Identification of the Donors for Blast Resistance from Traditional Rice Varieties of Kerala using Functional Markers

by

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DECLARATION

I, hereby declare that this thesis entitled **"Identification of the Donors for Blast Resistance from Traditional Rice Varieties of Kerala using Functional Markers"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

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Henry Nickolas

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LIST OF ABBREVIATIONS

µl	Microlitre
µM	Micro molar
^{0}C	Degree Celsius
A	Absorption
AICRP	All India Coordinated rice improvement programme
Avr	Avirulence gene
BC	Before Christ
bp	Base Pairs
CAPS	Cleaved Amplified Polymorphic Sequence
CD	Critical Difference
cM	Centimorgan
DAT	Days after Transplantation
dNTP	Deoxyribonucleotide triphosphate
DNA	Deoxyribonucleic acid
EDTA	Ethylene Diamine Tetra Acetic acid
et al.	And others
F:	Forward
Fig.	Figure
g	Gram
ha	Hectare
hrs	Hours
kb	Kilo base
M.	Magnaporthe
MAS	Marker Assisted Selection

ml	Millilitre
mM	Mill molar
mm	Millimetre
ng	Nanogram
NILs	Near Isogenic Lines
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait loci
R:	Reverse
R	Resistance gene
RBD	Randomized Block design
RFLP	Restriction Fragment Length Polymorphism
RM	Repeat Motif
RNA	Ribonucleic acid
rpm	Rotations per Minute
SDS	Sodium Dodecyl Sulphate
SSR	Simple Sequence Repeats
STMS	Sequence-Tagged Microsatellite Site
Taq	*Thermus Aquaticus*
TE	Tris EDTA buffer
U	Units of enzyme
V	Volt
viz.	Namely

Introduction

1. INTRODUCTION

Rice, *Oryza sativa* L., member of Poaceae family is the world's most important staple food crop, feeding more than half of the world's population. It is the second highest produced grain worldwide, predominant dietary energy source for 17 countries in Asia and provides 20 per cent of the world's dietary energy supply. Thus rice holds an indisputable position among the major crops from a point of view of global food security. In India, it is cultivated on an area of 42.8 million hectares with total production of 104.32 million tonnes in 2011-2012. And in Kerala, it is cultivated in an area of 2.34 lakh ha with a production of 6.25 Lakh tonnes during 2009-10 (Kumari, n.d.).

Rice the world's largest food crop providing caloric needs to millions of people daily, suffers attack by a large number of pests and pathogens, which under epidemic conditions, cause serious yield losses. Among these, rice blast caused by *Magnaporthe oryzae* is one of the most devastating and destructive diseases of rice worldwide. Under heavy dew, arial plant parts are affected, leaf surface becomes speckled with oval lesions and plants are liable to lodging if nodes are infected. If the panicles are infected, it results in severe yield losses.

The disease can be managed by the use of fungicides, resistant cultivars, agronomic practices and biotechnological methods. However, the use of resistant cultivars is the most economical and environment friendly method for the management of rice blast but the resistance is subject to break down due to appearance of new or more virulent races of the pathogen. Resistance to the *M. oryzae* is a classic gene-for-gene system, where a major resistance gene is effective against pathogen strains containing the corresponding avirulence gene.

DNA markers have been used effectively to identify resistance genes and marker assisted selection (MAS) has been applied for integrating different resistance genes into rice cultivars lacking them. In order to identify the new sources of resistance against blast, there is need for identification of resistance genes in genetically diversified rice material (Kumbhar *et al.*, 2013).

Kerala is considered as one of the centers of diversity of rice and the antiquity of rice cultivation here dates back to 3000 BC (Manilal, 1990). Rice covers a vast array of ecological niches in Kerala and a vast diversity of germplasm of both cultivated and wild rice exist here (Kumary and Francies, 2002). It is estimated that, nearly 2000 traditional varieties were predominantly cultivated in Kerala, across the eight agro-eco systems in the state. Many of these varieties possess several unique characteristics. The world renowned Pattambi rice varieties, which have contributed sources of resistance to many IRRI varieties, are all pureline selection of land races, hailing from the erstwhile Malabar province (Dev *et al.*, 1983).

Various scientists worked on identification of disease resistance genes in particular of blast resistance genes in rice. But, no pertinent studies have been completed in Kerala for the identification of these resistance genes at the genetic level or for a clear-cut utilisation of these, with the help of Marker Assisted Selection. This will help in developing donors for resistance genes for breeding under Kerala conditions.

This project to identify blast resistance gene donors from Kerala traditional varieties with the help of SSR markers was proposed with following objectives.

- Identifying the traditional rice varieties with blast resistance genes (*Pi 1, Pi 2, Pi kh*) using associated functional markers
- Field screening of selected accessions under disease stress condition for the conformation of resistance gene action

Review of Literature

2. REVIEW OF LITERATURE

Rice (*Oryza sativa* L.) is one of the most important cereals of the world and is consumed by one third of the world population. India ranks first in area (42.8 million hectare) and second in production (104.32 million tonnes) of rice globally. About 34 per cent of the total cultivated area of the nation is under rice cultivation and out of the total production of food grains; rice contributes 42 per cent (Krishnaveni *et al.*, 2012)

2.1 SIGNIFICANCE OF RICE BLAST DISEASE

Rice blast, the most common and serious disease which occurs in almost all rice growing regions of the world, attacks leaves, stem nodes, all parts of the panicle and grains (Chin, 1974). Blast disease of rice was first reported in China as 'rice fever disease' by Soong Ying Shin, in his book, 'Utilization of natural resources' (Ou, 1985). Rice blast is known to cause approximately 60 per cent to 100 per cent yield losses (Kihoro *et al.*, 2013). Blast disease caused by *Magnaporthe oryzae* has become an important limiting factor in rice production at global level. The fungus was first described from diseased rice plants in Italy (Cavara, 1891) and subsequently in Japan (Shirai, 1896). It has become an important limiting factor for rice cultivation in various parts of the world due to prevailing blast conducive environments during the crop season (Sharma *et al.*, 2002).

Several parameters can be analysed to justify the importance of this fungal disease. First, its geographic distribution is wide; rice blast is present in all rice growing areas worldwide. Second, yield losses can be very large; in upland conditions, complete yield loss is frequently observed, with dramatic impact on populations fed mainly on rice. Third, fungicides are often used to control rice blast and this generates additional costs in rice production (Yamaguchi, 2004).

In India, rice blast was first recorded in 1913, however a devastating epidemic occurred in Thanjavur delta of South India, during 1919

(Padmanabhan, 1965). Seven epidemics of blast occurred in four states of India, between 1980 and 1987, two each in Himachal Pradesh, Andhra Pradesh and Tamil Nadu and one in Haryana causing heavy losses (Nagarajan, 1988). Severe attack of blast disease causes epidemics in the field (Teng *et al.*, 1991) and complete loss of seedlings in the nursery (Chaudary *et al.*, 1994). During *kharif* season, the disease is prevalent throughout the rice growing areas in India especially in Himachal Pradesh, Uttarakhand, Kerala, Jharkhand, Madhya Pradesh, Chhattisgarh, Assam, Tripura, West Bengal, Orissa, Maharashtra, Andra Pradesh, Karnataka and Tamil Nadu (ICAR, 2013). The survey done by Yamini, (2012) on the incidence and intensity of blast disease of rice revealed that, blast disease incidence of rice was in the range of 34 to 80 per cent and severity in the range of 11.7 to 65.13 per cent in different rice growing areas of Palakkad district.

2.2 CAUSAL ORGANISM

Blast disease of rice caused by *Magnaporthe oryzae*, with the anamorphic stage *Pyricularia oryzae* Cavara [synonym *Pyricularia grisea* (Cooke)Sacc. the anamorph of *Magnaporthe grisea* (Herbert)] is considered as the principal disease because of its wide distribution and its destructiveness under favourable conditions (Gnanamanickam, 2009).

It is a potentially damaging disease in upland environment where, drought and soil stress pre-dispose the rice crop to severe attacks by the pathogen. Yield loss due to blast can be as high as 50 per cent when disease occurs in endemic proportions. *Magnaporthe oryzae,* a filamentous heterothallic ascomycetous fungus which comes under the genus *Magnaporthe* collectively parasitizes more than 50 hosts, individual isolates have limited host range and cross infectivity is relatively rare. The ability of this fungus to quickly overcome resistance within a short time after the release of a new cultivar has made breeding for resistance a constant challenge. An understanding of the structure and dynamics of pathogen population is essential for prudent implication of strategies for management of the disease (Babujee and Gnanamanickam, 2000).

The primary cycle of *Magnaporthe oryzae* may be initiated by conidia or other fungal structures. Survival structures of *Magnaporthe oryzae* are conidia, mycelia, chlamydospores and perhaps the perfect stage. Mycelia have been known to survive one year in dry straw and two to four years on nodal lesions. The blast fungus can also survive on seed and conidia can remain viable on seed and straw (Zeigler *et al.,* 1994). Conidia are produced on lesions only when relative humidity is above 89 per cent with an optimum of 93 per cent. Spore release typically follows a diurnal pattern. Release begins around midnight, probably as a result of conidial maturation coinciding with dew deposition (Gruramanis, 1995)

Blast reaction on the host varies according to varieties. Within the same variety, the severity varies according to the age of plant, host nutrition and meteorological factors. There are two distinct stages in plant growth in which rice is highly susceptible for foliar infection of blast disease, *viz.*, seedling stage and rapid tillering stage. Meteorological factors are the most important elements in epidemiology of blast disease of rice. Conidia are not produced below 88 per cent relative humidity and at least 90 per cent saturation is essential for their abundant production. Dispersal is highest during night hours when the temperature is in the range of 25 °C to 27 °C. Host nutrition markedly affects reaction of rice to blast, especially in susceptible varieties. Heavy manuring of nitrogen fertilizers augments disease proneness. Greater the accumulation of nitrogen in the leaf tissues under high levels of manuring, more susceptible the variety is (Singh, 1996).

2.3. ISOLATION OF PATHOGEN

Isolation of *Magnaporthe oryzae* from the diseased samples of leaves, neck and node of the infected rice plants was done by Padmanabhan *et al.* (1970) on Oat meal agar with traces of biotin and thiamine. Dhua (1986) proposed a selective method for isolating *Pyricularia,* in which the leaf samples collected at 4 pm were sterilized in sodium hypochlorite and incubated in Oat meal agar for 15 hours at 24 ± 2 °C to get a pure culture. For inducing sporulation before

isolation, neck blast symptoms were collected washed in sterile distilled water, placed on moist filter paper in petridishes at room temperature. Conidia from lesion surface was spread on 3 per cent water agar with a sterile loop and incubated overnight. Single germinating conidia was isolated and transferred to PDA medium (Xia *et al.*, 1993).

2.4. MOLECULAR DIAGNOSIS OF BLAST PATHOGEN

The recent advent of molecular biology has contributed to the diagnosis of plant pathogenic fungi by offering new revolutionary methods for quicker and more accurate detection, identification and quantification. As initial molecular markers, isoenzymes and DNA probes were used to detect and differentiate various fungal species. Subsequently, molecular diagnostics based on the PCR technique have further accelerated the process and facilitate a more sensitive method of detection. Fungi can be identified at the species level by primers designed on selected conserved sequences like the rRNA gene cluster followed by further characterization of the amplified fragment. The molecular genetic analysis of rice blast fungus was done as early as 1991 by Valent and Chumley. Harmon *et al.* (2003) developed and evaluated a polymerase chain reaction (PCR) based method to detect *Magnaporthe oryzae* in infected perennial rye grass tissue. The morphological and molecular variability of rice blast pathogen *Magnaporthe oryzae* was studied by Meena (2005). Kusaba *et al.* (2006) studied the molecular and genetic characterization and host specificity of pathogen isolates from annual rye grass in Japan. The entire genomes of the rice blast fungus and rice have been sequenced and that *Magnaporthe oryzae* is the first plant pathogenic fungus to have its genome sequenced and released to the public (TeBeest *et al.*, 2007).

2.5. IMPORTANCE OF HOST PLANT RESISTANCE FOR CONTROLLING BLAST DISEASE

Resistance is often short lived; with varieties released as resistant showing susceptibility after only a few seasons of wide spread cultivation. Although resistance conditioned by single major genes has often proved unstable, major gene resistance to blast has been useful and should continue

to be important in rice production if resistance genes are carefully selected and managed (Kiyosowa, 1980). It is common that resistant varieties became susceptible after a short time in production. Therefore, it is essential to study the durability in resistance of rice varieties to blast disease, to help in identifying the varieties which could sustain from the disease pressure in various ecosystems (Wang *et al.*, 1989).

Major genes are those that prevent completion of life cycle of incompatible races of *M. oryzae*. QTLs are those that reduce the sporulation of the pathogen within a compatible interaction. The deployment of major gene resistance will minimize selection pressure and there by prevent evolution of resistance in pathogen population (Bonman *et al.*, 1992).

Resistance to blast may be conditioned by major genes or by quantitative trait loci (QTLs) (McCouch *et al.*, 1994). The interaction between a plant resistance gene and the corresponding avirulence gene of the pathogen triggers a series of defence responses (Bent 1996; Hammond-Kosack and Jones, 1996). One of the most common features is localized cell death at the infection site, known as hypersensitive response (HR). The HR is correlated with a transient burst of active oxygen species, activation of specific defense- related genes, accumulation of antimicrobial compounds, and alterations of the plant cell wall (Hammond- Kosack and Jones, 1996; Dangl *et al.*, 1996). It has been documented in many plant species that HR is associated with increased resistance throughout the plant to subsequent infection by different pathogens (Ryals *et al.*, 1996).

Parlevliet (1998) describes partial resistance as an incomplete quantitative resistance based on minor genes. It is characterized by compatibility between the pathogen of the plant with reduced development of the disease compared to plants with no Partial resistance (Parlevliet, 1998). Genetic study indicates that partial resistance is under oligo or polygenic control and can be affected by the environment (Parlevliet, 1998).

The resistant genes are clustered in several regions of the rice genome. Nine loci have been reported on chromosome 11 and five loci have been reported on chromosome 6 (Babujee and Gnanamanickam, 2000).

The use of chemicals or additional operations to reduce disease incidence will lead to a tremendous increase in the cost of cultivation. As most rice farmers are resource poor, the increase in cultivation cost will be unbearable for them. Moreover, a hike in the chemical content in environment leads to numberless problems for the living world. Here comes the importance of resistance mechanism which can support farmers in disease control. Independent of the rice-growing system or area, breeding for resistance to blast is needed to control the disease despite the availability of effective fungicides (Ballini *et al.*, 2008). The use of resistant varieties is the most practical and economical approach to control blast. However, the use of those variety has not been completely successful due to the presence of lineages (that may consist of different physiologic races) overcoming host resistance. Effective resistance can be achieved by combining into the same cultivar different race-specific genes and genes conferring quantitative resistance. Another method is by deploying resistance genes in mixed plant populations. Recent studies indicated that use of cultivar mixture is an effective tool in blast management. IRRI scientists introduced the practice of interplanting glutinous rice varieties with blast-resistant hybrid varieties in Yunnan province, China. Blast caused great yield loss on traditional glutinous rice varieties and farmers were spraying fungicides for up to seven times. Interplanting has prevented the fungus from continuous build-up of inoculum that had previously occurred in the monoculture fields of the glutinous varieties (IRRI, 2008).

More than 400 high yielding commercial cultivars having blast resistance have been released in india since the use of host resistance is an effective and economical way to control the blast disease (Khush and Jena, 2009).

However, the pathogen is highly variable and many of these varieties have become susceptible over a period of time. To know the effectiveness of blast resistance genes, the multi - environment tests (METs) were done under AICRP which indicated that, the durable resistance of rice varieties Tadukan, Rasi, Tetep and IR-64 can be potential sources for blast resistance breeding (AICRP, 2011).

In a typical gene-for-gene system, the host resistance (R) gene product interacts with the pathogen avirulence (Avr) gene product, leading to an incompatible reaction. During this process, R-genes are believed to activate defence pathways involved in the resistance mechanism (Liu *et al.*, 2010).

