MOLECULAR MARKER ASSISTED PYRAMIDING OF *Pi9* AND *Pi54* BLAST RESISTANCE GENES IN RICE CULTIVAR K 343

By

USHA KIRAN

(J-14-D-21-Biot)

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Ph.D

MOLECULAR MARKER ASSISTED PYRAMIDING OF *Pi9* AND *Pi54* BLAST RESISTANCE GENES IN RICE CULTIVAR K 343

USHA
KIRAN

2020

CERTIFICATE-I

This is to certify that the thesis entitled **"Molecular Marker Assisted Pyramiding of *Pi9* and *Pi54* Blast Resistance Genes in Rice Cultivar K 343"**, submitted in partial fulfilment of the requirements for the degree of **Doctor of Philosophy in Biotechnology** to the faculty of Post-Graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu is a record of boniafide research carried out by **Ms. Usha Kiran,** Registration No. **J-14-D-21-Biot** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that such help and assistance received during the course of investigation have been duly acknowledged.

(Dr. Manmohan Sharma)
Major Advisor

Place: Jammu
Date: 12-10-2020

Endorsed

Coordinator
School of Biotechnology

Date:

CERTIFICATE-II

We, the members of the Advisory Committee of **Ms. Usha Kiran**, Registration No. **J-14-D-21-Biot,** a candidate for the degree of **Doctor of Philosophy in Biotechnology,** have gone through the manuscript of the thesis entitled **"Molecular Marker Assisted Pyramiding of *Pi9* and *Pi54* Blast Resistance Genes in Rice Cultivar K 343",** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the degree.

Dr. Manmohan Sharma
Associate Professor,
School of Biotechnology
(Major Advisor)

Place: Jammu

Date: 12-10-2020

Advisory Committee Members:

Dr. R.K Salgotra,
Professor and Coordinator,
School of Biotechnology
(Member from Major Subject) .....

Dr. Bikram Singh,
Professor
Division of Plant Breeding and Genetics
(Member from Minor Subject) .....

Dr. Sachin Gupta,
Associate Professor
Division of Plant Pathology
(Member from Supporting Subject) .....

Dr. Sanjay Guleria,
Professor and Head
Division of Biochemistry
(Dean's nominee) .....12.10.20.....

CERTIFICATE-III

This is to certify that thesis entitled **"Molecular Marker Assisted Pyramiding of *Pi9* and *Pi54* Blast Resistance Genes in Rice Cultivar K 343"** submitted by **Ms. Usha Kiran,** Registration No. **J-14-D-21-Biot** to the faculty of Post Graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu in partial fulfilment of the requirements for the degree of **Doctor of Philosophy in Biotechnology** was examined and approved by the Advisory Committee and External Examiner(s) on ....7th December 2020

Sharada 7/12/2020

External Examiner
Dr. Sharada M. Potukuchi
Head School of Biotechnology
SMVDU, Katra

Dr. Manmohan Sharma
Associate Professor
School of Biotechnology
Major Advisor

Coordinator
School of Biotechnology

Dean
Faculty of Agriculture

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ABSTRACT

Title of Thesis/Dessertation	: Molecular Marker Assisted Pyramiding of *Pi9* and *Pi54* Blast Resistance Genes in Rice Cultivar K 343
Name of the student	: Usha Kiran
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Major Subject	: Biotechnology
Name and Designation of Major Advisor	: Dr. Manmohan Sharma Associate Professor
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Name of University	: Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu.

Abstract

Rice blast disease caused by the fungus *Magnaporthe oryzae*, poses a serious threat to the rice production world over. Deployment of host-plant resistance, has proved to be the most economical and environment friendly option for managing the disease. The present investigation entitled "Molecular marker assisted pyramiding of *Pi9* and *Pi54* blast resistance genes in rice cultivar K 343" was carried out at School of Biotechnology, SKUAST-Jammu from 2015-2019 to pyramid blast resistance genes in the genetic background of rice variety K 343; to validate the introgressed genes in the target background using SSR markers; and to evaluate the pyramided lines for different traits to identify superior genotypes. K 343 was used as recipient parent which is an elite *Indica* rice cultivar while DHMAs and RML 22 were used as donor parents for *Pi54* and *Pi9* genes, respectively. For *Pi54* gene, closely linked SSR marker RM 206 (0.7 cM from locus) and for *Pi9* gene SSR marker AP5930 (0.05 cM from locus) were selected for foreground selection. A total of 101 genome wide polymorphic SSR markers were selected and used for background selection to find out the percent recovery of recurrent parent genome using GGT 2.0 software.

Foreground and background selection in BC_2F_1 stocks led to identification of three plants (P1, P3, P17) in K 343*3/DHMAS population and three plants (P3, P11, P28) in K 343*3/RML 22 population which were positive for target gene and had recurrent parent genome recovery more than 85 percent with broader similarity with recurrent parent with respect to agro-morphological traits. Screening of both BC_2F_1 populations with PLP-1 strain of *M. oryzae* depicted that these plants showed resistant to highly resistant reaction. Simultaneous foreground selection of *Pi54* and *Pi9* genes in F_2 convergent population (comprising of 4000 plants) depicted 45 plants positive for both *Pi54* and *Pi9* genes. Background selection of gene positive plants led to the identification of 8 pyramided plants (P4, P5, P6, P8, P16, P37, P42 and P45) which had recurrent parent genome recovery more than 90 percent and agronomical similarity with recurrent parent genome and highly resistant reaction to blast fungus. It indicated the strong resistance nature of *Pi54* and *Pi9* genes together to the predominant fungal strain (PLP-1) of *Magnaporthe oryzae*. The identified lines showed low amylose and moderate protein content. These pyramided plants can be further used as genetic stocks for identification of blast resistance varieties or can be used as donors of genes *Pi9* and *Pi54* in breeding programmes.

Key Words: *Magnaporthe oryzae*, foreground selection, background selection, SSR, *Pi54*, *Pi9*

UKiran

Sign. of student

Sign. of Major Advisor

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Introduction

CHAPTER -1

INTRODUCTION

Rice (*Oryza sativa* L.), belonging to the family Poaceae (Gramineae), is a self-pollinated diploid plant (2n=2x=24) with a genome size of 430 Mb. It is the staple diet of over three billion people around the world, particularly in Asia (Abdullah *et al*., 2006; Skamnioti and Gurr, 2009; Hosseyni-Moghaddam and Soltani., 2013). More than half of the world population i.e. more than 3.5 billion people depend on rice for more than 20 percent of their daily calories. Rice is grown across the world except Antarctica and has higher energy digestibility among the staple crops (Kumari *et al*., 2017). It is predominantly consumed dietary component in those areas of the world, where population densities are high and overall dietary levels are least adequate. Asia accounts for 90 percent of global rice consumption, and total rice demand continues to rise.

World over paddy was cultivated over an area of about 167.13 million hectares with production of about 771 million tons (509.4 million tons of milled rice) during 2017-18 (FAO, 2019). Of the total area under rice cultivation, 92 percent of the rice is grown in Asia, which is home to more than half of the world population (Sharma *et al*., 2012). India is one of the largest producers of rice producing 112.19 million tonnes of milled rice (168.45 million tons paddy) from an area of 43.7 million hectares during 2017-18 (Anonymous, 2019; Kulkarni and Peshwe, 2019). The current production level needs to be increased to 130 million tonnes by the year 2025, to meet the demand of increasing population in India and to sustain the self-sufficiency in rice production (Viraktamath, 2009 and Selvaraj *et al*., 2011)

In Jammu and Kashmir, rice is a predominant dietary component of majority of population; therefore it is important for livelihood security and economy of the region. Rice cultivation is an integral component of rich cultural heritage of the J&K. During 2016-17, it was grown over an area of about 283.44 thousand hectare with production and productivity of 5725 thousand quintles and 2.02 tonnes per hectare, respectively (Anonymous, 2018). Jammu Division occupies about 52 percent of the total area under rice while 48 percent of the total area is covered by Kashmir Division. In Jammu and Kashmir, rice is cultivated under diverse ecological conditions ranging from high altitude to temperate plain basins of valley in Kashmir and temperate hill

ecologies to intermediate and subtropical conditions of Jammu region, both under irrigated and upland conditions.

The major limitations in the cultivation of rice, particularly in hill and temperate ecologies, comprises of low temperature during flowering and maturity, widespread phosphorus and zinc deficiency and incidence of rice blast. The estimated annual requirement of rice in Jammu & Kashmir is about 11 lakh tonnes against current production of just 5.72 lakh tonnes (Anonymous, 2018). The deficit is met through imports from the neighbouring states of the country.

One of the important research strategies to achieve the targeted levels of production includes developing rice varieties to counter the major biotic and abiotic stresses. During the entire life cycle, rice plant is affected by as much as 36 different pathogens including bacteria, fungi, viruses and nematods etc. Among them blast, caused by the heterothallic ascomycete fungus *Magnaporthe oryzae* (Hebert), Barr (anamorph *Pyricularia oryzae*), is the most destructive disease of rice which is reported to cause yield losses up to 50 percent in Asia, Africa and some parts of America (Manandhar *et al*.,1992; Li *et al*., 2007; Khush and Jena, 2009; Skamnioti and Gurr, 2009; Helliwell *et al*., 2013; Kulkarni and Peshwe., 2019).

Rice blast disease is favoured by relative humidity over 90 percent and temperatures ranging between 20 to 30 ºC with the production of conidia on lesions. Frequent prevalence of dew, mist and drizzle are congenial conditions for proliferation and multiplication of the fungal pathogen. Soil fertility also affects disease severity i.e. high levels of organic matter or excessive use of nitrogen enhances the chance of fungal proliferation and hence increased incidence of the disease, when the cultivar resistance is not specific. However, nitrogen deficiency can also predispose and expose the plants to disease (Prabhu *et al*., 1996). Disease severity varies with various factors such as location, weather, crop growth stage and the inherent level of partial resistance acquired/expressed by various cultivars.

