as a key limiting factor in all rice producing regions due to its widespread dispersion and destructiveness under favourable conditions. According to reports, a single explosion can result in a yield loss of up to 85 per cent overall (Divya *et al*., 2014). More than 60 million people who are hungry will be fed with the amount of rice that is destroyed annually by blast (Sharma *et al*., 2012). This substantial drop in rice yield needs to be reduced. It is simple to design and cultivate rice varieties that confer resistance to biotic stress in order to meet these challenges and increase yield.

Blast disease can be controlled by using several fungicides that are available but they are costly and not eco-friendly. Therefore, to encounter this and prevent the yield loss occurring due to blast infection, it is essential to study the genetics of pathogen population to execute the techniques for management of disease (Babujee and Gnanamanickam, 2000). The laborious nature of blast pathogen virulence investigations on a variety of hosts is further highlighted by inoculation techniques used in favourable climatic settings. In these conditions, molecular techniques have become alternate methods for characterising blast isolates (Babujee and Gnanamanickam, 2000).

The valuable information on the diversity of pathogens across the globe, provided by the molecular diversity analysis of *M. oryzae* can be used to breed or to confer blast resistance into superior cultivars. The most environmentally friendly method of combating blast disease is the breeding of resistant cultivars. Efficient method of reducing the incidence of rice blast disease is to utilise resistant cultivars, which have low input costs for farmers (Hittalmani *et al*., 2000; Ronald, 2014).

A molecular marker is a portion of the genome's DNA sequence that is connected to a specific gene or the inheritance of a unique trait (Collard *et al*., 2005). As a result, specific plants can be chosen if the molecular marker is present because it denotes the desired qualities.

Vast data regarding various molecular markers, accelerated gene pyramiding and gene introgression strategies are accessible. PCR-based markers were created to recognise and screen resistance (R) genes as a result of new approaches including precision mapping and cloning of genes for blast resistance. The ability to more accurately identify genes and introduce blast resistance genes into elite cultivars is the biggest benefit of employing DNA markers (Wang *et al*., 2013). Resistance (R) genes such as *Pi*-1 (Hittalmani *et al*., 2000), *Piz*-5 and *Pita* (Narayanan *et al*., 2002), *Pi*1, *Pi*2 and *Pi*33 (Divya *et al*., 2014), Pi5 (Lee *et al*., 2009), *Pi-kh* (Madhavi *et al*., 2012), *Pi*-1 and *Piz*-5 (Gouda *et al*., 2012), *Pi1*, *Pi2* and *Pikh* (Harikrishnan, 2017), *Pi*46 and *Pita*

(Ming *et al*., 2018), *Pi*21 (Rosalyan *et al*., 2020), had been pyramided into many elite rice genotypes by using MAS strategy.

Jyothi (Ptb 39), one of the most popular high yielding rice variety of Kerala with very good cooking and nutritional qualities, is highly susceptible to blast disease. Similarly, Kanchana (Ptb 50), another high yielding variety suitable for both upland and wetland cultivation, is also highly susceptible to blast. Hence, this programme envisages transferring blast resistance to Jyothi and Kanchana through marker assisted strategy using the donor 'Parambuvattan (PTB7)', which is resistant to blast disease having two genes for resistance *Pi*-1 and *Pikh* with closely and tightly linked microsatellite markers.

In consideration with the above, efforts were made to introgress two genes for blast resistance (*Pi*-1 and *Pikh*) into Jyothi and Kanchana rice varieties from Parambuvattan (PTB7) through marker assisted breeding strategy. The attempt resulted in development of F_1 hybrids pyramided with the resistance genes. Hence, the present programme was framed with the following objectives:

I. Parental polymorphism study
II. Development of F_1
III. Screening of F_1 seedlings with molecular markers and artificial inoculation

Review of Literature

2. REVIEW OF LITERATURE

2.1. Rice and its importance

Rice (*Oryza sativa* L.) is the most important cereal crop which constitutes major part of diet for nearly half of the global population (Yu *et al*., 2002) and contributes 29 to 72 per cent calorie intake. It is a major cereal crop not only in India but all over the world. Due to green revolution rice production increased to greater extent but cannot meet the demands due to decrease in area of production and increase in population. According to various estimations, rice production should be increased by 30 per cent by 2030.

To meet this, there is need to develop varieties with higher yield and stability. Even though yield potential is high, final productivity is low due to various biotic and abiotic stresses. On rice, there have been reports of more than 70 diseases brought on by fungi, bacteria, viruses, or nematodes (Zang *et al*., 2004). Rice blast disease, caused by *Magnoporthe oryzae,* is the most damaging in all rice growing regions worldwide, according to Couch and Cohn (2002). Rice production can be increased by introducing new varieties possessing strong resistance to biotic and abiotic factors. Presently, DNA marker technology has contributed to a great extent to genetic improvement through the selection of desirable traits, such as resistance to diseases / pest. Molecular markers are relevant in marker assisted breeding to detect disease resistance genes. Many rice varieties resistant to various biotic and abiotic stresses were released and widely accepted by farmers for cultivation with the application of marker assisted selection (Xu and Crouch, 2008).

2.2. Taxonomy of rice

Rice belongs to the genus *Oryza*, tribe *Oryzeae*, subfamily Bambusoideae or Ehrhartoideae, of the family Poaceae (graminae). The genus *Oryza* has 25 known species, of which 23 are wild and two *viz., Oryza sativa* and *Oryza glaberrima*, are domesticated (Morishima, 1984; Brar and Khush, 2003). Both the domesticated species *O. sativa* and *O. glaberrima*, have different origins. *O. nivara* and *O. barthii* are thought to have

originated in tropical West Africa and South East Asia, respectively, whereas *O. glaberrima* is grown only in few regions of Africa. Morinaga (1968) identified three morphological groupings in *O. sativa* based on regional distribution as japonica, javanica, and indica. According to Oka (1958), the japonica and javanica groupings can be divided into two categories tropical and temperate japonicas. Tall versions of the former have dense panicles (Glaszmann and Arraudeau, 1986). Glaszmann (1987) made a significant contribution to our understanding of rice genetic diversity groups when he used isozymes to study 1,688 Asian traditional varieties and discovered six genetically different groups, designated as I–VI. Group I includes the species native to tropical areas that Oka (1958) categorised as "indicas". Group VI, which is at the other extreme, contains variants that are adapted to temperate regions and are referred to as "japonicas" by Oka (1958). The majority of the highland rice varieties belong to the latter group.

2.3. Rice genome

Rice (*Oryza sativa* L.) has 12 chromosomal pairs and is a diploid (2n=24) crop. Rice is regarded as a model crop due to its lower genome size (430 Mb), availability of a high density genetic map, and other factors (Jena and Mackill, 2008). The public now has access to the indica (LYP9) and japonica (Nipponbare) rice genome draft sequences (IRGSP, 2005).

The rice genome sequence is now widely available, breeding and biotechnology approaches may be effectively used to increase rice production per unit area (Stephen *et al*., 2002). Additionally, novel genes of relevance for biotic or abiotic stress can be discovered and used for crop improvement due to advancement of genomics and biotechnological technologies.

2.4. Major constraints of rice production

The productivity of rice is severely constrained by a number of biotic and abiotic stresses. The range of anticipated losses as a result of biotic stressors is 15 to 85 per cent (Rola and Widawsky, 1998). Blast, sheath blight and bacterial leaf blight are the three

most prevalent and harmful diseases that affect rice (Wopereis et al., 2009). 35 per cent of the yield loss caused by disease in rice is due to blast, 20 per cent by bacterial leaf blight, 20 per cent by sheath blight, ten per cent by tungro, and the remaining ten per cent by other diseases (Kumar, 2014).

2.5. Major Diseases of rice

There are a lot of diseases that limit rice output. Three fungal diseases *viz*., blast, sheath blight, and sheath-rot, the bacterial disease known as bacterial blight (BB) and the viral infection known as rice tungro disease (RTD), are considered to be major worldwide. These five diseases are the main production bottlenecks in Asia, where more than half of the world's rice is grown and consumed. Due to the havoc they wreak to rice production, these diseases have also been extensively studied, and there is abundant scientific information accessible for them (Gnanamanickam, 2009).

2.6. Blast disease of rice

In India, 72 of the 114 pathogens that influence rice cultivation are significant (Crop Protection Compendium 2004, http://www.cabi.org/compendia). Due to its widespread distribution and potential for destruction under favourable conditions, rice blast disease is caused by *Magnaporthe oryzae* [(Hebert) Yaegashi & Udagawa)] (Couch and Kohn, 2002) [anamorph *Pyricularia grisea* (Cooke) Saccardo] is regarded as one of the primary rice disease. Soong ying-shin first wrote about the rice blast disease in China during 1637 in his book use of natural resources (Rao, 1994), and in Japan in 1704. In the United States, Metcalf first noted the disease in South Carolina in 1876 and may have been the first to refer to it as "blast," a term that accurately describes the harm it causes (Rao, 1994). It was originally noted in India in 1918 in the Thanjavur (Tanjore) delta of South India. However, it was not until a deadly pandemic struck in 1919 that it came to public attention (Padmanabhan, 1965). In almost every part of the world where rice is produced, there have been reports of rice blast (Bonman, 1992; Ou, 1985), and there have been reports from 85 different nations (Flores, 2008). According to reports, rice blast

causes yield loss between 10 and 30 per cent per year (Talbot, 2003). According to conservative estimates, the rice blast disease annually destroys enough rice to feed 60 million people (Zeigler *et al*., 1994). Between 1975 and 1990, 157 million tonnes of rice were reportedly lost worldwide only due to blast disease (Baker *et al*., 1997).

2.6.1 Pathogen (*Magnaporthe oryzae*)

A haploid filamentous Ascomycete having a genomic size of about 40 Mb and seven chromosomes, the fungus *Magnaporthe oryzae* (Dean *et al*., 2005). *M. grisea* is becoming as a top model organism for research on the host-parasite interactions and fungal phytopathogenicity. When a three-celled conidium drops on a host leaf and attaches itself to the leaf cuticle through spore-tip mucilage, rice blast fungal infection begins. A germ tube extends as germination progresses, hooking and swelling at its tip before differentiating into an infectious structure known as the appressorium. Except for a clearly defined pore in between the appressorium and rice leaf, the appressorium turns melanized as it matures (Howard and Valent, 1996). The disease begins when this infectious structure forms on the host's surface. The infected hypha then infiltrates and develops through the rice leaf after a penetration peg is driven along the host surface (Talbot *et al*., 2003). At this phase, the symptoms are clearly present, and tiny oval lesions start to develop along with local chlorosis. Lesions developing eventually turn necrotic and may merge. Cavara originally identified the fungus *Pyricularia oryzae*, which causes the rice blast disease, in 1891, (cited by Ou, 1985). The filamentous ascomycete *Magnaporthe oryzae*, sometimes known as *M. grisea*, is now known as *P. oryzae* teleomorph (Herbert, 1971). The piriform-shaped conidia of the anamorph *P. oryzae* have one or two transversal septa, are little darked or hyaline, and are connected to the conidiophore through their bottom enlarged. Conidiophores are simple, branching rarely, septated, growing synchronously, and somewhat brownish. The teleomorph *M. oryzae* has not yet been observed in nature, and it has been created in a laboratory by crossing certain compatible isolates. Hyaline ascospores are produced during the teleomorph stage; they are generally fusiform, three-septate consisting of a unitunicate

asci (Scheuermann *et al*., 2012). The inoculum is made up of diseased seeds, agricultural waste, and wind-distributed spores from nearby farming areas. According to Kingsolver *et al*. (1984), the mycelium found on field leftover rice straw may endure for around six years. Infected seeds can disrupt the early stages of vegetative development, but they hardly result in disease epidemics. There are more than 50 *M. oryzae* hosts that are known which includes cultivated (Metha and Baier, 1998) as well as weed species (Mackill and Bonman, 1986), although there are very few instances of infections spreading from one host to another. The sporulating lesions on diseased leaves that are indicative of a policyclic disease serve as the primary inoculum sources for subsequent infections. When young seedlings have three to four leaves during flowering, the rice plant is highly vulnerable. Temperatures around 20 and 30°C and relative humidity exceeding 90% are also favourable for blast disease, with the development of conidia starting on lesions when humidity reaches 93%. Dew, mist, and drizzle are frequently observed, suggesting favourable circumstances for the infection. Fertility of the soil also has an impact on disease severity. When a cultivar lacks a particular resistance, high levels of organic matter or excessive nitrogen intake may cause the spread of disease more widely. However, lack of nitrogen can make plants more susceptible to diseases (Prabhu *et al*., 1996).

Host-Range: More than fifty other types of grasses are susceptible to damage by the blast fungus. Cultivated grasses like barley, wheat, pearl millet, and turfgrasses are susceptible to assault by certain strains. In South America, there have only been a few reports of wheat outbreaks (Valent and Chumley, 1994). Although, it is known to attack Arabidopsis through diverse mechanisms than how rice is infected (Park *et al*., 2009).

2.6.2 Symptoms and damage

All of the rice plant aerial portions are vulnerable to the blast pathogen. However, it can also be observed on the leaf sheaths, rachis, joints of the culm and even on the glume. The infection is most apparent when it attacks the leaf blades, panicle, neck and nodes.

2.6.2.1 Symptoms on leaves

The lesions or spots begin as tiny brown specks and develop into spindle-shaped growths that are 0.5–1.0 cm wide and several cm long, pointed at both ends. The margin is frequently brownish, with a greenish grey centre. However, varied climatic factors and varietal responses affect the size, colour, and shape of the lesions. On a cultivar that is sensitive, multiple greyish spots may form under favourable conditions, grow larger and broader, and consolidate, affecting the entire leaf (Rao, 1994).

2.6.2.2 Panicle blast

The neck infection symptom is a darkened node exactly behind the ear. The branches of panicles may be infected and transform brown to black, or the infection may spread beyond the neck area. The entire inflorescence may split off at the decaying neck and the infected panicles frequently break and fall off. Majority of the grains found on the panicle with the diseased neck are chaffy (Rao, 1994). The economic threshold point for panicle blast, above which chemical control should be used, was designated as 2%.

2.6.2.3 Nodal blast

If the culm is pulled out, the blackened area can be observed. Affected nodes on the lower part of the stem turn into black. On the rachis, especially where it branches and even on the glumes, brown to black spots can also be witnessed (Padmanabhan, 1965).

2.6.2.4 Other symptoms

Rice blast enhances the proportion of chalky kernels, reducing the calibre of rice processed. It is also reported that blast disease influences rice quality (Variar *et al*., 2009).

2.7. Genetics of disease resistance

Sasaki published the first studies on resistance inheritance in 1922. Takahashi (1965), Yamasaki, and Kiyosawa then followed (1966). Until 1960, the inheritance was

examined inadequately in view of pathogenic specialisation the causal fungus, making it challenging to come to a consensus on the topic. Only after Goto (1965) devised the differential system for blast fungus races in Japan were comprehensive research conducted. Kiyosawa and his colleagues studied the inheritance of resistance using seven Japanese blast fungal strains, and they reported 13 resistance related genes (Kiyosawa, 1981). These were given the identities *Pi-a, Pi-i, Pi-ks, Pi-k, Pi-z, Pi-ta, Pi-ta2, Pi-kp, Pi-km, Pi-kn, Pi-b,* and *Pi-t* respectively. Non-allelic genes were identified by the same base letter but were distinguished from one another phenotypically by a number or letter subscript which are separated by a hyphen (Kinoshita, 1986). Since then, a number of resistance genes have been identified by a base letter immediately followed by a number subscript, beginning with *Pi-1, Pi-2* and so on up to *Pi-44* (Mackill and Bonman, 1992).

Several genes for resistance to blast disease have been identified on specific chromosomes due to advancement of molecular markers. For instance, linkage with RFLP markers allowed *Pi*2 and *Pi*4 to be positioned on chromosomes 6 and 12, respectively (Yu *et al*., 1991). By breaking down complex genotypes and analysing the impact of individual genes in a similar genetic background, near-isogenic lines (NILs) were employed for genetic mapping. *Pi*1 was identified on chromosome 1 and *Pita* on chromosome 12 utilising a pair of NILs (Yu *et al*., 1996).

2.8. Mechanism of blast resistance

Both major as well as minor genes offer enduring resistance to *M. grisea* (Bonman and Mackill, 1998). Based on how the gene(s) impact the pathogen's ability to reproduce, blast resistance is often divided into two categories: qualitative (whole) and quantitative (partial). While partial resistance reduces the extent of pathogen multiplication in the perspective of a compatible interaction, qualitative resistance, governed by race specific major R genes called *Pyricularia* genes (*Pi*) and conditions like compatibility issue of the host and pathogen strain, hindering reproduction of the fungus (Wang *et al*., 1994). Partial resistance is frequently conditioned by more than one gene on different chromosomal regions, and it is noticed as durable resistance in many instances

(Bonman and Mackill, 1998). The adhesion of fungus spores to plant leaf surfaces initiates the blast infection. Fungal spores grow and produce infection-specific approssoria within 24 hours of immunisation, penetrating leaf cuticles and infecting epidermal cells (Ebbole, 2007). The rice *M. oryzae* interaction exhibits Flor's "gene-for-gene" theory upon fungal invasion (Flor, 1971). If rice R proteins are able to detect fungal effectors, effector-triggered immunity (ETI) is initiated, leading to a hypersensitive response (HR) that, within 48 hours, inhibits the development of the fungus. The detection of fungal pathogen-associated molecular patterns (PAMPs), such as chitin, triggers a limited PAMPs triggered immunity (PTI) response if fungal effectors are not detected, and fungal hyphae still continue to proliferate along plant tissues, resulting in disease symptoms after 4-5 days (Chen and Ronald, 2011). For rice blast resistance, the quick defensive response following *M. oryzae* invasion is crucial.

2.9. Breeding for resistance to blast

Since the commencement of rice development initiatives, notably in Japan, steps have been taken to generate rice types with blast resistance. Numerous breeding techniques have made use of blast resistance genes. Transferring these genes from background of one cultivar to another is simple. Thus, using traditional breeding programmes, *Pib, Pita, Pia, Pi1, Pikh, Pi*2 and *Pi*4 have been incorporated into various rice types. *Pita* (Rybka *et al*., 1997), *Pi*1 (Du *et al*., 2007), *Piz* (Conaway-Bormans *et al*., 2003), *Pi*35 (Nguyen *et al*., 2006), and *Pi*37 (Chen *et al*., 2005) are just a few that have employed for incorporating into new cultivars using MAS in recent years. Considerable amount of work has been achieved in locating and molecularly labelling blast resistance genes. Almost all the genes for leaf blast resistance that have been discovered so far. The neck blast, however, is more significant. Hence, efforts should be undertaken to find and tag the genes responsible for neck blast resistance for the development of resistant varieties to neck blast.

2.9.1 Traditional approaches of rice breeding for blast resistance

Plant breeding has traditionally been regarded as the most widely used technique for crop improvement. Breeders have created a number of blast resistant varieties that are suitable to various rice-growing locations across the world employing traditional plant breeding methods. In the past, crops have been bred for stress tolerance through breeding strategies such as pure line selection, recurrent selection, backcross selection, mass selection and mutation breeding.

Recurrent backcrossing is a conventional breeding technique that is frequently used to transfer alleles from a donor to a superior variety at one or more loci. Since over a century, backcross breeding has been a popular technique for transferring genes providing resistance for both biotic and abiotic stress into a superior or adapted variety that contains a wealth of beneficial agronomic characteristics but is prone to the target stress (Allard, 1999).

If the resistance is controlled by major genes, the pedigree technique is ideal for breeding rice that is resistant to insects and pathogens. In a short period, genes for resistance to six or seven main diseases and insects can be coupled (Khush and Jena, 2009). When IR20 was first introduced in 1969, it was immune to bacterial blight, blast, and green leafhopper. Since its first introduction in 1973, IR26 exhibited resistance to bacterial blight, blast, green leafhopper, and brown plant hopper. The first enhanced variety with various abiotic stress resistance and tolerance was called IR36 (Rockwood, 2001).

Numerous changes must be made to the world's food supply chain in order to feed the expanding population and to meet the need for agricultural production that is anticipated to rise by 70% as (Ronald, 2014). Therefore, in order to enhance agricultural output using standard breeding procedures, novel techniques are necessary (Collard and Mackill, 2008).

2.9.2 Molecular marker approaches in plant breeding

Utilisation of genomic and biotechnological techniques by rice breeders is being considered as a solution to several significant issues where the constraints of traditional breeding methods exist. DNA marker technology, a branch of biotechnology developed from molecular genetics and genomics research, has significant potential for plant breeding (Collard and Mackill, 2008).

2.9.2.1 Molecular Markers

Molecular markers are landmarks that are within or close to the genetic locus that is mainly accountable for a trait of interest. They are generally inherited or co-inherited with the specific locus in populations that are segregating over generations and are utilised to 'flag' the location of a specific gene or the inheritance of a specific trait (Collard *et al*., 2005). Additionally, the number of these markers is essentially limitless and they are not subject to environmental regulations (Collard and Mackill, 2008).

Molecular genetics and genomics-based DNA marker technology has a lot of potential for plant breeding. Through MAS, the use of DNA markers has the huge potential to improve the effectiveness and reliability of traditional plant breeding (Collard and Mackill, 2008).

Allelic variation within genes governing these characteristics can be identified using DNA markers. Reliability and accuracy in plant breeding might be significantly improved by employing DNA markers (Collard and Mackill, 2008). Database of molecular markers includes a lot about rice. In rice, high density map of SSR markers was generated using the physical rice map that was accessible (McCouch *et al*., 2002).

Today, a variety of contemporary plant breeding programme components, such as genetic analysis of populations, parental selection, Marker Assisted Selection (MAS), Marker Assisted Backcross Breeding (MABB), and gene pyramiding, are enabled by the use of molecular markers (Collard and Mackill, 2008).

2.9.2.2 Simple Sequence Repeats (SSR) markers

The class of molecular markers known as SSRs or Microsatellites relay on the presence of short oligonucleotide tandem repeats in the plant genome (Tautz and Rentz, 1994). Microsatellites are DNA motifs that are 1-6 nucleotides long and are constantly repeated 15–30 times in tandem (Bennet, 2000). SSR markers provide a simple, accurate, and measurable way to quantify the genetic diversity of agricultural plants since they are codominant and highly polymorphic (Litt and Luty, 1989; Tautz, 1989). Nearly 15,000 SSRs are already available for rice, and these are being utilised to produce high density genetic maps, genotype rice accessions, analyse diversity, improve the construction of core collections and practice marker-assisted breeding (Yu *et al*., 1991; Garris *et al*., 2005).

SSR markers have been employed to analyse the genetic diversity of both domesticated and wild species of rice for disease resistance, insect resistance and abiotic stress tolerance (Yu *et al*., 2002; Mc Couch, 2002).

2.9.3 Marker assisted selection (MAS)

MAS is the technique of identifying plants from a population that is segregating and have the relevant gene combinations employing DNA markers to aid in plant breeding (Mohan *et al*., 1997; Collard *et al*., 2005; Xu, 2003).

2.9.3.1 Marker assisted foreground selection

By tracking the genotype at flanking markers in order to trace the alleles of donor parent, it is possible to identify the existence of target trait in an individual. Foreground selection is a backcross breeding strategy that aims to introduce a gene from a ‘donor’ line into the molecular background of a ‘recipient’ line. Molecular markers may be used to detect if the introgressed gene is present (Hospital, 2001). This is an effective technique for altering oligogenic characteristics in a variety of plant breeding scenarios (Frisch, 1999). Foreground selection with marker assistance is helpful for features related to biotic and abiotic stresses that require laborious and time-consuming phenotypic

evaluation techniques. With this method, the desirable plants can be identified early for backcrossing. Moreover, this method may be used to select and introgress both dominant and recessive genes that impart stress tolerance (Panigrahi *et al*., 2013).

2.9.3.2 Marker assisted background selection

Since over a century, backcross breeding is already a common approach to transfer relevant genes inhering resistance to biotic and abiotic stresses into a superior or adapted variety that is endowed with a wealth of desirable agronomic characteristics but is vulnerable to the specific stress (Allard, 1999). In order to find genotypes with the most recurrent parent genome (RPG), a technique known as marker assisted background selection (MABS) can be used involving genotypic assessment of individuals in advanced generations (Hospital and Charcosset, 1997). This ultimately leads to an improved cultivar that is genetically identical to the common cultivar but for the added beneficial genes. This method is therefore very helpful for expediting the reconstruction of recurrent genome and the necessary donor segment. With traditional backcrossing, the reconstitution of the RP, which may contain a number of donor chromosomal segments extraneous to the target gene, nearly six backcross generations are required (Xu, 2003; Panigrahi, 2013).

Numerous marker loci encompassing the whole genome, MABS speeds up the recovery of the recurrent parent's RPG alleles. Meanwhile, MABS has become a standard technique in plant breeding (Frisch, 1999). By selecting against the donor genome except for the allele (s) to be introduced from the donor, it has been demonstrated that MAS may be beneficial in backcross breeding programmes for decreasing linkage drag and improving population sizes (Hospital *et al*., 2001).