2.6 GENES CONFERRING BLAST RESISTANCE

The rice blast resistance genes confer a high race specific resistance to blast fungus races. The specific interaction between resistance genes in rice plant and avirulence genes in the fungal pathogen can be well explained by the gene-for-gene system (Flor, 1971). Although major genes have often been cited as the underlying cause of resistance instability (Ahn and Ou, 1982), strategies for developing durable resistance mediated by major genes have been proposed earlier (Ou, 1985).

The inheritance of host resistance to rice blast has been studied extensively. Using near-isogenic lines (NILs) developed at IRRI, Mackill and Bonman (1992) identified four major genes and designated these as *Pi-1(t), Pi-2(t), Pi-3(t)* and *Pi-4(t)* with two alleles, *Pi-4a(t)* and *Pi-4b(t).* Durable resistance is contributed by both major and minor genes (Zhu *et al.*, 1993).

Several genes conferring complete and partial resistance to blast disease have been located on rice chromosome maps. Several genes for blast resistance have been mapped by using restriction fragment length polymorphism (RFLP) markers (Wang *et al.*, 1994). Most of the known blast-resistance genes are located on chromosomes 6, 11 and 12 (McCouch *et al.*, 1994). These approaches depend upon careful characterization of the resistance spectrum of the genes in question

and combining them such that the gene 'pyramid' is effective against the target pathogen population (Chen *et al.*, 1995; Zeigler *et al.*, 1995). The molecular analysis of the corresponding avirulence genes in blast fungus and its genome analysis are the most advanced in the pathogenic fungi. Several avirulence genes from the fungus have been cloned (Kang *et al.*, 1995; Sweigard *et al.*, 1995). Identification and isolation of both resistance genes from the host and avirulence genes from the pathogen can be expected to help clarify the molecular mechanisms underlying specific host - pathogen recognition in plants. The accumulation of information regarding resistance gene mediated host defense mechanisms will facilitate engineering of genes conferring durable resistance to a broad spectrum of pathogens. Plant resistance genes have been extensively studied, and many resistance genes in various plants have been cloned by map-based cloning or transposon tagging strategies (Baker *et al.*, 1997).

Combining several genes and monitoring their presence is difficult by conventional methods because of their epistatic effects and the simple masking of the effect of one gene by another. Mapping blast resistance genes and locating closely linked markers has made it possible to confirm the presence of given gene in a variety with multiple genes. Thus, even when the phenotype is ambiguous, the presence of a gene can be monitored over successive segregating generations. *Pi-2(t)* was allelic to *Pi-z* and *Pi-4a(t)* was allelic to *Pi ta*. Over the past few years, more than 40 rice blast-resistance genes (R-genes) have been identified, and over 30 of them have been mapped on various rice chromosomes (Ahn *et al.*, 2000). Fukuoka (2001) mapped the resistance gene, designated *pi 21*, on chromosome 4 as a single recessive gene between RFLP marker loci G271 and G317 at a distance of 5.0 cM and 8.5 cM respectively.

The expression patterns of defence response genes involved in rice – *Magnaporthe oryzae* interaction also have been widely studied (Kim *et al.*, 2001).

Pi-z, a dominant resistant gene located on the short arm of chromosome 6 that confers complete resistance to five races of blast was identified by

Conaway *et al.*, (2003). Genetic and molecular analysis localized a major resistance gene, *Pi 40(t)*, on the short arm of chromosome 6, and another four resistance genes (*Piz, .Piz-5, Piz-t*, and *Pi 9*) were also identified, in a study conducted by Jeung *et al.* (2007). Study also revealed that, the rice blast resistance (R) gene *Pi-ta* mediates gene-for-gene resistance against strains of the fungus *Magnaporthe oryzae* that express avirulent alleles of *AVR-Pi ta.* The completion of the genome sequencing of both rice and *Magnaporthe oryzae has* strengthened the position of rice blast disease as a model to study plant - pathogen interactions in monocotyledons (Ballini *et al.*, 2008).

However, none of the genes has been shown to have resistance potential against all the strains of *Magnaporthe oryzae.* Identification and characterization of appropriate blast resistance (R) genes is vital for the development of resistant rice varieties. Several attempts to utilize R-genes in rice blast by developing disease resistant cultivars have been futile owing to the lack of information regarding the intricate mechanisms involved in R-gene mediated resistance pathways and the high rate of variability in the corresponding avirulence (Avr) genes. Therefore, in order to develop sustainably successful blast resistant rice lines, a comprehensive dissection of the reactions downstream of R - Avr interactions is highly desirable. The co-expression patterns of bacterial disease resistance genes and their transcriptional regulators in transgenic rice have indicated that the resistance genes trigger an immune response (Sana *et al.*, 2010; Zhou *et al.*, 2010).

During the last few years, genetics of blast resistance in rice has been extensively studied and many dominant R - genes conferring complete resistance to *Magnaporthe oryzae* have been identified. Approximately seventy three major resistance genes and three hundred and fifty QTLs have been mapped on almost all the chromosomes of rice, except perhaps chromosome 10. Chromosome 6 of rice has been reported to contain many important genes and QTLs for blast resistance, many of which are closely mapped to each other (Ghaley *et al.*, 2012).

Wang *et al.* (2013) in their study inoculated, forty-four representative local blast isolates onto international monogenic differentials carrying twenty-four major blast resistance genes *Pia, Pib, Pii, Pik, Pikh, Pikm, Pikp, Piks, Pish, Pit, Pita, Pita 2, Piz, Pizt, Pi1, Piz 5, Pi 3, Pi 5*(t), *Pi 7*(t), *Pi 9, Pil 2*(t), *Pi 11*(t), *Pi 19*, and *Pi 20* and the susceptible recurrent parent Lijiangxin tuan heigu (LTH), under greenhouse conditions. The percentage of virulent reactions of monogenic lines to the forty-four isolates was found ranging from 8.3 per cent to 79.2 per cent. LTH was susceptible to all forty-four isolates. All twenty-four monogenic differential lines were resistant to at least 4 isolates of *M. oryzae*, and the frequency of resistant reactions of the monogenic lines carrying *Pi 9, Pi 19, Piz, Piz-5, Piz-t, Pil 2*(t), *Pi 5*(t), and *Pi kh* were 94.2 per cent, 84.1 per cent, 81.8 per cent, 81.8 per cent, 79.5 per cent, 72.7 per cent, 68.2 per cent, and 68.2 per cent, respectively. This study suggested that *Pi 9, Pi 19, Piz, Piz-5, Piz-t, Pil 2* (t), *Pi 5* (t) and *Pi kh* may be important R - genes for preventing blast disease.

2.6.1 **Gene *Pi 1***

Using near-isogenic lines (NILs) developed at IRRI, Mackill and Bonman (1992) identified four major genes and designated these as *Pi-1(t), Pi-2(t), Pi-3(t) and Pi-4(t).* Allelism tests by Inukai *et al.* (1994a) differentials indicated that the blast resistance genes *Pi-1(t)* and *Pi-3(t)* were different from any other blast resistance genes already reported. Yu *et al.* (1996) also reported the resistance capacity of the gene *Pi-l(t)* which is present on the chromosome 11. The near-isogenic line - C101LAC in the background of the susceptible recurrent parent CO 39, carries the major gene *Pi-1* (Bonman, 1998). Three major genes (*Pi 1, Piz-5 and Pita*) for blast resistance on chromosomes 11, 6 and 12, respectively, were fine-mapped and closely linked RFLP markers were identified (Hittalmani *et al.*, 2000).

2.6.2 Gene *Pi 2*

Yu *et al.,* 1991 explained the complete resistance of Both *Pi-2(t)* and *Pi-4(t)* genes of rice to the blast fungal pathogen *Magnaporthe oryzae.* From several studies of NILs in CO39 genetic background (Inukai *et al.,* 1994b; Zeigler *et al.,* 1995) the resistance spectrum of one gene, *Pi 2(t),* was found to be quite broad. As economically important plant genes, they have been characterized phenotypically. Near isogenic line (NIL) C101A51 is identified as a gene source for *Pi-2(t)* (Chen *et al.,* 1996). Blast resistance genes were conferred by independent dominant genes in the NILS C101Lac and C101A51 designated *Pi-1 and Pi 2* respectively (Bonman, 1998).

2.6.3 Gene *Pi kh*

Sharma *et al.* (2005a) articulates that, the rice blast resistance gene *Pi-kh* has been isolated from the indica rice line Tetep showing resistance to different *Magnaporthe oryzae* strains in the North-Western Himalayan region of India. The gene *Pi 54* is present in rice line Tetep and it was mapped with CAPS and STMS DNA markers at 1.6 and 1.1 cM distances respectively (Sharma *et al.*, 2005a). Gupta *et al.* (2011) also enunciates that *Pi 54* gene (*Pi-kh*) confers a high degree of resistance to diverse strains of the fungus *Magnaporthe oryzae.* Recently, Costanzo and Jia (2010) and Rai *et al.* (2011) reported that *Pi 54 (Pi-kh)* confers broad spectrum resistance against geographically diverse strains of *Magnaporthe oryzae* collected from various parts of India and the US. Later on, physical location of this gene was determined on long arm of rice chromosome 11 (Rai *et al.*, 2011).

2.7 MOLECULAR MARKERS LINKED TO BLAST RESISTANCE GENES

The uses of molecular markers are based on the naturally occurring DNA polymorphism, which forms basis for designing strategies to exploit for applied purposes. A marker must be polymorphic that is, it must exit in different forms so that chromosome carrying the mutant genes can be distinguished from the chromosomes with the normal gene by a marker it also carries. Genetic polymorphism is defined as the simultaneous occurrence of a trait in the same

population of two discontinuous variants or genotypes. DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Unlike protein markers, DNA markers segregate as single genes; and they will not be affected by environment. DNA is easily extracted from plant materials and its analysis can be cost and labour effective. The first such DNA markers to be utilized were fragments produced by restriction digestion - the restriction fragment length polymorphism (RFLP) based genes marker. Consequently, several markers system has been developed (Kumar *et al.*, 2009)

Microsatellite markers, for the identification of closely related cultivars developed from genomic libraries can belong to either the transcribed region or the non-transcribed region of the genome, and rarely is there information available regarding their functions. Microsatellite sequences are especially suited to distinguish closely related genotypes; because of their high degree of variability, they are, therefore, favoured in population studies (Smith and Devey, 1994). It seems that the QTLs reported by Wang *et al.* (1994) for blast lesion number and blast diseased leaf area and the one identified for blast resistance in present investigation might be the same, as blast lesion number and blast diseased leaf area are highly correlated to blast resistance in the field condition. In that study, they also reported QTLs for blast lesion number and blast diseased leaf area on the short arm of chromosome 6 with marker RZ398 as one closely linked to these QTLs.

Microsatellites, which is also known as Simple Sequence Repeats (SSRs), are sections of DNA, consisting of tandemly repeating mono-, di-, tri-, tetra- or penta-nucleotide units that are arranged throughout the genomes of most eukaryotic species. Microsatellite polymorphism can be detected by Southern hybridisation or polymerase chain reaction. Microsatellites represent tandem repeats, but their repeat motifs are shorter (1–6 base pairs). If nucleotide sequences in the flanking regions of the microsatellite are known, specific primers (generally 20–25 bp) can be designed to amplify the microsatellite by PCR (Powell *et al.*, 1996).

An RFLP map of rice chromosome 6, where the *Pi-2(t)* gene and RG64 marker are linked at a distance of 2.8 + 1.4 (S.E.) cM. *Pi-l(t)* was located within an introgressed region on chromosome 11, defined by marker loci RZ424, RZ536 and RG303. RZ536 was the closest marker, located at a distance of 14.0+4.5 cM. (Yu *et al.,* 1991). Identifying flanking DNA markers located within 5 - 10 cM from a gene of interest has yielded high level of selection accuracy for resistance (Hittalmani *et al.*, 1995). Chen *et al.* (1997) mapped the markers RZ398, RM204 and RM225 in the same genomic location on chromosome 6. SSR marker RM204 found associated with blast resistance has been mapped on the short arm of chromosome 6 at 17.2 cM, 31.1 cM by Temnykh *et al.* (2000) and 25.1 cM by Temnykh *et al.* (2001) in their respective genetic maps. They also mapped the marker RM225 on short arm of chromosome 6 at 1.1 cM away from the marker RM204. A microsatellite marker, MRG5836 was the most useful for identifying the gene *Pi-z* (Conaway-Bormans *et al.*, 2003). Sharma *et al.* (2005b) mapped the *Pi kh* gene between two SSR markers TRS26 and TRS33 which are 0.7 and 0.5 cM away respectively and can be used in Marker Assisted Selection for blast resistant rice cultivars. Eizenga *et al.* (2006) reported marker RM225 and RM204 to be close to the blast resistant genes *Pi 22* and *Pi 27.* Sharma *et al.* (2010) defined that, SSR marker RM206 is closely linked to the blast resistance gene *Pi kh*. Arunakanthi *et al.*, (2008) has confirmed the presence of *Pi-1* and *Pi-2* gene by genotyping analysis with SSR markers RM224 an RM527 respectively.

Materials and Methods

3. MATERIALS AND METHODS

The present study to identify blast resistance gene from traditional rice varieties of Kerala using functional markers was carried out in the department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during 2012-2014.

The investigation was carried in two experiments

1. Molecular marker analysis for detecting blast resistance genes
2. Field screening for the blast resistance

The experimental materials consisted of 30 traditional rice varieties selected based on the reports on the blast resistance. Name of the varieties, source of collection and other details are given in Table 1.

In the first experiment these varieties were analysed for the presence of resistance gene using specified SSR markers and corresponding check varieties – C101LAC (for gene *Pi 1*), C101A51 (for gene *Pi 2*), Tetep (for gene *Pi kh*) and BPT5204 (for susceptible gene).

The second experiment was conducted in a disease prone farmer's field at Pattambi, Palakkad District, Kerala. This experiment included thirty traditional varieties in the first experiment and 2 check varieties, Tetep (resistant check) and Jyothi (susceptible check).