In Jammu and Kashmir, rice blast is the most devastating disease and the most prominent reason for yield loss, limiting rice production specifically in the hill and temperate ecologies where this crop is cultivated in hundred percent irrigated and cool night ecology of *Kharif* season, which is conducive and suitable for proliferation and build up of blast fungus; and subsequently widely occurring blast epidemics in

rice (Anwar *et al*., 2009). Both leaf blast and neck blast are prevalent in the state. Blast frequently occurs on coarse grain *Japonica* and *Indica* cultivars recommended in the region. Most of the popular rice varieties under cultivation in Jammu region *viz*. K 39, Barkat (K 78), K 332, Chenab (K 343), Jehlum (K 448), China 1039, Kohsar (K 429) and Shalimar Rice 2; and Kashmir valley *viz*. K 39, Jehlum (K 448), Shalimar Rice 1, Shalimar Rice 2, Shalimar Rice 3, Mushk Budgi, Khutch, Kamad and China 972 show variable reaction to blast varying from moderately resistant to highly susceptible response depending on the prevailing weather conditions (Anwar *et al*., 2003; Anwar *et al*., 2009; Anwar *et al*., 2011; Najeeb *et al.*, 2016). Among the non-basmati rice cultivars, K 343 is the most popular *indica* rice variety in the hill zone of Jammu Region. However, studies have indicated susceptible to moderately resistant response of K 343 to blast fungus over the years depending upon the prevailing weather. Therefore, identification of resistant/tolerant donors against blast and their utilization in varietal improvement continues to be the integral part of rice breeding programme in Jammu and Kashmir.

The fungus (*M. oryzae*) is able to develop resistance to chemical treatments which poses a continuous threat to the effectiveness of high yielding rice varieties. The chemical treatment is not a sustainable, viable and bio-safe option for managing the disease. It not only pollutes the environment but also increases the cost and largely reduces the efficiency of rice production (Chen *et al*., 2020) Therefore, deploying host/genetic resistance is the most economical and environmentally friendly option for managing the disease (Manandhar *et al*., 1998 ; Hulbert *et al*., 2001). Pyramiding of multi-resistance genes into single genotype often confers wider spectrum of resistance and durability (Pradhan *et al*., 2015). In this context, major and minor genes can contribute towards durable resistance (Wang *et al*., 1994). Advances in rice genomics has enabled use of highly efficient DNA marker systems for selection of traits through indirect selection of resistance genes in segregating generations for developing resistant varieties by marker assisted selection (Jena and Mackill, 2008). These gene/allele-specific DNA markers play an important role in marker assisted selection (MAS) for accurate selection of target plants with minimum effort, time and cost. The advances made in the area of molecular markers have enabled tracking of the genes for disease resistance by following the path of markers that are linked/ tagged to each gene, thus making the identification of plants with two

or more genes simple.

Molecular markers have been used to identify and pyramid favourable (or deleterious) and multiple alleles for biotic and abiotic stress resistance in a collection of diverse genotypes (Jena and Mackill, 2008; Suh *et al*., 2009; Singh *et al*., 2012; Akos *et al.,* 2019*)*. Marker assisted selection (MAS) for pyramiding multiple genes along with rapid background recovery of the recurrent parent, while maintaining the exquisite quality characteristics of rice, could be an effective approach for rice improvement (Singh *et al*., 2001; Sundaram *et al.*, 2008; Xu and Crouch, 2008; Suh *et al*., 2009). Gene pyramiding is difficult using conventional breeding methods due to the dominance and epistatic effects of genes governing disease resistance. Moreover, genes with similar reactions to two or more races are difficult to identify and transfer through conventional approaches (Joseph *et al*., 2004) However, the availability of molecular markers closely linked to each of the resistance genes makes the identification of plants with two or three genes possible (Singh *et al*., 2001; Sundaram *et al*., 2008).

With the conclusion of the rice genome sequencing project (Goff *et al*., 2002; Yu *et al*., 2002), approximately 105 major rice blast R genes have been identified and 32 of them have been molecularly characterized including *Pib, Pib1*, *Pita, Pi9, Pi2, Pizt, Pid2, Pi33, Pii, Pi36, Pi37, Pikm, Pit, Pi5, Pid3, Pid3-A4, Pi54, Pish, Pik, Pikp, Pia, PiCO39, Pi25, Pi1, Pi21, P50* and *Pi65(t)* (Li *et al*., 2017; Wang *et al*., 2017; Zhao *et al*., 2018; Yadav *et al*., 2019). The emergence of new biotypes has demanded stacking of several resistance genes into high yielding cultivar backgrounds to confer a wide spectrum of resistance. These genes can be utilized using molecular breeding and transgenic approaches to introgress high degree of resistance in otherwise successful and well performing commercial cultivars which are susceptible to the fungus. This enhanced capability will enable them to survive attacks from several fungal biotypes at a time and also survive in unfavorable environmental conditions.

There are identified resistance genes such as *Pi1, Pita, Pi2, Pi9, Piz* and *Pi54* which have been reported to express resistance response under North Western Himalayan conditions. Of these, *Pi54* and *Pi9* genes are highly effective against the pathogen population of this region (Sharma *et al*., 2005a). Identified potential donors (DHMAS for *Pi54* and RML 22 for *Pi9*) as well as closely linked/gene derived markers for these genes are available (Sharma *et al*., 2005a; Fjellstrom *et al*., 2006

and Ramkumar *et al*., 2011). Thus, these genes can be successfully transferred from donor parents to the genetic background of elite rice varieties making them resistant to blast disease.

Keeping in view the economic importance of rice *viz-a-viz* losses caused by the blast fungus; and effectiveness of the *Pi54* and *Pi9* genes under North Western Himalayan ecologies, present study entitled **"Molecular Marker Assisted Pyramiding of *Pi9* and *Pi54* Blast Resistance Genes in Rice Cultivar K 343"** has been carried out with the following objectives:

1. To pyramid blast resistance genes in the genetic background of rice variety K 343
2. To validate the introgressed genes in the target background using SSR markers
3. To evaluate the pyramided lines for different traits to identify superior genotypes

Chapter-2

Review of Literature

CHAPTER-2

REVIEW OF LITERATURE

Rice (*Oryza sativa* L.) is one of the most important cereal crops. It belongs to the family Poaceae with a genome size of 430 Mb. It is the staple diet of over three billion people around the world, particularly in Asia (Abdull *et al*., 2006, Skamnioti and Gurr, 2009; Hosseyni-Moghaddam and Soltani., 2013). Asia contributes 90 percent of global rice production and consumption (Elert, 2014). India has the world's largest area of 43.7 million ha under rice and is the second largest producer (112.19 million tons) next only to China (Anonymous, 2019). In order to meet the future demand, the rice productivity in India has to be brought to 3.3 tonnes per ha from the current level of 2.5 tonnes per ha (Mohapatra *et al*., 2013). Bridging the gap between the potential yield and the actual yield obtained by the farmers is among the frontline strategies envisaged for enhancing rice production. This yield gap varies from 20 percent in irrigated ecosystems to 50 percent in upland and unfavourable ecologies (Viraktamath *et al*., 2011). Many biotic and abiotic factors influence production and productivity of rice. Among the biotic factors, major diseases like blast, sheath blight and bacterial blight; and emerging diseases such as bakanae and sheath rot are posing serious threats to rice production (Raghu *et al*., 2018). With the sequencing of whole rice genome and availability of various modern biotechnological tools and platforms, researchers found great opportunity to enhance the breeding efficiency using molecular approaches which are proving useful to enhance rice production. Genomic technologies have enabled use of highly efficient DNA marker systems to tag and track the gene of interest in target germplasm and segregating generations using marker assisted selection (MAS).

2.1 Blast disease of rice

Rice blast caused by the fungus *Magnaporthe oryzae* (Hebert) Barr, is one of the most devastating diseases that attack rice world over (Helliwell *et al*., 2013, Parinthawong and Suksiri, 2020). Several rice blast epidemics have occurred in different parts of the world, resulting in heavy yield losses upto 90 percent (He *et al*., 2012; Singh *et al*., 2015; Wu *et al.*, 2019) which threatens global food security (Liu *et al*., 2013). The amount of rice destroyed annually by blast is sufficient to give food to 60 million people world over. The incidence of the disease has been reported in 85

countries, particularly in the irrigated and rainfed lowlands of temperate and subtropical Asia, Latin America and Africa (Sharma *et al*., 2012). In India blast was first recorded in 1913 and the first devastating epidemic was reported in 1919 in the Tanjore delta of Madras state. A four percent reduction in yield due to blast was estimated for the first time in India. During 1960-1961, the total loss due to blast was 2,65,000 tonnes (Padmanabhan, 1965). Seven epidemics of Blast happened between 1980 and 1987 in the states of Himachal Pradesh, Andra Pradesh, Tamil Nadu and Haryana resulting in huge yield losses. The disease is a serious production constraint for rice in North Western Himalayan region of India comprising the Union Territory of Jammu and Kashmir, Uttrakhand and Himachal Pradesh (Sharma *et al*., 2002).

Total destruction of rice crop over large areas has been reported due to blast disease from Jammu and Kashmir (Anwar *et al.*, 2009). Rice blast disease is endemic to most rice growing areas of Jammu and Kashmir due to prevailing blast-conducive environmental conditions during the crop season. Rice blast took serious dimensions in Kashmir valley during 2007 with Mushk budgi, an early maturing aromatic variety of Kashmir, being completely destroyed (Husaini *et al*., 2011).

This is a polycystic disease spread by asexual spores (conidia) that infect above ground tissues of rice plants (Ou *et al*., 1985; Wilson and Talbot, 2009; Pennisi, 2010), the fungal spores germinates on leaf surface forming a germ tube which differentiates into a peculiar dome shaped structure called appressoria (Hamer *et al*.,1988). Further the cell cycle regulated autophagic programmed cell death occurs to initiate infection in the host (Howard *et al*., 1991; De Jong *et al*., 1997; Veneault-Fourrey *et al*., 2006).

Rice blast can infect rice from seedling to adult plant stages affecting leaves, nodes, collar, panicles and roots but cause the greatest losses when neck and panicles are infected. Most of the infections affect leaves causing diamond shaped lesions with grey or white center to appear, or on the panicles, which turn white and die before being filled with grain (Scardaei *et al*., 1997). Initial symptoms of rice blast are oval-shaped lesions that are 0.3 to 0.5 cm wide and 1.0 to 1.5 cm long, ranging from white to gray and surrounded by darker borders, and older lesions are typically larger and may coalesce to kill entire leaves (Lamari, 2009; Kumar *et al*., 2013).

Depending on the prevailing weather conditions, most of the popular rice varieties under cultivation in the hills of Jammu and Kashmir show variable reaction to blast varying from moderately resistant to highly susceptible response (Ali *et al*.,

2009). However, immune/complete resistance response was not observed in any of the cultivars. Blast frequently affects coarse grain Kashmiri *Japonica/Indica* rice cultivars. Disease severity varies with weather, location, crop growth stage and the innate level of partial resistance of cultivars (Anwar *et al*., 2009). In a survey it is revealed that almost all the rice production zones of Kashmir were affected with leaf blast disease within the average severity range of 3.7- 41.3 percent, the highest nodal blast was found in Kulgam (7.3%) followed by Khudwani(5.4%) and Larnoo(3.8%) areas of district Anantnag. Destructive phase of neck blast severity was observed in every district under survey, with an average range of 0.3 - 4 percent. Among the local coarse grain cultivars, Safed China, Jhelum and Chenab were observed susceptible to leaf blast but Mushk Budgi, Khutch and Kamad cultivars were found to be largely killed due to devastating occurrence of blast. It is also found that the genotypes K 627-7-1-11 and K-696-2-1-2-8 have shown immune to moderately resistance reaction to neck and leaf blast which further can be employed or used as local specific donors in 100 percent irrigated and temperate rice breeding programmes to access the benefit of disease resistance in the high yielding varieties.