2.9.4 Applications of marker assisted selection in rice

Tremendous effort has been expended into creating MAS and genetically improved rice types since rice output must be increased to meet rising demand. Farmers already have access to the rice types created by the use of MAS (Sundaram *et al*., 2008;

Krishnan *et al*., 2008). Numerous biotic and abiotic stresses in rice result in considerable yield loss. Many genes that may be effectively used in MAS to create superior varieties specific to the needs of the area have been recognised among the biotic stresses.

Neeraja *et al*. (2007) employed marker assisted backcross breeding method to create rice cultivars that were submergence tolerant. In backcrosses between a submergence-tolerant donor and the extensively cultivated recurrent parent Swarna, foreground, recombinant, and background selection, respectively, molecular markers closely connected with *Sub*1, flanking *Sub*1, and unlinked to *Sub*1 were utilised. A BC_3F_2 double recombinant plant that was homozygous for all Swarna type alleles except from a region between 2.3 and 3.4 Mb around the *Sub*1 gene was found. The outcomes demonstrated that in three backcross generations, the giant variety Swarna could be successfully transformed into a submergence-tolerant variety.

Table 1. Blast resistance genes and tightly linked markers in rice

Gene	Chr.	Tightly linked marker	Type	Reference
Pit	1	t311, t256, t8042	SNP	Hayashi *et al.*, 2006
Pi27(t)	1	RM151, RN259	SSR	Sallaud *et al.*, 2003
Pitp(t)	1	RM246	SSR	Barman *et al.*, 2004
Pi35(t)	1	RM1216, RM1003	SSR	Nguyen *et al.*, 2006
Pi37	1	RM302, RM212, FPSM1, FPSM2, FPSM4	SSR	Lin *et al.*, 2007
Pid1(t)	2	RM262	SSR	Chen *et al.*, 2004
Pitq5	2	RG520, RZ446B, RZ446A, RG654, RG256	RFLP	Zhou *et al.*, 2004
Piy1(t)	2	RM3248, RM20	SSR	Fukuta *et al.*, 2004
Piy2(t)	2	RM3248, RM20	SSR	Fukuta *et al.*, 2004
Pib	2	b213, b28, b2, b3989, Pibdom	SNP	Hayashi *et al.*, 2006
Pi16(t)	2	Amp-1	Isozyme	Pan *et al.*, 1996
Pi-Da(t)	2	RM5529, RM211	SSR	Lei *et al.*, 2005
Pi21	4	P702D03-#79	STS	Pan *et al.*, 1998

Pi39(t)	4	RM3843, RM5473	SSR	Shinoda *et al.*, 1971
Pi5(t)	4	RG788, RG498	RFLP	Wang *et al.*, 1994
Pi26(t)	5	RG313	RFLP	Ahn *et al.*, 1996
Pi10	5	OPF62700	InDel	Wu *et al.*, 2005
Pi26	6	K17, K2123	RFLP	Ahn *et al.*, 1996
Pi27(t)	6	Est-2	RFLP	Nguyen *et al.*, 2006
Piz-5	6	BS2-Pi9, NBS4-Pi9	STS	Deng *et al.*, 2006
Piz	6	z4794	InDel	Hayashi *et al.*, 2006
Piz-t	6	z4794	InDel	Deng *et al.*, 2006
Pi25(t)	6	A7-RG456	RFLP	Ahn *et al.*, 1996
Pid2	6	CAPS1, CAPS8	CAPS	Chen *et al.*, 2006
Pigm(t)	6	C26348	CAPS	Naqvi and Chattoo., 1996
Pitq1	6	RZ682, C236, RG653, RZ508	RFLP	Zhou *et al.*, 2004
Pi13(t)	6	Amp-3	Isozyme	Pan *et al.*, 1996
Pi13	6	R2123, R538	RFLP	Fjellstrom *et al.*, 2006
Pi2(t)	6	RG64	RFLP	Yu *et al.,* 1991
Pi2-2	6	AP5659-3, RM19817	SSR	Ballini *et al.*, 2008
Pi50(t)	6	GDAP51, GDAP16	InDel	Jiang *et al.*, 2012
Pi40(t)	6	RM3330, RM527 S2539	SSR, CAPS	Jeung *et al.*, 2007
Pi59(t)	6	RM19835	SSR	Zhou *et al.*, 2006
Pi33	8	RM72, RM44	SSR	Pan *et al.*, 1995
Pizh/Pi1(t)	8	RZ617	RFLP	Berruyer *et al.*, 2003
Pi29(t)	8	RZ617, RGA-IR86	RFLP	Nguyen *et al.*, 2006
Pi5(t)	9	94A20r, 76B14f, 40N23r	CAPS	Mackill and Bonman, 1992
Pi56(t)	9	RM24022	SSR	Jeon *et al.*, 2003
Pi28(t)	10	RZ500	RFLP	Nguyen *et al.*, 2006
PiCO39(t)	11	RGA8, RZ141, RGACO39	CAPS	Kwon *et al.*, 2008

Pilm2	11	L457B, G2132b, RZ536, RG1109	RFLP	Zhou *et al.*, 2004
Pi30(t)	11	OpZ11-f, RGA-IR14	RFLP	Nguyen *et al.*, 2006
Pi44(t)	11	AF348, AF349	AFLP	Chen *et al.*, 1996
Pikh/Pi54	11	RM206, RM144, RM1233	SSR	Fjellstrom *et al.*, 2006;
Pi1	11	CRG11-7, K28, RM224	SNP, SSR	Prasad *et al.*, 2009, Fuentes *et al.,* 2008.
Pi7(t)	11	RG103A, RG16	RFLP	Wang *et al.*, 1994
Pikm	11	k6861, k2167 RM254, RM144	InDel SSR	Li *et al.*, 2007
Pi18(t)	11	RZ536	RFLP	Li *et al.*, 2007
Pik	11	k6816, k2167	InDel	Hayashi *et al.*, 2006
Pik-p	11	k641, k39575, k403, k3957	SNP	Hayashi *et al.*, 2006
Pik-s	11	RM144, RM224, RM1233	SSR	Fjellstrom *et al.*, 2006
Pi-1(t)	11	RM12331, RM224	SSR	Sharma *et al.*, 2005
Pi6(t)	12	RG869	RFLP	Yu *et al*., 1991
Pi31(t)	12	O10-800	RFLP	Nguyen *et al.*, 2006
Pi32(t)	12	AF6	RFLP	Nguyen *et al.*, 2006
Ipi(t)	12	RG241X	RFLP	Causse *et al.*, 1994
IPi3(t)	12	RG241X	RFLP	Causse *et al.*, 1994
Pi39(t)	12	39M6, 39M7	CAPS	Bryan *et al.*, 2000
Pi20(t)	12	RM1337, RM5364, RM7102	SSR	Liu *et al.*, 2007
PiGD-3(t)	12	RM179	SSR	Liu *et al.*, 2007
Pi4(t)	12	RG869, RZ397	RFLP	Nguyen *et al.*, 2006
Pi58(t)	12	RM27954, RM27933, RM3103	SSR	Zhou *et al.*, 2004

Sundaram *et al*. (2008) introduced three main BB resistance genes (*Xa21, xa13*, and *xa5*) into Samba Mahsuri using marker aided backcross breeding (MABB). To create homozygous BC_4F_2 plants with various combinations of BB resistance genes, a selected BC_4F_1 plant was selfed. High degree of resistance to the BB pathogen was shown by the

two and three gene pyramid lines. The three gene pyramided genotypes excelled Samba Mahsuri in terms of yield when subjected to BB infection. The yield as assessed by multi-location experiments was not affected while these lines maintained the superior grain and cooking attributes of Samba Mahsuri.

2.9.5 MAS in rice for blast resistance

Rice blast disease may be controlled most successfully and economically by identifying and using widespread resistance genes (Mackill and Bonman, 1992). Conventional methods make it difficult to transfer blast resistance genes to varied genetic backgrounds, whereas MAS enables more accurate early selection (Miah *et al*., 2013).

Crop development has made breeding lines with broad-spectrum resistance a top objective. Utilising the wide range of resistance genes the main goal of breeding should be for the control of blast disease. Blast resistance genes have been recently introduced by MAS into the Luhui 17, G46B, Zhenshan 97B, Jin 23B, CO39, IR50, Pusa1602 and Pusa1603 lines (Miah *et al*., 2013).

A blast-resistant cultivar, 5173 was used to identify the *Piz*-5 (chromosome-6) gene, which was then introduced into a susceptible variety, 'CO39', to create the iso-genic line C101A51 (Mackill and Bonman, 1992). Since it has been in use for over ten years, the *Piz*-5 continues to remain efficient over blast in many rice cultivating areas (Liu *et al*., 2002). The fact that *Piz*-5 gene imparts broad spectrum resistance to many isolates is one of the likely explanations for the longevity of *Piz*-5 mediated blast disease resistance (Gouda *et al*., 2012).

Three genes *viz*., *Pi*-1, *Piz*-5, and *Pita* for blast resistance were fine-mapped by Hittalmani *et al*. (2000). They discovered RFLP markers for these genes that were coupled. In the Philippines and India, researchers evaluated two and three gene combinations that were resistant to leaf blast, and the results showed that possible combinations comprising *Piz*-5 showing greater resistance than when it is present alone.

Liu *et al*. (2002) performed fine-scale mapping for the two blast resistance (R) genes *Pi*9 (t) and *Pi*2 (t) to better understand the molecular mechanism of broad-spectrum resistance to rice blast. The *Pi*2 (t) and *Pi*9 (t) plants were inoculated with 43 blast isolates gathered from 13 different countries in order to investigate the resistance range of these R genes. *Pi*2 (t) carrying lines were resistant to 36 isolates, while *Pi*9(t) bearing lines were extremely resistant to every isolate examined, demonstrating the two gene broad-spectrum resistance to many blast isolates. According to their fine-scale data, a 100 kb area in which *Pi*9 (t) and *Pi*2 (t) are located either allelic or closely related.

Elite indica rice variety IR50 was strengthened by Narayana *et al*. (2002) against blast and BB. This was the first publication describing the molecular breeding technique of MAS and conversion to stack two important genes (*Piz*-5 + *Xa21*) in rice. The *Piz*-5 was introduced *via* MAS utilising the process of backcrossing, and the *Xa21* was delivered utilising particle bombardment to undergo genetic transformation. Under typical greenhouse circumstances at IRRI, the recurrent parent line responses to the rice blast isolates IK81-3 and IK81-25 were discovered to be very resistant, and the majority of the isolines with *Piz*-5 displayed reduced infection. In the blast nursery, 5-10 per cent of the leaf area was found to be infected under natural circumstances.

Eight blast resistant isogenic lines were produced in the commonly used rice variety Koshikari by Ishizaki *et al*. (2005). For blast and other agronomic parameters, they examined the isogenic lines. Koshikari isogenic lines had lower leaf and panicle blast scores than Koshikari. At a 1 per cent level for culm length, leaf blast score, panicle blast score, 1,000 grain weight, and grain quality, and a 5 per cent level for overall eating quality, significant variations between the isogenic lines were found. As a result, they recommended using Koshikari isogenic lines that are blast resistant for all-purpose farming.

The three SSR markers AP-5930, AP5659-3, and AP5659-5 were reported by Fjellstrom *et al*. (2006). *Piz*-5 resistance response co-segregated with the marker, AP005659. Zhou *et al*. (2006) used a map-based cloning approach to clone this gene, and

observed that it encodes a protein which consists of a leucine-rich repeat domain and a nucleotide-binding site. Incorporating these genes in long-term durable resistance-focused blast resistance breeding programmes will assist in reducing disease during rice production.

A map-based cloning technique was used by Lee *et al*. (2009) to clone the resistance (R) gene in order to comprehend the molecular basis of *Pi*5 mediated resistance to *M. oryzae*. The *Pi*5 locus was reduced to a 130-kb interval by genetic and phenotypic analysis of 2014 F_2 offspring from a mapping population obtained from a hybrid between IR50, a sensitive rice cultivar, and the RIL260 line harbouring *Pi*5. Two potential genes, *Pi5*-1 and *Pi*5-2, that encode proteins with three patterns typical of R genes an N-terminal coiledcoil (CC) motif and an NBS-LRR motif were discovered by sequence analysis of this genomic area. Their examination of gene expression showed that *Pi*5-2 is expressed constitutively whereas *Pi*5-1 transcripts increase in response to pathogen exposure. These findings showed that rice *Pi*5 mediated resistance to *M. oryzae* depends on the existence of these two genes.

Telebanco-Yanoria *et al*. (2010) created 20 rice NILs with 11 blast resistance genes by repeatedly backcrossing 19 donor varieties with the susceptible Lijiangxintuanheigu japonica-type variety (LTH). Using an unique avirulent blast isolate for each desired resistance gene, the resistant plants were chosen in each backcross generation. SSR markers that were positioned inside the chromosomal locations where the resistance genes had previously been mapped provided additional evidence that the targeted resistance genes had been incorporated. Genome analyses utilising SSR markers revealed that the NILs' genetic make-up was more closer to that of the LTH, the recurrent parent. These NILs, which have the genetic make-up of a variety resembling a japonica, were anticipated to be useful genetic resources for research and breeding initiatives aimed at developing blast resistance.

In All India Coordinated Plant Pathology experiments conducted over time and in various places in India, C101A51 containing the *Pi*2 gene was reported to show broad range disease resistance (Madhavi *et al*, 2011).

By employing MAS, Korinsak *et al*. (2011) created 83 BC_3F_2 backcross introgression lines (BILs) from a cross between the broad-spectrum blast resistant variety IR68835-98-2-B-2-1-1 and the susceptible variety KDML105.

Madhavi *et al*. (2011) enhanced Samba Mahsuri (B95-1) using MAS by incorporating blast and bacterial blight resistance in rice varieties. The susceptible recurrent parent was crossed with the donor line C101A51 harbouring the *Pi*-2 (t) gene. A pyramided line called 'Improved Samba Mahsuri' has three BB resistance genes. 248 plants were phenotyped with a highly pathogenic isolate of *M. grisea* (DRR isolate) in a homogeneous blast nursery and *X. oryzae pv. oryzae* in pot experiment investigations, plants bearing the *Pi*-2 and Xa21+ xa13+ xa5 genes were selfed and progressed to an F_2 generation. Results indicated that among 248 plants 188 were resistant and 60 susceptible.

In the backdrop of the indica type rainfed lowland superior rice cultivar IR-49830-7-12-2, Koide *et al*. (2011) created a series of NILs which targets nine genes for blast resistance by recurrent backcrossing. Reaction profiles to blast isolates, allelism tests, and DNA marker analyses all supported the introgression mechanism of each resistance gene in every NIL. These improved NILs might be employed to create a multiline variety that is ideal for tropical lowlands that get rain.

Selvaraj *et al*. (2011) investigated the genetic factors of variability, correlation, and path coefficient investigations for grain yield and certain other yield characteristics among rice genotypes that were resistant to the rice blast disease. The results of phenotypic correlation coefficient (PCV) were marginally higher than those of genotypic correlation coefficient (GCV), indicating minor environmental effect on the manifestation of character. The number of tillers per plant, followed by the number of productive tillers

per plant, plant height, and grain production per plant, all showed strong heritability along with high genetic progress and high GCV. Grain yield was significantly positively correlated with traits including plant height, number of productive tillers per plant, number of tillers per plant, panicle length, full grains per panicle, and test weight. The resistance genes *Pi*9, *Pi*2 and *Piz-t* were identified from the *Pi*2/9 region on chromosome 6 and provide broad-spectrum resistance to various *M. oryzae* isolates (Jiang *et al*., 2012).

Improved Samba Mahsuri cultivar was subjected to an effort by Madhavi *et al*. (2012) to introduce the blast resistant gene *Pi-kh* from donor source Tetep. Molecular markers used to screen the segregating populations were PTA248 and RM206 for bacterial blight resistance gene Xa21 and the blast resistance gene *Pi-kh* respectively. The resistant progenies that resulted from screening were then examined using phenotypic assays with the bacterial blight and blast. This study showed how molecular markers may be successfully used to introduce a dominant blast gene (*Pi-kh*) into a rice cultivar called Improved Samba Mahsuri that already possessed genes for resistance to bacterial blight.

In PRR78 which is a restorer line of the Pusa RH-10 Basmati rice hybrid, MAB with *Pi*-1 and *Piz*-5 genes conferred resistance to rice blast (Gouda *et al.*, 2012). Using MABB, two dominant, broad-spectrum resistance genes for blast disease, *Pi*-1 and *Piz*-5, from the CO39 pyramid were introduced into the PRR78 genome. Foreground selection was employed to create introgression lines using the microsatellite markers RM5926 and AP5659-5, which are intimately connected to the *Pi*-1 and *Piz*-5 genes, respectively. Along with PRR78, these lines were also assessed for characteristics related to cooking quality, disease response, and agronomic performance. For all the features assessed, with the exception of gelatinization temperature, majority of the improved lines were comparable to PRR78. Additionally, an RPG investigation found similarities between these lines and PRR78. In terms of yield, hybrids created with enhanced PRR78 lines outperformed Pusa RH10.

A rice restorer line called R8012 that is resistant to blast and bacterial blight was attempted to be bred by Xiao-deng *et al*. (2012). They were able to develop the top

restorer line, R8012, *via*., MAS, which had all four target genes (*Pi*25+ *Xa*21+ *Xa*13+ *Xa*5). In comparison to commercial checks, the hybrid Zhong 9A/R8012 developed from the chosen line demonstrated greater blast and BB resistance as well as better grain production. In order to demonstrate that MAS is a workable approach for efficiently pyramiding numerous resistance genes, this study offers a classic example.

Jiang *et al*. (2012) improved the blast resistance of Jin 23B and its descended hybrids, Jinyou 402 and Jinyou 207, by introducing the three major blast resistance genes *Pi*1, *Pi*2, and D12. Using natural inoculation techniques in a disease nursery, the enhanced Jin 23B lines were assessed for their resistance against rice blast. The findings demonstrated that the improved Jin 23B and hybrids possessed resistance to rice blast increased with the number of genes they contained. *Pi*1, *Pi*2, and D12 also demonstrated a strong dosage effect on blast resistance in the hybrid background during the whole growth period in field conditions, which was very helpful for developing blast resistant hybrid varieties. When there was no disease stress, examination of agronomic features revealed that the enhanced Jin 23B and its derivative rice hybrid were taller than that or identical to controls.

Pinta *et al*. (2013) employed MAS to introduce blast and bacterial leaf blight resistance genes into the high-quality, well-liked, and vulnerable RD6 rice cultivar, which is grown by many rice farmers in North and Northeast Thailand. It was possible to validate the genes for the blast and bacterial leaf blight using artificial inoculation in a greenhouse. Twelve pyramided lines were found after screening introgression lines harbouring five quantitative trait loci (QTLs) in the BC_2F_2 generation using five flanking sets of SSR markers. While in BC_2F_2 (2-8-236) revealed the greatest degree of bacterial leaf blight resistance with a high blast broad spectrum resistance (BSR), two plants displayed higher levels of BSR.

In order to add three blast resistance genes *Pi*1, *Pi*2, and *Pi*33 to a well-known susceptible rice cultivar ADT43, Divya *et al*. (2014) used MABB. Four lines from the BC_3F_1 generation were found to be introgressed with three genes and were analysed for

background information. Up to 95% of the genome was recovered, and these lineages were carried on to new generations. The features for yield, morphology, and grain quality were remarkably similar to those of ADT43. In two epiphytic localities, Coimbatore and Gudalur, blast nursery screening was conducted on pyramided lines with triple gene combination as opposed to genotypes containing single genes and susceptible checks.

Using the marker aided backcross breeding technique, Kumari *et al*. (2014) aimed to introduce BB and blast resistant genes into the short duration, high yielding, long slender rice variety MTU 1010. For *xa*13 and *Xa*21, Improved Samba Mahsuri was utilised as a donor, while NLR 145 was used as a donor for the *Pi*54 gene. Pyramided lines that were self-crossed and F_3 lines that were interconnected had their blast resistance tested. Agro-morphological traits of 14 lines with the three gene and the two gene combinations exhibited resistance to BB and blast were examined. In addition to demonstrating resistance to both BB and blast, line ICF3-16-59 demonstrated statistical parity with MTU 1010 in terms of yield and yield-related features. Regarding yield and features that are relevant to yield, ICF3-16-235 line showed a clear advantage over MTU 1010.

Ming *et al*. (2018) utilized MAS strategy to introgress two genes *Pi46* and *Pita* conferring resistance to blast along with *Wxb* gene which governs the trait endosperm amylose content (AC) in rice, into R8166 an superior indica restoring line having poor grain quality and also little resistance to blast disease. Eight improved lines were identified which are having 88.68 to 96.23% recurrent parent genome recovery among which two lines R163 and R167 were selected for further analysis. The improved line R167 showed the highest recovery ratio that is 96.23% was on par with recurrent parent with respect to agronomic traits whereas line R163 which exhibited lowest recovery ratio (88.68%) showed significant difference in characters like heading date and yield compared to recurrent parent. Hybrids derived from R163 and R167 expressed high resistance and grain quality traits *viz*., amylose content (AC), chalky grain rate (CGR),

gel consistency (GC) and degree of endosperm chalkiness (DEC). The study revealed efficiency of MAS in developing elite restoring lines.

Rosalyan *et al*. (2020) employed MABB to introgress blast resistance gene *Pi21* from the donor Sensho to both IR63307-4B-13-2, an indica breeding line as well as kinandang patong, a tropical japonica line. By using genotyping strategy in BC_4F_3 and BC_4F_4 generations, 192 improved lines carrying *Pi21* gene were identified by using specific InDel marker. Thirteen lines were randomly selected and checked for resistance by artificial inoculation of blast isolates under greenhouse conditions and also for characters like heading date, panicle length, primary branches per panicle and total grains per panicle. Results revealed that all the 13 lines selected were showing resistance to 11 blast isolates and maintained the characters of the reccurent parent indicating that *Pi21* gene can be deployed in rice cultivars.

Singh *et al*. (2021) utilised marker assisted forward breeding (MAFB) coupled with a simultaneous crossing approach to combine three drought tolerant quantitative trait loci (QTL) $qDTY_{1.1}$, $qDTY3_{.1}$ and $qDTY_{12.1}$, four BLB genes *Xa4, Xa5, Xa13* and *Xa21* and one blast resistance gene, *Pi9* in elite rice cultivar Lalat. At F_7 and F_8 generations, the introgression lines (ILs) created in the study were phenotypically evaluated for drought, BLB and blast resistance. The yield advantage of ILs with major-effect QTL (*qDTY*) over elite parent Lalat during the reproductive stage (RS) drought stress varies from 9 to 124 Per cent in DS2019 and from 7 to 175 per cent in WS2019. The ILs selected had blast scores of 1 to 3 and they were extremely resistant to BLB, with lesion lengths of 1.3 to 3.0 cm. Grain quality was examined in ILs that had yields comparable to or greater than Lalat and were tolerant to RS drought, resistant to BLB and resistant to blast disease. The grain quality features of six ILs, including hulling, milling, head rice recovery (HRR), chalkiness, alkali spreading value (ASV) and amylose concentration, were found to be comparable to those of Lalat (AC).

Feng *et al*. (2022) introduced *Pigm* gene into two geng rice cultivars (Wuyungeng 32/WYG32 and Huageng 8/HG8) using backcross and marker-assisted selection (MAS).

Five advanced backcross lines (ABL) with *Pigm* and the identical genotypes as the corresponding recurrent parent in the other 13 known R gene loci were established in each genetic background. All ABLs exhibited higher resistance in seedling inoculation assay carried out using 184 isolates collected from regions of Yangtze River. Regarding panicle blast resistance, all ABLs achieved great levels of resistance to the disease in experiments conducted over three years with the inoculation of a total of 7 mixed conidial suspensions gathered from various parts of Jiangsu province. The ABLs demonstrated noticeably more resistance than the recurrent parents in natural field nursery testing.

Materials and Methods

3. MATERIALS AND METHODS

Aiming to confer resistance to Kerala rice varieties Jyothi and Kanchana against blast disease, the varieties were hybridized with donor 'Parambuvattan (PTB7)' having two genes for blast resistance *Pi*-1 and *Pikh* under the programme entitled 'Incorporation of blast resistance into Jyothi and Kanchana rice (*Oryza sativa* L.) varieties through marker assisted breeding'. The present programme was conducted in the Department of Plant Breeding and Genetics, College of Agriculture, during 2018-2022 using Jyothi, Kanchana and Parambuvattan (PTB7) as parents.

The study comprised of three major experiments.

I) Parental polymorphism study
II) Development of F_1
III) Screening of F_1 seedlings by molecular markers and artificial inoculation

The details of the materials used and methods in the present programme are presented below.

3.1 Brief description of the experimental site

The experimental site was located at the College of Agriculture (COA), Kerala Agricultural University, Vellanikkara, Thrissur 680 656, Kerala, 40m above MSL between 10º 31'N latitude and 76º 13'E longitude and experiencing humid tropical climate. The laboratory and field facilities under Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara, Thrissur were used for the study.