3.1 MOLECULAR MARKER ANALYSIS

3.1.1 Isolation of Genomic DNA

Isolation of genomic DNA was done from all the 34 samples from leaves following the procedure of Regowsky *et al.* (1991) with required modifications. The procedure is as follows:

- Approximately 1 g of tender leaf sample was taken in a clean autoclaved mortar and crushed by freezing in liquid nitrogen

Table 1. Varieties used in the study and their characters

Sl. No.	Variety name	Source of Seed	Photosensitivity	Kernel colour
1	Aryan	RARS, Pattambi	Photo insensitive	Red
2	Ponnaryan	RARS, Pattambi	Photo insensitive	Red
3	Vellari	RARS, Pattambi	Photosensitive	Red
4	Velutharikayama	RARS, Pattambi	Photo insensitive	Red
5	Parambuvattan	RARS, Pattambi	Photo insensitive	Red
6	Thavalakkannan	RARS, Pattambi	Photo insensitive	Red
7	Veluthari Thavalakkannan	RARS, Pattambi	Photo insensitive	White
8	Thekkancheera	RARS, Pattambi	Photo insensitive	Red
9	Thekkan chitteni	RARS, Pattambi	Photosensitive	Red
10	Kayama	RARS, Pattambi	Photo insensitive	Red
11	Maskathi	RARS, Pattambi	Photo insensitive	White
12	Kavunginpoothala	RARS, Pattambi	Photosensitive	White
13	Jeddu halliga	RARS, Pattambi	Photo insensitive	Red
14	Eravapandi	RARS, Pattambi	Photosensitive	Red
15	Athikkiraya	RARS, Pattambi	Photosensitive	Red
16	Vadakkan chitteni	RARS, Pattambi	Photosensitive	Red
17	Thekkan	RARS, Pattambi	Photosensitive	Red
18	Veluthavattan	RARS, Pattambi	Photo insensitive	Red
19	Cheriya Aryan	RARS, Pattambi	Photo insensitive	Red
20	Kodiyan	RARS, Pattambi	Photosensitive	Red
21	Kattamòdan	RARS, Pattambi	Photo insensitive	Red
22	Karuthamodan	RARS, Pattambi	Photo insensitive	Red
23	Chuvannamodan	RARS, Pattambi	Photo insensitive	Red
24	Oorumundakan	RRS, Kayamkulam	Photosensitive	Red
25	Njavara from Kunnathoor	FSRS, Kottarakkara	Photo insensitive	White
26	Palakkadan njavara from Elampalloor	FSRS, Kottarakkara	Photo insensitive	White
27	Nadan njavara from Elampalloor	FSRS, Kottarakkara	Photo insensitive	White
28	Njavara from Karipra	FSRS, Kottarakkara	Photo insensitive	White
29	Cheradi from Kunnathoor	FSRS, Kottarakkara	Photosensitive	Red
30	Kumpa cheradi from Puthoor	FSRS, Kottarakkara	Photosensitive	Red
31	Jyothi	RARS, Pattambi	Photo insensitive	Red
32	Tetep	DRR, Hyderabad	Photosensitive	White
33	C101LAC	DRR, Hyderabad	Information not available	White
34	C101A51	DRR, Hyderabad	Information not available	White
35	BPT5204	DRR, Hyderabad	Photo insensitive	White

- The powder was transferred to 2 ml eppendorf tube and 1 ml of extraction buffer [1.00 g SDS (1 per cent), 1.576 g Tris HCl (100 mM), 0.584 g Sodium chloride (100 mM), 0.372 g EDTA (10 mM), volume made upto 100 ml with distilled water] was added
- The tubes were then placed in a water bath maintained at 60 °C for 30 minutes after homogenization
- The mixture was then centrifuged at 10000 rpm at 4 °C for 10 minutes
- The aqueous phase was collected and 400 µl of phenol chloroform (25:24) was added and again centrifuged at 10000 rpm at 4 °C for 10 minutes
- The supernatant was collected to which 200 µl of chloroform iso-amyl alcohol (24:1) was added and centrifuged at 10000 rpm at 4 °C for 10 minutes
- The aqueous phase was collected and 200 µl of chloroform iso-amyl alcohol (24:1) was added again and centrifuged at 10000 rpm at 4 °C for 5 minutes
- The supernatant was collected and 60 µl of 3 M sodium acetate and 600 µl ice-cold isopropanol were added and kept overnight at – 20 °C for precipitation
- The solution was centrifuged after about 16 hours at 12000 rpm for 10 minutes and the supernatant was discarded without dislodging the pellet
- The precipitate was then washed twice using 70 per cent ethanol and dried
- After drying, the precipitate was dissolved in 100 µl 0.1x TE buffer [tris buffer 0.12 g (10 mM), EDTA 0.037 g (1 mM)] and stored at -20 °C

3.1.2 Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out in a horizontal gel electrophoresis unit by following procedure.

- Required amount of agarose (0.8 per cent) was weighed and melted in 1x TAE buffer
- After cooling the solution to 42-45 °C, add ethidium bromide at the rate of 2 µl per 100 ml

- The solution was poured on to a preset, sealed gel casting tray with comb fixed in position, to a height of 3 mm – 5 mm
- The gel was solidified for 15-20 minutes
- Comb and sealing tapes were removed
- Then the tray was submerged in the electrophoresis tank filled with 1x TAE buffer ensuring that the buffer covered the gel to a height of 1 mm
- DNA sample and loading dye [glycerol 30 per cent + bromophenol blue] were mixed in 5:1 ratio
- DNA dye mixture was loaded into the slots of gel using a micropipette near the negative terminal
- After attaching cathode and anode of the electrophoresis unit to the power supply, a constant voltage of 60 V was used for the run
- Turned off the power supply when the loading dye moved about $3/4^{th}$ of the gel
- Documented the gel using gel documentation system

3.1.3 Quantification of DNA

Quality and the quantity of DNA was calculated using spectrophotometer reading. 5 µl of DNA dissolved in 0.1x TE was added to 3 ml of distilled water and absorbance at 260 and 280 nm was read against distilled water as blank, using UV spectrophotometer. The concentration of DNA in sample was calculated using formula

$$\text{Amount of DNA (µg/ml)} = \frac{A_{260} \text{ x } 50 \text{ x Dilution factor}}{1000}$$

Where, A_{260} = absorption at 260 nm

The quality of DNA was judged from ratio of absorbance values at 260 nm and 280 nm. A ratio of 1.8 - 2.0 indicates best quality DNA.

3.1.4 Primers

Reported SSR primers specific to genes under study were used for PCR amplification. Sequence of the primers and annealing temperature is given in Table 2.

3.1.4.1 PCR Aliquot

Procedure of Cordeiro *et al.* (2002) was used for the amplification of DNA. The reaction was carried out in 25 µl reaction mixture containing 25 ng genomic DNA, 1.5 mM MgCl2, 200 µM total dNTPs, 1 unit of Taq DNA polymerase, 1x PCR buffer and 0.2 µM each of forward and reverse primer.

3.1.4.2 PCR - Cycle

Amplification was done in programmable thermocycler (BioRad) that was programmed as follows:

An initial denaturation at 94 °C for 5 minutes followed by 35 cycles of 94 °C for 1 minute, at annealing temperature for 1 minute and 72 °C for 2 minutes; followed by a final elongation at 72 °C for 5 minutes and a 4 °C hold. Amplified products were separated by agarose gel electrophoresis using 1.8 per cent gel as described earlier and photographed using gel documentation system.

3.2 FIELD SCREENING

Experiment was conducted with thirty traditional varieties and two check varieties (one susceptible and one resistant) in an area of 5 cents during *kharif* season, 2013.

Nursery was prepared as per the recommendations of KAU (KAU, 2011). Seedlings were pulled out 30 days after sowing and transplanted to the main field.

Table 2. Sequence of the selected primers and respective annealing temperature

Sl. No.	Gene	Primer	Nucleotide Sequence	Annealing temperature (°C)
1	*Pi 1*	RM224	F: ATCGATCGATCTTCACGAGG	58
			R: TGCTATAAAAGGCATTCGGG	
2	*Pi 2*	RM527	F: GGCTCGATCTAGAAAATCCG	52
			R: TTGCACAGGTTGCGATAGAG	
3	*Pi kh*	RM206	F: CCCATGCGTTTAACTATTC	55
			R: CGTTCCATCGATCCGTAT	

3.2.1 Design of Experiment

Randomized Block Design was used for the field level screening of the varieties. 32 varieties were taken and they were arranged in lines, with each line containing 20 hills in 3 replications. Layout of uniform blast nursery was used (Fig. 1) (Prasad et al, 2011). The uniform blast nursery (UBN) method, approved by International Rice Research Institute (IRRI) was used for screening of rice germplasm against *Magnaporthe oryzae.* Local susceptible variety is sown as border rows on all sides of the bed. The susceptible check variety was sown after every twenty test entries. This helps to spread the inoculums. Test material was sown in rows perpendicular to the border rows. Scoring was done after 10 - 15 days of post infection depending on the severity of the infection on susceptible check using Standard Evaluation System (SES) (IRRI, 1996).

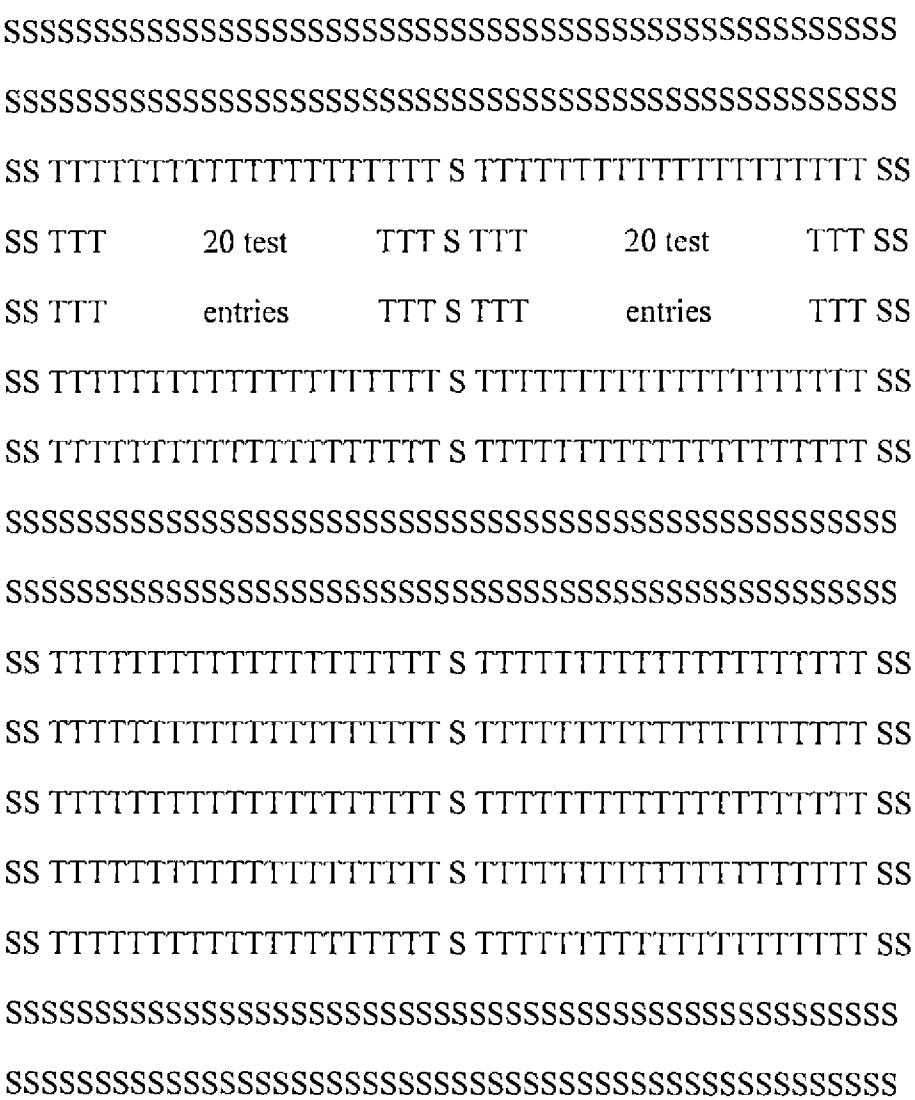
3.2.2 Isolation of Pathogen

The blast pathogen was isolated from paddy leaf samples collected from hot spot areas of farmer's field in Pattambi during December 2012, following the standard procedures (Tuite, 1969) using Potato Dextrose Agar (PDA) medium (Yamini, 2012).

The isolation of the pathogen was done from the leaves showing typical symptoms after keeping in moist chamber for 24 hours. The tissue isolation technique was done following the standard procedure. On the third day when mycelial growth was observed, the mycelial bits were aseptically transferred to PDA slants. The cultures were periodically sub cultured in PDA slants. The pathogenicity test of the isolate was conducted and proved following Koch's postulates.

3.2.3 Inoculation of the pathogen

Spore suspension was prepared from 7 day old blast culture grown on PDA and the mycelium was scraped in 10 ml of distilled water and the solution was filtered through two fold cheese cloth to remove the fungal debris. The spore concentration was adjusted to 1 x 10^5 spores per ml using



T- Each test entry in a single row 10 cm apart

S- Highly susceptible blast varieties

Figure 1. Layout of Uniform Blast Nursery (UBN) for Screening against Leaf Blast disease developed by IRRI (1996)

haemocytometer as described by Aneja, (2009). The spore suspension containing Tween – 20 (0.2 per cent) was sprayed uniformly over two month old plants. The inoculum was sprayed during evening till the entire plant surface become wet with spore suspension and left overnight. Water was sprayed three to four times during day time to maintain high humidity. All the agronomic practices were carried out, in time, as per the Package of Practices Recommendations by the Kerala Agricultural University (2011).

3.2.4 Disease Scoring

Periodical observations were made for the development of symptoms on leaves at 47 DAT and 79 DAT and disease intensity were recorded using 0-9 SES Scale (IRRI, 1996) (Table 3, Plate 1) and infection index of the disease was calculated by using following formula devised by McKinney (1923).

$$\text{Infection Index} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of plants}} \times \frac{100}{\text{Maximum disease category}}$$

3.3 DATA ANALYSIS

The data generated from the different experiments were subjected to analysis of variance (ANOVA) after appropriate transformation wherever needed. Correlation coefficients between various characters were also calculated (Cochran and Cox, 1965).

Table 3. 0-9 grade disease rating scale used for screening of blast nursery

Grade	Disease severity	Host response
0	No lesion observed	Highly Resistant
1	Small brown specks of pin point size	Resistant
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	Moderately Resistant
3	Lesion type same as in 2, but significant number of lesions on the upper leaves	Moderately Resistant
4	Typical susceptible blast lesions, 3 mm or longer infecting less than 4 per cent of leaf area	Moderately Susceptible
5	Typical susceptible blast lesions of 3mm or longer infecting 4-10 per cent of the leaf area	Moderately Susceptible
6	Typical susceptible blast lesions of 3 mm or longer infecting 11-25 per cent of the leaf area	Susceptible
7	Typical susceptible blast lesions of 3 mm or longer infecting 26-50 per cent of the leaf area	Susceptible
8	Typical susceptible blast lesions of 3 mm or longer infecting 51-75 per cent of the leaf area many leaves are dead	Highly Susceptible
9	Typical susceptible blast lesions of 3 mm or longer infecting more than 75 per cent leaf area affected	Highly Susceptible

1 2 3 4 5 6 7 8 9

Scores

Plate 1. Score chart of leaf blast of paddy

Results

4. RESULTS

In the present study, 30 Kerala traditional rice varieties along with 4 check varieties were analysed for the presence resistance gene with molecular markers and the field level evaluation of the varieties were conducted in comparison with known susceptible and resistant check varieties. Three genes were studied using three SSR markers and field level scoring of disease was done using SES scale by IRRI. The results are elaborated in this chapter.

4.1 MOLECULAR MARKER ANALYSIS

The genomic DNA isolated from the 34 varieties yielded good DNA, whose quality and quantity was checked by gel electrophoresis using 0.8 per cent agarose gel and by spectrophotometer, respectively. The quality of DNA obtained ranged from 1.22 to 2.80 (Table 4).

4.1.1 Simple Sequence Repeat

The polymerase chain reaction of 30 traditional varieties and check varieties was carried out with three SSR primers linked with respective genes. Markers exhibited polymorphism between the susceptible and resistant varieties (Table 5). For each gene, specific susceptible and resistant check varieties were also used for easy detection of the specific amplicons reported.

4.1.1.1 Gene Pi 1

Thirty varieties along with resistant and susceptible checks were genotyped with SSR primer RM224 closely linked to the gene *Pi 1*. The product amplified at 146 bp as in the gene source check C101Lac was taken as the standard. Among thirty traditional varieties, nine varieties showed the presence of the resistance gene. Among these, six varieties were heterozygous and three were dominant homozygous (Plate 2, Plate 3, Plate 4). Varieties, Jeddu Halliga, Chuvannamodan, Njavara from Karipra, showed the presence of dominant homozygous resistance gene. While, Thekkancheera, Thekkan Chitteni, Njavara from Kunnathoor, Parambuvattan, Kavunginpoothala, and Karuthamodan had this gene in heterozygous condition.