Anwar *et al.* (2011) conducted a study from monoconidial single lesion on international differential hosts and found that the physiological race IC-17 was the most common race in Kashmir. Along with that they also found that ID-1 and IC-25 races caused avirulence reactions(R) to Raminand Str-3 but virulence to Kanto-51 (s). Zenith was found to have resistant reaction to all the isolates except isolates from certain places of Pulwama, Budgam and Srinagar. It is reported that new physiological races appeared with a frequency of about one new race for every four monoconidial isolates tested in international differentials sets of rice cultivars.

The disease can be managed through agronomic practices, use of fungicides, planting resistant cultivars and biotechnological interventions (Ribot *et al*., 2008). The indiscriminate use of fungicides prompts the evolution of resistance, which in turn leads to disease resurgence. Use of host-plant resistance has been considered as one of the most appropriate approaches to combat this disease (Khush and Jena, 2009). In other words, breeding and cultivation of resistant rice cultivars remain the most efficient, economical and environmentally benign means to manage the disease, especially in resource-poor farmers field (Séré *et al*., 2007; Panda *et al*., 2017). The first step in developing resistant varieties is identification of broad spectrum resistance gene(s) and their incorporation into the background of agronomically superior

genotypes (Yadav *et al*., 2017). Conventional plant breeders have developed number of blast resistant varieties well adapted to different rice growing regions world-wide.

Although host resistance is the most economical viable and environmentally sound way to manage blast disease, resistance of rice cultivars is usually effective only for two to three years (Zeigler *et al.*, 1994). Such a rapid breakdown is due to the rapid adaptation of *M. oryzae* to specific host resistance genes (Huang *et al*., 2014). Convention breeding for disease resistance is tedious, time consuming and mostly dependent on environment as compared to molecular breeding particularly MAS, which is more precise and highly efficient.

2.2 The causal organism (*Magnaporthe oryzae*)

Rice blast is generally considered as the principle disease of rice and is caused by the fungus belonging to the Ascomycete, *Pyricularia grisea* Sacc. (= *Pyricularia oryzae* Cavara = teleomorph *Magnaporthe grisea* (Hebert) Barr Comb nov.) (Bussaban *et al*., 2005). *Magnaporthae oryzae* is separated from *Magnaporthae grisea* based on the phylogenetic analysis and inter-strain fertility test (Couch and Kohn, 2002; Ebbole, 2007). *M. oryzae* has genome size of ~ 40 Mb distributed among seven chromosomes (Dean *et al*., 2005). As Rice–*Magnapothae* pathosystem has been extensively analysed at the molecular level, rice blast has become a model in the study of plant-fungal interactions (Valent and Chumley,1991; Wilson and Talbot, 2009). The fungal pathogen *M. oryzae* has been placed among the top 10 fungal plant pathogens in the world based on its scientific and economic importance (Dean *et al*., 2012).

M. oryzae can replicate quickly and successively by mitosis, nuclear migration and death of conidia from which the infection originated and produce appressoria capable of infecting aerial structures and hyphae capable of infecting roots of young and old rice plants (Sesma and Osbourn, 2004; Wilson and Talbot, 2009; Fernandez and Wilson, 2012). Autophagic cell death of conidia is connected to cell cycle control and produces conidiophores that are dispersed to other tissues and plants by wind and water splash to reinitiate the infection cycle by attachment of a spore that germinates and forms an appressorium (Agrios, 2005; Wilson and Talbot, 2009). Rice blast conidia can spread within 230 m from their source; dispersal is favored in darkness and with high relative humidity and winds greater than 3.5 ms^{-1} (Kingsolver *et al*., 1984). The primary source of innoculum is infected residue and seeds of rice, and in the tropics, airborne conidia are present throughout the year, enabling stable

epidemics to occur year-round (Ou, 1985; Guerber and TeBeest, 2006; Raveloson *et al*., 2018).

M. oryzae serves an excellent model organism for studying fungal phyto-pathogenicity and host- parasite interactions. The *Magnapothae oryzae* pathosystem is of great interest because both the host and pathogen are experimentally amenable (Ebbole, 2007). The entire genomes of the rice blast fungus and rice have been sequenced and are released in the public domain (Dean *et al*., 2005). This disease has advanced to become one of the premier model fungal pathosystems for studying host-pathogen interactions because of the depth of comprehensive studies in both species using modern genetic, genomic, proteomic and bioinformatic approaches. Many investigators have considered it to be a model disease for the study of genetics, epidemiology, molecular pathology of host parasite interactions and biology (Huang *et al*., 2014; Lopez and Cumagun *et al*., 2019). *M. oryzae* genome is rich in retrotransposones and repetitive segments (Dean *et al*., 2005), which helps it to change its virulence to overcome the resistance provided by R- genes (Vasudevan *et al*., 2014).

Kumar and Singh (1996) concluded that *Pyricularia grisea* affect number of host plants belonging to the family Gramineae including cereals and grasses such as *Oryza sativa, Eleusine coracane*, *Eleusine indica*, *Digitaria sanguinalis*, *Pennisetum typhoides* and *Echinochloa colonum*.

Dar and Iqbal (2017) carried out an investigation to find perpetuation of rice blast pathogen (*M. oryzae*) under temperate conditions of Kashmir during the month of October to March on specimens like paddy straw, stubbles, weed straw, soil, rice residue(husk), seeds and undecomposed organic material . They found that in the samples collected, highest frequency (14.15%) of blast pathogen (*M. grisea*) was recorded in the month of October. However, month of March showed least occurrence (2.50%). Among collected samples, maximum perpetuation of blast pathogen (*M. grisea*) was exhibited by stubbles (14.35%) followed by paddy straw (12.37%) and the minimum were exhibited in undecomposed material (1.60%), followed by weed straw (3.40%) and soil (5.8%). So, they concluded that these studied components are the possible source of perpetuation for rice blast pathogen.

Kulkarni and Peshwe (2019) conducted an experiment for screening, isolation and molecular identification of *M. oryzae*. They collected blast infected leaf samples from lonavala susceptible paddy field. Morphological identification of blast fungus showed grayish colony with circular smooth margins and concentric rings on potato

dextrose agar. Spore Induction was performed using stem bits. Pathogenicity assay was performed in plastic pots and using detached leaf samples from susceptible paddy variety. Further desired blast pathogen i.e. *Magnaporthe oryzae* was identified with ITS region through Sanger sequencing.

2.3. Rice blast resistance (R) genes

Rice blast resistance genes were first described by Sasaki in Japan in 1923 and since the identification of first resistance gene *Pi-a* from *Japonica* rice variety Aichi Asahi, over 105 major rice blast R genes have been identified and 32 of them have been molecularly characterized (Li *et al*., 2017; Wang *et al*., 2017; Zhao *et al*., 2018). R genes are distributed on 11 chromosomes of rice genome, except chromosome 3; more than 64 percent are clustered in chromosome 6, 11 and 12, representing 18 percent, 25 percent and 21percent, respectively (Ashkani *et al*., 2016). Most of the resistance genes are dominant, except the recessive genes *pi21, pid-2 and pi66*(t) (Liang *et al*., 2016). Many R genes are clustered at the same locus and allelic to each other. For instance, *Pi2, Piz-t, Pi9* and *Pi50* are allelic at the *Pi2/9* locus (Qu *et al*., 2006; Zhou *et al*., 2006; Su *et al*., 2015). *Pik, Pikp, Pikm, Pikh, Pi1* and *Pi7* are allelic at the Pik locus (Ashikawa *et al*., 2008; Yuan *et al*., 2011; Zhai *et al*., 2011; Hua *et al*., 2012). *Pish, Pi35* and *Pi37* are allelic at *Pish* locus (Lin *et al*., 2007; Takahashi *et al*., 2010; Fukuoka *et al*., 2014). Moreover, a limited number of sequence differences between these R-gene alleles were found to determine their specificities against distinct sets of rice blast isolates (Zhou *et al*., 2006; Fukuoka *et al*., 2014; Su *et al*., 2015). It was also found that functional and nonfunctional R-gene haplotypes at the same locus from resistant and susceptible rice varieties were distinguished by a few sequence changes (Bryan *et al*., 2000; Su *et al*., 2015).

Most of the deployed R genes have often been identified in landraces, cultivars, or wild rice collections because of different physiological races of *M. oryzae* (Tanksley *et al*., 1997; Yadav *et al*., 2019) except for *Pi9, Pi54rh, Pi40(t),* and *Pirf2-1(t),* which were domesticated from *O. minuta, O. rhizomatis, O. australiensis,* and *O. rufipogon,* respectively. Most of the R-genes encode nucleotide-binding site-leucine-rich repeat (NBS-LRR) proteins except *pi21* and *pid2,* which encode proline-containing protein and receptor kinase (Fukuoka *et al*., 2009; Kouzai *et al*., 2013; Zheng *et al*., 2016; Zhu *et al*., 2016; Yadav *et al*., 2019). During interactions between rice and blast pathogens, products of the R gene can specifically recognize the corresponding elicitors of *M. Oryzae.* Among the identified blast resistance genes so

far *Pi1*, *Pi2*, and *Pi54* have been reported to provide broad-spectrum resistance and have been widely used in different combinations by breeders for improving blast resistance in the majority of the ruling varieties (Balachiranjeevi *et al*., 2015; Madhavi *et al*., 2016; Khan *et al*., 2018; Swathi *et al*., 2019). However, the resistance conferred by a single gene often breaks down in the varieties after a few years of their release due to the dynamic behavior of the blast pathogen (Hittalmani *et al*., 2000; Fukuoka *et al*., 2015).

The blast genes *Pi1* and *Pi2* are derived from *Indica* rice lines LAC23 and C101A51, respectively, which are highly effective against the pathogen population in Asia (Tacconi *et al*., 2010; Mackill and Bonman, 1992). The *Pi54* gene was identified and mapped on chromosome 11L from a rice variety Tetep and was reported to govern resistance against predominant races of the blast pathogens in India (Sharma *et al*., 2005; Sharma *et al*., 2010. The exploitation of these genes in the marker-assisted breeding programme is an effective and economic strategy for the development of blast resistant lines.