3.2 Experimental materials

3.2.1 Plant materials

3.2.1.1 Recurrent parents

a) Jyothi (PTB 39)

Parentage : (Ptb10 x IR8)
Duration : 115-120 days

Bran color and grain type : Red long and bold

Characteristics : High yielding and susceptible to blast disease of rice

b) Kanchana (PTB 50)

Parentage : (IR36 x Pavizham)

Duration : 105-110 days

Bran color and grain type : Red long and bold

Characteristics : High yielding and susceptible to blast disease of rice

3.2.1.2 Donor

Parambuvattan (PTB7)

The traditional rice variety of Kerala having two genes for blast resistance *Pi-1* and *Pikh* with closely and tightly linked microsatellite markers. The duration of crop is 120 days.

3.2.2 Molecular markers used

DNA markers which are closely linked to the blast resistance genes *viz., Pi*-1 and *Pikh* were used based on the previous studies. The sequence and annealing temperature of the molecular markers are given in Table 2. For *Pi*-1 and *Pikh* genes, RM224 and RM206 markers located at genetic distance of 0.0 cM and 0.7 cM, respectively, from the genes on chromosome 11 was used.

Table 2. Details of markers used for evaluation of blast resistance which are linked to resistance genes

Gene	Marker	Sequence	Annealing temp.(Tm)	Marker type
Pi-1	RM 224	F: ATCGATCGATCTTCACGAGG	63.2°C	SSR
		R: TGCTATAAAAGGCATTCGGG		
Pikh	RM 206	F: CCCATGCGTTTAACTATTCT	62.6°C	SSR
		R: CGTTCCATCGATCCGTAT		

Plate 1. Seed material of plant varieties used in the study

3.3 Methods

3.3.1 Experiment I: Parental polymorphism study

Parental polymorphism was analysed for Jyothi, Kanchana and Parambuvattan (PTB7) rice varieties using reported SSR markers RM224 and RM206 which are tightly linked to blast resistant genes *Pi*-1 and *Pikh*.

3.3.1.1 Marker assisted selection

3.3.1.1.1 Laboratory chemicals, glassware and equipment

The AR (analytical agents) grade chemicals (extra pure) and markers from Sisco Research laboratories (SRL) were used in this study. The constituents for PCR mixture like Taq buffer, dNTPs, $MgCl_2$, Taq DNA polymerase, *etc*. used in the present programme were purchased from Genei Pvt. Ltd., Bangalore. The plastic wares used were from Tarsons India Pvt Ltd. The SSR (Simple sequence repeats) primers manufactured by Sigma Aldrich Chemicals Pvt. Ltd., Bangalore were used and RNase was supplied by Thermo Scientific.

The present programme was carried out using the facilities in molecular biology lab available at Department of Plant Breeding and Genetics. High-speed refrigerated centrifuge (Eppendorf 5804 R) was used for centrifugation. NanoDrop spectrophotometer (Jenway- Genova Nano) was used for the assessment of DNA quality and quantity. For DNA amplification, Eppendorf Mastercycler® nexus gradient PCR machine was used and for carrying out Agarose gel electophorosis horizontal gel electrophoresis units by Bio- Rad, USA and Tarson India Ltd.

3.3.1.2 Isolation of genomic DNA and assessment of quality and quantity of extracted DNA

Total cellular DNA (deoxy ribonucleic acid) of parents and F_1 hybrids was extracted to determine their quality and quantity.

3.3.1.3 Extraction of genomic DNA

Young leaves were collected from the actively growing tip of plants early in the morning. The collected leaves were labeled, covered with aluminium foil and immediately taken to the laboratory after placing in an ice box in order to prevent deterioration. Leaf surface was cleaned by washing with sterile distilled water followed by wiping with 70 per cent ethanol. The samples were weighed and stored at -20ºC until used for DNA extraction.

Reagents used

1. CTAB buffer

Table 3. Composition of CTAB buffer

Contents	Concentration	Quantity for 100 ml
CTAB (W/V)	2%	2 g
Nacl	1.4 M	8.1816 g
EDTA (pH 8)	20 mM	7.445 g
Tris base (pH 8)	100 mM	1.2214 g
Distilled water	-	100 ml

2. Chloroform – isoamyl alcohol (100 ml)

Chloroform – 96 ml

Isoamyl alcohol – 4 ml

3. 70% ethanol

Ethanol- 70 ml

Distilled water- 30 ml

4. Chilled isopropanol (100%)

Procedure

CTAB (Cetyl-Tri Methyl Ammonium Bromide) method was used for DNA extraction as reported by Murray and Thompson (1980).

The DNA extraction was done for parents Jyothi, Kanchana, Parambuvattan (PTB7) and F_1 hybrids. Leaf samples that are fresh, healthy and young were collected from 15-25 days old seedlings and utilised for extraction of genomic DNA.

1. The collected samples were ground in liquid nitrogen into fine powder by adding 500 µl extraction buffer (CTAB), pinch of PVP (Poly vinyl pyrrolidine), and 50µl β-mercaptho ethanol using sterile, pre-chilled mortar and pestle.
2. The extracted suspension was transferred to a 2 ml polypropylene tubes having 500 µl of pre-warmed (65ºC) DNA extraction buffer (CTAB).
3. Suspension obtained was incubated at 65ºC for 30 min in waterbath with intermittent shaking at 10 minutes interval.
4. The tubes were placed on supports and allowed to cool to room temperature. Next, a micropipette was used to add nearly equal amount of chloroform : isoamyl alcohol (24:1) to the suspension. The solution was blended using a spinner or by gently inverting the tubes.
5. The mixture was then stored for centrifugation, which took place at 4°C for 10 minutes at 10,000 rpm. To prevent DNA shearing, the supernatant after centrifugation was carefully transferred and put to a new tube with a wide diameter tip.
6. To complete the second wash, an equal volume of chloroform : isoamyl alcohol (24:1) was added.
7. The content was then kept for centrifugation at 10000 rpm for 10 minutes at 4ºC. The supernatant was carefully taken and transferred to a fresh tube with wide bore tip inorder to avoid DNA shearing.

8. RNase (10 mg/ml) was mixed to the DNA voil @ of 2 µl /100 ul of crude DNA. Mixture was incubated for 30 min at 37ºC in a water-bath with intermittent shaking.
9. To complete the third wash, an equal volume of chloroform : isoamyl alcohol (24:1) was added.
10. The content was then kept for centrifugation at 10000 rpm for 10 minutes at 4ºC. The supernatant was collected carefully and transferred to a fresh tube with wide bore tip to avoid DNA shearing.
11. Then, 0.6 volume of pre-chilled iso-propanol was added, followed by a gentle inversion until a fibrous mass was apparent. Tubes were incubated for 2 hours or overnight at -20ºC.
12. After incubation for 2 hours or overnight tubes were then put for centrifugation at 10000 rpm for 10 minutes at 4ºC to get a precipitate. The supernatant was drained by gentle inversion of tubes. Then the tubes were left inverted with lids open on blotting paper to drain the residual iso-propanol.
13. After that, the DNA pellet obtained was washed twice with 100% ethanol by keeping for centrifuge at 10000 rpm for 10 minutes at 4ºC and later kept for 15-20 minutes at room temperature for drying the pellet.
14. After drying of pellet 100µl of TE buffer (pH 8.0) or double distilled water was added to dissolve the pellet.

3.3.1.4 Determination of quality and quantity of isolated DNA

3.3.1.4.1 Assessing quality by agarose gel electrophoresis

Agarose gel electrophoresis was used to visualize and quantify the isolated DNA samples with the help of a ladder.

Reagents used

1. Agarose
2. 50X TAE buffer

3. Tracking dye

4. Ethidium bromide (0.5µg /ml stock)

Table 4. Composition of TBE buffer

Contents	Quantity for 1 L
Tris base	242 g
EDTA	18.61 g
Glacial acetic acid	57.1 ml
Distilled H_2O	1000 ml

Procedure

Agarose gel (0.8 per cent) was prepared by melting 0.8 g agarose in 100ml of 1X TAE buffer. The solution was allowed to cool to the temperature of 42-45 ºC. Later ethidium bromide was added at the rate of 8µl for 100 ml and mixed throughly. The gel casting tray was wiped with 70 per cent alcohol. The solution was poured into the preset, sealed gel casting tray later the comb was placed to a height of 3-5 mm. the solution was allowed to set for 25-30 minutes. The comb and sealing tapes were then removed and the tray was then transferred into electrophoresis unit. 1X TBE buffer was added to the tray till the gel is fully submerged. The DNA sample was diluted with millipore water in the ratio of 1:9. The DNA sample (5µl) was mixed with 3 µl of tracking dye and then added into the wells carefully using a micropipette near the negative terminal. A DNA ladder having 100 bp molecular weight was loaded in one of the wells as a standard marker for easy identification and interpretation of results. The cathode and annode of the electrophoresis unit were connected to the power supply and allowed to run for 45 minutes at 80 Volts until the tracking dye reaches 2/3rd of the gel. After electrophoration, the gel was carefully transferred to gel documentation unit (Gel Doc Fire Reader

Documentation System, UVITEC, Merck, UK) and observed under UV exposure. Presence of highly resolved high molecular weight thick bands near the wells indicates the presence of DNA. RNA contamination can be observed as presence of thick bands around 100 bp region while a thick white patch observed inside the well indicated the presence of protein. UVITEC Fire reader software provided by Merck, UK was used to analyse the electrophoresed agarose gel.

3.3.1.4.2 Assessing quality and quantity by NanoDrop Spectrophotometer

Further confirmation of the quality and quantity of the DNA isolated was analysed using Nanodrop Spectrophotometer (Jenway–Genova Nano). The maximum absorbance of nucleic acids and proteins takes place at 260 nm and 280 nm respectively. Absorbance of the DNA samples (1µl) was measured at wavelengths 260 nm and 280 nm. The purity of DNA was analysed based on the OD_{260}/OD_{280} ratio. A ratio between 1.8 to 2.0 indicates good quality DNA whereas, ratio greater than 1.8 indicates contamination with protein and greater than 2.0 indicates RNA contamination. The quantity of DNA sample was calculated based on the relation that Optical density (OD) for a DNA sample with a concentration of 50 µg/ml (double stranded) at 260 nm equals one.

i.e., 1 OD 260 = 50 µg/ml (ds)

Therefore, Quantity of DNA (µg/ml) = Absorbance at OD260 x 50

Procedure

1. Lid of the spectrophotometer was opened followed by sampling arm, to remove any dust particles present on the pedestal it was wiped with soft laboratory wipe.

2. In order to set the reading to zero blanking was done by using (DDH_2O)

3. Then, 1µl of the sample to be tested was loaded onto the pedestal and option measure was selected and the obtained readings were recorded.

4. The pedestal was then wiped with 70 per cent ethanol using a soft laboratory wipe after recording the observation.

3.3.3 Dilution of DNA for PCR

The recorded DNA quantity (ng/µl) values and OD values were used for further dilution of the DNA samples. The DNA with a concentration of 50 ng is apt for PCR reactions. Therefore, the obtained concentration of DNA was diluted to obtain 100 µl, 50 ng DNA per sample by using the formula $V_1N_1 = V_2N_2$

3.3.4 Dilution of primers

Primer stocks were diluted with millipore H_2O to give 1 M stock solution. Primer dilutions were done by dissolving primer and Millipore H_2O in 1:9 ratio.

3.3.5 Polymerase Chain Reaction

PCR assay was carried out for foreground selection by using markers linked to gene and background selection was done by genome wide markers. PCR reaction mix contained 50 ng of DNA, 10x PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM MgCl2), 2 mM dNTPs, 5 pmol each of forward and reverse primer and 3 U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India) in a reaction volume of 20 µl.

The desired number of PCR cycles, time, annealing temperature (AT) and extension time were standardised based on the primers used and conditions were programmed and saved in the PCR thermal cycler (model-1 Master cycler® nexus gradient PCR, Eppendorf). For amplication, PCR reaction was carried out in 0.2 ml thin walled flat caps consisting of the following components (Table 5).

Table 5. PCR reaction mixture

Reagent	Stock concentration	Aliquot	Final concentration
DNA	25 ng/µl	1 µl	25 ng
PCR buffer	10x	2 µl	1x
dNTP mix	2mM	1.8 µl	0.2mM
Forward Primer	5 pM	1 µl	
Reverse Primer	5 pM	1 µl	
Taq DNA polymerase	3 U/µl	0.3µl	0.6 U
Milli-Q water		12.9 µl	
Total	**20 µl**		

Table 6. PCR thermal regimes and conditions

Step	Reaction	Temperature	Time	Cycle
I	Initial denaturation	94ºC	4	1
II	Denaturation	94ºC	1	35
	Annealing	52-65 ºC	1	
	Extension	72 ºC	2	
III	Final extension	72 ºC	7	1
Iv	Cold storage	4 ºC	Infinity	

3.3.5.1 Resolution of amplified PCR products

The most used method for analysing nucleic acids and proteins is gel electrophoresis. In this case, we separated the amplified DNA fragments by size using the same method with 3 % (w/v) agarose. Agarose gel was prepared by weighing 4g Agarose powder and then dissolving it in 100 ml of 1xTAE [490 ml double distilled water + 10 ml of 50x TAE buffer (242.2g Tris base: Mwt. 121.14; 100 ml of 0.5M EDTA: PH 8.0;

57.1ml Glacial acetic acid: Mwt. 61.83; made vol. to 1000 ml using deionized Milli-Q water)] in conical flask. The produced suspension was warmed in a microwave oven at 900 watts for about 8-10 minutes to produce a clear solution. After the solution had cooled, 10 l of Ethidium Bromide stock solution (0.05 l/ml of 1x TAE, 10 mg/ml of double-distilled water) were added. The gel was gently put onto a gel casting tray after a little shake. The gel was placed in the gel tank after 20 to 30 minutes, and the tank was filled with 1x TAE (PH 8.0), making sure the gel tray was buried in TAE buffer. Each 20 µl PCR product had 4 µl of 6x loading dye (0.25 Per cent bromophenol blue, 0.25 Per cent xylene cyanol FF, and 40 Per cent sucrose) added to it, which was then well mixed using a spinner. Using pippetes, the PCR result was combined with dye and placed into each individual well. As a reference, a ladder of 100 bp size was included (Fermentas, Lithuania, USA). The power pack was set at 80 Volts/cm of run, and the electrophoresis took between 1 to 1.5 hours.

3.3.5.2 Gel documentation

Electrophoresed gel was documented using UVITECH fire reader software (Merck, UK) for proper visualisation of bands.

3.3.5.3 Scoring of primers for all genotypes

The documented gel profiles of all the individual SSR primers were carefully observed and scored the data for further analysis.

3.3.5.4 Molecular weight analysis

Molecular weight analysis of the obtained PCR images was done using MAX software, UVITECH Cambridge. In comparison to the known molecular weight of the marker, the location of amplicon position and its molecular weight were assessed.

3.3.6 Marker assisted foreground selection

The selection for target donor alleles *Pi-1* and *Pikh* in parents and F_1 hybrids was carried out with tightly linked microsatellite SSR markers RM224 and RM20

Table 7. Primer sequence of markers used for foreground selection (Gramene marker database)

Gene	Marker name	F/ R	Primer sequence	Chr.	Linkage distance
Pi-1	RM 224	F	5'-ATCGATCGATCTTCACGAGG -3'	11	(0.0cM)
		R	3'-TGCTATAAAAGGCATTCGGG -5'		
Pikh	RM 206	F	5'-CCCATGCGTTTAACTATTCT -3'	6	(0.7cM)
		R	3'-CGTTCCATCGATCCGTAT -5'		

3.3.7 Marker assisted background selection

For the recovery of recurrent parent genome in the segregating generations, polymorphic markers were identified by screening parents with the primers listed below.

Table 8. List of primers used for background selection (Gramene marker database)

Marker name	Forward/ Reverse	Primer sequence	Annealing temp. (ºC)	Chr.
RM9	F	GGTGCCATTGTCGTCCTC	64.8	1
	R	ACGGCCCTCATCACCTTC		
RM212	F	CCACTTTCAGCTACTACCAG	58.7	1
	R	CACCCATTTGTCTCTCATTATG		
RM302	F	TCATGTCATCTACCATCACAC	59.0	1
	R	ATGGAGAAGATGGAATACTTGC		
RM10346	F	GCTTGATCTGCCCTTGTTTCTTGG	70.8	1
	R	CAACGATGACGAACACAACC		
RM495	F	AATCCAAGGTGCAGAGATGG	59.1	1
	R	CAACGATGACGAACACAACC		
RM3340	F	TCTTGGCAAGCTCTCCTCTC	63.8	2
	R	CCATCATCTCGATCTTGACG		

RM7076	F	TGGTTCGATTCGGATTTC	60.2	3
	R	AAGCTATTCACAAGCAGCTC		
RM251	F	GAATGGCAATGGCGCTAG	64.1	3
	R	ATGCGGTTCAAGATTCGATC		
RM520	F	AGGAGCAAGAAAAGTTCCCC	55.1	3
	R	GCCAATGTGTGACGCAATAG		
RM518	F	CTCTTCACTCACTCACCATGG	59.0	4
	R	ATCCATCTGGAGCAAGCAAC		
RM163	F	ATCCATGTGCGCCTTTATGAGGA	59.0	4
	R	CGCTACCTCCTTCACTTACTAGT		
RM5749	F	GTGACCACATCTATATCGCTCG	58.0	4
	R	ATGGCAAGGTTGGATCAGTC		
RM26212	F	GTCGCTCCTCTCCTCCAATCC	68.4	4
	R	GCTCGCTGCTTCTAATCTCTTGC		
RM252	F	TTCGCTGACGTGATAGGTTG	64.0	4
	R	ATGACTTGATCCCGAGAACG		
RM280	F	ACACGATCCACTTTGCGC	65.3	4
	R	TGTGTCTTGAGCAGCCAGG		
RM164	F	TCTTGCCCGTCACTGCAGATATC	68.8	5
	R	GCAGCCCTAATGCTACAATTCTT		
RM169	F	TGGCTGGCTCCGTGGGTAGCTG	76.5	5
	R	TCCCGTTGCCGTTCATCCCTCC		
RM225	F	TGCCCATATGGTCTGGATG	62.8	6
	R	GAAAGTGGATCAGGAAGGC		
RM6836	F	TGTTGCATATGGTGCTATTTGA	62.8	6
	R	GATACGGCTTCTAGGCCAAA		
RM527	F	CAGTTCCGAGCAAGAGTACTC	63.5	6
	R	GGATCGGACGTGGCATATG		

RM5930	F	CATGAAAGAAAGGAGTGCAG	62.1	6
	R	CACGAATTGACCAGCCAAG		
AP5659-5	F	CTCCTTCAGCTGCTCCTC	60.3	6
	R	TGATGACTTCCAAACGGTAG		
RM85	F	CCAAAGATGAAACCTGGATTG	62.6	7
	R	GCACAAGGTGAGCAGTCC		
RM336	F	CTTACAGAGAAACGGCATCG	63.4	7
	R	GCTGGTTTGTTTCAGGTTCG		
RM223	F	GAGTGAGCTTGGGCTGAAAC	54.0	8
	R	GAAGGCAAGTCTTGGCACTG		
RM515	F	TAGGACGACCAAAGGGTGAG	56.0	8
	R	TGGCCTGCTCTCTCTCTCT		
RM447	F	CCCTTGTGCTGTCTCCTCTC	63.9	8
	R	ACGGGCTTCTTCTCCTTCTC		
RM5545	F	CAGCACTCCTCCCCTACCAG	65.0	8
	R	GGCTAAGTCAGCGTGAGACC		
RM256	F	GACAGGGAGTGATTGAAGGC	64.6	8
	R	GTTGATTTCGCCAAGGGC		
RM201	F	CTCGTTTATTACCTACAGTACC	58.1	9
	R	CTACCTCCTTTCTAGACCGATA		
RM242	F	GGCCAACGTGTGTATGTCTTC	63.8	9
	R	TATATGCCAAGACGGATGGG		
RM566	F	ACCCAACTACGATCAGCTCG	64.0	9
	R	CTCCAGGAACACGCTCTTTC		
RM6100	F	TCCTCTACCAGTACCGCACC	63.6	10
	R	GCTGGATCACAGATCATTGC		
RM258	F	TGCTGTATGTAGCTCGCACC	64.6	10
	R	TGGCCTTTAAAGCTGTCGC		

RM147	F	TACGGCTTCGGCGGCTGATTCC	76.4	10
	R	CCCCCGAATCCCATCGAAACCC		
RM3701	F	GAGCTAGAGGGAGGAGGTGC	64.5	11
	R	TTGACTGATAGCCGATTGGG		
RM552	F	CGCAGTTGTGGATTTCAGTG	64.2	11
	R	TGCTCAACGTTTGACTGTCC		
RM254	F	AGCCCCGAATAAATCCACCT	64.5	11
	R	CTGGAGGAGCATTTGGTAGC		
RM5926	F	ATATACTGTAGGTCCATCCA	53.2	11
	R	AGATAGTATAGCGTAGCAGC		
RM1233	F	AATAGGCCTGGAGAGAATTTCC	63.4	11
	R	CCTTATAAGCCGTCTCGATCC		
RM19	F	CAAAAACAGAGCAGATGAC	59.9	12
	R	CTCAAGATGGACGCCAAGA		
RM17	F	TGCCCTGTTATTTTCTTCTCTC	62.4	12
	R	GGTGATCCTTTCCCATTTCA		

3.3.3 Experiment II Development of F_1

Jyothi and Kanchana varieties of rice which are susceptible to blast disease were crossed with Parambuvattan (PTB7), a blast resistant variety by using it as male parent or pollen donor. Seed interval was calculated based on duration of the parents and staggered sowing was taken for synchronisation of flowering.

3.3.3.1 Hybridisation

The varieties Jyothi and Kanchana were crossed with donor Parambuvattan (PTB7) for blast resistance to develop F_1 hybrids. Hand pollination of the female spikelets, which were emasculated through clipping method.

3.3.3.2 Emasculation

Panicles of female parents (Jyothi and Kanchana) showing fifty to sixty per cent panicle emergence from the flag leaf were selected for carrying out emasculation. Later the leaf sheath was detached slightly from panicle inorder to expose the spikelets and for the purpose of emasculation. Emasculation was performed in the evening (after 4 pm) By cutting, the very immature florets at the foot of the panicle that had anthers that were shorter than half of the floret were eliminated. For performing emasculation, florets that are expected to open the following day (with the height of the anthers equal to or greater than half the florets) were chosen. To reveal the anthers, the top third of the chosen florets was removed with scissors. The anthers were then taken out carefully using the forceps tips prong by gently pressing them against the side of the floret and lifting out. The panicles which bare emasculated were bagged in butter paper bags, tagged and labeled with date**.** Later the butter paper bags were held securely in place by folding its bottom edge against the peduncle before tagging.

3.3.3.3 Pollination

During 8-10 am on the subsequent day of emasculation, panicles about to dehisce were selected from the male parent variety Parambuvattan (donor parent) and enclosed in a petridish. Pollen grains were collected by gently tapping the top of the petridish. The collected pollen grains were then transferred to the stigma of Jyothi and Kanchana (recurrent parents) with the help of a thin camel brush. The pollinated panicles were re-bagged to avoid contamination by foreign pollen. Seed set was checked on the sixth day after hybridisation. The harvested seeds were dried to moisture of 13 per cent.

3.3.4 Experiment III Screening of F_1 seedlings

3.3.4.1 Marker assisted screening

3.3.4.1.1 Foreground selection

Foreground selection was exercised on F_1 plants to identify heterozygous individuals by using gene based SSR markers RM224 and RM206 for the genes *Pi-1* and *Pikh*.

3.3.4.2 Artificial screening

3.3.4.2.1 Evaluation for resistance to blast

3.3.4.2.2 Culture preparation, inoculation and scoring for Blast disease

1. *Pyricularia orzae* stock isolates were revived from storage using pure agar slants and streptomycin at a concentration of 10 mg/250 ml of medium.

2. Mycelia from plants that were 7 days old were transferred and aseptically macerated in a tube with 10 ml of rice extract under aseptic conditions.

3. The resultant mycelia and conidia suspension was put onto 2 ml of oat meal agar each plate, and it was cultured for 7 days at 28ºC.

4. To encourage sporulation, the plates were parafilm-sealed and left at room temperature under constant illumination from fluorescent lights for 3-4 days. The plates were periodically inspected for growth.

5. The incubated plates were removed, 15-20 ml of distilled water were poured over them, and then conidia were gently scraped off from the medium's surface of medium and mixed with the suspension using a sterile glass slide.

6. Spore suspension from each plate was collected in a wide-mouth autoclaved container, and then it was filtered through guaze mesh and transferred to a new bottle.

7. A hemocytometer was used to count the number of spores present. This was determined by covering the hemocytometer counting chamber with a cover slip. A drop of the conidia suspension was pipetted into the V-shaped furrow using a micro-pipette, where it was allowed to be pulled into the chamber by capillary action. A volume of 10^{-4} ml was represented by one of the nine 1 mm squares. The conidia of a 1 mm square area were counted using a tally counter and a 10X objective. Per 1mm square region, there were more than 100 conidia. The formula used to determine the other four squares was C = n/v, where C is the conidia concentration expressed as conidia/ml, n is the average number of conidia per square mm, and v is the volume measured, which is 10^{-4}. So, C = $nx10^4$

8. Just prior to inoculation, Tween20 was added to the spore suspension @ 0.02 per cent.

9. Six seedlings were planted in each of the 5x4 plastic pro-trays four wells, each of

which had a diameter of 5 cm.