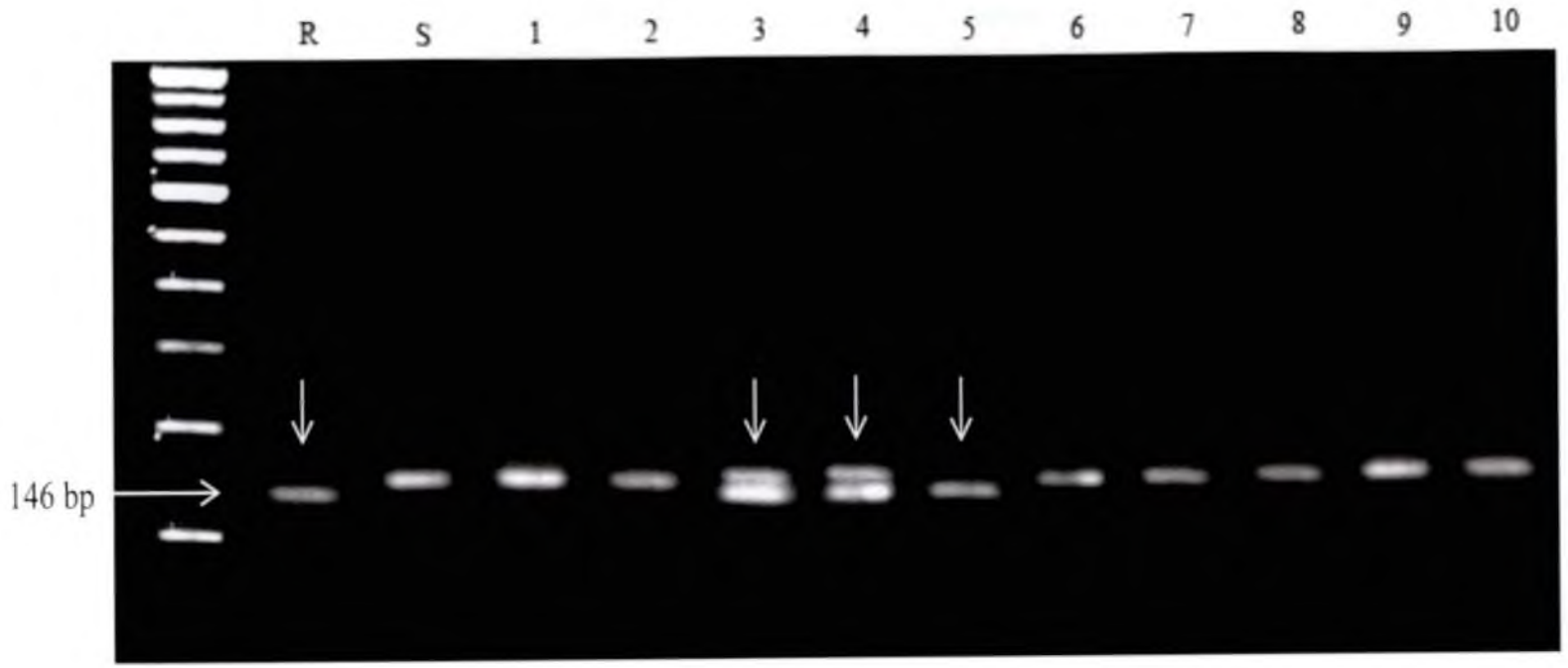


Plate 2. Amplification profile of the DNA of ten varieties and two check varieties using the primer RM224

R- C101Lac, S- BPT5204, 1- Aryan, 2- Velutharikayama, **3- Thekkancheera**, **4- Tekkan Chitteni**, **5- Jeddu Halliga**, 6- Vadakkan Chitteni, 7- Thekkan, 8- Veluthavatta, 9- Kodiyan, 10- Kattamodan

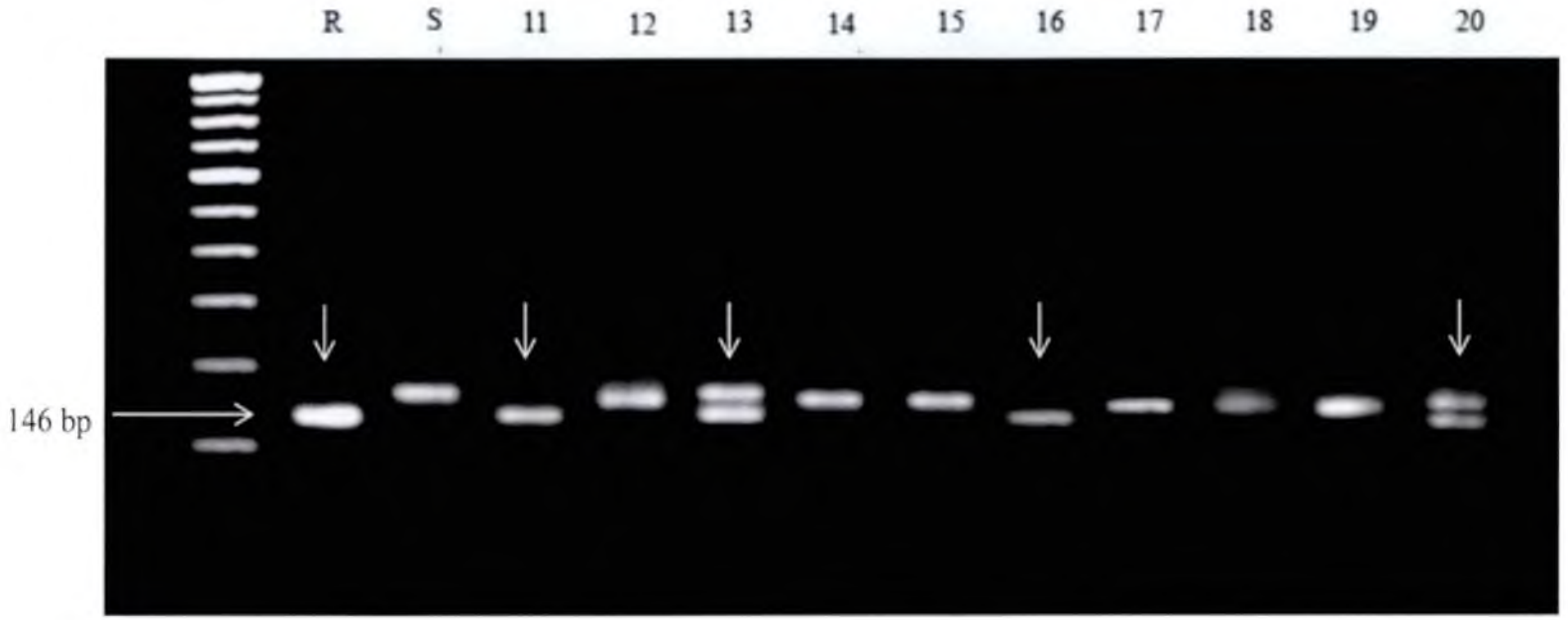


Plate 3. Amplification profile of the DNA of ten varieties and two check varieties using the primer RM224

R- C101LAC, S- BPT5204, **11- Chuvannamodan**, 12- Oorumundakan, **13- Njavara from Kunnathoor**, 14- Palakkadan Njavara from Elampalloor, 15- Nadan Njavara from Elampalloor, **16- Njavara from Karipra**, 17- Cheradi from Kunnathoor, 18- Kumpa Cheradi from Puthoor, 19- Ponnaryan, **20- Parambuvattan**

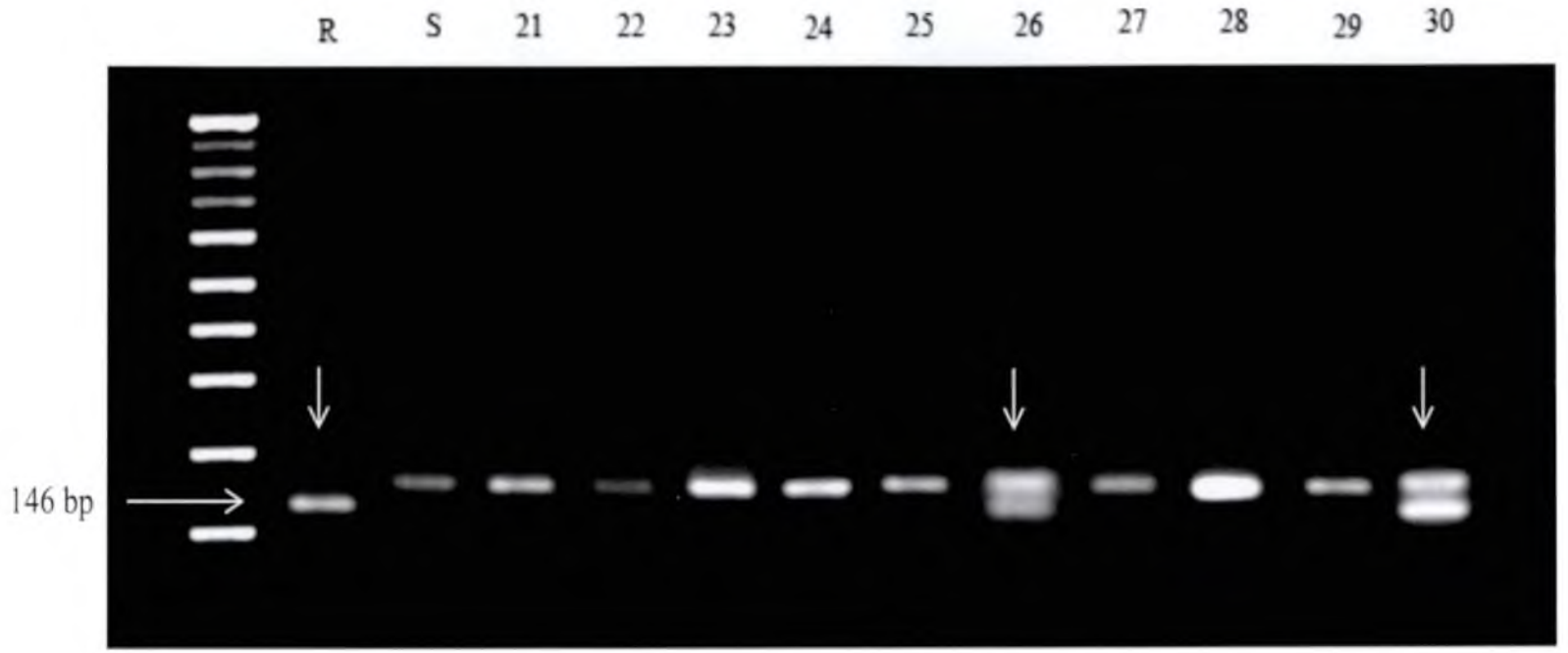


Plate 4. Amplification profile of the DNA of ten varieties and two check varieties using the primer RM224

R- C101LAC, S- BPT5204, 21- Vellari, 22- Thavalakkannan, 23- Veluthari Thavalakanan, 24- Kayama, 25- Maskathi, **26- Kavunginpoothala**, 27- Eravapandi, 28- Athikkiraya, 29- Cheriya Aryan, **30- Karuthamodan**

Table 4. Quality and Quantity of DNA of 30 traditional rice varieties used in the study

Sl. No.	Varieties	A 260 nm	A 280 nm	A260/A280	DNA Yield (ng/µl)
1	Aryan	0.014	0.007	2.00	420
2	Ponnaryan	0.004	0.003	1.33	120
3	Vellari	0.011	0.009	1.22	330
4	Velutharikayama	0.012	0.007	1.71	360
5	Parambuvattan	0.010	0.006	1.67	300
6	Thavalakkannan	0.004	0.003	1.33	120
7	Veluthari thavalakkannan	0.019	0.012	1.58	570
8	Thekkancheera	0.006	0.003	2.00	180
9	Thekkan chitteni	0.015	0.007	2.14	450
10	Kayama	0.003	0.002	1.50	90
11	Maskathi	0.026	0.014	1.86	780
12	Kavunginpoothala	0.004	0.002	2.00	120
13	Jeddu halliga	0.014	0.005	2.80	420
14	Eravapandi	0.010	0.007	1.43	300
15	Athikkiraya	0.011	0.009	1.22	330
16	Vadakkan chitteni	0.024	0.015	1.60	720
17	Thekkan	0.008	0.005	1.60	240
18	Veluthavattan	0.016	0.010	1.60	480
19	Cheriya Aryan	0.002	0.001	2.00	60
20	Kodiyan	0.006	0.004	1.50	180
21	Kattamodan	0.012	0.008	1.50	360
22	Karuthamodan	0.011	0.006	1.83	330
23	Chuvannamodan	0.028	0.015	1.87	840
24	Oorumundakan	0.027	0.015	1.80	810
25	Njavara from Kunnathoor	0.017	0.009	1.89	510
26	Palakkadan njavara from Elampalloor	0.023	0.012	1.92	690
27	Nadan njavara from Elampalloor	0.016	0.009	1.78	480
28	Njavara from Karipra	0.013	0.008	1.63	390
29	Cheradi from Kunnathoor	0.009	0.006	1.50	270
30	Kumpa cheradi from Puthoor	0.010	0.005	2.00	300
32	Tetep	0.003	0.002	1.50	90
33	C101LAC	0.016	0.010	1.60	480
34	C101A51	0.010	0.007	1.43	300
35	BPT5204	0.004	0.002	2.00	120

Table 5. Presence of various genes in selected varieties

Treatment No.	Varieties	Genes		
		Pi 1	*Pi 2*	*Pi kh*
1	Aryan	A	P	A
2	Ponnaryan	A	P	A
3	Vellari	A	P	A
4	Velutharikayama	A	P	A
5	Parambuvattan	P	A	P
6	Thavalakkannan	A	P	P
7	Veluthari Thavalakkannan	A	P	P
8	Thekkancheera	P	P	A
9	Thekkan Chitteni	P	A	A
10	Kayama	A	P	A
11	Maskathi	A	A	A
12	Kavunginpoothala	P	A	P
13	Jeddu Halliga	P	P	A
14	Eravapandi	A	A	A
15	Athikkiraya	A	A	A
16	Vadakkan Chitteni	A	P	A
17	Thekkan	A	P	A
18	Veluthavattan	A	P	A
19	Cheriya Aryan	A	A	A
20	Kodiyan	A	P	A
21	Kattamodan	A	P	A
22	Karuthamodan	P	P	A
23	Chuvannamodan	P	P	A
24	Oorumundakan	A	P	P
25	Njavara from Kunnathoor	P	A	A
26	Palakkadan njavara from Elampalloor	A	P	A
27	Nadan njavara from Elampalloor	A	P	A
28	Njavara from Karipra	P	P	A
29	Cheradi from Kunnathoor	A	A	P
30	Kumpa cheradi from Puthoor	A	A	A
31	Tetep	A	A	P
32	C101LAC	P	A	A
33	C101A51	A	P	A
34	BPT5204	A	A	A

A - Absent

P - Present

4.1.1.2 Gene Pi 2

Presence of the *Pi 2* gene was confirmed by the presence of the product at 233 bp on amplification with the primer RM527. This was confirmed by the amplification profile of the resistance gene source check C101A51. Among the selected varieties, twenty varieties were having gene of resistance. Among that, except one variety, all the remaining varieties carried resistance gene in dominant homozygous condition (plate 5, Plate 6, Plate 7). Varieties Aryan, Velutharikayama, Jeddu Halliga, Vadakkan Chitteni, Thekkan, Veluthavattan, Kodiyan, Kattamodan, Chuvannamodan, Oorumundakan, Palakkadan njavara from Elampalloor, Nadan njavara from Elampalloor, Njavara from Karipra, Ponnaryan, Vellari, Thavalakannan, Veluthari thavalakkannan, Kayama and Karuthamodan were confirmed with the presence of dominant homozygous alleles. Thekkan cheera was the only variety which was having the gene *Pi 2* in heterozygous condition.

4.1.1.3 Gene Pi kh

Variety Tetep was used as the resistance source for the identification of the presence of *Pi kh* gene among studied varieties. Gene specific SSR marker RM206 produced an amplicon at 145 bp showing the presence of this gene. Four dominant homozygotes and two heterozygotes were identified (Plate 8, Plate 9, Plate 10). Oorumundakan, Thavalakkannan, Veluthari Thavalakkanan, and Cheradi from Kunnathoor were the varieties having the gene in homozygous condition. Two varieties Parambuvattan and Kavunginpoothala had the gene in heterozygous condition.