Zhou *et al.* (2006) studied the rice blast resistance (*R*) genes *Pi2* and *Piz-t* and reported that these genes confer broad spectrum resistance against different sets of *Magnaporthe grisea* isolates. They first identified the *Pi2* gene using a map-based cloning strategy. Fine genetic mapping, molecular characterization of the *Pi2* susceptible mutants, and complementation tests indicated that *Nbs4 Pi2* is the *Pi2* gene. The *Piz-t* gene, a *Pi2* allele in the rice cultivar Toride 1, was isolated based on the *Pi2* sequence information. Complementation tests confirmed the family member *Nbs4-Piz-t* is *Piz-t*.

Owing to the huge potential of Tetep in resistance breeding for the effective management of rice blast in the North-Western region of India, the *Pi-kh* (*Pi54*) gene has been found highly effective against the pathogen population found in the North Western Himalayan region of India (Sharma *et al*., 2002) and mapped in the same cultivar Tetep using different types of DNA markers (Sharma *et al*., 2009). Because of its effectiveness against many strains of *M. oryzae* and availability of closely linked and also gene based markers, the *Pi-kh* (*Pi54*) gene has been introgressed in Indian cultivars of rice using marker assisted back cross breeding (Singh *et al*., 2011). *Pi54* gene is confirmed to have broad-spectrum resistance against predominant races found in India (Thakur *et al*., 2015).

Earlier, blast resistance gene *Pitp(t)* was been mapped in cultivar Tetep by using

simple sequence length polymorphism markers (Barman and Chattoo, 2004). Besides, *Pi38* was identified in *Indica* rice Tadukan (Gowda *et al*., 2006) and *Pi-42(t)* from *Indica* cultivar DHR9 (Kumar *et al*., 2010). *Pi9* is a paralogue of *Pi2/Piz-t.* Both *Pikm-1* and *Pikm-2*, which are present as a head-to-head pair at the *Pik* locus, are required to confer *Pik-m*-specific resistance, and *Pi5*-mediated resistance entails the cooperation of two independent, non-homologous NBS-LRR genes (Ashikawa *et al*., 2008; Lee *et al*., 2009). Markers closely linked to blast resistance genes *Pi1* (Npb181and RZ536), *Pi2* (RG64) and *Pi9* (RG 16, Nbs2Pi-9, AP4791, AP5930, AP5650-3 and AP5650-5) genes have been validated in different studies (Yu *et al*., 1991; Qu *et al*., 2006; Fjellstrom *et al*., 2006). Another rice blast resistance gene *'Piz'* present in rice genotypes Zenith and Fukunishiki, represents a potential source of blast resistance for the NorthWestern Himalayan region of India (Rathour *et al*., 2008).

The recessive *pi21* allele is a quantitative blast resistance gene that was isolated from the *Japonica* rice cultivar Owarihatamochi on chromosome 4 (Fukuoka *et al*., 2009). Deletions in the proline-rich motifs of *pi21* accelerate the defense response of the plant under blast attack, although the response is neither as strong nor as fast as that of a qualitative resistance gene. This type of non-specific, weaker response presumably contributes to the reduction of selective pressure for pathogens to overcome host resistance, rendering *pi21* more durable and broad-spectrum (Fukuoka and Okuno, 2001; Fukuoka *et al*., 2009, 2012). Previous studies on the introgression of *pi21*, alone or in combination with other quantitative resistance genes in select Japanese rice cultivars confirmed the effectiveness of *pi21* in conferring durable resistance against blast isolates in Japan (Fukuoka *et al*., 2009, 2015; Yasuda *et al*., 2015; Horo *et al*., 2016). Combining *pi21* with another quantitative resistance gene, *Pi35*, in the *Japonica* rice cultivar Koshihikari, results in the resistant reaction of the cultivar against the Japanese blast isolate Ao-92-06-2. Even without *Pi35*, *pi21* is able to significantly reduce the diseased leaf area caused by the blast isolate (Yasuda *et al*., 2015). Similarly, near-isogenic lines of *pi21* in the *Japonica* rice cultivar Aichiasahi exhibit significant reduction in leaf lesion size when challenged with nine Japanese blast isolates (Fukuoka *et al*., 2015). Although the results of these studies strongly indicate the efficiency of *pi21* in conferring blast resistance in rice, the effects have only been assessed in the genetic background of temperate *Japonica* cultivars against the blast isolates from Japan. There are identified sources of resistance genes such as *Pi1, Pita, Pi2, Pi9, Piz* and *Pi54* which have been reported to express

resistance response under North Western Himalayan conditions. Of these, *Pi54* and *Pi9* genes are highly effective against the pathogen population of this region (Sharma *et al.,* 2005b). Identified potential donors (DHMAS for *Pi54* and RML 22 for *Pi9*) as well as closely linked/gene derived markers for these genes are available (Sharma *et al*., 2005b; Fjellstrom *et al*., 2006; Ramkumar *et al*., 2011). Alternatively, quantitative resistance conferred by quantitative trait loci (QTLs) is also a valuable resource for the improvement of rice disease resistance (Ashkani *et al.,* 2016).

Six R genes, *Pi2, Pi9, Pi40, Pigm, Piz-t* and *Piz* harbor alleles of the *Piz* locus located on the short arm near the centromere of rice chromosome 6 (Qu *et al*., 2006; Zhou *et al*., 2006; Jeung *et al*., 2007; Deng *et al*., 2017). *Pi40* gene from Australia's wild rice showed broad- spectrum resistance to rice blast races from South Korea (Suh *et al*., 2009). *Pigm,* a resistance gene from the local variety, Gumei 4, in China, is resistant to 50 isolates from all over the world (Deng *et al*, 2017). *Bsr-d1*, cloned from the local variety Digu, is a typical resistance gene that encodes a C2H2 transcription factor protein, which exhibits similar phenotypic incomplete resistance to several races of rice blast (Li *et al*., 2017).

The identification of number of resistance genes in rice provided the researchers with a good foundation for the breeding of resistant varieties with molecular marker assisted selection (MAS). Using MAS, blast resistance genes have been introgressed into Luhui 17, G46B, Zhenshan 97B, Jin 23B, CO 39, IR 50, Pusa 1602 and Pusa 1603, Pusa 1637-18-7-6-2 lines through marker assisted selection (Miah *et al*., 2013, Singh *et al*., 2015). MAS-breeding (MAB) has proved as an accurate, simpler, and low-cost tool compared to the traditional breeding methods for breeding resistant varieties (Chen *et al*., 2020).

The rice and rice blast system belong to a typical gene-for-gene system (Flor., 1971), in which the host resistance (R) genes show functional correspondence to their cognate pathogen avirulence (Avr) genes (Orbach *et al*., 2000; Valent and Khang., 2010). The co-evolution and interaction of R and Avr gene raises the possibility of a gene-specific arms race leading to diversification of both R and Avr genes (Dodds *et al*., 2006). Nine rice blast Avr genes have been cloned (Wang *et al*., 2017). The direct and indirect interaction models between R and Avr proteins were illustrated (Li *et al*., 2009; Yoshida *et al*., 2009; Kanzaki *et al*., 2012; Wu *et al*., 2015; Zhang *et al*., 2015; Ray *et al*., 2016). Field efficacy of any R gene in rice varieties is proposed to depend on the frequency of its cognate Avr gene in the rice blast pathogen population, which

provides a basis of Avr-gene based diagnosis for the deduction of effectiveness of R genes (Selisana *et al*., 2017)

Singh *et al. (*2015) conducted an experiment on molecular screening and genetic diversity studies of major rice blast resistance genes in 192 rice germplasm accessions using simple sequence repeat (SSR) markers. The genetic frequencies of the 10 major rice blast resistance genes varied from 19.79 percent to 54.69 percent. Seven accessions IC337593, IC346002, IC346004, IC346813, IC356117, IC356422 and IC383441 had maximum eight blast resistance genes, while FR13B, Hourakani, Kala Rata 1-24, Lemont, Brown Gora, IR87756-20-2-2-3, IC282418, IC356419, PKSLGR-1 and PKSLGR-39 had seven blast resistance genes. Twenty accessions possessed six genes, 36 accessions had five genes, 41 accessions had four genes, 38 accessions had three genes, 26 accessions had two genes, 13 accessions had single R gene but only one accession IC438644 did not possess any blast resistance gene. 17 out of 192 accessions harboured 7 to 8 blast resistance genes.

Yang *et al*. (2017) carried out studies in which they collected 358 rice varieties from different ecotypes in China and evaluated them for the neck blast resistance under natural conditions favoring disease development in Jining, Shandong Province. Their results showed that 124 (34.6%) and 234 (65.4%) varieties were resistant and susceptible to *M. oryzae* under natural field conditions, respectively. Among all the 358 rice varieties that were screened for the presence of 13 major blast resistances (*R*) genes against *M. oryzae* by using functional markers, 259 varieties contained one to seven *R* genes. Along with that, the relationship between the presence of *R* genes and the disease reactions was also investigated by integrative analysis of phenotyping and genotyping based on functional markers. Their results showed that *Pi2* gene was significantly correlated with neck blast resistance. Furthermore, any of the 13 major blast *R* genes was absent from 32 rice varieties exhibiting obvious neck blast resistance, which would be the potential materials for identifying novel neck blast *R* genes. Their results provided an insight into the distribution of the 13 major blast *R* genes in the tested Chinese rice germplasm resources, which could serve as elite germplasm and basis for developing rice blast resistant varieties in future.

2.4 Marker assisted selection (MAS) for introgression of blast resistance genes

Conventional rice breeding is a slow process, typically requires 10–12 years from initiation to varietal release. Conventional breeding mostly depends on environmental conditions and development of new varieties takes long time (Werner

et al., 2005; Zhang *et al*., 2006). MAS offers better selection strategies in rice breeding with a shorter period of time. It is more efficient, effective and reliable than phenotypic selection. MAS also allows the breeding of complex traits which is not feasible through conventional methods.

For efficient and successful implementation of MAS approach in a crop, availability of basic molecular tools such as molecular markers, genetic maps, etc. is a pre-requisite. Molecular markers are now very useful in tracking loci and genome regions in crop breeding programmes, as large numbers of molecular markers that are tightly linked to disease resistance genes are available in most of the major crop species (Philips and Vasil., 2001; Jain *et al*., 2002 and Gupta and Rastogi, 2004). Since the idea of indirect selection using genetic markers was first reported over 80 years ago (Sax *et al*., 1923), and particularly in the last few decades, new technologies have emerged that allow breeders to more easily select changes at the DNA level. Much of the progress to date has centered on marker-assisted backcrossing or the pyramiding of genes against rice blast. Molecular markers are essential for mapping genes of interest, marker-assisted breeding, and cloning genes using mapping-based cloning strategies (Hayashi *et al*., 2004). Of the different types of molecular markers, microsatellites have been utilized most extensively, because they can be readily amplified by PCR and detect large amount of allelic variation at each locus. These markers are abundant, distributed throughout the genome and are highly polymorphic compared with other genetic markers. Recent advances in rice genomics have now made it possible to identify and map a number of genes through linkage to existing DNA markers. The development of molecular methods to efficiently identify novel resistance genes has the potential to greatly improve modern cultivars, and such methods would help accelerate the application of MAS and marker-assisted backcross breeding (MAB) in rice improvement programmes.