10. In accordance with recommendations, well-decomposed organic matter, N_2 and P_2O_5 were applied to the clean soil in the field one week prior to sowing.

11. To guarantee seedlings grew luxuriantly, pots were maintained in a suitable and healthy atmosphere. As and when necessary, intermittent watering was carried out. Seedlings that were 21 days old and in the third to fourth leaf stage were infected using the Bonman method (1986). Using a hand atomizer, seedlings were evenly sprayed (100Kpa). Each pro-tray received a spray of about 40 ml of spore solution for each fungus isolate.

12. The inoculated plants were moved to dew chambers and maintained at 25°C and 95–100% RH 13 for 24 hours in the dark. . The plants were then moved to a mist chamber and kept there for 6-7 days at a temperature of 25 to 28 °C with regular lighting.

14. Inoculated seedlings were observed on 6 and 10 DAI.

Observation

Disease scoring

Disease scoring was done according to standard disease scoring scale developed by International Rice Research Institute (IRRI) using a scale of 0-9 (Table 9).

Table 9. Scale for disease scoring of rice blast disease (IRRI, 2013)

Grade	Disease severity	Host response
0	No lesions observed	Resistant (R)
1	Small brown specks of pin point size or larger brown specks without sporulating centre	Resistant (R)
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are mostly found on the lower leaves	Resistant (R)
3	Lesion type same as in 2, but significant number of lesions on the upper leaves	Resistant (R)
4	Typical susceptible blast lesions, 3 mm or longer infecting less than 4 per cent of the leaf area	Moderately Resistant (MR)
5	Typical susceptible blast lesions, 3 mm or longer infecting less than 4-10 per cent of the leaf area	Moderately Resistant (MR)

Grade	Disease severity	Host response
6	Typical susceptible blast lesions, 3 mm or longer infecting less than 11-25 per cent of the leaf area	Moderately Susceptible (S)
7	Typical susceptible blast lesions, 3 mm or longer infecting less than 26-50 per cent of the leaf area	Susceptible (S)
8	Typical susceptible blast lesions, 3 mm or longer infecting less than 75 per cent of the leaf area	Susceptible (S)
9	More than 75 per cent of the leaf area affected	Susceptible (S)

Per cent Disease Severity (PDS)

Per cent Disease Severity will be scored according to standard disease scoring scale developed by International Rice Research Institute (IRRI).

$$\text{Per cent Disease Severity(PDS)} = \frac{\text{Sum of all numerical rating}}{\text{Total no. of seedlings observed x 9}} \text{ x } 100$$

3.4 Observations recorded

3.4.1 Morphological observations of parents

The parents selected for the present programme (Jyothi, Kanchana and Parambuvattan) were germinated on petriplates using germination paper on the same date. Later the germinated seedlings were transferred to tray. After 15 days the seedlings were transferred to pots and labelled using tags. The individual plants were evaluated for traits plant height, tillers, panicle number and length, days to flowering, sterile and filled grains, 100 seed weight and grain weight. The observations were recorded on the basis of individual plants.

1. Plant height (cm)

The height of the plant was calculated in centimetres (cm) from the plant base to the tip of the primary panicle.

2. Number of tillers per plant

For each plant, number of panicles at the time of harvest were counted and recorded. **3. Number of panicles per plant**

At the time of harvest, the number of tillers for each plant was counted and noted.

4. Panicle length (cm)

From the base to the tip of the primary panicle on each plant, the length was measured and recorded in centimeters (cm).

5. Days to flowering

The number of days taken by each plant from the date of sowing to first flowering was recorded.

6. Filled grains per panicle

For each plant, number of filled grains per panicle after the harvest were counted and recorded.

7. Sterile grains per panicle

For each plant, number of sterile grains per panicle after the harvest were counted and recorded.

8. 100 seed weight (g)

Total of 100 filled grains were taken and counted from 5 plants and weighed using electronic balance and means were expressed in grams (g).

9. Grain weight per plant

Total grains harvested from a plant were dried to optimum moisture levels and weighed on electronic balance. This was expressed as grams per plant.

3.4.2 Molecular observations

3.4.2.1 Quantity and quality of DNA isolated

Nanodrop was used to assess the quantity and quality of DNA isolated. Based on A260/A280 ratio purity of DNA samples was assessed. The ratio between 1.8-2.0 indicated pure DNA. Value greater than 2 indicates RNA contamination and less than 1.8 indicates contamination with protein.

3.4.2.2 Amplification pattern of markers

UVITECH Fire reader software gel documentation system was used for capturing the image as well as to analyse the band pattern of the amplicons resolved by gel electrophoresis.

Results and Discussion

4. RESULTS AND DISCUSSION

Rice is the staple food crop and also major source of carbohydrate for the people of Kerala. Supply of rice in the state is not able to meet the existing demand. Blast, the most devastating and destructive disease of rice, caused by *Magnoporthe oryzae,* is the most widespread in rice growing areas all over the world (Ou, 1985; Srinivasprasad *et al*., 2009). Rice crop is highly vulnerable to this pathogen in all the growth stages, starting from seedlings to maturity stage, thereby affecting leaves, nodes, collar, panicles and roots (Sharma *et al*., 2012). All over the world, rice blast disease causes 50-80 per cent yield losses depending on epidemic proportions and in India the yield loss is upto 54.3 per cent (Scardaci, 1997; Rajarajeshwari and Muralidharan, 2006).

The most effective strategy to combat this is development of resistant varieties (Gouda *et al*., 2012). It is beneficial economically as well as eco-friendly. Hence, development of blast resistant cultivars gained utmost importance in breeding programmes. Nearly 100 major genes which confer resistance to various strains of the pathogen *Magnaporthe oryzae* have been identified and successfully utilised in various rice breeding programmes. Incorporation of single major gene conferring resistance may not be effective as it may turn into susceptible in long run if it is cultivated on a large scale. Therefore, pyramiding of multiple genes conferring resistance to various strains of pathogens into a single cultivar by using marker assisted breeding strategy in order to ensure durability (Hospital *et al*., 1992; Hari *et al*., 2013).

Kush and Jena (2009) reported that introgression of resistant genes (R genes) is cost effective and environmentally sustainable method to control plant diseases. Therefore, many crop breeding programmes are involved in transfer of resistant genes into elite cultivars. Long term resistance is not provided by most of the R genes as most of the pathogens can eventually overcome them due to genetic flexibility (Krattinger *et al*., 2009; Lee *et al*., 2009 ; Skamnioti and Gurr, 2009 ; Liu *et al*., 2010). In order to ensure longevity in resistance, the ideal resistant (R) gene should have broad spectrum resistance and high durability. To develop resistance against various stains of *M. oryzae*

in elite cultivars, marker assisted selection strategy is very helpful which rely upon molecular markers (Jiang *et al*., 2012).

Breeding programmme utilising both phenotypic and genotypic markers are more accurate and fast. Development of resistant cultivars using conventional breeding is not precise, time consuming and require intensive work. Instead, we can use molecular approaches to incorporate the genes conferring resistance to *M. oryzae* (Lin *et al*., 1995).

Marker assisted selection (MAS) is an effective tool in breeding for rapid and precise selection of the target gene (Tanksley *et al*., 1989). MAS exhibits higher reliability due to its selective effects, that are independent of gene effects and environmental factors. MAS enables selection of genotype in early generation, therefore hastens the breeding programme (Chen *et al*., 2005). Utilization of DNA markers in marker assisted backcross breeding (MABB) increases the efficiency of selection to a greater extent (Collard and Mackill, 2008).

Huge number of molecular markers are available in various crops due to recent advancement in genomics field. Therefore, MAS can be efficiently employed in breeding programmes due to its consistency, reliability, target specificity in selection of complex traits and saving the time (Jena and Mackill, 2008).

The cultivar 'LAC23', an upland cultivar from Liberia with broad spectrum resistance to blast isolates of China (Chen *et al*. 2001 a and b), and isolates of southern India, was the first to carry the blast resistance gene Pi-1, which is located on chromosome 11. (Srinivasachary *et al*., 2002). *Pi*-1 gene was acknowledged as helpful for incorporation and pyramiding of *Pi*-1 gene into blast-susceptible rice cultivars due to its broad-spectrum resistance (Liu *et al*., 2003, Prasad *et al*., 2009). According to Hittalmani *et al*. (2000), the blast resistance gene *Pi*-1 is found on chromosome 11 and confers high levels of resistance to several *M. oryzae* races.

In the present study, presence of the gene *Pi*-1 was identified by using SSR primer RM224. Fuentes *et al*. (2008) conducted a study on identification of molecular

markers which are linked to *Pi*-1 and reported two SSR markers RM1233*I and RM224 that are mapped on the same position regarding to *Pi*-1 gene (0.00 cM).

Nickolas (2014) conducted a study on identification of donors for blast resistance from traditional rice varieties of Kerala using molecular markers reported that traditional rice varities Parambuvattan and Kavunginpoothala with gene combination *Pi*-1 and *Pikh* were exhibiting more resistance to races of *M. oryzae.* and they were having specific grain qualities of Kerala rice varieties. Therefore, popular high yielding varities which are susceptible to blast disease can be easily reconstituted by pyramiding the resistance genes *Pi*-1 and *Pikh.*

In the present study, Parambuvattan (PTB7) the traditional rice variety of Kerala carrying two genes *Pi*-1 and *Pikh* with dominant broad spectrum resistance genes was used as donor for transferring resistant genes to restorer lines Jyothi and Kanchana to ensure blast resistance in hybrids.

Jyothi (Ptb 39), one of the most popular high yielding rice variety of Kerala with very good cooking and nutritional qualities, is highly susceptible to blast disease. Similarly, Kanchana (Ptb 50), another high yielding variety suitable for both upland and wetland cultivation, is also highly susceptible to blast. Hence, this programme envisages transferring of blast resistance to Jyothi and Kanchana through marker assisted strategy using donor 'Parambuvattan', the traditional rice variety of Kerala carrying two genes for resistance *Pi*-1 and *Pikh* with closely and tightly linked microsatellite markers.

Popular rice varieties of Kerala, Jyothi and Kanchana, being susceptible to the blast pathogen, huge economic loss is incurred during their cultivation each year in the state by the farming community. Understanding the impact of blast disease on rice production and productivity, the present programme was planned to incorporate two blast resistance genes (*Pi*-1 and *Pikh*) into Jyothi and Kanchana varieties from the donor, Parambuvattan (PTB7), followed by the development of F_1 and screening of F_1 hybrid seedlings with artificial inoculation as well as molecular markers. It also aimed to

evaluate the morphological characteristics of parents and molecular characteristics of the F_1 hybrids thus generated.

Parents were raised in pots and F_1 hybrids were produced by hand pollination method. F_1 seedlings obtained from Jyothi and Kanchana were subjected to screening for blast resistance with artificial inoculation as well as molecular markers along with the respective parents. The results obtained from the study has been enumerated and discussed in this chapter.

4.1 Morphological characteristion of parents

4.2 Validation of genes for blast resistance in parental genotypes

4.3 Marker assisted background selection

4.4 Hybridisation between donor and recurrent parents

4.5 Screening of F_1 seedlings with artificial inoculation and molecular markers

4.1 Morphological characterisation of parents

The parents used in the present study Parambuvattan, Jyothi and Kanchana were evaluated for their agro-morphological characteristics. The results obtained are detailed below (Table 10, 11 and 12).

4.1.1 Plant height (cm)

The plant height of parents Jyothi, Kanchana and Parambuvattan was 86.4 cm, 83.1 cm and 89.5 cm respectively.

4.1.2 Number of tillers / plant

The number of productive tillers per plant in parents Jyothi, Kanchana and Parambuvattan was 29.6, 7.4 and 7.5 respectively.

4.1.3 Panicles / plant

The number of panicles per plant in parents Jyothi, Kanchana and Parambuvattan were 19.9, 6.2 and 5.7 respectively.

4.1.4 Panicle length (cm)

The panicle length in the recurrent parents Jyothi and Kanchana was 22.9 and 17.2 cm wheareas, in donor Parambuvattan it was 20.0 cm respectively.

4.1.5 Days to flowering

The average days to 50 per cent flowering, in the recurrent parents Jyothi and Kanchana was 87.0 and 80.8. Wheareas, in donor Parambuvattan it was 89.1 respectively.

4.1.6 Filled grains / panicle

The average number of filled grains per panicle recorded in parents Jyothi, Kanchana and Parambuvattan was 85.5, 86.8 and 73.0 respectively.

4.1.7 Sterile grains / panicle

The average number of sterile grains per panicle recorded in parents Jyothi, Kanchana and Parambuvattan was 15.1, 12.5 and 27.1 respectively.

4.1.8 100 seed weight (g)

The 100 seed weight of recurrent parents Jyothi and Kanchana was 2.7 and 2.7 g wheareas, in donor Parambuvattan it was 2.6 respectively.

4.1.9 Grain weight / plant (g)

Average grain yield per plant in Jyothi, Kanchana and Parambuvattan was 38.5, 12.4 and 9.1 g respectively.

Table 10. Morphological characters of Jyothi

JYOTHI									
Plant No.	**PH**	**T/P**	**P/P**	**PL**	**DTF**	**FG/P**	**SG/P**	**100SW**	**GW/P**
1	79.6	34	19	22.5	84	81	18	2.21	31.73
2	87.1	35	24	23.9	88	87	16	2.43	39.65
3	89.0	28	21	24.8	85	86	16	2.62	38.43
4	84.6	27	20	23.7	87	88	15	3.01	28.42
5	85.3	32	18	23.9	89	85	17	2.82	31.74
6	84.2	31	21	22.6	90	84	14	2.36	42.02
7	94.1	28	24	23.1	88	83	13	2.93	51.13
8	86.2	26	25	24.3	87	89	17	2.84	49.98
9	87.3	29	17	22.1	85	87	12	2.82	31.87
10	85.4	28	16	20.9	89	84	14	2.71	35.24
11	94.4	36	18	21.7	90	85	15	2.88	46.98
12	89.1	32	15	24.9	83	87	11	2.91	26.14
13	85.6	29	20	23.9	86	88	13	3.03	32.42
14	87.4	27	19	21.8	88	86	16	2.68	38.57
15	88.5	29	23	22.6	85	82	15	2.95	43.24
16	86.9	32	22	20.8	87	84	18	2.78	43.34
17	81.5	30	21	23.4	89	87	20	2.89	32.76
18	84.6	25	17	21.6	87	89	17	2.79	32.91
19	87.3	26	19	23.2	89	81	12	2.92	55.48
20	80.4	28	20	22.8	85	88	14	2.64	37.88
Average	**86.4**	**29.6**	**19.9**	**22.9**	**87.0**	**85.5**	**15.1**	**2.7**	**38.5**

Plant height (PH), tillers per plant (T/P), panicles per plant (P/P), panicle length (PL), days to flowering (DTF), fertile grains per panicle (FG/P), sterile grains per panicle (SG/P), 100 seed weight (100 SW), grain weight per plant (GW/P)

Table 11. Morphological characters of Kanchana

KANCHANA									
Plant No.	**PH**	**T/P**	**P/P**	**PL**	**DTF**	**FG/P**	**SG/P**	**100SW**	**GW/P**
1	80.2	7	7	16.9	78	85	15	2.67	12.75
2	83.7	8	7	18.2	81	87	10	2.85	11.63
3	81.2	6	5	15.7	80	85	9	2.58	7.97
4	79.8	9	8	19.1	82	88	11	2.63	14.82
5	84.5	8	6	17.8	79	85	12	2.72	11.91
6	86.3	9	7	16.7	80	87	16	2.81	14.28
7	81.9	7	6	17.5	81	86	13	2.64	11.98
8	82.8	7	5	15.9	79	84	14	2.72	10.65
9	79.9	9	8	17.9	78	87	11	2.87	16.72
10	85.3	8	8	18.4	82	84	10	2.65	14.23
11	83.4	6	5	16.8	81	88	15	2.84	13.93
12	82.6	9	7	17.7	82	90	12	2.82	14.99
13	85.7	7	5	18.2	78	88	13	2.71	10.55
14	82.9	5	4	16.5	79	86	15	2.74	8.28
15	83.7	8	7	18.5	81	85	11	2.69	13.89
16	84.3	5	4	16.8	82	84	16	2.81	7.64
17	82.8	8	6	17.3	78	89	13	2.89	11.98
18	83.4	9	8	17.4	79	89	10	2.78	16.56
19	86.5	8	6	16.3	81	91	11	2.81	13.28
20	81.4	6	5	15.9	80	88	13	2.83	9.86
Average	**83.1**	**7.4**	**6.2**	**17.2**	**80.0**	**86.8**	**12.5**	**2.7**	**12.4**

Plant height (PH), tillers per plant (T/P), panicles per plant (P/P), panicle length (PL), days to flowering (DTF), fertile grains per panicle (FG/P), sterile grains per panicle (SG/P), 100 seed weight (100 SW), grain weight per plant (GW/P)

Table 12. Morphological characters of Parambuvattan (PTB7)

PARAMBUVATTAN (PTB7)									
Plant No.	**PH**	**T/P**	**P/P**	**PL**	**DTF**	**FG/P**	**SG/P**	**100SW**	**GW/P**
1	91.4	7	6	21.3	90	74	26	2.58	8.46
2	87.2	8	6	20.9	89	73	25	2.61	9.52
3	88.9	6	4	19.8	86	71	27	2.67	5.56
4	92.3	9	7	21.2	88	73	28	2.62	10.99
5	87.8	6	5	20.3	87	75	29	2.55	8.68
6	90.2	7	6	18.9	89	72	30	2.57	8.58
7	89.6	8	5	19.4	90	74	27	2.56	8.72
8	86.8	9	7	20.2	91	73	28	2.54	11.67
9	88.8	9	6	21.1	88	71	29	2.63	10.77
10	90.7	8	7	17.8	89	70	24	2.59	10.85
11	91.7	7	5	19.7	88	72	25	2.65	7.49
12	87.9	5	4	20.5	89	73	27	2.53	6.51
13	89.5	6	4	21.7	90	74	28	2.63	6.63
14	91.8	9	6	16.9	91	76	25	2.57	8.42
15	90.7	5	3	21.9	88	72	26	2.74	4.56
16	86.6	9	7	20.6	91	74	25	2.76	11.63
17	93.1	7	6	19.5	89	76	29	2.65	10.77
18	86.9	9	7	20.5	89	71	27	2.58	10.75
19	87.6	8	7	17.6	91	72	28	2.67	12.69
20	91.5	8	6	18.7	90	74	29	2.55	8.86
Average	**89.5**	**7.5**	**5.7**	**20.0**	**89.1**	**73.0**	**27.1**	**2.6**	**9.1**

Plant height (PH), tillers per plant (T/P), panicles per plant (P/P), panicle length (PL), days to flowering (DTF), fertile grains per panicle (FS/P), sterile grains per panicle (SS/P), 100 seed weight (100 SW), grain weight per plant (GW/P)

4.2 Validation of genes for blast resistance in the parental genotypes

4.2.1 Isolation of genomic DNA

The plant materials used in the present study included traditional donor Parambuvattan (PTB7) having two major blast resistance genes *Pi*-1 and *Pikh* was collected from RARS (Regional Agricultural Research Station), Pattambi, KAU.

The recurrent parents Jyothi and Kanchana used in the study were collected from the Department of Seed Science and Technology, Kerala Agricultural University (K.A.U), Vellanikkara, Thrissur.

The genotypes collected were grown in the Kerala Agricultural University (K.A.U), College of Agriculture, Vellanikkara, field of Department of Plant Breeding and Genetics, Thrissur. DNA was extracted from young leaves (21 days old seedlings) from donor and recurrent parents using Qiagen Dneasy Mini Kit (Germany). The quality and quantity of the obtained DNA samples were checked by Agrose gel electrophoresis as well as Nano Drop Spectrophotometer.

4.2.2 Quantity and quality estimation of extracted genomic DNA

The concentration of extracted DNA of the recurrent parents Jyothi, Kanchana and donor parent Parambuvattan (PTB7) ranged as 675.15 µg/ml, 828.4 µg/ml and 676.2 µg/ml respectively.

The quality of DNA in these parents was estimated as 1.95, 1.94 and 1.91 respectively.

Table 13. Quantity and quality of DNA isolated

Individuals	Quantity of DNA (µg/ ml)			Quality of DNA(Ratio of A_{260}/A_{280})		
	Mean	Range		Mean	Range	
		Min.	Max.		Min.	Max.
Jyothi (Recurrent parent)	675.15	190.4	1159.9	1.95	1.82	2.09
Kanchana (Recurrent parent)	828.4	460.7	1130.5	1.94	1.83	2.07
Parambuvattan (donor parent)	676.2	166.1	1409.4	1.91	1.81	2.06

4.2.3 Dilution of DNA for PCR

The recorded values of DNA quantity (ng/µl) and OD were used for further dilution of the DNA samples. The DNA with a concentration of 50 ng is apt for PCR reactions. Therefore, the obtained concentration of DNA was diluted to obtain 100µl, 50ng DNA per sample by using the formula $V_1N_1 = V_2N_2$

Table 14. Dilution of DNA samples of parents

Sample	Concentration (µg/ ml)	DNA (µl)	DDH_2O (µl)
Jyothi	675.15	3.7	46.3
Kanchana	828.4	3.0	47.0
Parambuvattan	676.2	3.7	46.3

4.2.4 Dilution of primers

Primer stocks were diluted with millipore H_2O to give 1 M stock solution. Primer dilutions were done by dissolving primer and millipore H_2O in 1: 9 ratio.

4.2.5 Parental polymorphism study using PCR (Polymerase Chain Reaction)

DNA isolated from the parents was diluted and used for PCR amplification by utilising the specific microsatellite markers which are linked to the target gene. Two SSR markers linked to blast resistance genes were used for the identification of parental polymorphism between recurrent parents (Jyothi, Kanchana) and Donor (Parambuvattan) at molecular level. The markers used were RM224 for *Pi*-1 gene and RM206 for *Pikh* gene.

The amplification profile generated by the marker RM224 which is specific to the blast resistance gene *Pi*-1 revealed that the traditional donor Parambuvattan (PTB7) produced the PCR product at 148 bp whereas in the susceptible parents, Jyothi and Kanchana, it was at 170 bp respectively (Plate 3).

The amplification profile generated by the marker RM206 which is specific to the blast resistance gene *Pikh* revealed that Parambuvattan (PTB7) produced the PCR product at 146 bp whereas in the susceptible parents, Jyothi and Kanchana produced PCR product at 160 bp respectively (Plate 4).

The polymorphic SSR markers selected for our present study (RM224 for *Pi-1* and RM206 for *Pikh*) were able to differentiate between the recurrent parents and donor with the expected amplicon product sizes of 148 and 146 bp in resistant parent and the product size in susceptible parents are 170 bp and 160 bp respectively (www. gramene.org).

In the present breeding programme, SSR markers RM224 and that are tightly linked to *Pi*-1 and *Pikh* genes were used to assess parental polymorphism between the recurrent parents and donor.. RM224 and RM206 markers exhibited clear polymorphism between the genotypes which showed the size variation for the marker alleles for all the genotypes selected. Therefore, the markers selected for screening are appropriate. The SSR marker RM224 was chosen as it is mapped on the same position (0.0 cM) with target gene *Pi*-1. Similarly, marker RM206 was selected due to its close linkage and

mapping distance (0.7 cM) from *Pikh* gene position. Hence, the markers linked to target genes are recognized as valuable source for introgression into blast susceptible rice cultivars.

The blast resistant gene *Pi*-1 which is located on chromosome 11 was had broad spectrum of resistance against blast isolates of china (Chen *et al*., 2001 a, b) and also to isolates of southern India (Srinivasachary *et al*., 2002)

Selection of plants carrying the trait of interest in further generations is not possible unless the parents are polymorphic for the trait of interest. The SSR marker RM224 was selected as it is mapped in the same position (0.0 cM) with *Pi*-1 gene. It is recognised as valuable marker for pyramiding and introgression of *Pi*-1 gene into blast susceptible rice cultivars (Fuentes *et al*., 2008, Liu *et al*., 2003, Prasad *et al*., 2009). Nickolas (2014) had reported the presence of Pi-1 gene in Parambuvattan (PTB7).

Results obtained are similar to earlier reports where RM224 was used as foreground marker to incorporate *Pi*-1 gene (Suh *et al*., 2009, Divya *et al*., 2014 Fuentes *et al*., (2003); Fjellstrom *et al*., (2006); Sharma *et al*., (2010); Gouda *et al*., (2013); Harikrishnan (2017).