4.2 FIELD SCREENING OF GENOTYPES

After completion of the genotyping of selected varieties, a field level screening was done for scoring the varieties for blast disease incidence. Farmer's field at Pattambi in Palakkad district was selected for conducting this part of experiment, since it is a hot spot of *Magnaporthe oryzae*. The experiment was conducted in Randomized Block Design (RBD) with three replications. 32 varieties (30 traditional varieties and 2 check varieties – Tetep, reported

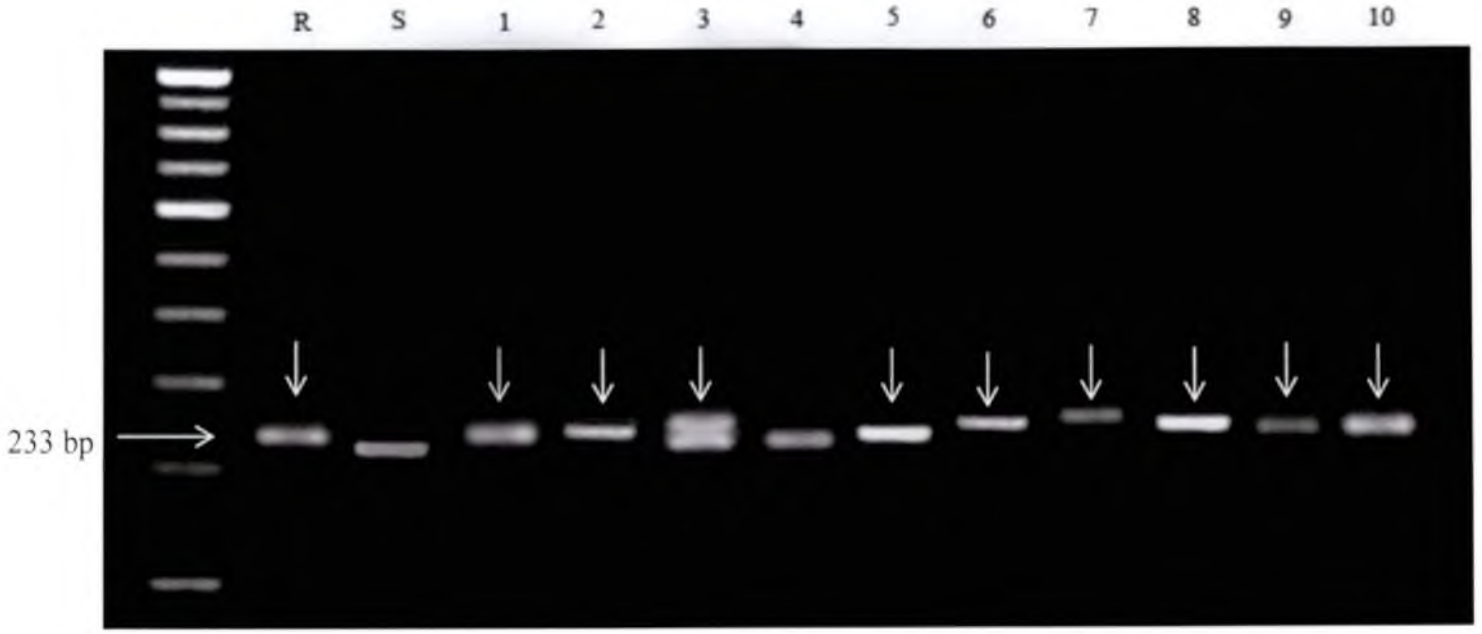


Plate 5. Amplification profile of the DNA of ten varieties and two check varieties using the primer RM527

R- C101A51, S- BPT5204, 1- Aryan, 2- Velutharikayama, 3- Thekkan cheera,4- Tekkan Chitteni, 5- Jeddu Halliga, 6- Vadakkan Chitteni, 7- Thekkan, 8- Veluthavattan, 9- Kodiyan, 10- Kattamodan

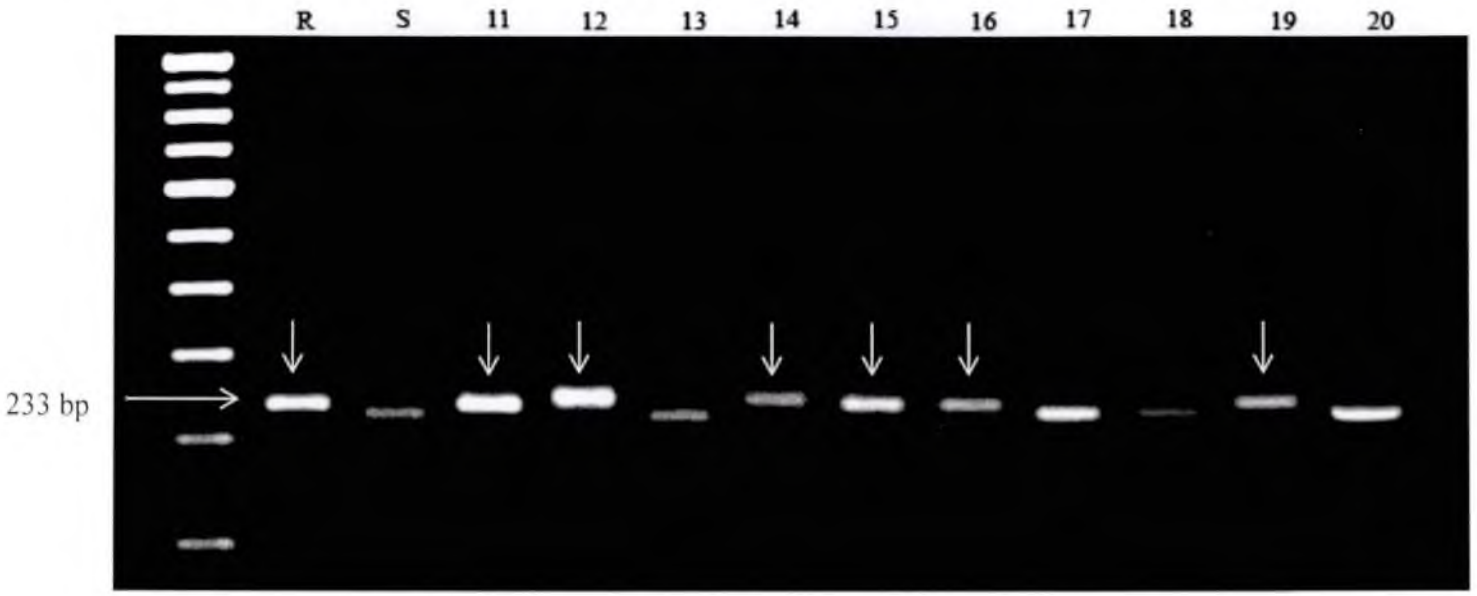


Plate 6. Amplification profile of the DNA of ten varieties and two check varieties using the primer RM527

R- C101A51, S- BPT5204, **11- Chuvannamodan**, **12 –Oorumundakan**, 13- Njavara from Kunnathoor, **14- Palakkadan Njavara from Elampalloor**, **15- Nadan Njavara from Elampalloor**, **16- Njavara from Karipra**, 17- Cheradi from Kunnathoor, 18- Kumpa Cheradi from Puthoor, **19- Ponnaryan**, 20- Parambuvattan

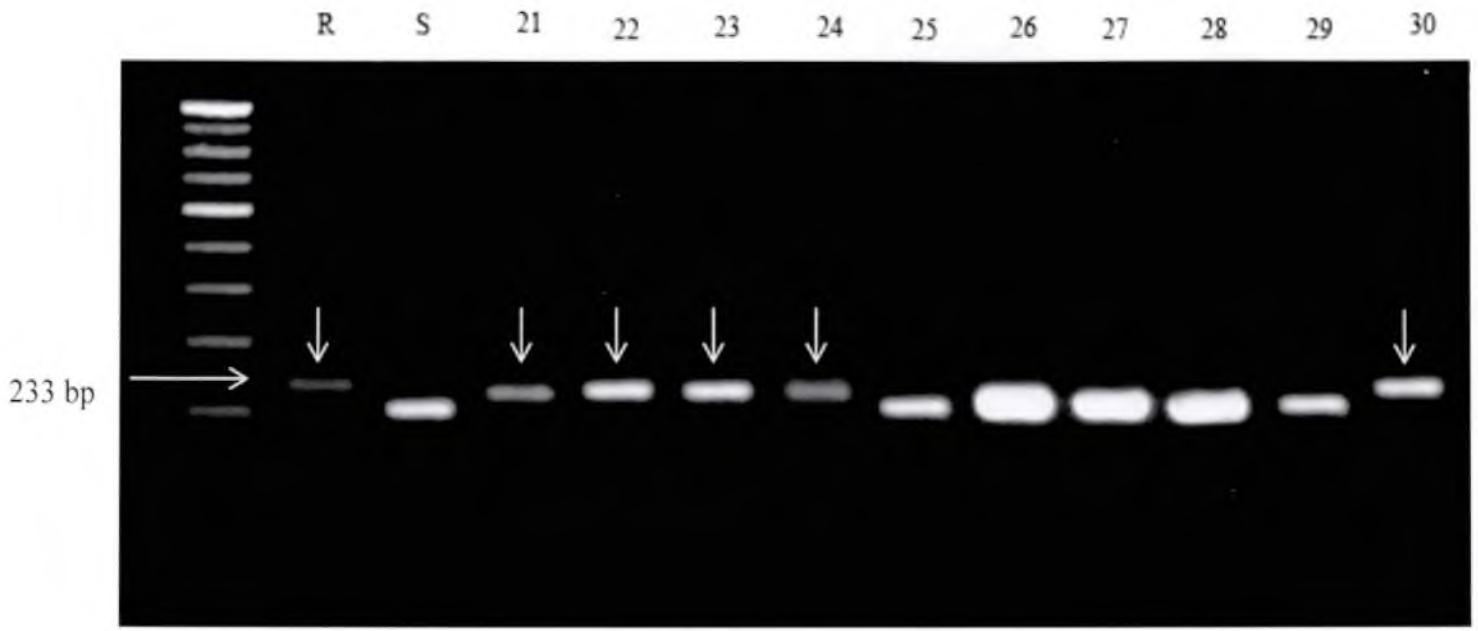


Plate 7. Amplification profile of the DNA of ten varieties and two check varieties using the primer RM527

R- C101A51 , S- BPT5204, **21- Vellari, 22- Thavalakkannan, 23- Veluthari Thavalakanan, 24- Kayama**, 25- Maskathi, 26- Kavunginpoothala, 27- Eravapandi, 28- Athikkiraya, 29- Cheriya Aryan, **30- Karuthamodan**

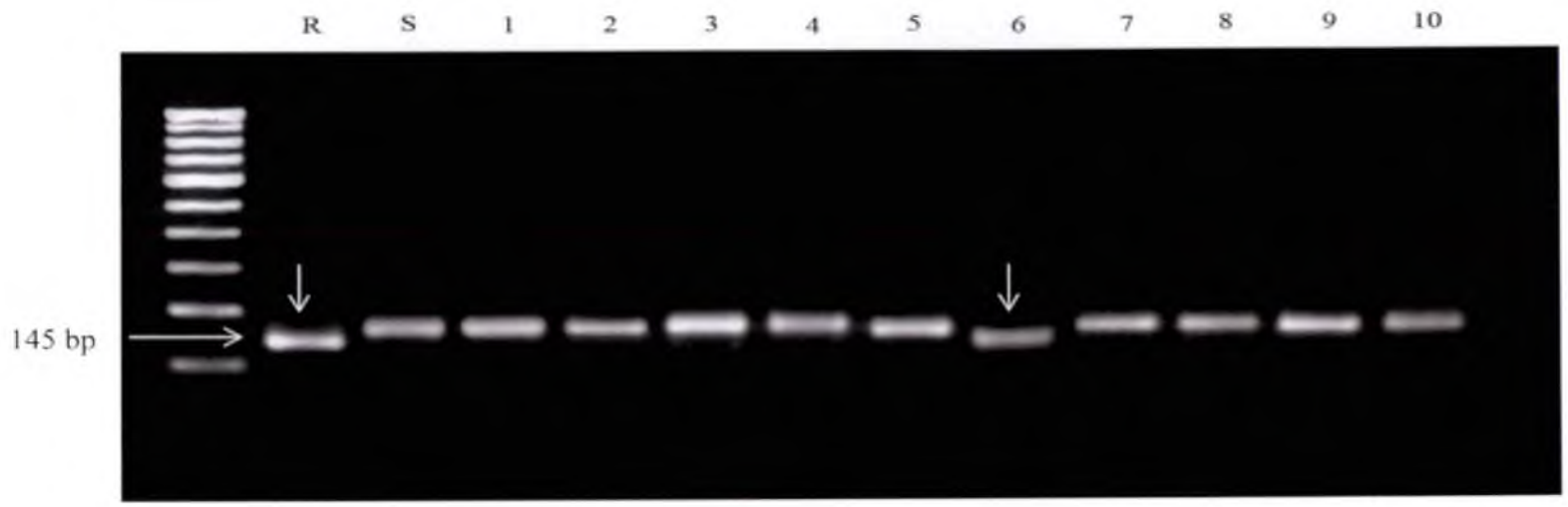


Plate 8. Amplification profile of the DNA of ten varieties and two check varieties using the primer RM206

R- Tetep, S- BPT5204, 1- Athikkiraya, 2- Veluthavattan, 3- Kodiyan, 4- Kattamodan, 5- Chuvannamodan, **6- Oorumundakan** 7- Njavara from Kunnathoor, 8- Palakkadan Njavara from Elampalloor, 9- Nadan Njavara from Elampalloor, 10- Njavara from Karipra

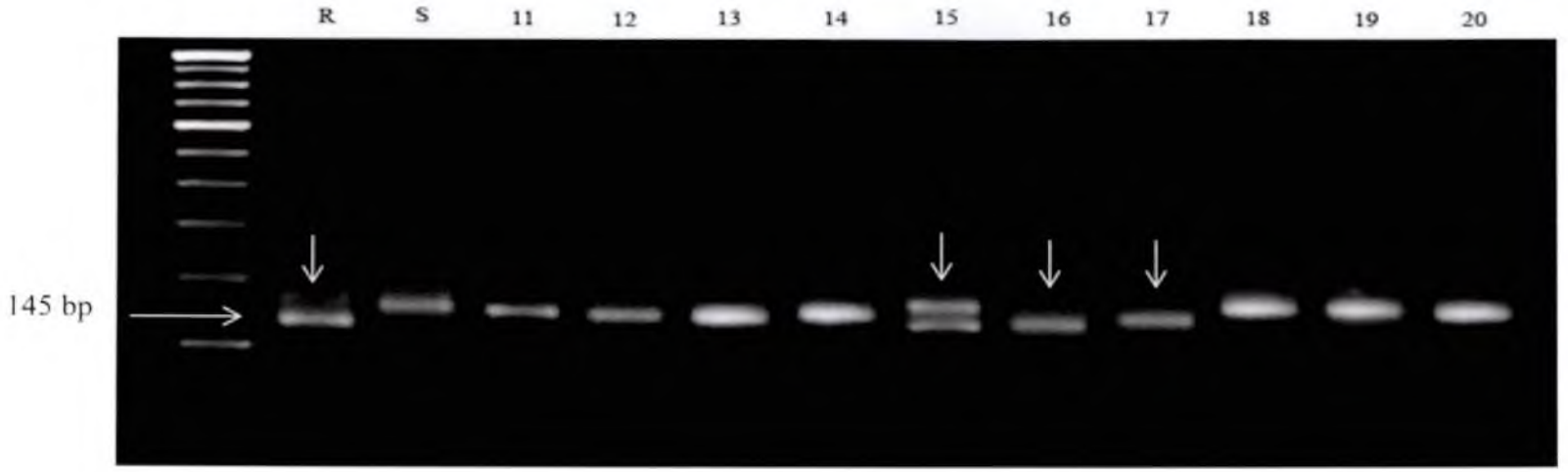


Plate 9. Amplification profile of the DNA of ten varieties and two check varieties using the primer RM206

R- Tetep, S- BPT5204, 11- Aryan, 12- Ponnaryan, 13- Velutharikayama, 14- Vellari, **15- Parambuvattan**, **16- Thavalakkannan**, **17- Veluthari Thavalakanan**, 18- Thekkan cheera, 19- Tekkan Chitteni, 20- Kayama

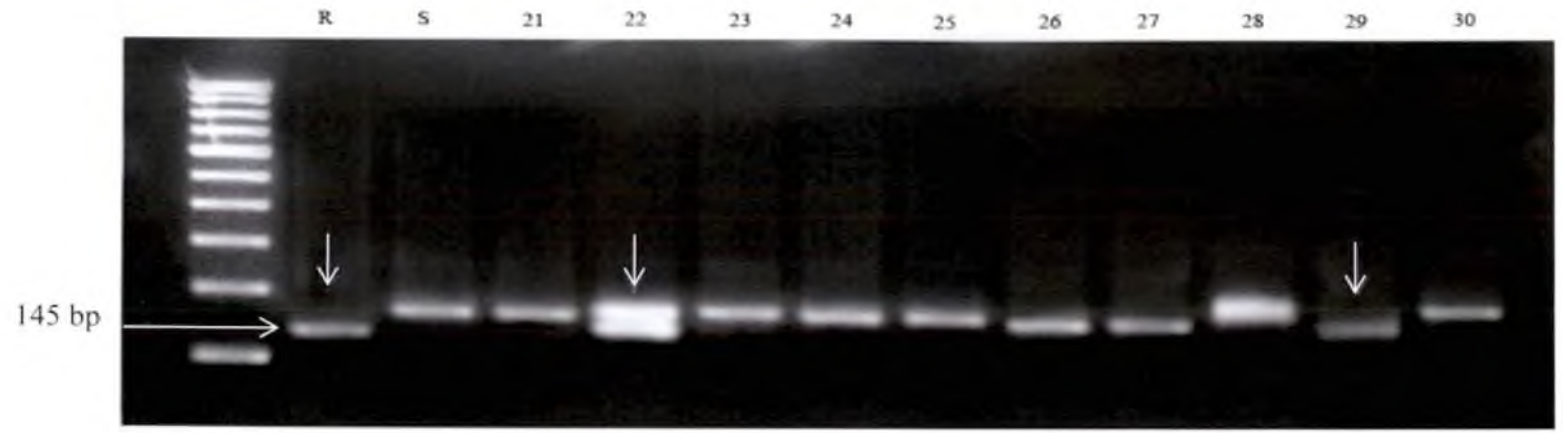


Plate 10. Amplification profile of the DNA of ten varieties and two check varieties using the primer RM206

R- Tetep, S- BPT5204, 21- Maskathi, **22- Kavunginpoothala**, 23- Jeddu Halliga, 24- Eravapandi, 25- Vadakkan Chitteni, 26- Thekkan, 27- Cheriya Aryan, 28- Karuthamodan, **29- Cheradi from Kunnathoor**, 30- Kumpa Cheradi from Puthoor

resistant variety and Jyothi, reported susceptible variety) were taken for the study (Plate 11).