Narayanan *et al*. (2002) improved an elite *Indica* rice line IR50, by molecular breeding approach involving marker assisted selection (MAS) and genetic transformation for resistance against blast (BL) and bacterial blight (BB). They used resistant CO39 near-isogenic line (NIL) C101A51 carrying *Piz5* as the donor parent and IR50 served as the recurrent parent in backcrossing up to four generations. BC_4F_1 plants were finally selfed to produce BC_4F_2 seeds. Sequence-tagged site (STS) marker RG64 was used to identify *Piz 5* in the segregating population and the resultant resistant progenies were obtained through phenotypic assays and MAS. DNA

markers for *Pita* have been used to follow its introgression into advanced breeding lines (Johnson *et al.*, 2003). The PCR-based allele-specific and InDel marker sets are available for nine blast resistance genes, and they provide an efficient marker system for MAS for blast resistance breeding (Hayashi *et al.*, 2006).

Hari *et al*. (2008) and Srinivasarao *et al*. (2009) conducted an experiment in which they introgress *Pi54* gene into susceptible rice varieties through a closely linked SSR marker, RM206, via marker-assisted introgression, which can be resolved through agarose gel electrophoresis.

Miah *et al.* (2014) used marker assisted backcrossing (MABC) approach to introgress (a) blast resistance gene (putative *Piz)* from the donor parent Pongsu Seribu 1, the blast-resistant local variety in Malaysia, into the genetic background of MR219, a popular high-yielding rice variety that is blast susceptible, to develop MR219 improved variety, resistant to blast. The recurrent parent genome recovery was analyzed in early generations of backcrossing using simple sequence repeat (SSR) markers. 70 out of 375 SSR markers were found polymorphic between the parents, and these markers were used to evaluate the plants in subsequent generations. Background analysis revealed that the extent of RPG recovery ranged from 75.40 percent to 91.3 percent and from 80.40 percent to 96.70 percent in BC_1F_1 and BC_2F_1 generations, respectively. In this study, the recurrent parent genome content in the selected BC_2F_2 lines ranged from 92.7 percent to 97.7 percent. The average proportion of the recurrent parent in the selected improved line was 95.98 percent. Marker assisted selection allowed identification of the plants that were more similar to the recurrent parent for the loci evaluated in backcross generations. The application of MAS with the MABC breeding programme accelerated the recovery of the RP genome, reducing the number of generations and the time for incorporating resistance against rice blast.

Many rice researchers have developed improved rice varieties for resistance against blast through marker assisted selection (MAS) and genetic transformation (Narayanan *et al*., 2002; Swamy and Sarla, 2011). The PCR-based allele-specific markers provide efficient system for blast resistance breeding (Hayashi *et al*., 2006; Latif *et al*., 2011). Jiang *et al*. (2012) have improved the blast resistance of rice cultivar Jin23B by MAS technology.

2.5 Gene pyramiding for development of blast resistant varieties

Among the biotic stresses blast disease is the most harmful threat to high productivity of rice (Kwon and Lee, 2002; Li *et al*., 2007), due to its wide distribution and ability to survive in wide range of environmental conditions. Due to this disease, yield loss ranged from 1 to 50 percent, meaning each year this disease destroys abundant rice to feed more than 60 million people and economic losses over $70 billion of dollar (Scheuermann *et al*., 2012). This loss in rice yield should be minimized in order to help the marginal and poor farmers of developing countries (Latif *et al*., 2011). The fungus is able to develop resistance to both chemical treatments and genetic resistance which is continuous threat to the effectiveness of blast-resistant rice varieties. Hence, it is urgent to find out strategies for developing durable resistance varieties to the disease. In this perspective, major and minor genes can contribute to producing durable resistance (Wang *et al*., 1994).

Based on the molecular marker assisted selection (MAS) approach, the identifications of R genes donors and linked molecular markers have greatly facilitated R genes transferring in rice breeding programmes to improve resistance against blast disease (Khanna *et al*., 2015; Xiao *et al*., 2015). Moreover, some broad-spectrum resistant varieties were identified to harbor multiple R genes, including Tetep (Barman *et al*., 2004), IR64 (Sallaud *et al*., 2003), Sanhuangzhan 2 (Liu *et al*. , 2004), Digu (Chen *et al*., 2004; Shang *et al*., 2009) and Gumei2 (Wu *et al*., 2005). This suggest that the combination of multiple race-specific R genes is an effective strategy to develop cultivars with broad-spectrum resistance to blast disease (Hittalmani *et al*., 2000; Tacconi *et al*., 2010; Khanna *et al*., 2015; Xiao *et al*., 2016). However, multiple R genes mean intensive pressure to promote the evolution of *M. oryzae* races, which is not desirable in a population due to a high level of genomic instability of the pathogen (Dean *et al*., 2005; Ballini *et al*., 2008). It is expected that the super races could arise in an ecological region and result in severe blast epidemics via defeating the multiple major R genes. Therefore, it is crucial to slow down the evolution rate of the pathogen towards virulence for the plant disease managements (Ballini *et al*., 2008; Miah *et al*., 2013). Some strategies i.e. multilines (Abe., 2004), mixtures (Zhu *et al*., 2000) and pyramiding (Bonman *et al*., 1992) are based on the use of complete and specific resistance genes and others are based on the accumulation of partial resistance (Bonman *et al*., 1992) for breeding blast resistance.

The development of efficient molecular marker systems and their applications

in MAS has led to the emergence of a new strategy for combating major biotic and abiotic stresses, which is called marker assisted gene pyramiding. Pyramiding entails stacking multiple genes leading to the simultaneous expression of more than one gene in a variety to develop durable resistance expression. In a gene pyramiding scheme, strategy is to cumulate into a single genotype, genes that have been identified in multiple parents. The use of DNA markers, increases the speed of pyramiding process (Joshi and Nayak, 2008). Gene pyramiding is gaining considerable importance as it would improve the efficiency of plant breeding leading to the development of genetic stocks and precise development of broad spectrum resistance capabilities. The success of gene pyramiding depends upon several critical factors, including the number of genes to be transferred, the distance between the target genes and flanking markers, the number of genotype selected in each breeding generation, the nature of germplasm, etc. With MAS based gene pyramiding, it is now possible for the breeder to conduct many rounds of selections in a year. Gene pyramiding with marker technology can integrate into existing plant breeding programmes all over the world to allow researchers to access, transfer and combine genes at a rate and with a precision not previously possible (Joshi *et al*., 2008) .

In diagnostic laboratories the use of PCR is limited by cost and sometimes the availability of adequate test sample volume. To overcome these short comings and also to increase the diagnostic capacity of PCR, a variant termed multiplex PCR has been described. Multiplex PCR genotyping method is a technique which uses two or more pairs of specific primers in a single tube to amplify multiple target genes simultaneously. It requires that primers lead to amplification of unique regions of DNA, both in individual pairs and in combinations of many primers under a single set of reaction conditions (Yap *et al*., 2016). Multiplex PCR has the potential in considerable savings of time and effort within the laboratory without compromising test utility. Since its introduction, multiplex PCR has been successfully applied in many areas of nucleic acid diagnostics, including gene deletion analysis (Chamberlain *et al*., 1988; Chamberlain *et al*., 1989), mutation and polymorphism analysis (Shuber *et al*., 1993; Rithidech *et al*., 1997), quantitative analysis (Zimmermann *et al*., 1996; Sherlock *et al*., 1998), and RNA detection (Jin *et al*., 1996; Zou *et al*., 1998).

Hu *et al*. (2012) pyramided Bph14 and Bph15 into rice Minghui63 by MAS, and the hybrids bred from the improved Minghui63 showed enhanced resistance to BPH.

Singh *et al.* (2012a) introgressed blast resistance gene *Pi54* and ShB resistance quantitative trait loci using the rice cultivar "Improved Pusa Basmati 1" (carrying the BB resistance genes *xa13* and *Xa21*) as the recurrent parent and cultivar "Tetep" (carrying the blast resistance gene *Pi54* and ShB resistance quantitative trait loci) as the donor. Marker-assisted foreground selection was employed to identify plants possessing resistance alleles in the segregating generations along with stringent phenotypic selection for faster recovery of the recurrent parent genome (RPG) and phenome (RPP). Background analysis was carried with molecular markers to estimate the recovery of RPG in improved lines. Foreground selection along with stringent phenotypic selection identified plants homozygous for the genes: *xa13, Xa21* and *Pi54*, which were advanced to BC_2F_5 through pedigree selection. Marker-assisted selection for qSBR11-1 in BC_2F_5 using flanking markers identified seven homozygous families. Background analysis revealed that RPG recovery was up to 89.5 percent. Screening with highly virulent isolates of BB, blast and ShB showed that the improved lines were resistant to all three diseases and were at par with the parent "Improved Pusa Basmati 1" for yield, duration and Basmati grain quality. This was the first report of marker assisted transfer of genes conferring resistance to three different diseases in rice wherein genes *xa13* and *Xa21* for BB resistance, *Pi54* for blast resistance, and a major QTL qSBR11-1 were combined through marker-assisted backcross breeding. In addition to offering the potential for release as cultivars, the pyramided lines have served as useful donors of gene(s) for BB, blast and ShB in future Basmati rice breeding programmes.

Divya *et al.* (2014) pyramided three blast resistance genes *Pi1***,** *Pi2* and *Pi33* to a popular susceptible rice variety ADT43 using marker assisted selection. Gene pyramiding process was facilitated by marker aided selection using molecular markers for both foreground as well as background genotyping . Previously reported linked molecular markers were used for the survey of resistant and susceptible genotypes. In the BC_3F_1 generation four lines viz, AC- B3-11-7, AC-B3-11-36, AC-B3-11-57 and AC-B3-11-83 were identified to be stacked with three genes and subjected to background analysis and a genome recovery up to 95 percent was observed and carried forward to further generations. Morphological, yield and grain quality traits were significantly similar to ADT43 parent. The introgressed lines with three gene combinations were highly resistant to the blast pathogen compared to genotypes with single genes and the susceptible checks under blast nursery screening at two hotspot

locations i,e Coimbatore and Gudalur. The selected three gene pyramided backcross lines in the desirable background were further advanced in breeding programmes to obtain an improved ADT 43 with resistance to fungal blast disease.