According to Guptha *et al*. (2011), the *Pikh* gene located on chromosome 11 imparts a high level of resistance to several strains of *M. oryzae*. According to Sharma *et al.* (2002) blast resistance gene *Pikh* may be successfully cloned, physically mapped, and used to combat disease strains in India's northwestern Himalayan region. SSR primer RM206 was used for checking the presence of the gene *Pikh.* Sharma *et al*. (2010) reported that RM206 which is a gene specific SSR marker is tightly linked to *Pikh* gene on chromosome 11. Nickolas (2014) had reported the presence of *Pikh* gene in Parambuvattan (PTB7).

Results obtained are similar to earlier reports of Harikrishnan (2017) where RM206 was used as forground marker to incorporate *Pikh* gene into Uma rice variety.

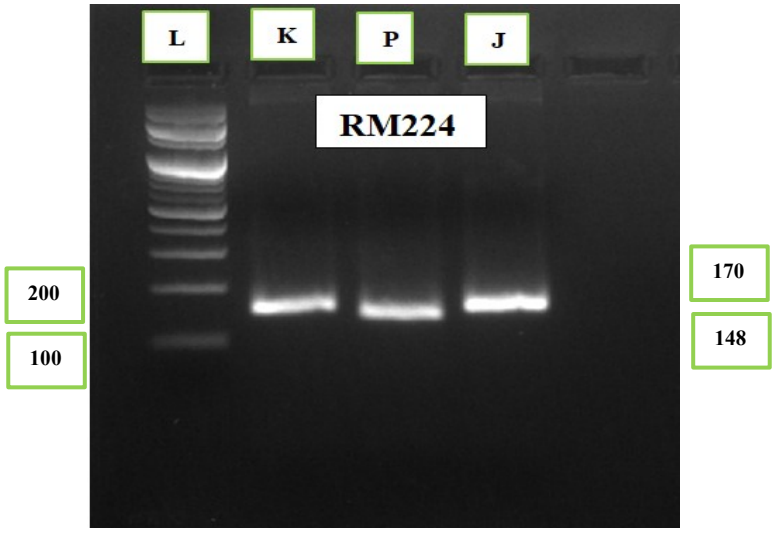


Plate 2. Validation of *Pi*-1 gene in donor parent (PTB-7) and recurrent parents Jyothi and Kanchana with marker RM224

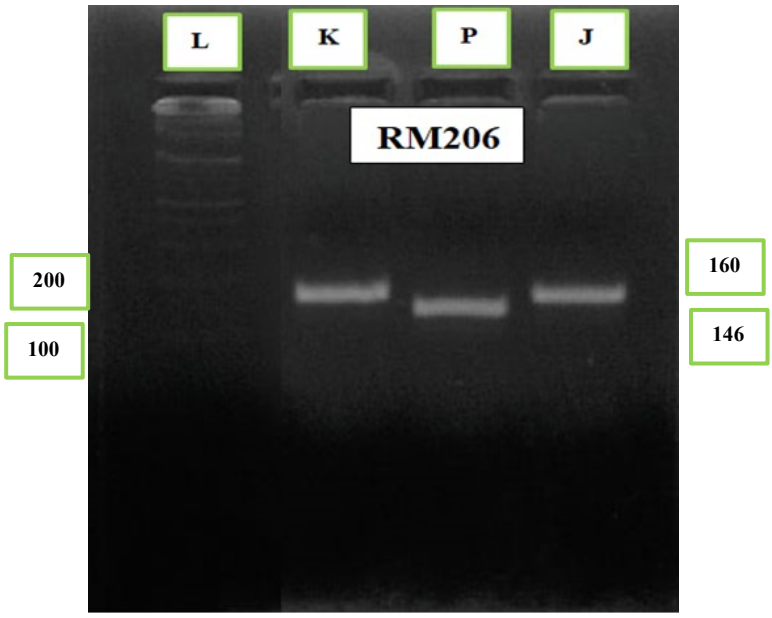


Plate 3. Validation of *Pi*kh gene in donor parent (PTB-7) and recurrent parents Jyothi and Kanchana with marker RM206

L: Ladder K: Kanchana P: Parambuvattan J: Jyothi

Nickolas (2014) reported that the traditional rice variety Parambuvattan having a gene combination *Pi*-1 and *Pikh,* exhibiting more resistance to races of *M. oryzae.* Therefore, popular high yielding varities Jyothi and Kanchana which are susceptible to blast disease can be improved with the resistance genes *Pi*-1 and *Pikh* using Parambuvattan as the donor.

4.3 Marker assisted background selection

Parental polymorphism analysis is a pre-requisite to initiate marker assisted breeding. MAS is reliable when the parents show polymorphism for the trait of interest. DNA molecular markers are particularly useful when they identify differences between genotypes and are called as polymorphic whereas, that are unable to identify variations are monomorphic markers. The markers which are polymorphic can also be represented as dominant or codominant based on whether marker can differentiate homozygote and heterozygote. Codominant makers represent variation in size whereas, in case of dominant it may or may not exhibit size variation. Variation in size of the bands on the gel indicates the marker alleles (Collard *et al*., 2005)

In all genomic areas other than the target locus, background markers are utilised to choose the recurring parent marker alleles while the target locus is chosen using foreground markers. Eliminating the undesired or potentially harmful genes transferred from the donor during crossing is crucial. By utilising traditional breeding techniques, it is challenging to eliminate linkage drag and undesirable donor alleles inherited in the same genomic areas as the target locus, but molecular markers make this task simple and effective.

Phenotypic selection together with marker assisted background selection have the potential to make the breeding protocol economic and effective. Background markers are essential because expect the target locus, all remaining genomic regions can be selected using them. To lessen the linkage drage, this method of choosing individuals having

recombination events between the target locus and closely linked flanking markers might be used (Collard and Mackill, 2008).

From the last few decades, utilization of molecular markers has been playing an increasing role in rice breeding. Among all the markers used in molecular studies microsatellites have gained utmost importance and have been extensively utilised (Miah *et al*., 2013). SSR markers are more advantageous than remaining markers because when amplified by PCR product, they create huge amount of allelic variations at each locus, distributed throughout the genome. Moreover they are abundantly available, highly polymorphic, co-dominant, species-specific and cost effective.

For recovering the recurrent parent genome in the segregating generations polymorphic markers were identified by screening the parents with 42 SSR primers (distributed all over the chromosomes). Results obtained are listed below (Table 15). Molecular weight analysis of the obtained PCR images of all the primers was done using MAX software, UVITECH Cambridge. To know the difference in expression of molecular marker, the location of amplicon position and its molecular weight were assessed. Results indicated that eleven primers were polymorphic and produced distinct reproducible amplicon patterns for Parambuvattan and Jyothi, while fifteen were polymorphic for Parambuvattan and Kanchana (Plate 5.1 to Plate 5.16).

RM markers that were polymorphic between the recurrent parents and donor are used in subsequent generations to recover the recipient genome. Similar studies were carried by earlier researchers. Fourty eight polymorphic SSR markers were used by Gouda *et al*. (2012), 56 SSR markers by Neeraja *et al*. (2007), 85 SSR markers by Khanna *et al*. (2015), 79 SSR markers by Balachiranjeevi *et al*. (2015) and 123 SSR markers by Hari *et al*. (2013), for recovery of recurrent parent genome.

Plate 4. Validation of markers distinguishing between donor PTB-7 and recurrent parents Jyothi and Kanchana

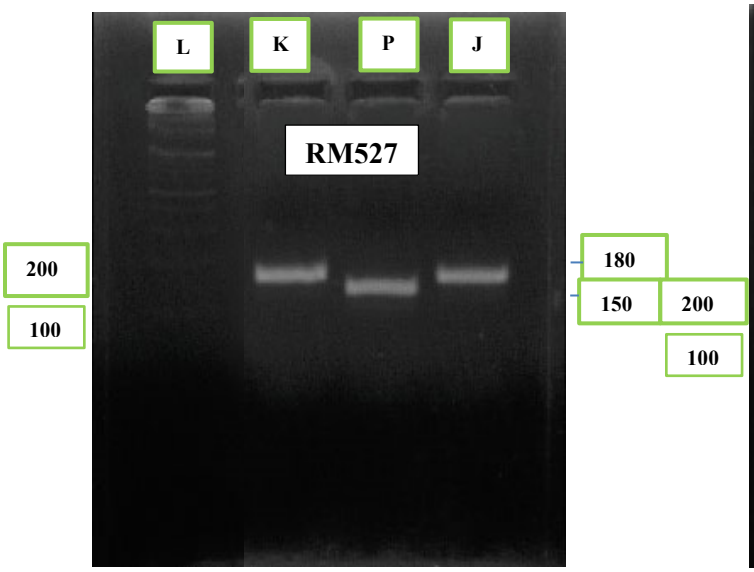


Plate 4.1. Screening with RM527

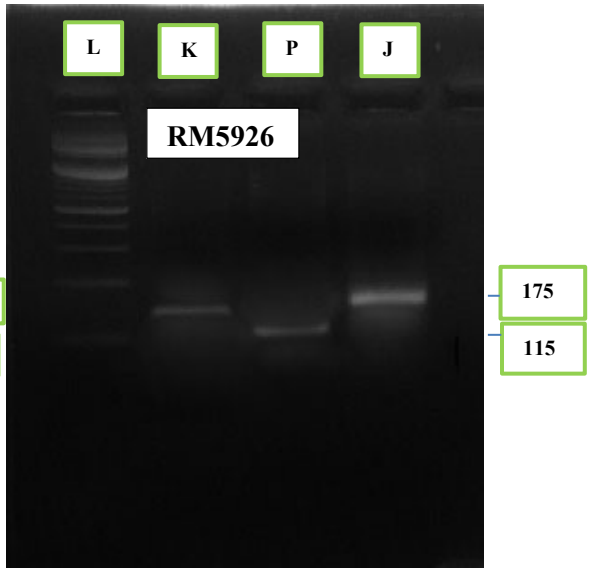


Plate 4.2. Screening with RM5926

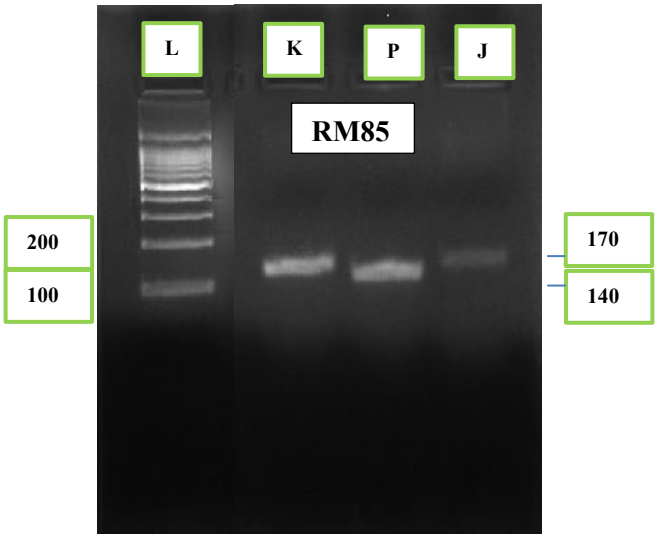


Plate 4.3. Screening with RM85

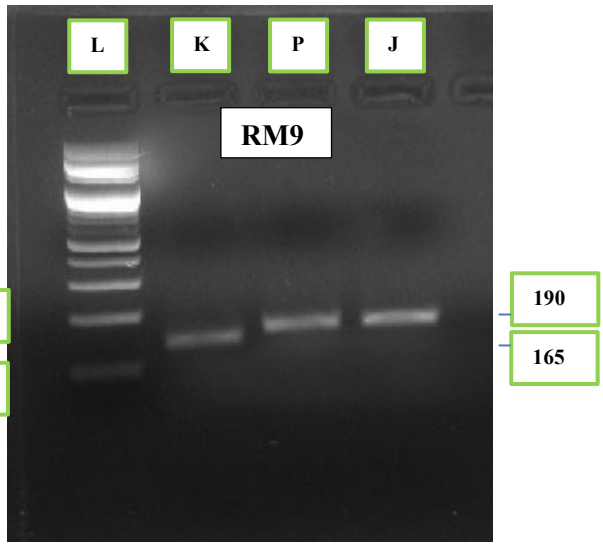


Plate 4.4. Screening with RM9

L: Ladder K: Kanchana P: Parambuvattan J: Jyothi

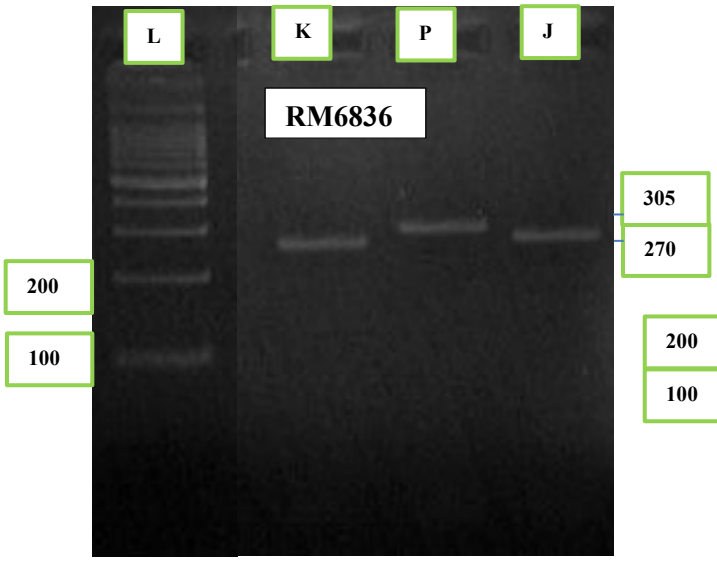


Plate 4.5. Screening with RM6836

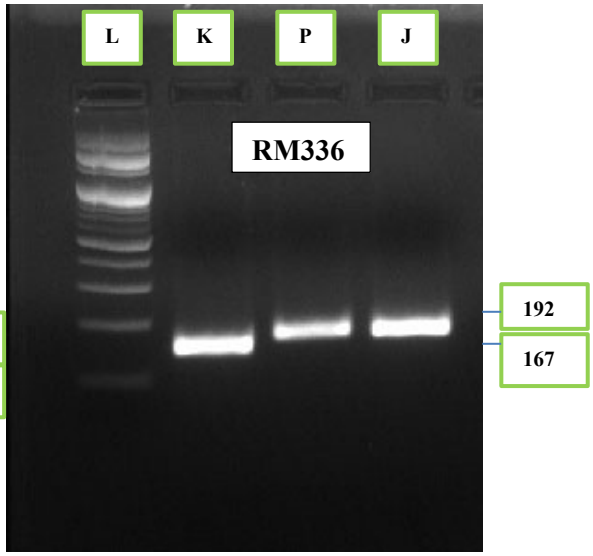


Plate 4.6. Screening with RM336

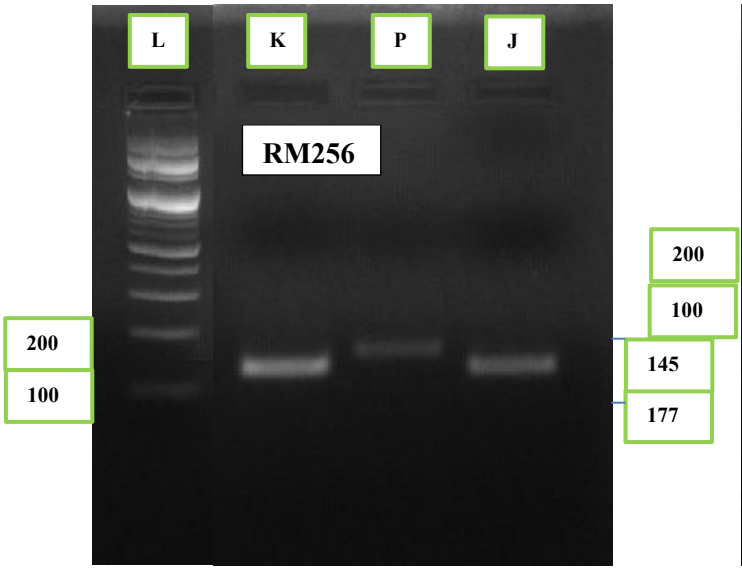


Plate 4.7. Screening with RM256

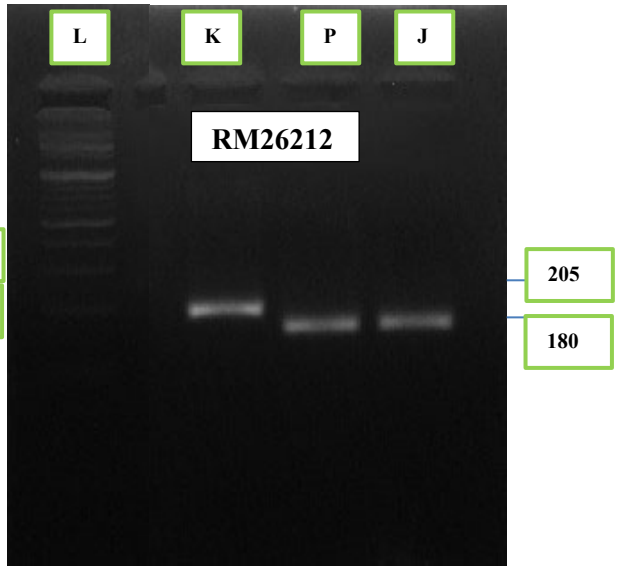


Plate 4.8. Screening with RM26212

L: Ladder K: Kanchana P: Parambuvattan J: Jyothi

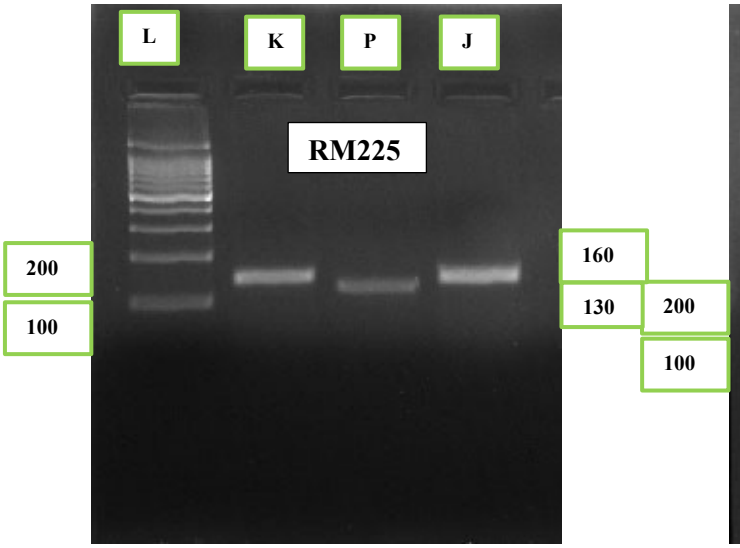


Plate 4.9. Screening with RM225

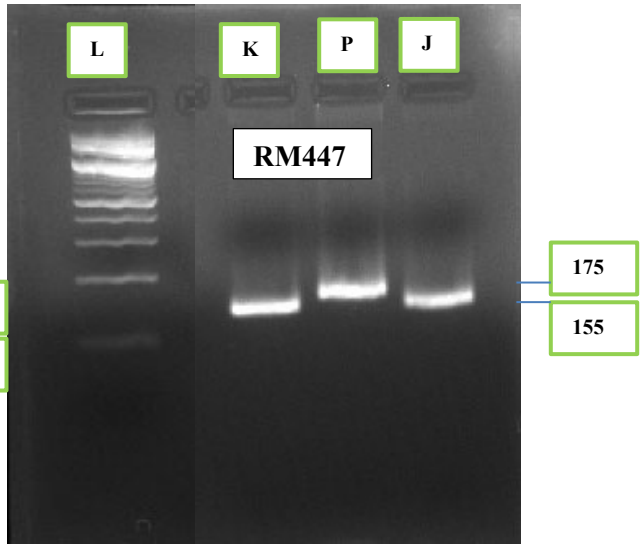


Plate 4.10. Screening with RM447

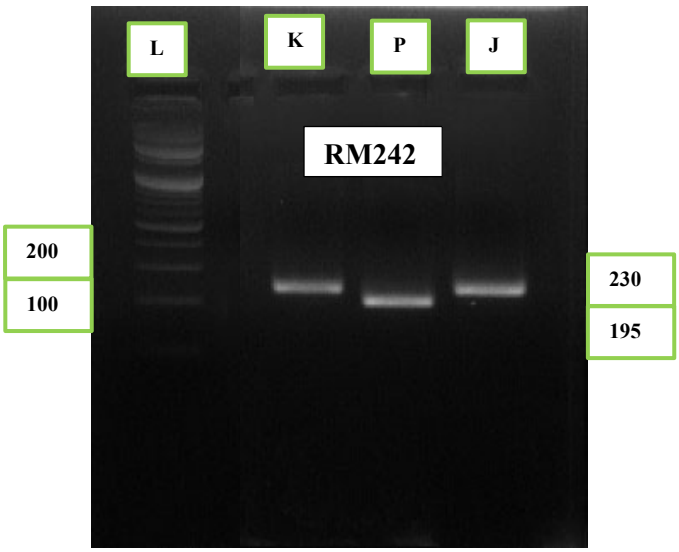


Plate 4.11. Screening with RM242

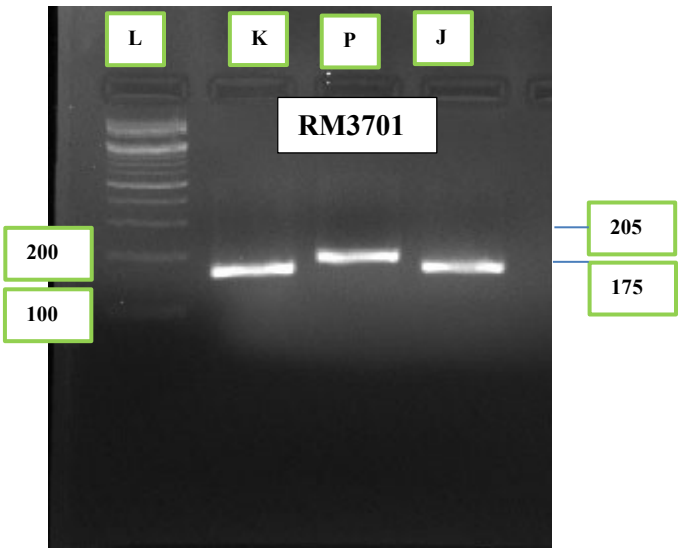


Plate 4.12. Screening with RM3701

L: Ladder K: Kanchana P: Parambuvattan J: Jyothi

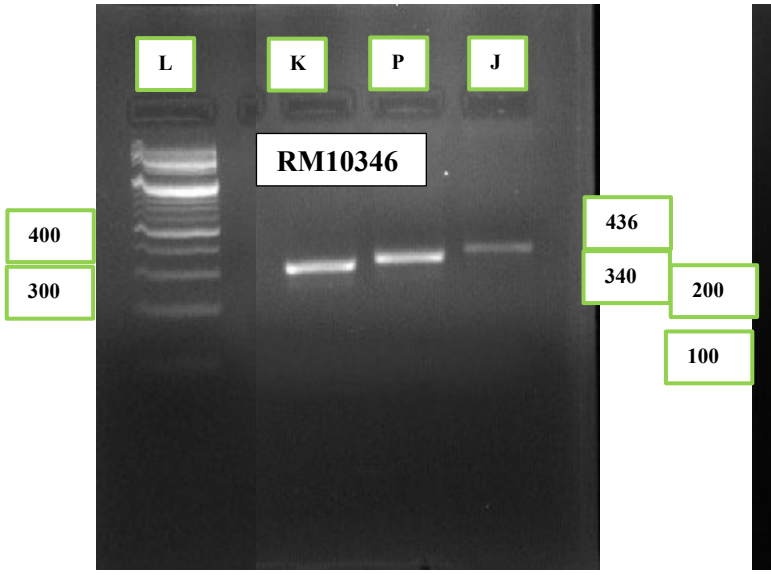


Plate 4.13. Screening with RM10346

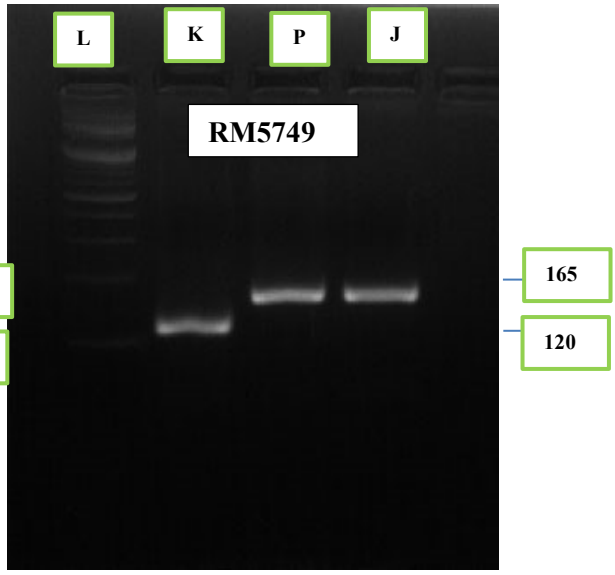


Plate 4.14. Screening with RM5749

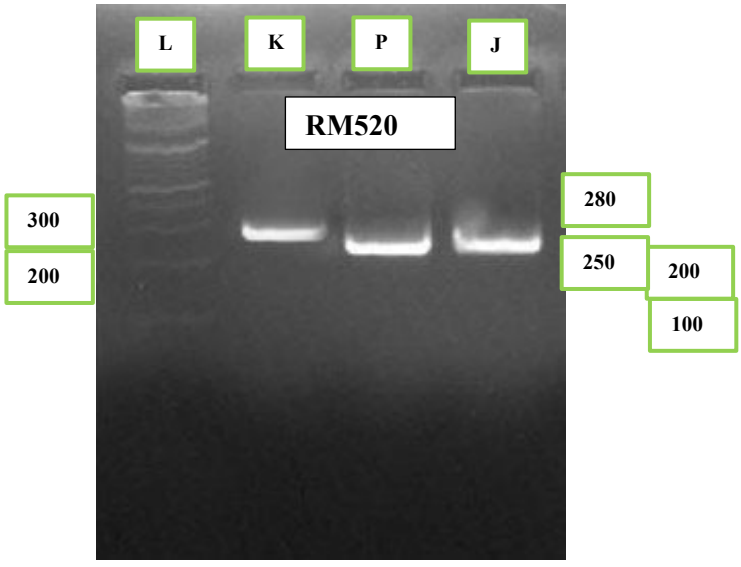


Plate 4.15. Screening with RM520

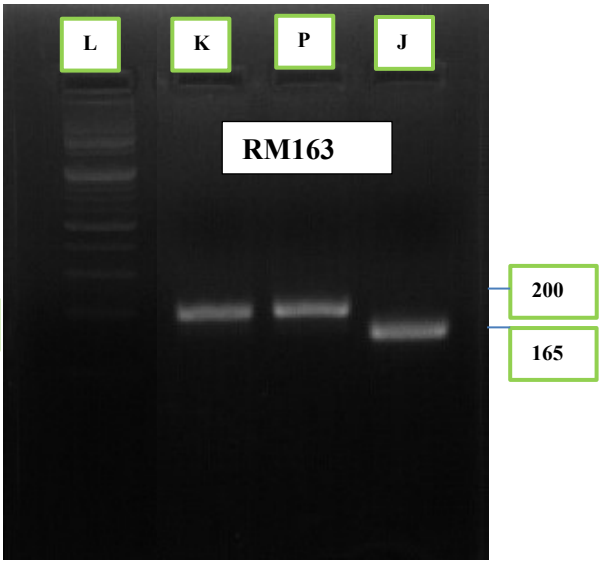


Plate 4.16. Screening with RM163

L: Ladder K: Kanchana P: Parambuvattan J: Jyothi

Table 15. List of background markers showing polymorphism

Sl. No.	Marker	Chromosome	Polymorphism between Parambuvattan (PTB7) and	
			Jyothi	Kanchana
1	RM527	6	Present	Present
2	RM5926	11	Present	Present
3	RM85	7	Present	Present
4	RM9	1	Absent	Present
5	RM6836	6	Present	Present
6	RM336	7	Absent	Present
7	RM256	8	Present	Present
8	RM26212	4	Absent	Present
9	RM225	6	Present	Present
10	RM447	8	Present	Present
11	RM242	9	Present	Present
12	RM3701	11	Present	Present
13	RM10346	1	Present	Present
14	RM5749	4	Absent	Present
15	RM520	3	Absent	Present
16	RM163	4	Present	Absent

4.4 Hybridisation between donor and recurrent parents

4.4.1 Raising the parents for hybridisation

Jyothi and Kanchana varieties of rice which are susceptible to blast disease were crossed with Parambuvattan (PTB7) a blast resistant variety by using it as male parent (pollen donor). Sowing interval was calculated based on duration of the parents and staggered sowing was ubdertaken for ensuring synchronisation of flowering.