4.2.1 Symptomatology

The causal organism of blast disease in rice, *Magnaporthe oryzae* was isolated from the infected leaf samples showing typical symptoms in the hot spot areas of farmer's field in Pattambi. The fungus was sub cultured on Potato Dextrose Agar slants, stored in a refrigerator at 4°C and was used for further studies.

The pathogenicity test of the isolate was conducted and proved following Koch's postulates. Inoculation of pathogen was done using the isolated culture. The symptoms were observed as minute brown specks which subsequently enlarged to become spindle shaped spots with pointed ends, several cm long and about 0.5 - 1.0 cm wide. The centre of the spots was grey or whitish, and the margin was usually brown or reddish brown. In severe conditions, the spots coalesced and resulted in blighting of the whole leaf (Plate 12). Periodical observations were made for the development of symptoms on leaves at 47 DAT and 79 DAT, disease intensity were recorded using 0 - 9 SES Scale

4.2.2 Disease Score

Thirty traditional varieties and two check varieties were scored twice with a gap of one month. Scores varied from 1 - 7 (Table 6). Varieties were classified into four divisions based on the scores obtained (Table 7). Ten varieties, Thekkan chitteni, Njavara from Kunnathoor, Cheradi from Kunnathoor, Chuvannamodan, Njavara from Karipra, Thekkancheera, Oorumundakan, Parambuvattan, Kavunginpoothala and Karuthamodan were moderately resistant. Fifteen varieties Cheriya Aryan, Aryan, Ponnaryan, Vellari, Velutharikayama, Kayama, Jeddu halliga, Vadakkan chitteni, Thekkan, Veluthavattan, Kodiyan, Palakkadan njavara from Elampalloor, Nadan njavara from Elampalloor, Thavalakkannan and Veluthari thavalakkanan showed moderately susceptible response. Remaining six varieties Maskathi, Eravapandi, Athikkiraya, Kumpa cheradi from Puthoor,

Plate 11. General view of the experimental field

Plate 12. Symptomatology of rice blast disease

Table 6. Selected varieties scored for two months

Treatment No.	Varieties	Average Score	
		47 DAT	79 DAT
1	Aryan	4	4
2	Ponnaryan	5	5
3	Vellari	5	5
4	Velutharikayama	4	5
5	Parambuvattan	4	3
6	Thavalakkannan	6	5
7	Veluthari thavalakkanan	5	4
8	Thekkancheera	4	3
9	Thekkan chitteni	3	2
10	Kayama	4	4
11	Maskathi	5	6
12	Kavunginpoothala	3	2
13	Jeddu halliga	3	4
14	Eravapandi	6	7
15	Athikkiraya	5	6
16	Vadakkan chitteni	4	4
17	Thekkan	4	4
18	Veluthavattan	5	5
19	Cheriya Aryan	5	5
20	Kodiyan	4	4
21	Kattamodan	6	6
22	Karuthamodan	4	3
23	Chuvannamodan	3	3
24	Oorumundakan	4	3
25	Njavara from Kunnathoor	3	3
26	Palakkadan njavara from Elampalloor	6	4
27	Nadan njavara from Elampalloor	4	4
28	Njavara from Karipra	4	3
29	Cheradi from Kunnathoor	3	3
30	Kumpa cheradi from Puthoor	4	6
31	Jyothi	4	6
32	Tetep	1	1

Table 7. Classification of the varieties based on the overall reaction to rice blast disease

Division	Score	Varieties
Highly resistant	0	Nil
Resistant	1	Tetep
Moderately resistant	2-3	Thekkan chitteni, Njavara from Kunnathoor, Cheradi from Kunnathoor, Chuvannamodan, Njavara from Karipra, Thekkancheera, Oorumundakan, Parambuvattan, Kavunginpoothala, Karuthamodan
Moderately Susceptible	4-5	Cheriya Aryan, Aryan, Ponnaryan, Vellari, Velutharikayama, Kayama, Jeddu halliga, Vadakkan chitteni, Thekkan, Veluthavattan, Kodiyan, Palakkadan njavara from Elampalloor, Nadan njavara from Elampalloor, Thavalakkannan, Veluthari thavalakkanan
Susceptible	6-7	Maskathi, Eravapandi, Athikkiraya, Kumpa cheradi from Puthoor, Kattamodan, Jyothi
Highly susceptible	8-9	Nil

Kattamodan and Jyothi showed susceptible response. None of the varieties were immune or highly susceptible.

4.2 STATISTICAL ANALYSIS

Using the scores obtained, an infection index was calculated and their variances were analysed (Table 8). Analysis of variance was also done for the scores sorted with respect to the number of genes present.

4.2.1 Infection Index

From the statistical analysis for infection index, it was interpreted that, there exist a significant difference between the varieties with respect to susceptibility (Table 8). Variety Tetep was found to be with minimum infection index of 7.34 and it was significantly different from all the remaining varieties. Variety Tetep was followed by the varieties Thekkan chitteni and kavunginpoothala, and they were on par with Njavara from Karipra, Thekkancheera, Karuthamodan, Parambuvattan, Cheradi from Kunnathoor, Chuvannamodan and Oorumundakan.

4.2.2 Correlating Infection Index with Number of Genes

Scores were analysed by classifying genotypes according to the number of genes present in them. From the analysis of variance table, it was interpreted that, there exists high significant difference between groups (Table 9). It evidently showed that, there was an effect due to the change in number of genes. The correlation coefficient between number of genes and respective score averages showed a high negative significant relation (Table 10).

Table 8. Infection index of various treatments calculated using scores obtained 79 DAT

Treatment No.	Varieties	Infection index mean
1	Aryan	44.71 (41.96)
2	Ponnaryan	59.90 (50.71)
3	Vellari	53.31 (46.90)
4	Velutharikayama	51.51 (45.87)
5	Parambuvattan	37.00 (37.46)
6	Thavalakkannan	58.41 (49.84)
7	Veluthari thavalakkanan	45.52 (42.43)
8	Thekkancheera	35.15 (36.36)
9	Thekkan chitteni	25.15 (30.10)
10	Kayama	45.14 (42.21)
11	Maskathi	61.09 (51.41)
12	Kavunginpoothala	27.01 (31.31)
13	Jeddu halliga	46.24 (42.84)
14	Eravapandi	73.69 (59.14)
15	Athikkiraya	65.53 (54.05)
16	Vadakkan chitteni	45.51 (42.42)
17	Thekkan	40.76 (39.68)
18	Veluthavattan	60.46 (51.04)
19	Cheriya Aryan	59.27 (50.34)
20	Kodiyan	42.15 (40.48)
21	Kattamodan	66.08 (54.38)
22	Karuthamodan	35.52 (36.58)
23	Chuvannamodan	37.93 (38.02)
24	Oorumundakan	38.10 (38.12)
25	Njavara from Kunnathoor	38.48 (38.34)
26	Palakkadan njavara from Elampalloor	42.25 (40.54)
27	Nadan njavara from Elampalloor	41.21 (39.94)
28	Njavara from Karipra	32.73 (34.90)
29	Cheradi from Kunnathoor	37.37 (37.68)
30	Kumpa cheradi from Puthoor	61.46 (51.63)
31	Jyothi	61.85 (51.85)
32	Tetep	07.34 (15.72)
CD (0.05)		(8.142)

Figures in parenthesis are transformed data

Table 9. Average scores obtained during 79 DAT classified with respect to the number of genes

Gene	Average score
No gene	5.68
Pi 1	2.98
Pi 2	4.44
Pi kh	3.37
Pi 1 + Pi 2	3.19
Pi2 + Pi kh	3.77
Pi1 + Pi kh	2.88

Table 10. Correlation coefficient between number of genes and respective average scores

	Average score	No. of genes	Critical correlation Coefficient (0.05)
No. of genes	-0.51641	1	0.208

Discussion

5. DISCUSSION

World rice requirements are expected to increase 1.7 per cent annually between 1990 and 2025, which requires an additional 13 million tons of rice per year. Rice crop is affected by various abiotic and biotic stresses, which is resulting in a huge yield loss wherever rice is growing. Of the various biotic stresses like bacterial leaf blight, sheath blight and stem borer limiting rice productivity, blast disease caused by *Magnaporthe oryzae* is a serious constrain in rice production at global level. It has become an important limiting factor for rice cultivation in various parts of the world due to prevailing blast conducive environments during the crop season (Sharma *et al.,* 2002). Rice blast is a major disease often resulting in a significant yield loss, as high as 70 to 80 per cent during an epidemic (Ou, 1985).

The Hindu (2007) reported that, blast disease is spreading over an alarming condition in Kerala covering the northern districts like Thrissur, Palakkad, Wyanad, Malappuram and Kozhikode. The survey done by Yamini (2012) on the incidence and intensity of blast disease of rice, also revealed that, blast disease incidence of rice was in the range of 34 to 80 per cent and severity in the range of 11.7 to 65.13 per cent in different rice growing areas of Palakkad district. Krishnaveni *et al.* (2012) also reported that, Pattambi is a hot spot for rice blast disease.

Independent of the rice-growing system or area, breeding for resistance to blast is needed to control the disease despite the availability of effective fungicides (Ballini *et al.,* 2008). Traditional accessions are reservoirs of unique genes, which confer resistance to biotic and abiotic stresses. The land races and wild or weedy species together constitute 80 per cent of the rice germplasm, but they are not exploited appropriately. Even though portions of land races have been collected and maintained in the form of gene banks in several germplasm programmes, there have been very few reported studies designed to assess the various genes of resistance present in them.

Traditional varieties of Kerala stand as a rich source for disease resistance genes. The present study was thus designed to identify the donors for blast resistance from traditional rice varieties of Kerala using functional markers. The results are discussed elaborately in this chapter.

5.1. LOCATING GENES FOR RESISTANCE

Thirty traditional varieties were selected for identifying the presence or absence of three blast resistance genes *Pi 1, Pi 2 and Pi kh* using specific markers linked to them. The genes were reported to give a broad spectrum resistance to different races of *Magnaporthe oryzae.*

5.1.1 Screening for *Pi 1* Gene

Hittalmani *et al.* (2000) had reported *Pi 1* as one of the major genes for imparting broad spectrum resistance to many of the races of *M. oryzae* and they also reported that, it is located on the chromosome 11. In this study, presence of the *Pi 1* gene was searched using the SSR primer RM224. Fuentes *et al.* (2008) in their study for the identification of molecular markers linked to the gene *Pi 1,* reported two markers RM1233*I and RM224 which is mapped in the same position with *Pi 1* gene (0.00 cM) (Fig 2).

Varieties Jeddu Halliga, Chuvannamodan, Njavara from Karipra, Thekkancheera, Tekkan Chitteni, Njavara from Kunnathoor, Parambuvattan, Kavunginpoothala, and Karuthamodan showed the presence of resistant allele marker at 146 bp in the PCR profile with primer RM224. This was confirmed by the amplification profile of the C101LAC, the resistant gene source for *Pi 1.* Prasad *et al.* (2011) had reported that, the resistant fragment allele of *Pi 1* gene amplified at 146 bp in the PCR with RM224 as primer in the accession C101LAC. Among the lines identified for the presence of the gene *Pi 1,* Njavara from Kunnathoor and Njavara from Karipra are two medicinal rice varieties specific to the locality.

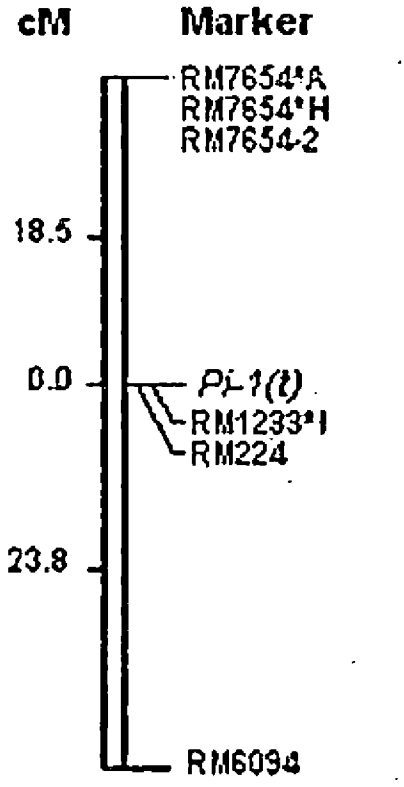


Figure 2. Map position of the marker RM224 (Fuentes *et al.*, 2008)

5.1.2 Screening for *Pi 2* Gene

Chen *et al.,* 1996 reported a broad spectrum resistance imparted by *Pi 2* gene. They also reported C101A51 as a donor for this particular gene. In this study, the presence of *Pi 2* gene was searched using the SSR primer RM527. Arunakanthi *et al.* (2008) reported that, the Presence of this gene can be confirmed by SSR marker RM527 located in the chromosome 6 (Fig. 3). Yu *et al.*, 1991 has also reported that the gene *Pi 2* of rice confer complete resistance to the blast fungal pathogen *M. oryzae.* In the PCR analysis with the SSR primer RM527 varieties Aryan, Velutharikayama, Jeddu Halliga, Vadakkan Chitteni, Thekkan, Veluthavattan, Kodiyan, Kattamodan, Chuvannamodan, Oorumundakan, Palakkadan njavara from Elampalloor, Nadan njavara from Elampalloor, Njavara from Karipra, Ponnaryan, Vellari, Tavalakannan, Veluthari thavalakkannan, Kayama, Karuthamodan and Thekkan cheera produced the resistant fragment allele at 233 bp confirming the presence of the gene *Pi 2* as in the resistant gene donor C101A51. The amplification of resistant fragment allele for this marker was specified as 233 bp by Prasad *et al.* (2011).