Khanna *et al*. (2015) intercrossed monogenic near isogenic lines Pusa 1637-18-7-6-20 and Pusa 1633-3-8-8-16-1 carrying P*i9* and *Pita*, respectively and genotyped them using marker assisted foreground, background and phenotypic selection for recurrent parent phenotype. The pyramided lines carrying *Pi9+Pita* were found to be either at par or superior to the recurrent parent Pusa Basmati 1 for all the traits like agro-morphologial , grain, and cooking quality traits. These pyramided line were also found to be resistant against three virulent pathotypes of *M. oryzae* namely, Mo-nwi-kash 1, M0-nwi-lon2 and Mo-ei-ran1.

Kumari *et al*. (2017) demonstrated the effectiveness of the strategy for rapid single step gene stacking using co-transformation approach to engineer durable resistance against rice blast disease and they were the first to report the stacking of two blast R genes using co-transformation approach. They stacked two R genes, *Pi54* and *Pi54rh* having broad spectrum resistance against multiple isolates of *M.oryzae.* and are transferred into the blast susceptible *Japonica* rice Taipei 309(TP309) using particle gun bombardment method. qRT-PCR analysis has shown *M. oryzae* induced expression of both the R genes in stacked transgenic lines. Higher level of resistance was observed in stacked transgenic lines via functional complementation analysis.

Jiang *et al.* (2019) introduced nine cloned blast resistance genes into four rice varieties through MAS tool, and developed lines harboring two blast resistance genes which were highly resistant to leaf and neck blast under natural infection conditions.

Wu *et al*. (2019) performed comprehensive evaluation of resistance effect of pyramiding lines with different broad spectrum resistance genes against *M. oryzae* in rice. They showed that the seedling blast and panicle blast resistance levels of poly gene pyramiding lines (PPLs) were significantly higher than that of monogenic lines. Set of 15 different PPLs were constructed using marker assisted selection (MAS). They evaluated systematically the resistance effects of different alleles of *Piz* locus (*Pigm, Pi40, Pi9, Pi2 and Piz*) combined with *Pi1, Pi33* and *Pi54*, respectively, and the interaction effects between different R genes. In addition they found that gene combinations, *Pigm/Pi1, Pigm/Pi54* and *Pigm/Pi33* displayed broad-spectrum resistance in artificial inoculation at seedling and heading stage, and displayed stable broad-spectrum resistance under different disease nursery situations. Besides,

agronomic traits evaluation also showed PPLs with these three gene combinations were at par to the recurrent parent.

Patroti *et al*. (2019) developed Swarna-Sub1 line having multiple blast resistant genes with superior quality traits using marker assisted backcrossing strategy coupled with phenotypic selection. They stacked three major blast resistant genes viz., *Pi1, Pi2*, and *Pi54* though marker assisted selection using donors Swarna-LT (having *Pi1* and Pi54) and Swarna-A51 (having *Pi2*). They selected the plants via foreground selection using molecular markers tightly linked to three blast resistance genes and also submergence tolerance for retaining the Sub1 gene in the recurrent parent at BC_3F_5 generation. The stringent recurrent parent genome recovery analysis using SSR markers limited the linkage drag in all the three-gene pyramided lines The two and three blast resistance genes pyramided lines, i.e., SS30-24-82, SS30-24-73, and SS30-24-46, displayed a high level of blast resistance and submergence tolerance. The successful use of marker-assisted backcrossing strategy coupled with phenotypic selection helped in the development of Swarna-Sub1 lines having multiple blast resistance genes with superior agro-morphological and grain quality traits.

Chen *et al*. (2020) developed improved photo-thermo-sensitive genetic male sterile (PTGMS) lines of C815S in which 1-3 homozygous rice blast resistance genes and two BPH genes are pyramided. To breed PTGMS rice with resistance to both rice blast and Brown planthopper (BPH), they introgressed four broad-spectrum blast resistance genes *Pi9*, *Pi47*, *Pi48*, and *Pi49* and two BPH resistance genes *BPH14* and *BPH15* into a PTGMS line C815S through backcrossing and gene pyramiding coupled with molecular marker assisted selection (MAS). These improved PTGMS lines were found resistant, the improved lines had similar major agronomic traits like C815S and some traits were even better. These newly developed PTGMS lines could be used for breeding of superior two-line hybrid rice or advanced PTGMS lines.

Orasen *et al*. (2020) pyramided 5 R resistance genes *Pib, Piz, Pik, Pita2* and *Piz-t* in temperate *Japonica* rice varieties. They produced two lines, SJKK and SJKT-2, that have four pyramided genes and were fully resistant to rice blast when tested in the field and green house.

Sagar *et al*. (2020) introgressed two genes each governing resistance to major disease of rice namely bacterial blight (BB) (*xa13* and *Xa21*) and blast (*Pi2* and *Pi54*) into a popular Basmati cultivar, Pusa Basmati 1509 (PB 1509) through marker assisted backcross breeding (MABB). Foreground selection was done for genotyping

using molecular markers, seven plants out of large BC_2F_2 population of 1832 plants were found to be homozygous for all the four genes and were subjected to background selection coupled with phenotypic selection for agronomic and grain quality traits of the recurrent parent. BC_2F_2 selections were further advanced to BC_2F_4 generation to produce NILs. Six NILs with maximum RPG recovery were selected and evaluated for resistance to BB and blast disease and all the NILs were found similar to the donor parent. So, the NILs were similar to PB 1509 for major agronomic and grain quality traits with advantage of resistance to bacterial blight and blast disease.

Materials and Methods

CHAPTER-3

MATERIALS AND METHODS

The present investigation entitled " **Molecular Marker Assisted Pyramiding of *Pi9* and *Pi54* Blast Resistance Genes in Rice Cultivar K 343 "** was undertaken at the Experimental Farm, Greenhouse and Genomics Laboratory of School of Biotechnology, Faculty of Agriculture, SKUAST-J, Chatha, Jammu from 2015 to 2019. Experimental materials used and methodology adopted in this study are elucidated under the following headings:

3.1 Research materials

3.1.1 Plant material used

3.1.2 Plant material developed

3.1.3 Primers used

a. Foreground selection primers

b. Background selection primers

3.1.4 Reagents and solutions

3.2 Methods applied

3.2.1 Methodology of work

3.2.1.1 Development of BC_2F_1, F_1 complex and F_2 convergent population

3.2.2 Laboratory protocols

3.2.2.1 Isolation of genomic DNA

3.2.2.2 Quantification and quality analysis of genomic DNA

3.2.2.3 PCR amplification

3.3 Genotyping of research material generated

3.3.1 Foreground selection for *Pi54* and *Pi9* genes in BC_2F_1 plants and F_2 convergent population

3.3.2 Background selection of *Pi54* and *Pi9* positive BC_2F_1 and F_2 gene positive pyramided plants using polymorphic SSR markers

3.4 Evaluation of BC_2F_1 plants and F_2 gene positive pyramided plants to identify superior plants

3.4.1 Evaluation of recurrent parent genome recovery in BC_2F_1 plants and F_2 gene positive pyramided plants using GGT 2.0 software

3.4.2 Phenotyping for agro-morphological traits in BC_2F_1 plants and F_2 gene positive pyramided plants

3.1 Research materials

3.1.1 Plant material used

The plant material consisted of two *Indica* rice donor genotypes namely DHMAS and RML22; and one *Indica* rice recipient cultivar namely K 343. Two BC_1F_1 genetic stocks namely K343*^{2}/DHMAS and K343*^{2}/RML22 with high recurrent parent genome (RPG) recovery (> 75%) developed at School of Biotechnology (SBT), SKUAST-J were used for carrying out the present study. The detailed description of the material used is given here under:

a. **K 343**: An *Indica* rice cultivar, developed and released by SKUAST-Kashmir for hill and temperate ecologies of J&K in 1996. It is a bold grain, long duration (130- 140 days) rice cultivar with yield potential of 50-60 q/ha. Nucleus seed of this cultivar was obtained from Mountain Rice Research Station, SKUAST-K, Khudwani, Kashmir and maintained at School of Biotechnology, SKUAST Jammu. It is a predominant rice cultivar in the hill zone of Jammu and Kashmir. It is moderately to highly susceptible to blast, brown leaf spots and false smut; and cold tolerant (Plate 3.1).

b. **DHMAS**: It is an *Indica* rice line developed through doubled haploid breeding. It is pyramided with three blast resistance genes (*Pi1*, *Pi54* and *Pita)* against the prevalent races of *Magnaporthe oryzae* using marker assisted selection. In the present study it was used as donor parent for *Pi54* gene. It is medium duration (120-125 days) genotype (Plate 3.1).

c. **RML 22**: It is an *indica* rice line developed at IRRI, Philippines. It is donor of blast resistance gene (*Pi9)* against the prevalent races of *Magnaporthe oryzae*. It is long duration (130-140 days) genotype (Plate 3.1).

d. **BC_1F_1 (K343*^{2}/DHMAS):** An *Indica* rice genetic stock developed at SBT, SKUAST-J, Chatha, Jammu and Kashmir (Hangloo, 2018). It was produced by backcrossing DHMAS with K 343 twice. It has blast resistance gene (*Pi54)*

introgressed with higher recurrent parent genome recovery (>75 percent) and is moderately to highly resistant to blast disease. (Plate . 3.1)

e. **BC_1F_1 (K343*^{2}/RML22):** An *indica* rice genetic stock developed at SBT, SKUAST-J, Chatha, Jammu (Hangloo, 2018). It was developed by backcrossing RML22 with K 343 twice. It is has blast resistance gene (*Pi9)* introgressed with higher recurrent parent genome recovery (>75 percent) and was moderately to highly resistant to blast disease.

3.1.2 Plant material developed

Plant materials developed in the present study included BC_2F_1 genetic stocks (K343*^{3}/ DHMAS) and (K343*^{3}/ RML 22) and F_2 convergent population (K343*^{3}/ DHMAS x K343*^{3}/RML22) (Plate Nos. 3.2, 3.3, 3.4, 3.5, 3.6 and 3.7) following genotyping through foreground and background selection, phenotyping and pathotyping. The detailed methodology used for development of research material is described later in this chapter.