Seeds were germinated in petriplates by using germination paper. Later the germinated seedlings were transferred into trays and allowed to grow for 21 days. The seedlings were then transplanted to pots. During flowering time emasculation was carried out by using clipping method. The emasculated panicles were bagged in butter

paper bags, tagged and labeled with date**.** Hand pollination was done on next day morning using the pollen from donor Parambuvattan.

The F_1 seeds developed by crossing were harvested on maturity. A total of 52 F_1 seeds were developed from the cross Jyothi x Parambuvattan while the cross Kanchana x Parambuvattan yielded 48 seeds.

4.5 Screening of F_1 hybrid seedlings with molecular markers and artificial inoculation

4.5.1 Screening of F_1 seedlings with molecular markers

4.5.1.1 Foreground selection

4.5.1.1.1 Foreground selection in Jyothi x Parambuvattan F_1 seedlings

To confirm the presence of the resistant allele *Pi*-1 gene in F_1 seedlings the linked SSR marker RM224 was used. The amplified PCR product was resolved on 3% agarose gel to check the presence of resistance genes.

Among the eighteen F_1 seedlings of the cross Jyothi x Parambuvattan, seven plants (Plant No. 2, 3, 9, 13, 15, 16 and18) showed the presence of gene *Pi*-1 in heterozygous condition for the marker RM224 (Table 16 and plate 6.1) whereas, among fifteen F_1 seedlings of cross Kanchana x Parambuvattan seven plants (Plant No. 1 ,2, 4, 7, 9, 12 and 13) showed presence of *Pi*-1 gene in heterozygous condition for the RM224 marker (Table17 and plate 7.1).

4.5.1.1.2 Foreground selection for *Pikh*

The SSR marker RM206 was used to confirm the presence of the resistant allele *Pikh* in F_1 seedlings. The amplified PCR product was resolved on 3 % agarose gel to check the presence of resistance genes.

Among the eighteen F_1 seedlings of the cross Jyothi x Parambuvattan eight plants (Plant No. 1, 3, 5, 7, 9, 10, 13 and 18) showed the presence of gene *Pikh* in heterozygous

Germination of seeds

15 days after germination

45 days after transplantation

Emasculation, seed set

Bagging of panicles

Tagging of panicles

Plate 5. Hybridisation between donor and recurrent parents

condition using marker RM206 (Table 16 and plate 6.2) whereas, among the 15 F_1 seedlings of cross Kanchana x Parambuvattan six plants (Plant No. 1, 4, 5, 6, 10 and 13) showed presence of *Pikh* gene in heterozygous condition using RM206 marker (Table 17 and plate 7.2).

Therefore, among the eighteen F_1 seedlings of Jyothi x Parambuvattan, only four plants (Plant No. 3, 9, 13 and 18) exhibited the presence of both *Pi*-1 and *Pikh* genes in heterozygous condition (Table 18) whereas, among the fifteen F_1 seedlings of Kanchana x Parambuvattan only three plants (Plant No. 1, 4 and 13) exhibited the presence of both *Pi*-1 and *Pikh* genes in heterozygous condition (Table20). Hence, these plants can be forwarded for next generation.

Breeders choose plants with the donor parent's marker allele at the target locus through the process of "foreground selection." The major goal is to keep the target locus heterozygous in the next generations (one allele from the donor and another from the recurrent parent). Although referred to as positive selection, this is known as foreground selection (Hospital and Charcosset, 1997; Takeuchi et al., 2006).

Similar works were conducted by Singh *et al.* (2012) where MABB was utilised for transferring two blast resistant genes, *Pi*-54 and *Piz*-5, from rice cultivars C101A51, into PRR78. Similar studies were carried by Gouda *et al*. (2012), Divya *et al*. (2014), Hari *et al*. (2013), Ellur *et al*. (2015), Khanna *et al*. (2015), Balachiranjeevi *et al*. (2015), Miah *et al*. (2015), Harikrishnan (2017) for foreground selection of blast resistance genes.

Table 16. Distribution of alleles of PCR marker loci linked to blast resistance (R) genes in the Jyothi x Parambuvattan F_1 seedlings and parents

Target genes	***Pi*-1**	***Pikh***
Marker	**RM224**	**RM206**
Donor parent (Parambuvattan)	+	+
Recurrent parent (Jyothi)	–	–
Jyothi x Parambuvattan F_1 seedlings		
1	–	+
2	+	–
3	+	+
4	–	–
5	–	+
6	–	–
7	–	+
8	–	–
9	+	+
10	–	+
11	–	–
12	–	–
13	+	+
14	–	–
15	+	–
16	+	–
17	–	–
18	+	+
Total	**7**	**8**

Table 17. Distribution of alleles of PCR marker loci linked to blast resistance (R) genes in the Kanchana x Parambuvattan F_1 seedlings and parents

Target genes	***Pi*-1**	***Pikh***
Marker	**RM224**	**RM206**
Donor parent (Parambuvattan)	+	+
Recurrent parent (Kanchana)	_	_
Kanchana x Parambuvattan F_1 seedlings		
1	+	+
2	+	_
3	_	_
4	+	+
5	_	+
6	_	+
7	+	_
8	_	_
9	+	_
10	_	+
11	_	_
12	+	_
13	+	+
14	_	_
15	_	_
Total	**7**	**6**

4.5.1 Artificial inoculation of F_1 seedlings for blast resistance

Seeds of parents Jyothi, Kanchana and Parambuvattan and F_1 seeds of both the crosses were germinated in petriplates using germination paper. Later the germinated seedlings were transferred into trays and allowed to grow for 21 days. Then the seedlings were inoculated with blast isolate artificially to check the blast incidence in parents and the presence of resistance genes in F_1 hybrids. The inoculated seedlings were observed twice *i.e*., 6 and 10 DAI.

Disease scoring was done using 0-9 scores as described in IRRI-SES scale. Per cent Disease Severity (PDS) was calculated for parents by taking total number of seedlings whereas, for F_1 seedlings total number of leaves is taken into consideration.

Per cent Disease Severity was scored according to standard disease scoring scale developed by International Rice Research Institute (IRRI).

$$\text{Per cent Disease Severity(PDS)} = \frac{\text{Sum of all numerical rating}}{\text{Total no. of seedlings observed x 9}} \text{ x } 100$$

For caluculating PDS, ten plants of each parents were taken and observations were recorded. The resistant parent Parambuvattan having *Pi*-1 and *Pikh* genes for disease resistance was found to have a PDS of 26.6. whereas, Jyothi and Kanchana were found to be highly susceptible with a PDS of 68.8 and 78.8 respectively (Table 16). The results obtained are similar to the study conducted by Nickolas (2014) for the identification of donors for blast resistance from traditional rice varieties of Kerala using molecular markers.

Table 18. Disease scoring for parents

Variety	PDS
Jyothi	68.8
Kanchana	78.8
Pambuvattan	24.6

Plate 6. Screening of Jyothi x Parambuvattan F_1 seedlings with molecular markers

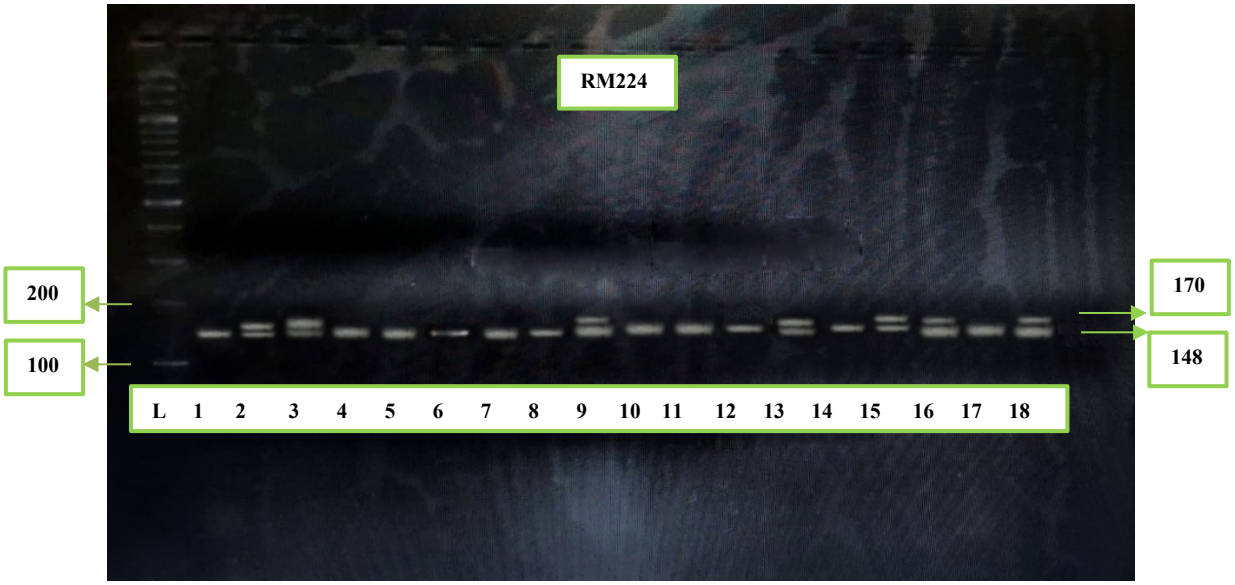


Plate 6.1. Screening of Jyothi x Parambuvattan F_1 seedlings with RM224 marker linked to *Pi*-1 gene

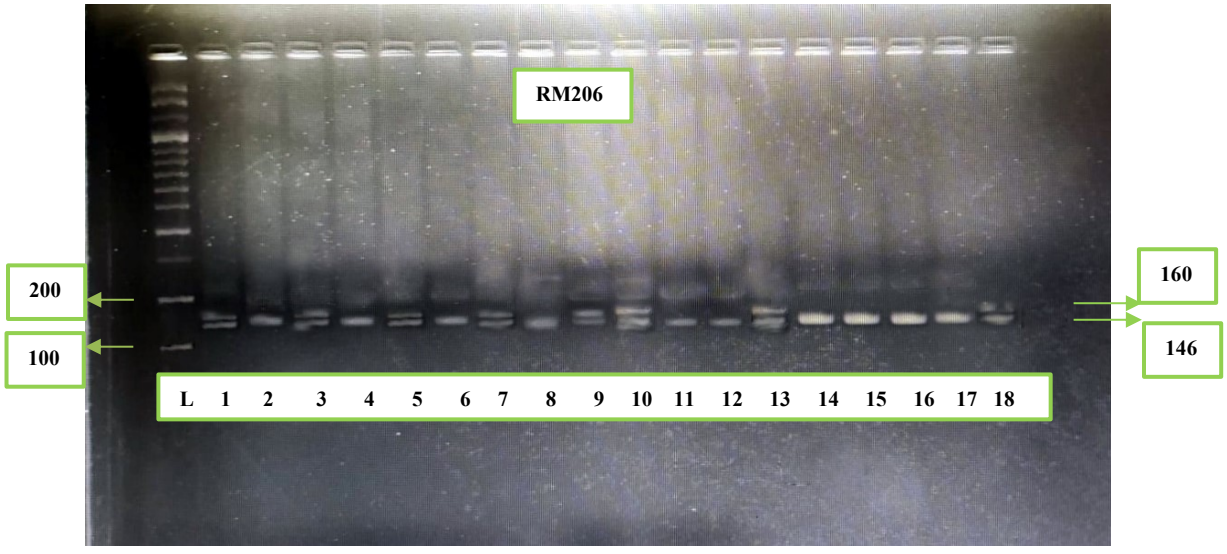


Plate 6.2. Screening of Jyothi x Parambuvattan F_1 seedlings with RM206 marker linked to *Pikh* gene

Plate 7. Screening of Kanchana x Parambuvattan F_1 seedlings with molecular marker

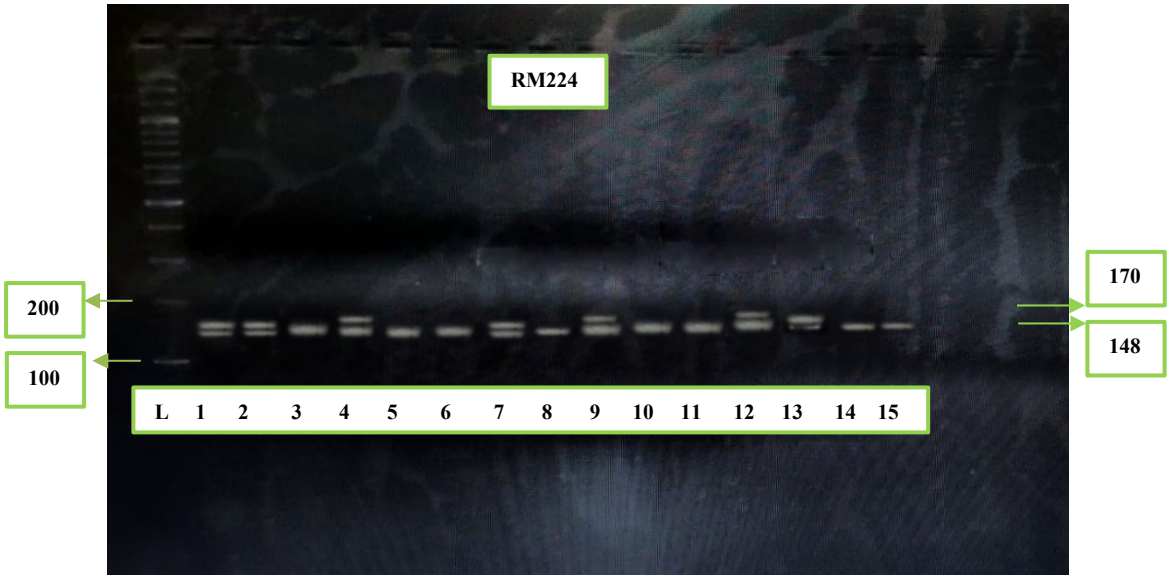


Plate 7.1. Screening of Kanchana x Parambuvattan F_1 seedlings with RM224 marker linked to *Pi*-1 gene

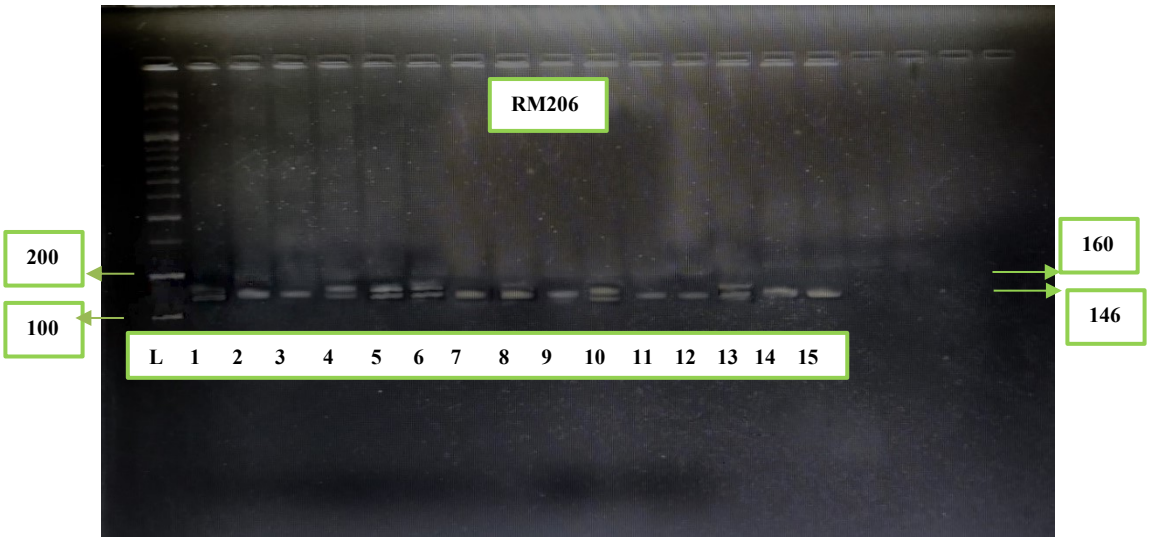


Plate 7.2. Screening of Kanchana x Parambuvattan F_1 seedlings with RM206 marker linked to *Pikh* gene

Among the 52 F_1 seeds developed from the cross Jyothi x Parambuvattan, eighteen were germinated whereas, 48 seeds were developed from the cross Kanchana x Parambuvattan among which fifteen germinated.

Mean PDS score of F_1 seedlings of the cross Jyothi x Parambuvattan was 34.3. whereas in Kanchana x Parambuvattan it was 32.4. Results indicated that the PDS score of F_1 seedlings was near to donor Parambuvattan that *ie*., are exhibiting partial resistance.

Young rice seedlings were inoculated as they contain low amount of silica in the cells which makes them sensitive for infection (Ou, 1985). Similar research was conducted

by earlier researchers to confirm the presence of target genes in F_1 seedlings and mapping population by challenge inoculation with *M. oryzae* isolates (Suh *et al*., 2009).

Similar studies were conducted by Jeung *et al*. (2007), Ashkani *et al*. (2011) and Zhu *et al*. (2012). The seedlings showing resistance were selected for further evaluation with molecular markers to confirm the presence of blast resistance genes using molecular markers.

Table 19. Distribution of foreground markers and PDS of the F_1 seedlings selected in Jyothi x Parambuvattan

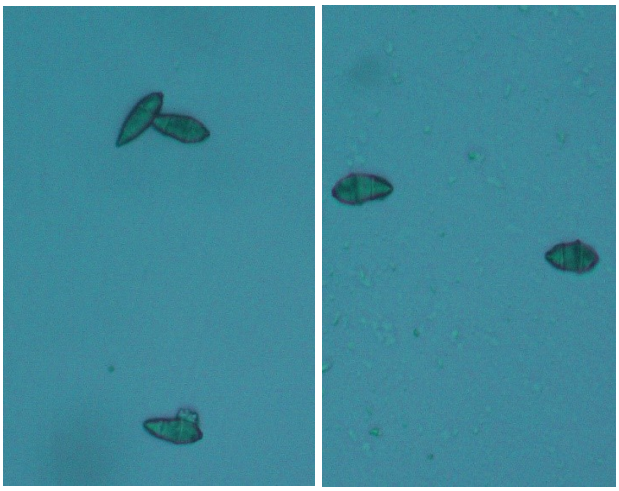
Plant No.	PDS	*Pi*-1	*Pikh*
3	24.8	Present	Present
9	30.1	Present	Present
13	26.8	Present	Present
18	29.7	Present	Present

Table 20. Distribution of foreground markers and PDS of the F_1 seedlings selected in Kanchana x Parambuvattan

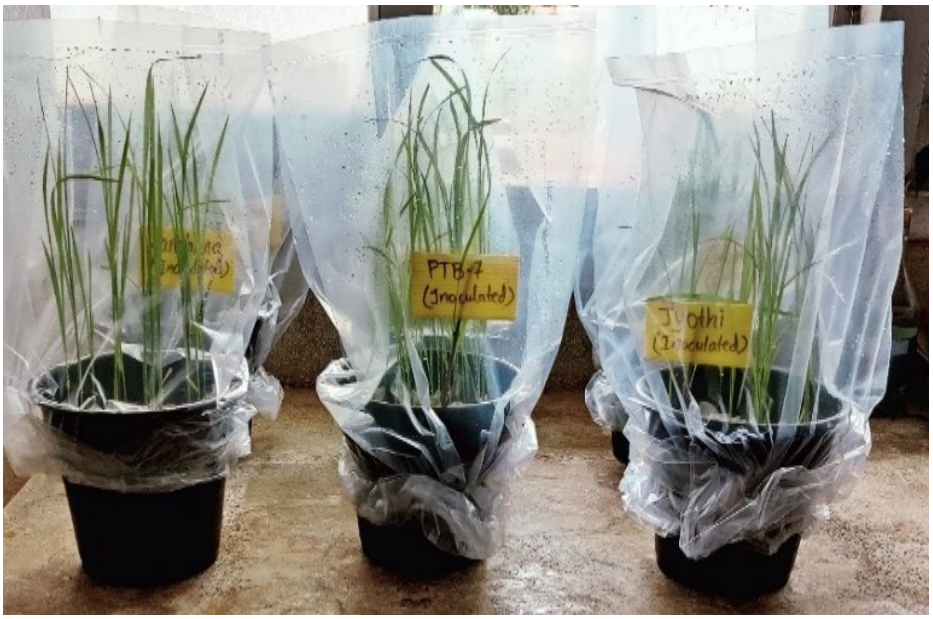
Plant No.	PDS	*Pi*-1	*Pikh*
1	26.6	Present	Present
4	29.3	Present	Present
13	28.9	Present	Present

Four F_1 seedlings of Jyothi x Parambuvattan (Plant No. 3, 9, 13 and 18) were showing the PDS 24.8, 31.1, 28.8 and 29.7 (Table 19). Three F_1 seedlings of Kanchana x Parambuvattan (Plant No. 1, 4 and 13) has the PDS of 26.6, 31.3 and 28.9 (Table 20). Therefore, the results indicated that the plants selected were showing partial resistance when inoculated and exhibited the presence of both the resistance genes *Pi*-1 and *Pikh*. Hence these selected plants can be forwarded for further generations.