5.1.3 Screening for *Pi kh* Gene

Gupta *et al.* (2011) reported that, the gene *Pi 54 (Pi kh)* confers a high degree of resistance to diverse strains of the fungus *M. oryzae.* Screening of the varieties for the presence of the gene *Pi kh* was done in comparison with the variety 'Tetep' as the resistant gene source. Sharma *et al.* (2002) reported that genetic and physical mapping and cloning of the rice blast resistance gene *Pi kh*, is highly effective against the pathogen population found in the Northwestern Himalayan region of India and an indica rice line Tetep was described as resistance gene source. Presence of the *Pi kh* gene was analysed by the PCR reaction with SSR primer RM206. Sharma *et al.* (2010) reported the gene specific SSR marker RM206 linked closely to the *Pi kh* gene on chromosome 11 (Fig. 4). Varieties Oorumundakan, Thavalakkannan, Veluthari Thavalakanan, Cheradi from Kunnathoor, Parambuvattan and Kavunginpoothala produced amplicon at

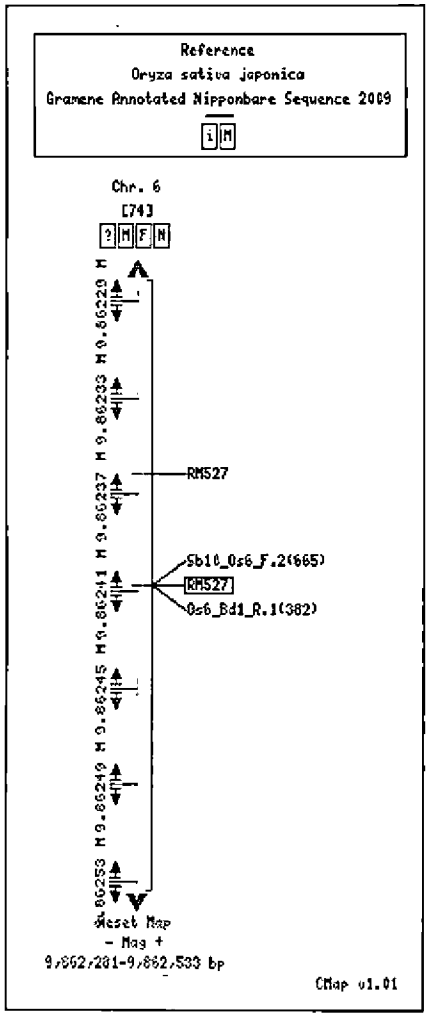


Figure 3. Map position of the marker RM527 (Gramene, 2006)

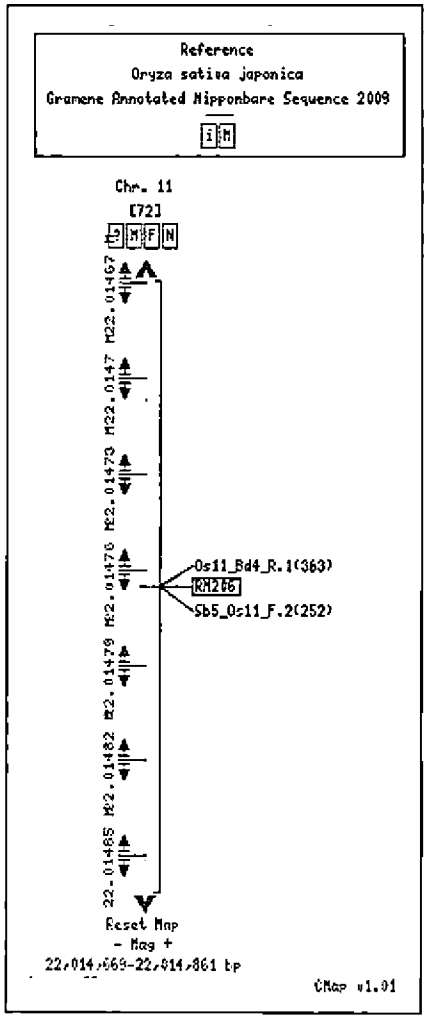


Figure 4. Map position of the marker RM206 (Gramene, 2006)

145 bp conforming the presence of the gene. The resistant allele for this gene was reported to be at 145 bp by Prasad *et al.* (2011).

The varieties having these three gene *Pi 1*, *Pi 2* and *Pi kh* were assessed for locating varieties with these three genes in combination (*Pi 1 + Pi 2, Pi 2 + Pi kh, Pi 1 + Pi kh*) (Figure 5). Chuvannamodan, Njavara from Karipra, Thekkancheera, Jeddu Halliga and Karuthamodan are having the resistant genes *Pi 1* and *Pi 2*. Among that, Chuvannamodan, Jeddu Halliga, Karuthamodan and Thekkancheera were collected from RARS, Pattambi (Central Kerala) and Njavara variety was collected from FSRS, Kottarakkara (South Kerala). Thavalakkannan, Veluthari thavalakanan and Oorumundakan were having a gene combination of *Pi 2 + Pi kh*. In that, variety Oorumundakan was collected from RRS, Kayamkulam (Onattukara region) and remaining two varieties were collected from RARS, Pattambi. A gene combination of *Pi 1 + Pi kh* was present in two varieties Parambuvattan and Kavunginpoothala which were collected from RARS, Pattambi.

5.2. FIELD SCREENING

Field screening for blast resistance was conducted in the hot spot region for blast disease in Pattambi. Symptoms on leaf are having more relation to the levels of resistance of host cultivar (Krishnaveni *et al.*, 2012). In this study leaf blast scores were taken as the indication for resistance using the 0-9 SES scale of IRRI. Varieties were classified based on the scores, into different groups as reported by Ghazanfar *et al.* (2009). Scores on classification showed that, ten varieties Tekkan Chitteni, Njavara from Kunnathoor, Cheradi from Kunnathoor, Chuvannamodan, Njavara from Karipra, Thekkancheera, Oorumundakan, Parambuvattan, Kavunginpoothala and Karuthamodan were moderately resistant.

The susceptible check Jyothi had scored 6 in correspondence to the level of infection. KAU (2011) reported that variety Jyothi is moderately tolerant to blast disease.

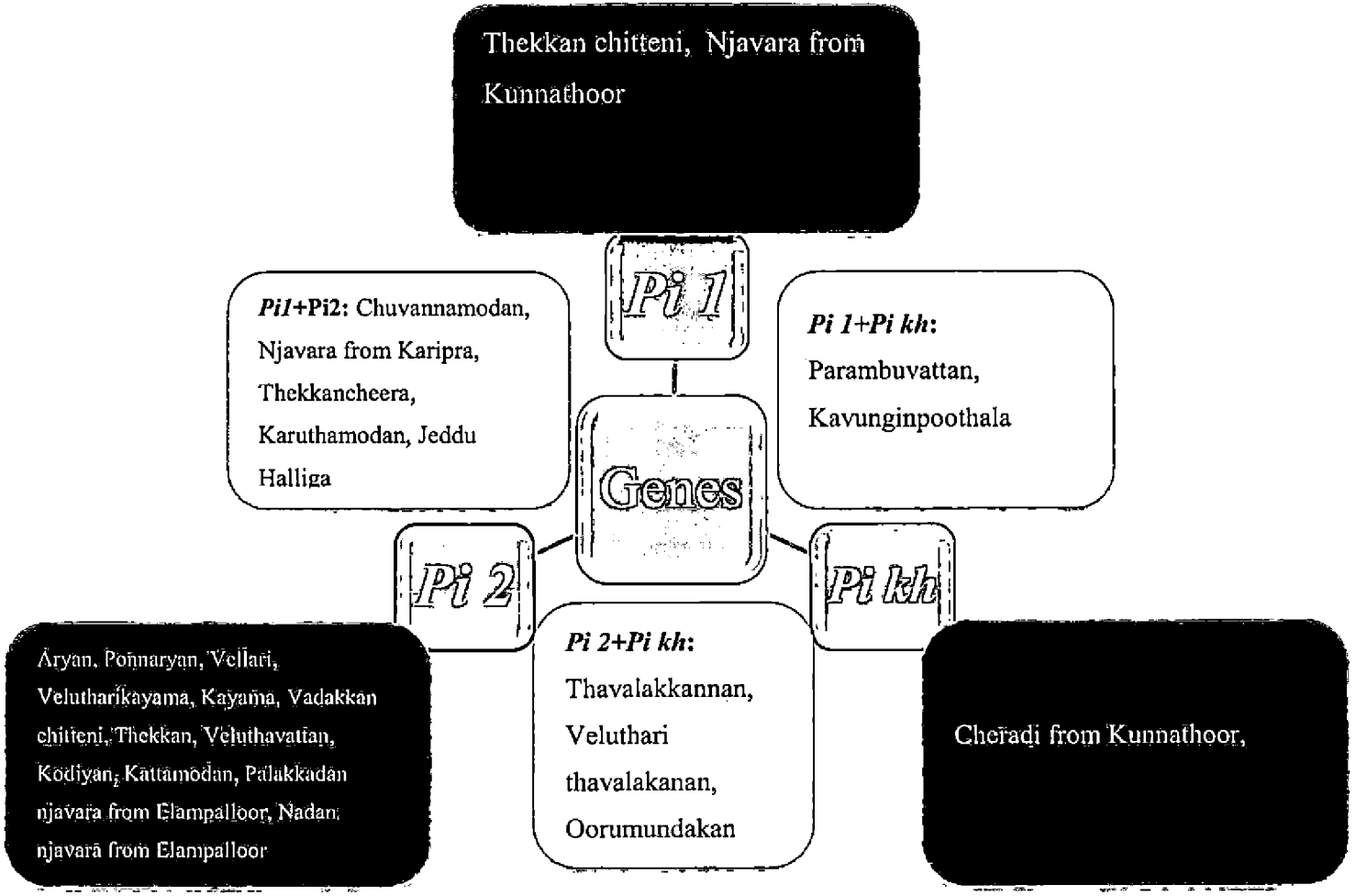


Figure 5. Varieties with respective genes of resistance

Infection index for each variety were calculated and this was in concurrence with the classification obtained from disease score. All the moderately resistant varieties had low infection index among the varieties under study. Calculation of infection index was done using the formula proposed by McKinney (1923) to assess the infection of wheat seedlings by *Helminthosporium sativum* based on a 0-9 disease grading scale (IRRI, 1996).

5.2.1 Comparison of Disease Score with Presence of Gene

The disease score of the varieties with single gene *Pi 1, Pi 2* and *Pi kh* is compared in figure 6. This clearly shows that, the disease score of the varieties having the gene *Pi 1* had a less disease score compared to other two genes *Pi 2* and *Pi kh*. Li *et al.* (2012) reported that resistance gene *Pi 1* is a broad-spectrum blast resistance gene. When the genes are taken in combination, the combination of the genes *Pi* and *Pi kh* showed low disease score (Fig. 7).

The average disease score calculated on single gene basis and combination basis is given in table 9. The scores show that the combination of genes *Pi* and *Pi kh* imparted more resistance to the races of *M. oryzae* prevalent in the location. Fuentes *et al.* (2008) has also reported gene *Pi 1* is capable of imparting resistance to many races of *M. oryzae* prevailing in Colombia. Recently, it was reported in a separate study that *Pi kh* confers broad spectrum resistance against geographically diverse strains of *M. oryzae* collected from various parts of India and the United States (Costanzo and Jia, 2010; Rai *et al.*, 2011).

Tetep, the resistant gene source for *Pi kh* gene was under the resistant group after classification. Several studies were conducted on the genetics of blast resistance in Tetep, and it has been shown that four complete resistance genes including *Pi kh and Pi 1* genes are present in this cultivar (Inukai *et al.*, 1995). They also report the presence of another pair of gene *Pi ta* and an unknown blast resistance gene in Tetep. So the total resistance shown by Tetep may be due to the presence of these two other genes. Both major and minor genes can contribute to the durable resistance (Hittalmani *et al.*, 2000).

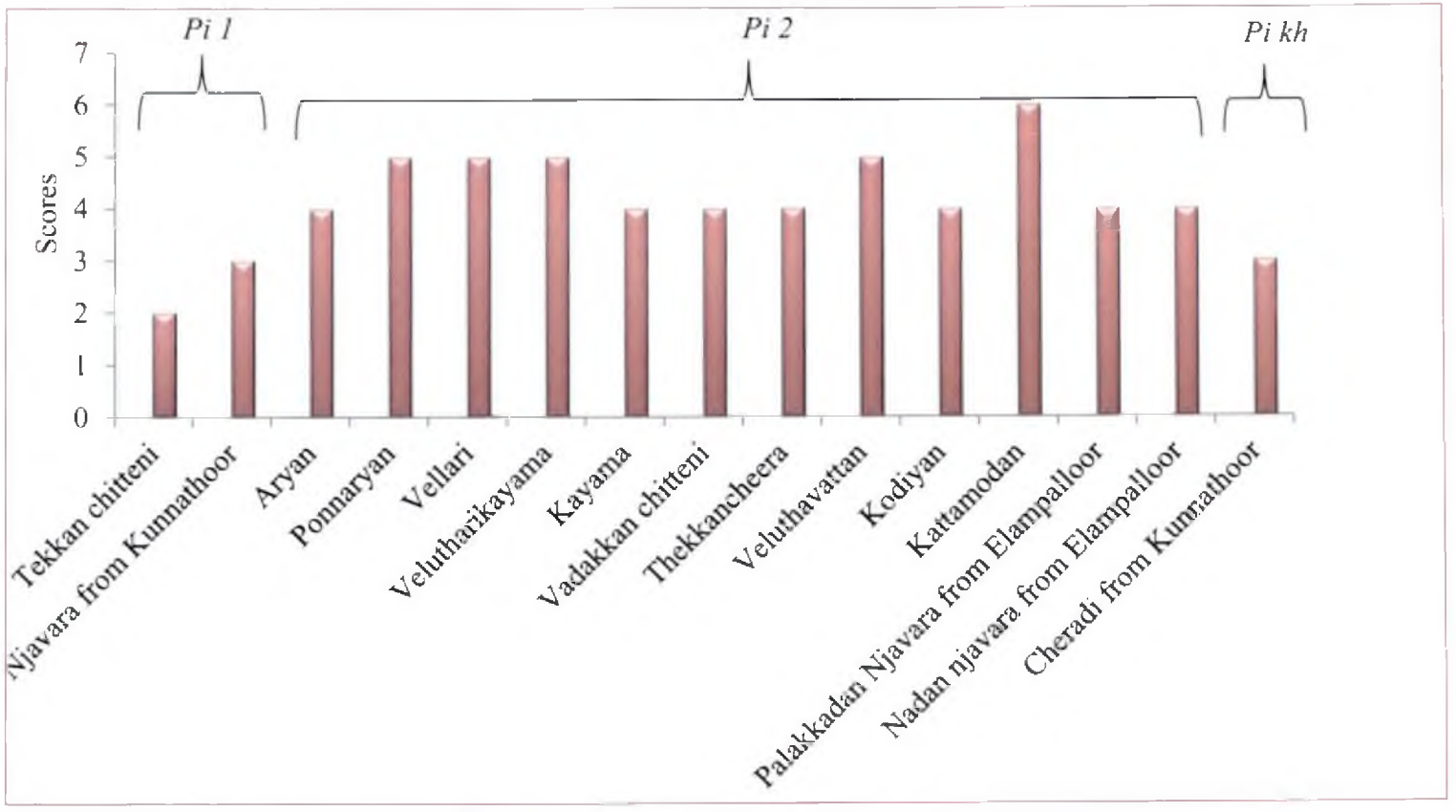


Figure 6. Representation of disease score of genotypes with single resistance gene