3.1.3 Primers used

a. Foreground selection primers

Keeping in view the effectiveness of genes against prevalent races of *Magnaporthe oryzae* under North Western Himalayan region, two genes namely *Pi54* and *Pi9* were selected for developing genetic stocks in the genetic background of elite recipient variety K 343. The markers reported to be closely linked and observed to cosegregate with target genes were selected for the present study. For foreground selection of *Pi54* gene, SSR marker RM206 (0.7 cM) was selected while for *Pi9* gene SSR marker AP5930 (0.05cM) was used for foreground selection in BC_2F_1 and F_2 convergent population based on earlier studies (Sharma *et al.,* 2005a & b; Fjellstrom *et al.,* 2006; Hangloo, 2018). Details are presented in Table 3.1.

b. Background selection primers

A total of 450 genome wide SSR markers that are uniformly distributed all over the rice genome were used for screening of the parental genotypes for background screening of recurrent parent genome (Hangloo, 2018). Out of these, 50 SSR markers which had shown parental polymorphism between the parents K 343 and DHMAS were used for genotyping of the BC_2F_1 population (K 343*^{3} /DHMAS) and F_2 convergent population for evaluating recovery of recurrent parent genome. Similarly, 51 SSR markers which had shown parental polymorphism between the parents K 343 and RML 22 were used for genotyping of the BC_2F_1 population (K

343*[3] /RML 22) and F_2 convergent population for background selection. The oligos/primers for these markers were got synthesized by Integrated DNA Technologies, USA. The detail of polymorphic SSR markers used for background selection in the present study are presented in Table 3.2 and Table 3.3

K 343 **DHMAS** **RML 22**

K 343 **DHMAS** **RML22**

F_2

(K 343*^{3}/DHMAS × (K343*^{3}/RML 22)

Plate 3.1: Recipient parent, donor parents and F_2 seeds

(K 343^{*3}/DHMAS) **(K 343^{*3}/RML 22)**

Plate 3.2: Growing and intercrossing of identified plants of two backcross stocks (K 343^{*3}/DHMAS and K 343^{*3}/RML 22) to develop F_1 complex seeds

Plate 3.3: Sprouting of F_1 complex rice grains (at NRRI Cuttack, Odisha)

Plate 3.4: Planting and maintaining F_1 complex population at NRRI, Cuttack

Plate 3.5: Sowing and transplanting of F_2 convergent population (K 343*^{3}/DHMAS × K343*^{3}/RML 22) in pots and field

Plate 3.6: Field view of F_2 convergent population (K 343*^{3}/DHMAS × K343*^{3}/RML 22)

Table 3.1 Detail of markers used for foreground selection of resistance genes

Resistance Gene	Chromosomal Location	Donor Lines	Marker for Selection	Type of Marker	Marker sequence	T_m (°C)	Expected product size (bp)	Reference
Pi54	11	DHMAS-a derivative of Tetep	RM206	SSR	F 5′CCCATGCGTTTAACTATTCT 3′ R 3′ CGTTCCATCGATCCGTATGG 5′	55	147	Sharma *et al.* (2005); Hangloo (2018)
Pi9	6	RML 22	AP5930	SSR	F 5′CATGAAAGAAAGGAGTGCAG 3′ R 3′ ACAGAATTGACCAGCCAAG 5′	55	180	Fjellstrom *et al.* (2006) Hangloo (2018)

Table 3.2: Polymorphic SSR markers for parental pair K 343/DHMAS

S. No	Marker	Marker Sequence	T_m (ºC)	Expected product size (bp)
1.	RM528	F 5′GGCATCCAATTTTACCCCTC3′ R 3′AAATGGAGCATGGAGGTCAC5′	55	232
2.	RM13838	F 5′CCCAACTGCTAGGTTTCTGATCC 3′ R 3′ACTGTGTTACTGTGTGCCGTTGC5′	55	129
3.	RM262	F 5′CATTCCGTCTCGGCTCAACT 3′ R 3′CAGAGCAAGGTGGCTTGC5′	55	154
4.	RM227	F 5′ACCTTTCGTCATAAAGACGAG 3′ R 3′GATTGGAGAGAAAAGAAGCC 5′	55	106
5.	RM6832	F 5′GTTGTAAATGCCTGAGTGC 3″ R 3″ AAAGAGCTAAACCGCTAGG 5′	55	182
6.	RM15838	F 5′ CGATGTCATTCGGTAGAAACAAGC3′ R 3′ CCTAGTCAAGGCATGGTCAATCC 5′	55	262
7.	RM223	F 5′GAGTGAGCTTGGGCTGAAAC 3′ R 3′GAAGGCAAGTCTTGGCACTG 5′	55	165
8.	RM3524	F 5′CGGAGCTGGTCTAGCCATC 3′ R 3′GTCTCCGTCTTCCTCACTCG 5′	55	129
9.	RM4A	F 5′TTGACGAGGTCAGCACTGAC 3′ R 3′AGGGTGTATCCGACTCATCG 5′	55	159
10.	RM7492	F 5′AGATGGTTGCCAAGAGCATG 3′ R 3′GTCACGTGGCGATTTAGGAG 5′	55	145
11.	RM517	F 5′GGCTTACTGGCTTCGATTTG 3′ R 3′CGTCTCCTTTGGTTAGTGCC 5′	55	266
12.	RM263	F 5′CCCAGGCTAGCTCATGAACC 3′ R 3′GCTACGTTTGAGCTACCACG 5′	55	199
13.	RM580	F 5′GATGAACTCGAATTTGCATCC 3′ R 3′CACTCCCATGTTTGGCTCC 5′	55	221
14.	RM5699	F 5′ATCGTTTCGCATATGTTT 3′ R 3′ATCGGTAAAAGATGAGCC 5′	55	167
15	RM240	F 5′CCTTAATGGGTAGTGTGCAC 3′ R 3′TGTAACCATTCCTTCCATCC 5′	55	132
16	RM1370	F5′AAACGAGAACCAACCGACAC3′ R 3′GGAGGGAGGAATGGGTACAC 5′	55	173

17	RM1282	F 5′AAGCATGACAGCTGCAAGAC3′ R 3′GGGGATGAAGGGTAATTTCG5′	58	157
18	RM3874	F5′TGGGTGATCTTAGTTTGGCC3′ R 3′AATGTGCCTGCACATGTCAC 5′	55	206
19	RM232	F 5′CCGGTATCCTTCGATATTGC 3′ R 3′CCGACTTTTCCTCCTGACG 5′	55	158
20	RM28048	F5′TTCAGCCGATCCATTCAATTCC3′ R 3′GCTATTGGCCGGAAAGTAGTTAGC 5′	55	93
21	RM7300	F 5′TCCGTATCCTAGTCGCGATC3′ R 3′CGCCGTCATGACTCATACTC5′	58	102
22	RM3	F5′ACACTGTAGCGGCCACTG3′ R 3′CCTCCACTGCTCCACATCTT 5′	55	145
23	RM220	F5′GGAAGGTAACTGTTTCCAAC3′ R 3′GAAATGCTTCCCACATGTCT 5′	55	127
24	RM110	F5′TCGAAGCCATCCACCAACGAAG3′ R 3′TCCGTACGCCGACGAGGTCGAG 5′	55	156
25	RM231	F5′CCAGATTATTTCCTGAGGTC3′ R 3′CACTTGCATAGTTCTGCATTG 5′	58	182
26	RM168	F5′TGCTGCTTGCCTGCTTCCTTT3′ R 3′GAAACGAATCAATCCACGGC5′	58	116
27	RM545	F 5′CAATGGCAGAGACCCAAAAG3′ R 3′CTGGCATGTAACGACAGTGG 5′	58	226
28	RM204	F 5′GTGACTGACTTGGTCATAGGG3′ R 3′GCTAGCCATGCTCTCGTACC5′	55	169
29	RM324	F5′CTGATTCCACACACTTGTGC3′ R 3′GATTCCACGTCAGGATCTTC5′	55	175
30	RM80	F 5′TTGAAGGCGCTGAAGGAG3′ R 3′CATCAACCTCGTCTTCACCG5′	55	142
31	RM218	F5′TGGTCAAACCAAGGTCCTTC3′ R 3′GACATACATTCTACCCCCGG5′	55	148
32	RM413	F 5′GGCGATTCTTGGATGAAGAG3′ R 3′TCCCCACCAATCTTGTCTTC5′	58	79
33	RM202	F 5′CAGATTGGAGATGAAGTCCTCC3′ R 3′CCAGCAAGCATGTCAATGTA 5′	58	189
34	RM242	F5′GGCCAACGTGTGTATGTCTC3′ R 3′TATATGCCAAGACGGATGGG5′	55	225

35	RM167	F5′GATCCAGCGTGAGGAACACGT3′ R 3′AGTCCGACCACAAGGTGCGTTGTC5′	55	128
36	RM219	F5′CGTCGGATGATGTAAAGCCT3′ R 3′CATATCGGCATTCGCCTG5′	55	202
37	RM144	F 5′TGCCCTGGCGCAAATTTGATCC3′ R3′GCTAGAGGAGATCAGATGGTAGTGCATG5′	55	237
38	RM225	F 5′TGCCCATATGGTCTGGATG3′ R 3′GAAAGTGGATCAGGAAGGC5′	55	140
39	RM216	F5′GCATGGCCGATGGTAAAG3′ R 3′TGTATAAAACCACACGGCCA5′	55	146
40	RM169	F 5′TG GCTGGCTCCGTGGGTAGCTG3′ R 3′TCCCGTTGCCGTTCATCCCTCC5′	55	167
41	RM286	F 5′GGCTTCATCTTTGGCGAC3′ R 3′CCGGATTCACGAGATAAACTC5′	55	110
42	RM447	F 5′CCCTTGTGCTGTCTCCTCTC3′ R 3′ACGGGCTTCTTCTCCTTCTC5′	55	111
43	RM3295	F 5′TCGTGTCATGCGATCGAC3′ R 3′GCTTCGACTCGACCAAGATC5′	55	92
44	RM7	F 5′TTCGCCATGAAGTCTCTCG3′ R 3′CCTCCCATCATTTCGTTGTT5′	58	180
45	RM208	F 5′TCTGCAAGCCTTGTCTGATG3′ R 3′TAAGTCGATCATTGTGTGGACC5′	58	173
46	RM310	F 5′CCAAAACATTTAAAATATCATG3′ R 3′GCTTGTTGGTCATTACCATTC5′	55	105
47	RM7102	F5′TTGAGAGCGTTTTTAGGATG3′ R3′RTCGGTTTACTTGGTTACTCG5′	55	169
48	RM149	F 5′GCTGACCAACGAACCTAGGCCG3′ R 3′GTTGGAAGCCTTTCCTCGTAACACG5′	55	253
49	RM471	F 5′ACGCACAAGCAGATGATGAG3′ R3′GGGAGAAGACGAATGTTTGC5′	55	106
50	RM13840	5′CGGTCTTTAGTAATGGTGCTTTGC3′ 3′GAGGCAGGTGTTTGTCGTCTAGC5′	55	195