Culture of *Pyricularia oryza* (*Magnaporthe grisea*)



Pyricularia oryza (*Magnaporthe grisea*) under microscope



Inoculated plants

Plate 8. Artificial inoculation

Plate 8 (contd…)

Parambuvattan Inoculated

Parambuvattan Control

Jyothi Inoculated

Jyothi Control

Plate 8 (contd…)

Kanchana Inoculated

Kanchana Control

Jyothi x Parambuvattan F_1 seedlings inoculated

Kanchana x Parambuvattan F_1 seedlings inoculated

Summary

5. SUMMARY

The research study entitled 'Incorporation of blast resistance into Jyothi and Kanchana rice (*Oryza sativa* L.) varieties through marker assisted breeding' was carried out at Kerala Agricultural University (K.A.U), Vellanikkara during 2018-2021. The present study aimed to transfer blast resistant genes to popular rice varieties of Kerala (Jyothi and Kanchana) using traditional rice variety Parambuvattan (PTB7) as donor which carries two genes for blast resistance *Pi*-1 and *Pikh*. The study was executed in five phases *viz.,*

I) Morphological characterisation of parents

II) Validation of genes for blast resistance in parental genotypes

III) Marker assisted background selection

IV) Hybridisation between donor and recurrent parents

V) Screening of F_1 seedlings with molecular markers and artificial inoculation

The salient research findings of the study conducted are briefly presented below

5.1. Morphological characterisation of parents

The parents used in the present study Jyothi, Kanchana and Parambuvattan, were evaluated for their agro-morphological characteristics.

- The plant height of parents Jyothi, Kanchana and Parambuvattan was 86.4, 83.1 and 89.5 cm, respectively.
- The number of productive tillers per plant in parents Jyothi, Kanchana and Parambuvattan was 29.6, 7.4 and 7.5, respectively.
- The number of panicles per plant in parents Jyothi, Kanchana and Parambuvattan were 19.9, 6.2 and 5.7, respectively.
- The panicle length in the recurrent parents Jyothi and Kanchana was 22.9 and 17.2 cm. Wheareas, in donor Parambuvattan it was 19.9 cm, respectively.
- The average days to 50 per cent flowering, in the recurrent parents Jyothi and Kanchana was 87.0 and 80.8 wheareas, in donor Parambuvattan it was 89.1 respectively.

- The average number of fertile grains per panicle recorded in parents Jyothi, Kanchana and Parambuvattan was 85.5, 86.8 and 73.0, respectively.
- The average number of sterile grains per panicle recorded in parents Jyothi, Kanchana and Parambuvattan was 15.1, 12.5 and 27.1, respectively.
- The 100 seed weight of recurrent parents Jyothi and Kanchana was 2.7 and 2.7g. Wheareas, in donor Parambuvattan it was 2.6g, respectively.
- Average grain weight per panicle in Jyothi, Kanchana and Parambuvattan was 38.5, 12.4 and 9.1 g, respectively.

5.2. Validation of genes for blast resistance in parental genotypes

The polymorphic SSR markers selected for our present study (RM224 for *Pi*-1 and RM206 for *Pikh*) are differentiating the recurrent parents and donor with the expected amplicon product sizes of 148 and 146bp in resistant parent whereas, in reccurent parents the amplicon size is 170bp and 160bp.

5.3. Marker assisted background selection

For recovering the recurrent parent genome in the segregating generations polymorphic markers were identified by screening the parents with 42 primer.

Results indicates that 27 primers were polymorphic and produced distinct reproducible amplicon patterns for Parambuvattan and Jyothi while 32 were polymorphic for Parambuvattan and Kanchana.

5. 4. Hybridisation between donor and recurrent parents

Jyothi and Kanchana varieties of rice which are susceptible to blast disease were crossed with Parambuvattan (PTB7) a blast resistant variety by using it as male parent or pollen donor. The F_1 seeds developed by crossing were harvested on maturity. A total of 52 F_1 seeds were developed from the cross Jyothi x Parambuvattan while the cross Kanchana x Parambuvattan yielded 48 seeds.

5.5. Screening of the F_1 seedlings with artificial inoculation and molecular markers

5.5.1 Screening of the F_1 seedlings with molecular markers

To confirm the presence of the resistant allele *Pi*-1 and *Pikh* gene in F_1 seedlings the linked SSR markers RM224 and RM206 were used. The amplified PCR product was resolved on 3% agarose gel to check the presence of resistance genes.

Among eighteen F_1 seedlings of Jyothi x Parambuvattan only four plants (Plant No. 3, 9, 13 and 18) exhibited the presence of both *Pi*-1 and *Pikh* genes in heterozygous condition whereas, among the fifteen F_1 seedlings of Kanchana x Parambuvattan only three plants (Plant No. 1, 4 and 13) exhibited the presence of both *Pi*-1 and *Pikh* genes in heterozygous condition

5.5.2 Screening of the F_1 seedlings with artificial inoculation

Seeds of the parents Jyothi, Kanchana and Parambuvattan and F_1 seeds of both the crosses were germinated in petriplates by using germination paper. Later the germinated seedlings were transferred into trays and allowed to grow for 21 days. Then the seedlings were inoculated with blast isolate artificially to check the blast incidence in parents and the presence of resistance genes in F_1 hybrids.

The donor Parambuvattan had a Per cent Disease Severity (PDS) of 26.6. Whereas, Jyothi and Kanchana were found to be highly susceptible with a PDS of 68.8 and 78.8 respectively.

Mean PDS score of F_1 seedlings of the cross Jyothi x Parambuvattan was 34.3 while Kanchana x Parambuvattan had 32.4. PDS score of F_1 seedlings was similar to the donor Parambuvattan, indicating their partial resistance.

Four F_1 seedlings of Jyothi x Parambuvattan (Plant No. 3, 9, 13 and 18) had the PDS 24.8, 31.1, 28.8 and 29.7. Three F_1 seedlings of Kanchana x Parambuvattan (Plant No. 1, 4 and 13) had the PDS of 26.6, 31.3 and 28.9, respectively. The results indicated that the selected plants were showing presence of both *Pi*-1 and *Pikh* genes and partial resistance when inoculated. Hence, these selected plants can be forwarded for further generations to develop high yielding blast resistant rice lines.

References

REFERENCES

Ahn, Y., Kim, S., Han, H., Choi, H., Moon, S. and McCouch. 1996. Molecular mapping of a gene for resistance to a Korean isolate of rice blast. *Rice Genetics*. *Newsl.* 13: 74-76.

Allard, R. W. 1999. History of Plant Population Genetics. Ann. Rev. Genet. 33: 1-27. Anonymous, 2013. FAOSTAT [Food and Agricultural Organization Statistical Database]. Statistical database of the food and agriculture of the United Nations, FAO, Rome.

Anonymous, 2014. FAOSTAT [Food and Agricultural Organization Statistical Database]. Statistical database of the food and agriculture of the United Nations, FAO, Rome.

Ashkani, S., Rafii, M. Y., Ibrahim, R., Meon, S., Abdulla, S. A., Rahim, H. A. and Latif, M. A. 2011. SSRs for Marker Assisted Selection for blast resistance in rice. *Plant Mol. Biol.* 30(1): 79-86.

Babujee, L. and Gnanamanickam, S. S. 2000. Molecular tools for characterization of rice blast pathogen (*Magnaporthe grisea*) population and molecular marker-assisted breeding for disease resistance. *Curr. Sci.* 78(3): 248-257.

Baker, B., Zambryski, P., Staskawicz, B. and Kumar, D. S. P. 1997. Signalling in plant microbe interactions. *Sci.* 276: 726-733.

Balachiranjeevi, C. H., Naik, S., Abhilash, V., Akanksha, S., Viraktamath, B. C., Mahav, M. S., Hariprasad, A. S., Laha, G. S., Prasad, M. S. and Neeraja, C. N. 2015. Marker assisted introgression of bacterial blight and blast resistance into DRR17B, an elite, fine-grain type maintainer line of rice. *Mol. Breed*. 35: 151p.

Ballini, E., Morel, J. B., Droc, G., Price, A., Courtois, B., Notteghen, J. L., and Tharreau, D. 2008. A genome-wide meta-anaylsis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. *Mol. Plant Microbe Interact.* 21(7): 859-868.

Barman, M., Gowda, R., Venu, B., and Chattoo, S. 2004. Identification of a major blast resistance gene in the cultivar 'Tetep'. *Plant Breed.* 123(3): 300-302.

Bennet, P. 2000. Microsatellites. *J. Clinical Pathol*. 53: 177-183.

Berruyer, R., Adreit, H., Milazzo, J., Gailard, S., Berger, A., Dioh, W., Lebrun, M. H. and Tharreau, D. 2003. Identification and fine mapping of *Pi*33, the rice resistance gene corresponding to the *Magnapothe grisea* avirulence gene ACE1. *Theor. Appl. Genet.* 107: 1139-1147.

Bishwajit, G., Sarker, S., Kpoghomou, M., Gao, H., Jun, J., Yin, D. and Ghosh, S. 2013. Self-sufficiency in rice and food security: A South Asian perspective. *Agric. Food Secur.* 2: 2-6.

Bonman, J. M. 1992. Durable resistance to rice blast environmental influences. *Euphytica* 63: 115-123.

Bonman, J. M. and Mackill, D. J. 1998. Durable resistance to rice blast. *Oryza* 25: 103-110.

Bonman, J. M., Dios, V. D. and Khin, M. M. 1986. Physiologic specialisation of *Pyricularia oryzae* in the Philippines. *Plant Dis*. 70: 767-769.

Brar, D.S. and Khush, G. S. 2003. Utilization of wild species of genus *Oryza* in rice improvement. In: *Monograph on genus Oryza*. Science publishers, Enfield, pp. 283-309.

Bryan, G. T., Wu, K., Farrall, L., Jia, Y., Hershey, H. P., McAdams, S. A., Faulk, K. N., Donaldson, G. K., Tarchini, R. and Valent, B. 2000. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistancegene *Pi-ta*. *Plant Cell* 12: 2033-2045.

Causse, M. A., Fulton, T. M., Cho, Y. G., Ahn, S. N., Chunwongse, J., Wu, K., Xiao, J., Yu, Z., Ronald, P. C. and Harrington, S. E. 1994. Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* 138(4): 1251-1274.

Chen, D. H., Zeigler, R. S., Ahn, S. W. and Nelson, R. J. 1996. Phenotypic characterization of the rice blast resistance gene *Pi*-2 (t). *Plant Dis.* 80: 52-56.

Chen, S., Lin, J., Xu, W., Zhai, Z., Ling, B., Ma, Y., and Ma, Y. 2004. Identification of two blast resistance genes in a rice variety, Digu. *J. Phytopathol.* 152(2): 77-85.

Chen, S., Wang, L., Que, Z., Pan, R. and Pan, Q. 2005. Genetic and physical mapping of *Pi*37(t), a new gene conferring resistance to rice blast in the famous cultivar St. No. 1. *Theor. Appl. Genet.* 111: 1563-1570.

Chen, S., Xu, C, G., Lin, H. X. and Zang, Q. 2001a. Improving bacterial blight resistance of '6078', an elite restorer line of hybrid rice, by molecular marker assisted selection. *Plant breed*. 120: 133-137.

Chen, H. L., Chen, B. T., Zhanga, D. P., Xie, Y. F. and Zhang, Q. 2001b. Pathotpes of *Pyricularia grisea* in rice fields of central and southern China. *Plant Dis*. 8: 842-850.

Chen, X. and Ronald, P. C. 2011. Innate immunity in rice. *Trends Plant Sci*. 16: 451-459.

Chen, X. W., Shang, J., Chen, D., Lei, C., Zou, Y., Zhai, W., Liu, G., Xu, J., Ling, Z.,. Cao, G. and Ma, B. 2006. AB-lectin receptor kinase gene conferring rice blast resistance. *Plant J.* 46: 794-804.

Collard, B. C. Y. and Mackill, D. J. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Phil. Trans. R. Soc.* 363: 557-572.

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B. and Pang, E. C. K. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker assisted selection for crop improvement: The basic concepts. *Euphytica* 142: 169-196.

Conaway-Bormans, C. A., Marchetti, M. A., Johnson, C. W., McClung, A. M. and Park, W. D. 2003. Molecular markers linked to the blast resistance gene *Pi-z* in rice for use in marker assisted selection. *Theor. Appl. Genet.* 107(6): 1014-1020.

Couch, B. C. and Cohn, L. M. 2002. A multilocus genealogy concordant with host preference indicates segregation of new species, *Magnaporthe oryzae* from *M. grisea*. *Mycologia* 94(4): 683-693.

Dean, R. A., Talbot, N. J., Ebbole, D. J., Farman, M. L., Nicol, R., Purcell, S., Nusbaum, C., Galagan, J. E. and Birren, B. W. 2005. The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* 434: 980-986.

Deng Y., Zhu, X., Shen, Y., and He, Z. 2006. Genetic characterisation and fine mapping of the blast resistance locus *Pigm(t)* tightly linked to *Pi*2 and *Pi*9 in a broad spectrum resistant Chinese variety. *Theor. Appl. Genet.* 113: 705-713.

Divya, B., Robin, S., Rabindran, R., Senthil, S., Raveendran, M., and John-Joel, A. 2014. Marker assisted backcross breeding approach to improve blast resistance in Indian rice (*Oryza sativa*) variety ADT43. *Euphytica* 200: 6177.

Du, P. V., Loan, L. C. and Sang, N. D. 2007. Blast research in Mekong river delta of Vietnam. In: *Japan International Research Center for Agricultural Sciences*, Tsukuba, Japan, 53: 53-63.

Ebbole, D. J. 2007. Magnaporthe as a model for understanding host-pathogeninteractions. *Ann. Rev. Phytopathol.* 45: 437-456.

Ellur, K. R., Khanna, A., Yadav, A., Pathania, S., Rajasheksara, H., Singh, V. K., Krishnan, G. S., Bhowmick, P. K., Nagarajan, M. and Vinod, K. K. 2015. Improvement of basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding. *Plant Sci*. 242: 330-241.

Fahad, S., Nie, L., Chen, Y., Hussain, S., Wu, C., Xiong, D., Jing, D., Li, Y., Khan, F. and Huang, J. 2014. Disease resistance in rice and the role of molecular breeding in protecting rice crops against diseases. *Biotechnol. Lett*. 36: 1407-1420.

Feng, Z., Li, M., Wang, X., Wu, K., Wang, J., Xu, Z., Wu, Y., Wang, G., Zhao, J. and Hu, K. (2022). Development of rice variety with durable and broad spectrum resistance to blast disease through marker assisted introduction of *Pigm* gene. *Research square,* pp. 1-17.

Fjellstrom, R., McClung, A. M. and Shank, A. R. 2006. SSR markers closely linked to the *Pi-z* locus are useful for selection of blast resistance in a broad array of rice germplasm. *Mol. Breed.* 17: 149-157.

Flor, H. H. 1971. Current status of gene-for-gene concept. *Ann. Rev. Phytopathol.* 9: 275-296.

Flores, A. 2008. New DNA markers for rice blast resistance. *Agric. Res.* 5: 22.

Frisch, M., Bohn, M. and Melchinger, A. E. 1999. Comparison of selection strategies for marker assisted backcrossing of a gene. *Crop Sci.* 39: 1295-1301.

Fuentes, J. L., Jose, F., Victoria, C., Escobar, F., Prado, G., Aricapa, G., Duque, M. C. and Tohme, J. 2008. Identification of microsatellite markers linked to blast resistance gene *Pi-1(t)* in rice. *Euphytica* 160: 295-304.

Fuentes, J. L., Jose, F., Victoria, C., Escobar, F., Prado. G., Aricapa, G., Duque, M. C. and Tohme, J. 2003. Simple Sequence Repeat (SSR) markers linked to blast resistance gene *Pi*-1 in rice for marker assisted selection. Available at: http//webapp.ciat.cgiar.org/epmr-ciat/pdf/poster-15-empr07.pdf.

Fukuta, Y., Araki, E., Telebanco-Yanoria, M. J., Ebron, L., Mercado-Escueta, D., Takai, T. and Khush, G. S. 2004. Identification of blast resistance gene, *Pish* in rice (*Oryza sativa* L.). In *Proceedings on Plant and Animal Genome, Conference XII*, 10 January 2004, San Diego, California; pp. 401-406.

Garris, A. J., Tai, T. H., Coburn, J., Kresovich, S. and McCouch, S. 2005. Genetic structure and diversity in *Oryza sativa* L. *Genet.* 169: 1631-1638.

Glaszmann, J. C. 1987. Isozymes and classification of Asian rice varieties. *Theor. Appl. Genet.* 74: 21-30.

Glaszmann, J. C. and Arraudeau, M. 1986. Varietal diversity and reproductive barriers. I. Rice plant type variation, japonica-javanica relationships. *Rice Genet. Newsl.* 3: 41-43.

Gnanamanickam, S. 2009. Biological control of rice disease. *Prog. Biol. Contol* 8:13-42.

Goto, K. 1965. Physiological races of *Pyricularia oryzae* in Japan. In: *Proceedings on Rice Blast Disease at International Rice Research Institute* (IRRI) *conference XI*, 3 August 1965, Baltimore, Maryland, pp. 237-242.

Gouda, P. K., Saikumar, S., Varma, C. M. K., Nagesh, K., Thippeswamy, S., Shenoy, V., Ramesha, M. S. and Shashidhar, H. 2012. Marker-assisted breeding of *Pi*-1 and

Piz-5 genes imparting resistance to rice blast in PRR78, restorer line of PusaRH-10 Basmati rice hybrid. *Plant Breed.* 132: 61-69.

Gupta, S. K., Rai, A. K., Kanwar, S. S., Chand, D., Singh N. K. and Sharma T. R. 2011. The single functional blast resistance gene *Pi54* activates a complex defense mechanism in rice. *J. Exp. Bot.* 63: 757-772.

Hari, K., Srinivasarao, B. C., Viraktamath, A. S. H., Prasad, G. S., Laha, M. I., Ahmed, P., Natrajkumar, K., Sujatha, M. S., Prasad, M. and Pandey, M. S. 2013. Marker assisted introgression of bacterial blight and blast resistance into IR58025B, an elite maintainer line of rice. *Plant Breed.* 132(6): 586-594.

Harikrishnan, P. J. 2017. Development of near isogenic lines of rice variety Uma for blast resistance genes through molecular marker assisted backcross breeding. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur p. 68.

Hayashi, H., Yoshida, I., and Ashikawa, K. 2006. Development of PCR-based allele-specific and InDel marker sets for nine rice blast resistance genes. *Theor. Appl. Genet.* 113(2): 251-260.

Hebert, T. T. 1971. The perfect stage of *Pyricularia grisea*. *Phytopathol.* 61(1): 83-87.

Hittalmani, S., Parco, A., Mew, T. V., Ziegler, R. S. and Huang, N. 2000. Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. *Theor. Appl. Genet.* 100: 1121-1128.

Hospital, F. 2001. Size of donor chromosome segments around introgressed loci and reduction of linkage drag in marker-assisted backcross programs. *Genet.* 158: 1363-1379.

Hospital, F. and Charcosset, A. 1997. Marker assisted introgression of quantitative trait loci. *Genet.* 147: 1469-1485.

Hospital, F., Chavalet, C. and Mubsant, P. 1992. Using markers in gene introgression breeding programmes. *Genet.* 132: 1119-1210.

Howard, R. J. and Valent, B. 1996. Breaking and entering: host penetration by the fungal rice blast pathogen *Magnaporthe grisea. Ann. Rev. Microbiol.* 50: 491-512.

International Rice Genome Sequencing Project. 2005. The map-based sequence of the rice genome. *Nature*. 436(7052): 793-800.

IRRI 2013. Standardization evaluation system of rice. International Rice Research Institute, Manila, Philippines. pp. 5-18.

Ishizaki, K., Hoshi, T., Abe, S., Sasaki, Y., Koboyashi, K., Kasanaeyama, T., Matsui, T. and Azuma, S. 2005. Breeding of blast resistance isogenic lines in rice variety Koshikari and evaluation of their characters. *Breed. Sci.* 55: 371-377.

Jena, K. K. and Mackille, D. J. 2008. Molecular markers and their use in marker assisted selection in rice. *Crop Sci.* 48: 1266-1276.

Jeon, J. S., Chen, D., Yi, G. H., Wang, G. L. and Ronald, P. C. 2003. Genetic and physical mapping of *Pi*5(t), a locus associated with broad-spectrum resistance to rice blast. *Mol. Genet. Genomics* 269: 280-289.

Jeung, J. U., Kim, B. R., Cho, S. S., Han, H. P., Moon, Y. T., Lee, K. K., and Jena, A. 2007. A novel gene, *Pi*40(t), linked to the DNA markers derived from NBS-LRR motifs confers broad spectrum of blast resistance in rice. *Theor. Appl. Genet.* 115(2): 1163-1177.

Jiang, N., Li, Z., Wu, J., Wang, X., Wu, L., Wang, S., Wang, D., Wen, T., Liang, Y. and Sun, P. and Liu, J. 2012. Molecular mapping of the *Pi*2/9 allelic gene *Pi*2-2 conferring broad- *Magnaporthe oryzae* in the spectrum resistance to rice cultivar Jefferson. *Rice* 5(29): 1-7.

Khanna, A., Sharma, V., Ellur, R. K., Shikari, A. B., Krishnan, G. S., Singh, U. D., Prakash, In: G., Sharma, T. R., Rathour, R. and Varier, M. 2015. Development and evaluation of near isogeneic lines for major blast resistance gene(s) in Basmathi rice. *Theor. Appl. Genet*. 128: 1243-1259.

Khush, G. S. 2013. Strategies for increasing the yield potential of cereals: Case of rice as an example. *Plant Breed.* 132: 433-436.

Khush, G. S. and Jena, K. K. 2009. Current status and future prospects for research on blast resistance in rice (*Oryza sativa* L.). In: *Advances in Genetics, Genomics and Control of Rice Blast Disease*. Springer Science Media, Berlin, Germany. pp. 1-10.

Kingsolver, C. H., Barksdale, T. H., and Marchetti, M. A. 1984. *Rice BlastEpidemiology*. Pennsylvania State University, Pennsylvania. 853p.

Kinoshita, T. 1986. Standardization of gene symbols and standard maps in rice. *Proceedings on Rice Genetics at International Rice Research Institute (IRRI)*, 12 May 1986, Philippines. pp. 215-228.

Kiyosawa, S. 1967. Genetic studies on host-pathogen relationship in rice blast disease. In Proc. Symposium of Rice disease and their control by growing resistant varieties and other measures. *Agric. Fisheries Res*. pp. 137-153.

Kiyosawa, S. 1981. Genetic analysis of blast resistance. *Oryza* 18: 196-203.

Koide, Y., Telebanco-Yanoria, M. J., Peña, F. D., Fukuta, Y. and Kobayashi, N. 2011. Characterization of Rice Blast Isolates by the Differential System and their Application for Mapping a Resistance Gene, *Pi*19(t). *J. Phytopathol.* 159(2): 85-93.

Korinsak, S., Sirithunya, P., Meakwatanakarn, P., Sarkarung, S., Vanavichit, A. and Toojinda, T. 2011. Changing allele frequencies associated with specific resistance genes to leaf blast in backcross introgression lines of Khao Dawk Mali 105 developed from a conventional selection program. *Field Crops Res.* 122: 32-39.

Krattinger, S. G., Lagudah, E. S., Meyer, W. S., Singh, R. P., Espino, J., Fadden, H., Bossolini, E., Selter, L. and Keller, B. 2009. Gene specific markers for the wheat gene Lr34/Yr18/Pm38 which confers resistance to multiple fungal pathogens. *Theor. Appl. Genet.* 119(5): 889-890.

Krishnan, G. S., Sharma, R. K., Rajkumar, K. A., Joseph, M., Singh, V. P., Singh, A. K., Bhat, K. V., Singh, N. K. and Mohapatra, T. 2008. Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. *Plant Breed.* 127: 131-139.

Kumar, S. 2014. Plant disease management in India: Advances and challenges. *Afr. J. Agric. Res.* 9(15): 1207- 1217.

Kumari, K. A., Rani, C. V. D., Sundaram, R. M., Vanisree, S., and Seshumadhav, M. 2014. Marker assisted introgression of BB and blast resistant genes in mega rice variety MTU1010. *Agrotechnol.* 2: 1-16.

Kwon, S., Cho, Y., Kim, Y., Suh, J., Jeung, J., Roh, J., Lee, S., Jeon, J., Yang, S., and Lee, Y. 2008. Development of Near-isogenic Japonica Rice Lines with Enhanced Resistance to *Magnaporthe grisea. Mol. Cell* 25(3): 407-416.

Lee, S., Song, M., Seo, Y., Kim, H., Ko, S., Cao, P., Suh, J., Yi, G., Roh, J., Lee, S. and An, G. 2009. Rice Pi5-Mediated Resistance to *Magnaporthe oryzae* requires the Presence of Two Coiled-Coil-Nucleotide-Binding Leucine-Rich Repeat Genes. *Genet.* 181: 1627-1638.

Lei, D., Huang, W. Li, J., and Wang, Z. 2005. Molecular mapping of a blast resistance gene in an indica. Rice cultivar Yanxian No.1. *Rice Genet*. *Newsl.* 22: 76-77.

Li, L., Wang, Y. L., Jing, J. X., and Li, Z. Q. 2007. The Pik-m gene, conferring stable resistance to isolates of *Magnaporthe oryzae,* was finely mapped in a crossover-cold region on rice chromosome 11. *Mol. Breed.* 20: 179-188.