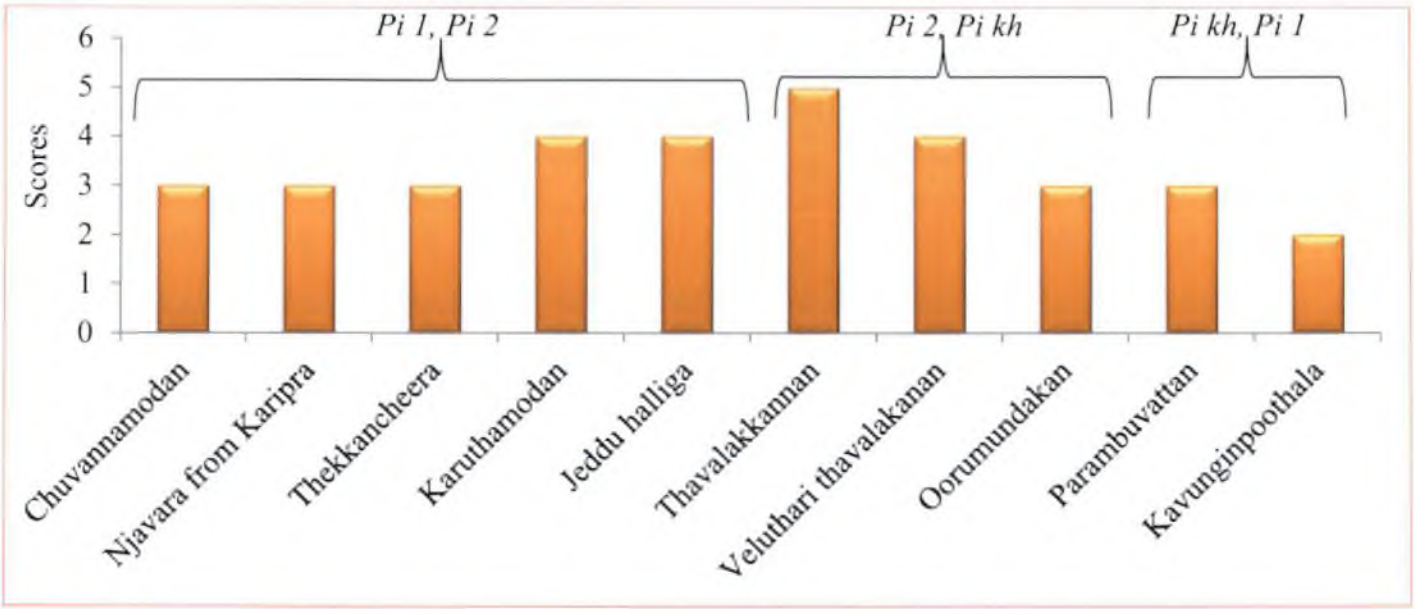


Figure 7. Representation of disease scores of genotypes with resistance genes in combination

Varieties Parambuvattan and Kavunginpoothala having genes *Pi 1 and Pi kh* can be used as donors for the pyramided genes in the breeding programmes. Varieties Chuvannamodan and Njavara from Karipra having the gene *Pi 1* in homozygous condition and having score of 3, which is included in the class moderately resistant, can be used as donors for gene *Pi 1*. Varieties Oorumundakan and Cheradi from Kunnathoor having the gene *Pi kh* in homozygous condition and having the score 3 which is included in the class moderately resistant can be used as donors for *Pi kh* gene.

The correlation coefficient between disease scores and number of genes showed that, there exists a significant negative correlation. So, as the number of genes increases, resistance increases as depicted in the figure 9. Gene Pyramiding entails stacking multiple genes leading to the simultaneous expression of more than one gene in a variety to develop durable resistance expression (Joshi and Nayak, 2010). So the pyramiding of *Pi 1* and *Pi kh* can impart a better resistance under Kerala condition.

Disease scoring of the leaf blast was done twice with an interval of one month. The initial score of all the varieties irrespective of the genes present were on par. The graphical representation of the disease score in two months for the three genes in separate and in combination is given in figure 8, 9. These figures show that, disease scores were drastically reduced in the second scoring when two genes of resistance were present in combination. This implies that, the expression of genes of resistance is activated after the initial infection. Sharma *et al.* (2005b) reported that gene *Pi kh* is induced in response to pathogen attack. Ward *et al.* (1991) also reported that, the expression of several defense related genes is up regulated in plants after pathogen infection.

From the study on resistance to the blast disease caused by *M. oryzae*, traditional varieties which are having specific genes for resistance alone and in combination were identified. These varieties can be used as donors for respective genes in further breeding programmes. The traditional varieties Kavunginpoothala

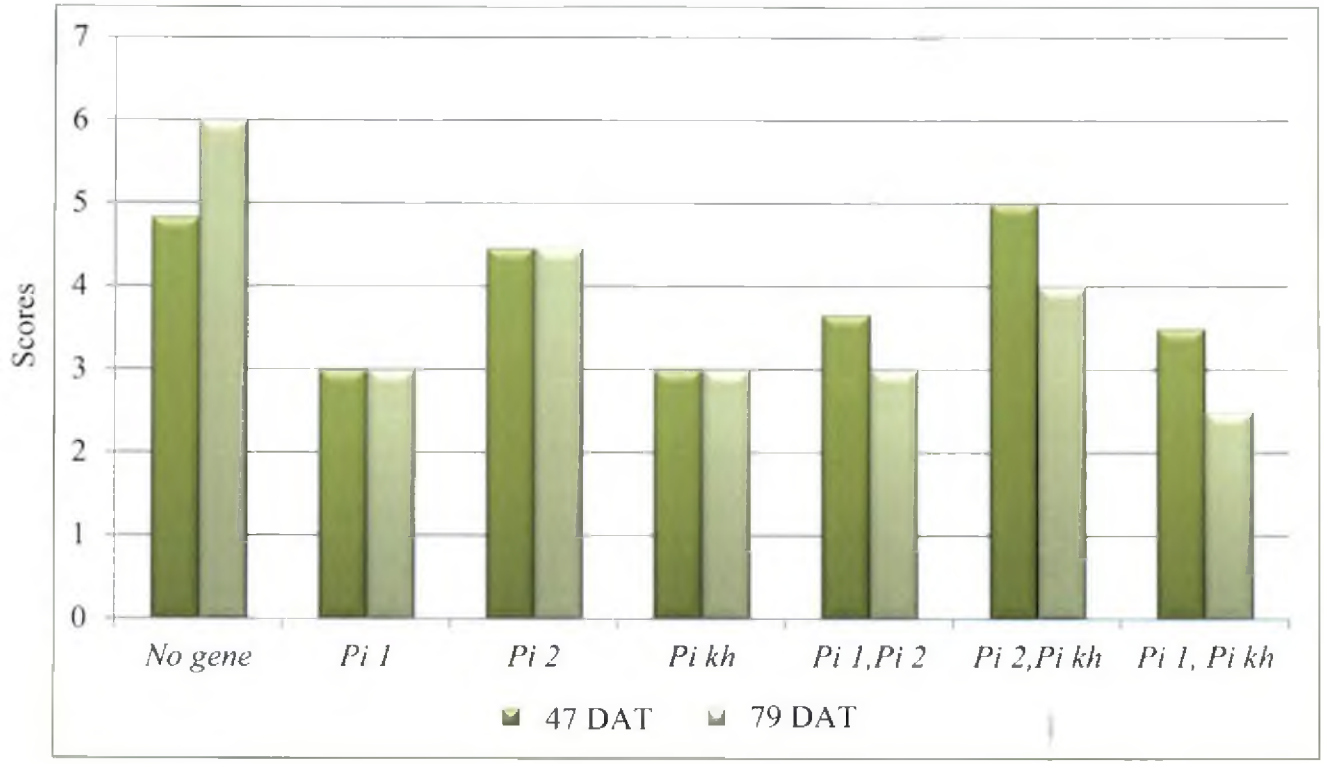


Figure 8. Representation of disease score of Periodical observations

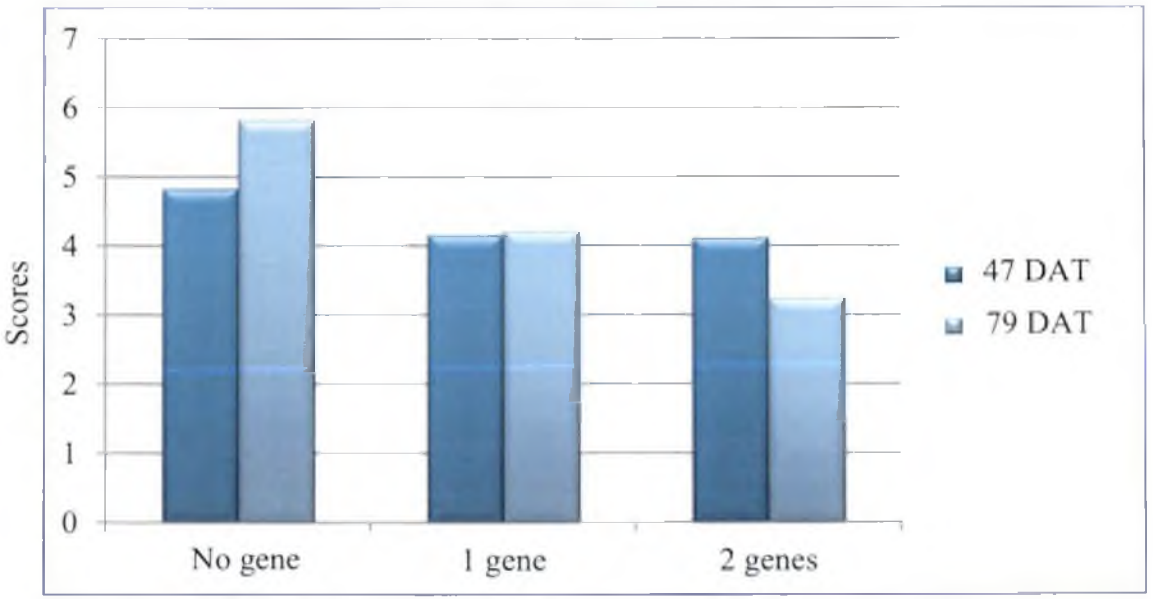


Figure 9. Representation of disease score of varieties of Periodical observations

and Parambuvattan identified as donors for the *Pi 1* + *Pi kh* combination are having specific grain qualities of Kerala varieties - Parambuvattan with red bold and Kavunginpoothala with white bold grains. Rice cultivation throughout Kerala is dominated by two varieties Uma and Jyothi. Jyothi was released from RARS, Pattambi and Uma was released from RRS, Moncompu during 1974 and 1998 respectively. In the field evaluation, these two varieties were found highly susceptible with a disease score of 6. By using the identified donors, essentially derived varieties can be developed from these popular varieties by reconstituting them by pyramiding the genes of resistance *Pi 1* and *Pi kh*. Since the donors have been identified from the traditional varieties with specific grain qualities of Kerala, reconstitution of the lines by maintaining the grain quality can be easily achieved.

Summary

6. SUMMARY

The present study "Identification of the donors for blast resistance from traditional rice varieties of Kerala using functional markers" was conducted in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during 2012 - 2014. The molecular studies were done at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani and the field study was carried out in farmer's field at Pattambi, Palakkad district. The major objectives of the study were to identify the traditional rice varieties with blast resistance genes (*Pi 1, Pi 2, Pi kh*) using associated functional markers and the field scoring of the lines under disease stress condition.

In the present study, thirty traditional rice varieties of Kerala were taken for resistance gene source identification and field level evaluation. The selected rice varieties were screened for the presence of the reported blast resistance genes *Pi 1, Pi2* and *Pi kh* using three SSR markers RM224, RM527 and RM206 respectively. C101Lac (*Pi 1*), C101A51 (*Pi 2*) and Tetep (*Pi kh*) from DRR, Hyderabad were taken as the resistant gene source check.

Functional marker analysis showed the presence of gene *Pi 1* in nine varieties. 20 varieties showed the presence of gene *Pi 2* and six varieties showed the presence of gene *Pi kh*. Among these, 3 varieties were having a gene combination of *Pi 1* and *Pi 2*. Three varieties were having genes *Pi 2* and *Pi kh* and two varieties had *Pi 1* and *Pi kh* gene combination. Five varieties did not show the presence of molecular marker linked to any of the genes under study.

Field screening was done for scoring the varieties for blast disease resistance by growing in the disease prone farmer's field at Pattambi. From screening, ten varieties were found to be moderately resistant and fifteen varieties displayed moderately susceptible response. Analysis of variance of the infection index calculated from disease score showed that, nine varieties were having low infection index among the selected varieties and they were on par. All the remaining varieties showed susceptible response. None of the varieties were

immune or resistant. Popular rice varieties Uma and Jyothi showed a high infection index.

Comparing the disease score, the scores showed that the combination of genes *Pi 1* and *Pi kh* imparted more resistance to the races of *M. oryzae* prevalent in the location. Parambuvattan and Kavunginpoothala having genes *Pi 1 and Pi kh* can be used as donors for the combinational genes in the breeding programmes. Considering single gene effect, gene *Pi 1* imparted moderate resistance. Varieties Thekkan chitteni, Karuthamodan and Njavara kunnathoor are having this gene showed a low infection index. Pyramiding of genes *Pi 1* and *Pi kh* can impart durable resistance to rice varieties of Kerala. Parambuvattan and Kavunginpoothala having these two genes in combination can be used as donors for the genes.

From the study on resistance to the blast disease caused by *M. oryzae,* traditional varieties which are having specific genes alone and in combination were identified. These lines can be used as donors for respective genes in further breeding programmes. The traditional varieties kavunginpoothala and Parambuvattan identified as donors for the *Pi 1* + *Pi kh* combination are having specific grain qualities of Kerala varieties - Prambuvattan with red bold and Kavunginpoothala with white bold grain. Rice cultivation throughout Kerala is dominated by two varieties Uma and Jyothi. Jyothi was released from RARS, Pattambi and Uma was released from RRS, Moncompu during 1974 and 1998 respectively. In the field evaluation, these two varieties were found highly susceptible with a disease score of 6. By using the identified donors, essentially derived varieties can be developed from these popular varieties by reconstituting them by pyramiding the genes of resistance *Pi 1* and *Pi kh*. Since the donors have been identified from the traditional varieties with specific grain qualities of Kerala, reconstitution of the lines by maintaining the grain quality can be easily achieved.

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*Original not seen

Identification of the Donors for Blast Resistance from Traditional Rice Varieties of Kerala using Functional Markers

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ABSTRACT

The research project entitled "Identification of the donors for blast resistance from traditional rice varieties of Kerala using functional markers" was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram and farmer's field at Pattambi, Palakkad district during 2012 - 2014. The major objectives of the study were to identify the traditional rice varieties with blast resistance genes (*Pi 1, Pi 2, Pi kh*) using associated functional markers and the field scoring of the lines under disease stress condition.

In the present study, thirty traditional rice varieties of Kerala were taken for resistance gene source identification and field level evaluation. The selected rice varieties were screened for the presence of the reported blast resistance genes *Pi 1, Pi 2* and *Pi kh* using three SSR (Simple Sequence Repeat) markers RM224, RM527 and RM206 respectively. C101Lac (*Pi 1*), C101A51 (*Pi 2*) and Tetep (*Pi kh*) from DRR, Hyderabad were taken as the resistant gene source check.

Functional marker analysis showed the presence of gene *Pi 1* in nine varieties. Twenty varieties showed the presence of gene *Pi 2* and six varieties showed the presence of gene *Pi kh*. Among these, three varieties were having a gene combination of *Pi 1* and *Pi 2*. Three varieties were having genes *Pi 2* and *Pi kh* and two varieties had *Pi 1* and *Pi kh* gene combination. Five varieties did not show the presence of molecular marker linked to any of the genes under study.

Field screening was done for scoring the varieties for blast disease resistance by growing in the disease prone farmer's field at Pattambi. From screening, ten varieties were found to be moderately resistant and fifteen varieties displayed moderately susceptible response. Analysis of variance of the infection index calculated from disease score showed that, nine varieties were having low index and they were on par. All the remaining varieties showed susceptible

response. None of the varieties were immune or resistant. Popular rice varieties Uma and Jyothi showed a high infection index.

Comparing the disease score and the presence of genes, it was inferred that, genes *Pi 1* and *Pi kh* in combination, imparted moderate resistance under Kerala condition. Varieties Parambuvattan and Kavunginpoothala having these two genes showed low infection index in the field screening. Considering single gene effect, gene *Pi 1* imparted moderate resistance. Varieties Thekkan chitteni and Njavara from Kunnathoor are having this gene showed a low infection index. Pyramiding of genes *Pi 1* and *Pi kh* can impart durable resistance to rice varieties of Kerala. Parambuvattan and Kavunginpoothala having these two genes in combination can be used as donors for the genes.