Lin, S., Chen, Z., Que, L., Wang, X., and Liu, Q. 2007. The blast resistance gene *Pi*37 encodes a nucleotide binding site-leucine rich repeat protein and is a member of a resistance gene cluster on rice chromosome1. *Genet.* 177(3): 1871-1880.

Litt, M. and Luty, J. A. 1989. A hypervariable microsatellite revealed by in vitro amplification of dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Genet.* 44: 397-401.

Liu, G., Lu, G., Zeng, L. and Wang, G. L. 2002. Two broad-spectrum blast resistance genes, *Pi*9(t) and *Pi*2(t) are physically linked on rice chromosome 6. *Mol. Genet. Genomics* 267: 472-480.

Liu, Q., Yang, F., Lin, L., Hua, C., Wang, L., and Wang, Q. 2010. Identification and fine mapping of *Pi*39(t), a major gene conferring the broad spectrum resistance to *Magnoporthe oryzae. Mol. Genet. Genomics* 278 (4): 403-410.

Liu, S. J., Jin, X. Y., Zhu, F., Wang, J. H., Li, Z. R., Liu, Y. B., and Liu, W. G. 2007. Improving blast resistance of a thermo-sensitive genic male sterile rice line GD-8S by molecular-assisted selection. *Rice Sci.* 15 (3): 179-185.

Liu, S. P., Li, X., Wang, C. Y., Li, X. H., and He, Y. Q. 2003. Improvement of resistance to rice blast in Zhenshan 97 by molecular markeraided selection. *Acta Bot. Sin.* 45: 1346-1350.

Mackill, D. J. and Bonman, J. M. 1986. New hosts of *Pyricularia oryzae*. *Plant Dis.* 70: 125-127.

Mackil, D. J. and Bonman, J. M. 1992. Inheritance of blast resistance in near isogenic lines of rice. *Phytopathol.* 82: 746-749.

Madhavi, R. K., Prasad, M. S., Laha, G. S., Mohan, K. M., Madhav, M. S. and Viraktamath, B. C. 2011. Combining blast and bacterial blight resistance in rice cultivar, Improved Samba Mahsuri. *Indian J. Plant Prot.* 39(2): 124 -129.

Madhavi, R. K., Prasad, M. S., Madhav, M. S., Laha, G. S., Mohan, K. M., Sundaram, R. M., Jahnavi, B, Vijitha, S., Rao, P. R. and Viraktamath, B. C. 2012. Introgression of blast resistance gene *Pi-kh* into elite indica rice variety Improved Samba Mahsuri. *Indian J. Pl. Prot*. 40(1): 52-56.

McCouch, S. R., Teytelman, L., Xu, Y., Labos, K.B., Clare, K. and Walton, M. 2002. Development and mapping of 2240 new SSR markers in rice (*Oryza sativa* L.). *DNA Res.* 9: 199-207.

Metha, Y. R. and Baier, A. 1998. Characterization of *Magnaporthe grisea* from BlackOat in Brazil. *Phytopathol.* 24(2): 119-125.

Miah, G., Rafii, M. Y., Ismail, M. R., Puteh, A. B., Rahim, H. A., Asfaliza, R. and Latif, M. A. 2013. Blast resistance in rice: A review of conventional breeding to molecular approaches. *Mol. Biol. Rep.* 40: 2369-2388.

Ming, W. X., Xin, L., Hui, W., Tao, G., Zhu, L., Yong, Z., Yuan, Z., Yun, Y. and Qiang, C. 2018. Pyramiding of *Pi*46 and *pita* to improve blast resistance and to evaluate the resistance effect of the two R genes. *J. integr. agric*. 15(10): 2290-2298.

Mohan, M. S., Nair, A. and Bhagwa. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. *Mol. Breed.* 3: 87-103.

Morishima, H., 1984. Species relationships and the search for ancestors. In Tsunoda, S. and Takahashi, N. (Ed). Biol. Rice. pp. 3-30, Japan. Sci. Soc. Press, Tokyo/Elsevier, Amsterdam.

Murray, M. G. and Thompson, W. F. 1980. Rapid isolation of higher weight DNA. *Nucl. Acids Res*. 8: 4321-4325.

Naqvi, N. I. and Chattoo, B. B. 1996. Development of a sequence characterised amplified region (SCAR) based indirect selection method for a dominant Blast-resistance gene in rice. *Genome* 39: 26-30.

Narayanan, N. N., Baisakh, N., Veracruz C. M., Gnanamanickam, S. S., Datta, K., and Datta, S. K. 2002. Molecular breeding for the development of Blast and Bacterial Blight resistance in Rice cv. IR50. *Crop Sci.* 42: 2072-2079.

Neeraja, C. N., Maghirang-Rodriguez, R., Pamplona, A., Heuer, S., Collard, B. C. Y., Septiningsih, E. M., Vergara, G., Sanchez, D., Xu, K., and Ismail, 2007. Amarker-assisted backcross approach for developing submergence-tolerant rice cultivars. *Theor. Appl. Genet.* 115: 767-776.

Nguyen, T. T. T., Koizumi, S., La, T. N., Zenbayashi, K. S., Ashizawa, T., Yasuda, N., Imazaki, I., and Miyasaka, A. 2006. *Pi*35(t), a new gene conferring partial resistance to leaf blast in the rice cultivar Hokkai 188. *Theor. Appl. Genet.* 113: 697-704.

Nickolas, H. 2014. Identification of the donors for blast resistance from traditional rice varieties of Kerala using functional markers. M.Sc. (Ag) thesis, KeralaAgricultural University, Thrissur, p. 91.

Oka, H. I. 1958. Intervarietal variation and classification of cultivated rice. *Indian J. Genet.* 18: 79-89.

Ou, S. H. 1985. Rice diseases. 2nd edtion. Commonwealth Mycological Institute, Kew, UK, p.109-201.

Padmanabhan, S. Y. 1965. Breeding for blast resistance in India. *Proc. Indian Academic Sci.* 49: 349-362.

Pan, L., Wang, H., and Ikehashi, T., and Tanisaka, S. 1996. Identification of a new blast resistance gene in the indica rice cultivar Kasalath using Japanese differential cultivars and isozyme markers. *Phytopathol.* 86(10): 1071-1075.

Pan, Q. H., Tanisaka, T. and Ikehashi, H. 1995. Studies on the genetics and breeding of blast resistance in rice IV Gene analysis for the blast resistance of a indica variety Kasalath. *Breed. Sci*. 45(2): 170.

Pan, Q., Hu, Z., Tanisaka, T., and Wang, L. 2003. Fine mapping of blast resistance gene *Pi*15, linked to Pii on chromosome 9. *Acta. Bot. Sin*. 45: 871-877.

Panigrahi, J., Mishra, R. R., Sahu, A. R., Rath, S. C., Seth, S., and Mishra, S. P. 2013. Marker assisted breeding for simple inherited traits conferring stress resistance in crop plants. *Ecoscan* 3: 217- 233.

Park, J. Y., Jin, J., Lee, Y., Kang, S., and Lee, Y. 2009. Rice Blast Fungus (*Magnaporthe oryzae*) infects Arabidopsis via a Mechanism Distinct from that required for the Infection of Rice. *Plant Physiol.* 149: 474-486.

Pinta, W., Toojinda, T., Thummabenjapone, P., and Sanitchon, J. 2013. Pyramiding of blast and bacterial leaf blight resistance genes into rice cultivar RD6 using marker assisted selection. *Afr. J. Biotechnol.* 12: 4432-4438.

Prabhu, A. S., Filippi, M. C., and Zimmermann, J. P. 1996. Genetic control of blast in relation to nitrogen fertilization in upland rice. *Pesquisa Agropecuária Brasileira* 31(5): 339-347.

Prasad, S. M., Kanthi, B. A., Balachandran, S. M., Seshumadhav, M., Madhan, M. K. and Viraktamath, B. C. 2009. Molecular mapping of rice blast resistance gene Pi-1(t) in the elite indica variety Samba mahsuri. *World J. Microbiol. Biotechnol.* 25:1765-1769.

Rajarajeshwari, K. and Muralidharan, N. V. L. 2006. Assessments of farm yield and district production loss from bacterial leaf blight epidemics in rice. *Crop Prot*. 25(3): 244-252.

Rao, K. M. 1994. *Rice Blast Disease*. Daya Publishing House, Delhi, 197p.

Rockwood, W. G. 2001. Rice research and production in the 21st century, Symposium IRRI, Philippines: 117-136.

Rola, A. C. and Widawsky, D. A. 1998. Impact of rice research. In : *Proc. Int. Conf. Impact Rice Res*., TDRI, IRRI: 135-158.

Ronald, P. C. 2014. Lab to farm: Applying research on plant genetics and genomics to crop improvement. *Biol.* 12(6): 1-6.

Rosalyan, B., Shim, A., Vincent, P. R., Marilyan, M., Ruby, S., Shim, J. L., Sunohara, H., Jena, K. K., Ashikari, M., and Doi, K. 2020 Marker assisted intogression of qualitative resistance gene *Pi*21 confers broad spectrum resistance to rice blast. *Rice sci.* 27(2): 113-123.

Rybka, K., Miyamoto, M., Ando, I., Saito, A., and Kawasaki, S. 1997. High resolution mapping of the indica-derived rice blast genes II. *Pita*2 and *Pita* and a consideration of their origin. *Mol. Plant Microbe Interact.* 10: 517-524.

Sallaud, C., Lorieux, M., Roumen, E., Tharreau, D., Berruyer, R., Svestasrani, P., Garsmeur, O., Ghesquiere, A. and Notteghem, J. L. 2003. Identification of five new blast resistance genes in the highly blast-resistant rice variety IR64 using a QTL mapping strategy. *Theor. Appl. Genet*. 106: 794-803.

Scardaci, S. C. 1997. Rice Blast: A New Disease in California. *Agronomy Fact Sheet Series* 1997, Department of Agronomy and Range Science, University of California, Davis, 18 May 2000.

Scheuermann, K. K., Raimondi, J. V., Marschalek, R., Andrade, A. and Wickert, E. 2012. *Magnaporthe oryzae* genetic diversity and its outcomes on the search for durable resistance, The molecular basis of plant genetic diversity, Prof. Mahmut Caliskan *(Ed*.), ISBN: 978-953-51- 0157-4.

Selvaraj, C. I., Nagarajan, P., Thiyagarajan, K., Bharathi, M. and Rabindran, R., 2011. Identification of microsatellite (SSR) and RAPD markers linked to rice blast disease resistance gene in Rice (*Oryza sativa* L.). *Afr. J. Biotechnol*. 10(17): 3301-3321.

Sharma, T. R., Chauhan, R. S., Singh, B. M., Paul, R., Sagar, V. and Rathour, R. 2002. RAPD and Pathotype Analyses of *Magnaporthe grisea* Populations from the north-western Himalayan Region of India. *J. Phytopathol.* 150: 649-656.

Sharma, T. R., Madhav, M. S., Singh, B. K., Shanker, P., Jana, T. K., Dalal, V., Pandit, A., Singh, A., Gaikwad, K., Upreti, H.C. and Singh. N. K. 2005. High-resolution mapping, cloning and molecular characterization of the Pi-kh gene of rice, which confers resistance to *Magnaporthe grisea. Mol. Genet. Genomics* 274: 569-578.

Sharma, T. R., Rai, A. K., Gupta, S. K., Vijayan, J., Devanna, B. N., and Ray, S. 2012. Rice blast management through host plant resistance: Retrospect and prospects. *Agric. Res.* 1: 37-52.

Sharma, T.R. Rai, A.K., Gupta, S.K., and Singh, N.K. 2010. Broad spectrum blast resistance gen *Pikh* cloned from rice line Tetap designated as *Pi54*. *J. Plant Biochem. Biotechnol.* 19: 87-89.

Sharma, T.R., Chauhan, R.S., Singh, B.M., Paul, R., Sagar, V., and Rathour, R. 2002. RAPD and Pathotype Analyses of Magnaporthe grisea Populations from the north-western Himalayan Region of India. *J Phytopathol.* 150: 649–656.

Shinoda, H. 1971. Studies on the varietal resistance of rice blast. Linkage relationship of blast resistance genes. *Bull. Chugoku Agric. Exp. St. Ser.* A 20: 1-25.

Singh, A., Singh, V. K., Singh, S. P., Pandian, R. T. P., Ellur, R. K., Singh, D., Bhowmick, P. K., Krishnan, G. S., Nagarajan, M., Vinod, K. K. and Singh, U. D. 2012. Molecular breeding for the development of multiple disease resistance in Basmati rice. *AoB Plants*: pls029; doi:10.1093/aobpla/ pls029.

Singh, U. M., Dixit, S., Alam, S., Yadav, S., Prasanth, V. V., Singh, K. A.,Venkateshwarlu, C., Abbai, R., Vipparala, A. K. and Badri, J. 2021. Marker assisted forward breeding to develop a drought, bacterial blight and blast resistant cultivar. *Plant Genome.* pp. 1-17.

Singh, V. K., Singh, A., Singh, S. P., Ellur, R. K., Choudhary, V., Sarkhel, S., Singh, D., Krishnan, G. S., Nagarajan, M., Vinod, K. K. and Singh, U. D. 2012. Incorporation

of blast resistance into 'PRR78', an elite Basmati rice restorer line, through marker assisted backcross breeding. *Field Crops Res.* 128: 8-16.

Skamnioti, P. and Gurr, S. J. 2009. Against the grain: safeguarding rice from rice blast disease. *Trends Biotechnol.* 27(3): 141-150.

Spence, C., Alff, E., Johnson, C., Ramos, C., Donofrio, N., Sundaresan, V. and Bais, H. 2014. Natural rice rhizospheric microbes suppress rice blast infections. *BMCPlant Biol.* 14(130): 1-17.

Srinivasachary, S., Hittalmani, S., Shivayogi, S., Vaishali, M. G., Shashidhar, H. E. and Girish, K. K. 2002. Genetic analysis of rice blast fungus of southern Karnataka using DNA markers and reaction of popular rice genotypes. *Curr. Sci.* 82: 732- 735.

Srinivasprasad, B., Aruna, K., Balachandran, M. and Seshumadhav, K. 2009. Molecular mapping of rice blast resistance gene *Pi*-1(t) in the elite indica variety. *World J. Microbiol. Biotechnol.* 25(10): 1765-1769.

Stephen, A. Goff, S. A., Ricke, D., Lan, T. H., Presting, G., Wang, R., Dunn, M., Glazebrook, J., Sessions, A., and Oeller. 2002. A draft sequence of the ricegenome (*Oryza sativa* L. *ssp. japonica*). *Science* 296: 92-100.

Suh, J. P., Roh, J. H., Cho, Y. C., Han, S. S., Kim, Y. G. and Jena, K. K. 2009. The *Pi*40 gene for durable resistance to rice blast and molecular analysis of *Pi*40 advanced backcross breeding lines. *J. Phytopathol*. 99: 243-250.

Sundaram, R. M., Vishnupriya, R. M., Biradar, S. K., Laha, G. S., Reddy, A. G., Rani, S. N., Sarma, N. P., and Sonti, R. V. 2008. Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica* 160: 411-422.

Tabien, R.E., Pinson, S.R.M., Marchetti, M.A., Li, Z., Park, W.D., Paterson, A.H and Stanasel, J.W. 1996. Blast resistance genes from Teqing and Lemont. *In: Rice genetics III.* Khush, G.S. (Ed.), Proceedings of Third International Rice Genetics Symposium. Oct 16-20 1995, Manila, Philippines. pp. 451-455.

Takahashi, Y. 1965. Genetics of resistance to the rice blast disease. In: *The Rice Blast Disease*. The Johns Hopkins Press, Baltimore: 303-329.

Takashi, M., Qiaoping, Y., Ying, K., Nathalie, C., Yeisoo, Y., Lance, E., Palmer, Teh-Yuan Chow, V., Raghuvanshi, K. and Gaikwad. 2005. The map based sequence of the rice genome. *Nature* 436: 783-800.

Talbot, 2003. On the trail of a cereal killer: Exploring the biology of *Magnaporthegrisea*. *Ann. Rev. Microbiol.* 57: 177-202.

Tanksley, S. D., Yound, N. D., Paterson, A. H., and Bonierbale, M. W. 1989. RFLP mapping in plant breeding: new tools for an old science. *Bio-Technology* 7: 257-263.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.* 17: 6463-6471.

Tautz, D. and Renz, M. 1994. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Res.* 12: 4127- 4138.

Telebanco-Yanoria, M. J., Koide, Y., Fukuta, Y., Imbe, T., Kato, H., Tsunematsu, H. and Kobayashi, N. 2010. Development of near-isogenic lines of Japonica-type rice variety Lijiangxintuanheigu as differentials for blast resistance. *Breed. Sci.* 60: 629638.

Valent B., and Chumley F. G. 1994. Avirulence genes and mechanisms of genetic instabilities in the rice blast fungus. In: Zeigler, R.S., Leong, S.A., and Teng, P.S (eds), *Rice Blast Disease*, Wallingford, CAB International, pp.111-134.

Variar, M., Vera-Cruz, C. M., Carrillo, M. G., Bhatt, J. C. and Sangar, R. B. S. 2009. Rice Blast in India and Strategies to Develop Durably Resistant Cultivars. In: *Advances in Genetics, Genomics and Control of Rice Blast Disease*. (Eds.) Wang, G.L. and Valent, B. Springer Science+Business MediaBV: 359-373.

Wang, G. L., Mackill, D. J., Bonman, J. M., McCouch, M. C., and Champoux, M. 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. *Genet.* 136: 1421-1434.

Wang, J. C., Jia, Y ., Wen, J. W., Liu, W. P., Liu, X. M., Li, L., Jiang, Z. Y., Zhang, J. H., Guo, X. L., and Ren, J. P. 2013. Identification of rice blast resistance genes using international monogenic differentials. *Crop Prot.* 45: 109-116.

Wopereis, M. C. S., Defoer, T., Idinoba, P., Diack, S. and Dugue, M. J. 2009. Curriculum for participatory learning and action research (PLAR) for integrated rice management (IRM) in inland valleys of Sub-Saharan Africa. pp. 105-109.

Wu, J. L., Fan, Y. Y., Li, D. B., Zheng, K. L., Leung, H., and Zhuang, J. Y. 2005. Genetic control of rice blast resistance in the durably resistant cultivar Gumei 2 against multiple isolates. *Theor. Appl. Genet.* 111: 50-56.

Xiao-deng, Z., Hai-peng, Z., Rong-yao, C., Jie-yun, Z., Shi-hua, C. and Li-yong, C. 2012. Breeding of R8012, a rice restorer line resistant to blast and bacterial blight through marker-assisted selection. *Rice Sci.* 19(1): 29-35.

Xu Y., and Crouch, J. H. 2008. Marker-assisted selection in plant breeding: from publications to practice. *Crop Sci.* 48: 391-407.

Xu, Y. 2003. Developing marker assisted selection strategies for breeding hybrid rice.*Plant Breed. Rev.* 23: 73-174.

Yamasaki, Y. and Kiyosawa, S. 1966. Studies on inheritance of resistance of rice varieties to blast. Inheritance of resistance of Japanese varieties to several strains of the fungus. *Bull. Natl. Inst. Agril. Sci.* 14: 39-69.

Yu, J., Songnian, H., Wang, J., Wong, G., Ka-shu, G., Li, S., Liu, B., Deng, Y., Zhang. and Ziuqing. 2002. A draft sequence of rice genome (*Oryza sativa* L. *ssp. Indica*).*Sc*i. 296: 79-92.

Yu, Z. H., Mackill, D. J., Bonman, J. M. and Tanksley, S. D. 1991. Tagging genes for blast resistance in rice via linkage to RFLP markers. *Theor. Appl. Genet*. 81: 471-476.

Yu, Z. H., Mackill, D. J., Bonman, J. M., McCouch, S. R., Guiderdoni, E., Notteghem, J. L. and Tanksley, S. D. 1996. Molecular mapping of genes for resistance to rice blast (*Pyricularia grisea Sacc.*). *Theor. Appl. Genet.* 93: 859-863.

Zeigler, R. S., Tohme, J., Nelson, R., Levy, M. and Correa, F. 1994. Linking blast population analysis to resistance breeding: A proposed strategy for durable resistance. In: *Rice Blast Disease*. RS Zeigler, S Leong, PS Teng, eds. Commonwealth Agricultural Bureaux International, Wallingford, England, pp: 267-292.

Zhang, G. Q., Zeng, R. Z., Zhang, Z. M., Ding, X. H., Li, W.T., Liu, G. M., He, F. H., Tulukdar, A., Huang, C. F., and Xi *et al*. 2004. The construction of a library of single segment substitution lines in rice (*Oryza sativa* L.). *Rice Genet. Newsl*. 21: 85-87.

Zhou, B., Qu, S., Liu, G., Dolan, M. and Sakai, H. 2006. The eight amino-acid differences within three leucine-rich repeats between *Pi*2 and *Piz-t* resistance proteins determine the resistance specificity to *Magnaporthe grisea. Mol. Plant Microbe Interactions* 19: 1216-1228.

Zhou, J. H., Wang, J. L., Xu, J. C., Lei, C. L. and Ling, Z. Z. 2004. Identification and mapping of a rice blast resistance gene *Pi-g(t)* in the cultivar Guangchangzhan. *Plant Pathol.* 53: 191-196.

Incorporation of blast resistance into Jyothi and Kanchana rice (*Oryza sativa* L.) through marker assisted breeding

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ABSTRACT OF THE THESIS

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ABSTRACT

Rice blast caused by *Magnoporthe oryzae* is one of the most devastating diseases that is reported repeatedly from the rice growing areas of Kerala. The most effective strategy to combat this is development of resistant varieties.

Jyothi (Ptb39), one of the most popular high yielding rice variety of Kerala, with very good cooking and nutritional qualities, is highly susceptible to blast disease. Similarly, Kanchana (Ptb50), another high yielding variety suitable for both upland and wetland cultivation, is also highly susceptible to blast. The present study entitled 'Incorporation of blast resistance into Jyothi and Kanchana rice (*Oryza sativa* L.) varieties through Marker Assisted breeding' was attempted to transfer blast resistant genes from 'Parambuvattan', the traditional rice variety of Kerala, carrying two genes for resistance *Pi*-1 and *Pikh* genes for resistance using tightly linked microsatellite markers.

Studies were conducted during January 2020 to February 2022. The parents were evaluated for the agro-morphological characters from 20 plants for each variety. Observations recorded were plant height, number of tillers per plant, panicles per plant, panicle length, days to flowering, filled grains per panicle, sterile grains per panicle, 100 seed weight and grain weight per plant.

The polymorphic SSR markers, RM224 for *Pi-1* and RM206 for *Pikh*, were able to differentiate between the parents with the expected amplicon sizes of 148 and 146 bp in donor parent and the product size in susceptible parents was 170 and 160 bp, respectively.

For recovering the recurrent parent genome in the segregating generations, background markers were identified by screening the parents with 42 primer (distributed all over the chromosomes). Results indicated that eleven primers were polymorphic and produced distinct reproducible amplicon patterns for Parambuvattan and Jyothi while fifteen were polymorphic for Parambuvattan and Kanchana.

F_1 seeds were developed by crossing Parambuvattan with Jyothi and Kanchana to transfer the blast resistance genes. A total of 52 F_1 seeds were developed from the cross Jyothi x Parambuvattan and 48 from Kanchana x Parambuvattan.

Among eighteen F_1 seedlings of Jyothi x Parambuvattan only, four plants (Plant No. 3, 9, 13 and 18) exhibited the presence of both *Pi*-1 and *Pikh* genes in heterozygous condition whereas, among the fifteen F_1 seedlings of Kanchana x Parambuvattan only, three plants (Plant No. 1, 4 and 13) exhibited the presence of both *Pi*-1 and *Pikh* genes in heterozygous condition.

Seeds of the parents and F_1s of both the crosses were germinated and artificially inoculated. The inoculated seedlings were observed 6 and 10 DAI. Disease scoring was done using 0-9 scores as described in IRRI-SES scale. Per cent Disease Severity (PDS) was calculated for parents by taking total number of seedlings and for F_1 seedlings total number of leaves were taken into consideration. The donor Parambuvattan had a PDS of 26.6 whereas Jyothi and Kanchana were highly susceptible with PDS of 68.8 and 78.8, respectively.

PDS score of F_1 seedlings of the cross Jyothi x Parambuvattan was 34.3 while Kanchana x Parambuvattan had 32.4. PDS score of F_1 seedlings was similar to the donor Parambuvattan, indicating their partial resistance.

Four F_1 seedlings of Jyothi x Parambuvattan (Plant No. 3, 9, 13 and 18) had the PDS 24.8, 31.1, 28.8 and 29.7, respectively. Three F_1 seedlings of Kanchana x Parambuvattan (Plant No. 1, 4 and 13) had the PDS of 26.6, 31.3 and 28.9. The results indicated that the selected plants were showing presence of both *Pi*-1 and *Pikh* genes and partial resistance when inoculated. Hence, these selected plants can be forwarded for further generations to develop high yielding blast resistant rice lines.