Table 3.3: Polymorphic SSR markers for parental pair K 343/RML22

S. No.	Marker	Marker Sequence	T_m (ºC)	Expected product size (bp)
1	RM475	F 5′CCTCACGATTTTCCTCCAAC3′ R 3′ACGGTGGGATTAGACTGTGC5′	55	235
2	RM430	F 5′AAACAACGACGTCCCTGATC3′ R 3′GTGCCTCCGTGGTTATGAAC5′	55	173
3	RM440	F 5′CATGCAACAACGTCACCTTC3′ R 3′ATGGTTGGTAGGCACCAAAG5′	55	169
4	RM334	F 5′GTTCAGTGTTCAGTGCCACC3′ R 3′GACTTTGATCTTTGGTGGACG5′	55	182
5	RM583	F 5′AGATCCATCCCTGTGGAGAG3′ R 3′GCGAACTCGCGTTGTAATC5′	55	192
6	RM162	F 5′GCCAGCAAAACCAGGGATCCGG3′ R 3′CAAGGTCTTGTGCGGCTTGCGG5′	55	229
7	RM225	F5′TGCCCATATGGTCTGGATG3′ R 3′GAAAGTGGATCAGGAAGGC5′	55	140
8	RM587	F 5′ACGCGAACAAATTAACAGCC3′ R 3′CTTTGCTACCAGTAGATCCAGC5′	55	217
9	RM11	F 5′TCTCCTCTTCCCCCGATC3′ R 3′ATAGCGGGCGAGGCTTAG5′	55	140
10	RM286	F 5′GGCTTCATCTTTGGCGAC3′ R 3′CCGGATTCACGAGATAAACTC5′	55	110
11	RM218	F5′TGGTCAAACCAAGGTCCTTC3′ R3′GACATACATTCTACCCCCGG5′	55	148
12	RM220	F5′GGAAGGTAACTGTTTCCAAC3′ R 3′GAAATGCTTCCCACATGTCT5′	55	127
13	RM408	F 5′CAACGAGCTAACTTCCGTCC3′ R 3′CAACGAGCTAACTTCCGTCC5′	55	128
14	RM234	F5′ACAGTATCCAAGGCCCTGG3′ R 3′CACGTGAGACAAAGACGGAG5′	55	156
15	RM263	F 5′CCCAGGCTAGCTCATGAACC3′ R 3′GCTACGTTTGAGCTACCACG5′	55	199
16	RM333	F 5′GTACGACTACGAGTGTCACCAA3′ R 3′GTCTTCGCGATCACTCGC5′	55	191

17	RM304	F5′TCAAACCGGCACATATAAGAC3′ R 3′GATAGGGAGCTGAAGGAGATG5′	55	160
18	RM231	F5′CCAGATTATTTCCTGAGGTC3′ R 3′CACTTGCATAGTTCTGCATTG5′	55	182
19	RM240	F 5′CCTTAATGGGTAGTGTGCAC3′ R 3′TGTAACCATTCCTTCCATCC5′	55	132
20	RM167	F5′GATCCAGCGTGAGGAACACGT3′ R3′AGTCCGACCACAAGGTGCGTTGTC5′	55	128
21	RM82	F5′TGCTTCTTGTCAATTCGCC3′ R 3′CGACTCGTGGAGGTACGG5′	55	186
22	RM274	F5′CCTCGCTTATGAGAGCTTCG3′ R 3′CTTCTCCATCACTCCCATGG5′	55	160
23	RM242	F5′GGCCAACGTGTGTATGTCTC3′ R 3′TATATGCCAAGACGGATGGG5′	55	225
24	RM324	F5′CTGATTCCACACACTTGTGC3′ R 3′GATTCCACGTCAGGATCTTC5′	55	175
25	RM5488	F 5′CTCCCTCTTCCTCTGTGTGC3′ R 3′CTCAGAGGAACAGCTGGGTC5′	55	136
26	RM1112	F 5′TCAGGACACATGGCCCTTAC3′ R 3′CAGCTCCTGACAGAGCACAC5′	55	107
27	RM556	F 5′ACTCCAAACCTCACTGCACC3′ R 3′TAGCACACTGAACAGCTGGC5′	55	93
28	RM160	F5′AGCTAGCAGCTATAGCTTAGCTGGAGATCG3′ R 3′TCTCATCGCCATGCGAGGCCTC5′	55	131
29	RM4601	F 5′CATACATGTGAACCTGACTG3′ R 3′CTAGCTTAGCATCTCCTCAA5′	55	118
30	RM262	F 5′CATTCCGTCTCGGCTCAACT3′ R 3′CAGAGCAAGGTGGCTTGC5′	55	154
31	RM6	F 5′GTCCCCTCCACCCAATTC3′ R 3′TCGTCTACTGTTGGCTGCAC5′	55	163
32	RM3732	F5′ATCCACAAACTCAGATGGGC3′ R3′TGCCACGCGATTGAAGAC5′	55	106
33	RM245	F 5′ATGCCGCCAGTGAATAGC3′ R 3′CTGAGAATCCAATTATCTGGGG5′	55	150
34	RM1370	F 5′AAACGAGAACCAACCGACAC3′ R 3′GGAGGGAGGAATGGGTACAC5′	55	173

35	RM232	F 5′CCGGTATCCTTCGATATTGC3′ R 3′CCGACTTTTCCTCCTGACG5′	55	158
36	RM3	F 5′ACACTGTAGCGGCCACTG3′ R 3′CCTCCACTGCTCCACATCTT5′	55	145
37	RM547	F 5′TAGGTTGGCAGACCTTTTCG3′ R 3′GTCAAGATCATCCTCGTAGCG5′	55	235
38	RM3874	F 5′TGGGTGATCTTAGTTTGGCC3′ R 3′AATGTGCCTGCACATGTCAC5′	55	206
39	RM70	F 5′GTGGACTTCATTTCAACTCG3′ R 3′GATGTATAAGATAGTCCC5′	55	170
40	RM16	F 5′CGCTAGGGCAGCATCTAAA3′ R 3′AACACAGCAGGTACGCGC5′	55	181
41	RM471	F 5′ACGCACAAGCAGATGATGAG3′ R 3′GGGAGAAGACGAATGTTTGC5′	58	106
42	RM480	F 5′GCTCAAGCATTCTGCAGTTG3′ R 3′GCGCTTCTGCTTATTGGAAG5′	58	225
43	RM310	F 5′CCAAAACATTTAAAATATCATG3′ R 3′GCTTGTTGGTCATTACCATTC5′	55	105
44	RM25003	F 5′GATTGATCCGAGAGACAAATCC3′ R 3′TCGATCAATAGTAGCAGCAGTAGG5′	55	115
45	RM247	F 5′TAGTGCCGATCGATGTAACG3′ R 3′CATATGGTTTTGACAAAGCG5′	55	160
46	RM171	F 5′AACGCGAGGACACGTACTTAC3′ R 3′ACGAGATACGTACGCCTTTG5′	55	328
47	RM5095	F 5′CTATATGACTATGCGAATGG3′ R 3′ACAAATGCAACTAAGGTAGA5′	55	182
48	RM201	F 5′CTCGTTTATTACCTACAGTACC3′ R 3′CTACCTCCTTTCTAGACCGATA5′	55	158
49	RM259	F 5′TGGAGTTTGAGAGGAGGG3′ R 3′CTTGTTGCATGGTGCCATGT5′	55	162
50	RM562	F 5′CACAACCCACAAACAGCAAG3′ R 3′CTTCCCCCAAAGTTTTAGCC5′	55	243
51	RM1347	F 5′AACAAATTAAACTGCCAAG 3′ R 3′GTCTTATCATCAGAACTGGA 5′	55	119

3.1.4 Reagents and solutions

A. Stock solutions for DNA extraction

1. **1M Tris HCl pH 8.0:** For this, 24.22g of 1M Trizma base having molecular weight 121.1g/mol was dissolved in the 150 ml of distilled water and pH was adjusted to 8.0 with 1N NaOH. The volume of the stock was finally made upto 200 ml with distilled water. Then solution was autoclaved and stored at room temperature.
2. **0.5 M EDTA:** To prepare this stock, 37.22g of ethyl diamine tetra acetic acid (EDTA) having molecular weight 372.24g/mol was dissolved in 150 ml of distilled water, pH was adjusted to 8.0 and volume was made upto 200ml. The 0.5 M EDTA was autoclaved and stored at room temperature.
3. **5 M NaCl:** For preparing this stock, 29.22g of NaCl (molecular weight 58.44g/mol) was dissolved in 100 ml of distilled water. The solution was autoclaved and stored at room temperature.
4. **TE Buffer pH 8.0**: The buffer was prepared with the following constituents:

   Tris-HCl pH8.0 = 2.0 ml

   EDTA pH8.0 = 0.4 ml

   They were mixed properly and the volume was made upto 200 ml by adding distilled water.
5. **DNA extraction buffer:** The extraction buffer was prepared with the following constituents:

   1 M Tris = 15.0 ml

   0.5 M EDTA = 6.0 ml

   5 M NaCl = 42.0 ml

   CTAB = 2.0 g

   They were mixed and dissolved properly and then the volume was made upto 200 ml by adding distilled water. ß-merceptoethanol 0.2% in 100ml of extraction buffer was added freshly and extraction buffer was pre-warmed before use.
6. **TBE Buffer (10X):** It was prepared with the following constituents:

Tris Base = 108.0 g

Boric acid = 55.0 g

0.5 M EDTA = 40.0 ml

They were dissolved properly and the volume was made upto 100 ml by

distilled water with final concentration 10X.

7. **Chloroform: Isoamyl alcohol (C:I) :** For preparing 100 ml of C:1 stock solution, 96ml of chloroform and 4 ml of isoamyl alcohol were taken and mixed well.
8. **RNase:** It was prepared by dissolving 10mg RNase powder in 1ml of 10mM Tris-HCl and 15mM NaCl and heated to 100°C for 15min. It was then cooled slowly to room temperature and stored at - 20°C.
9. **Working stocks of Primers:** The primers were supplied in the lyophilized form. Stocks were prepared by adding the double distilled Mili-Q water and from the stocks working concentration of 10 picomol of each primer set was prepared.

B. Stocks solutions for gel electrophoresis

1. **DNA loading dye (6X) [For 10ml]:** Following ingredients were used to prepare loading dye:

Bromophenol blue (0.25% w/v) = 0.025 g

Glycerol (40%) = 4.0 ml

They were dissolved properly and the volume was made upto 10ml by 1X TAE and stored at 4°C.

2. Electrophoresis buffer (TBE 50X) [For 100ml]

Tris base = 24.2 g

Boric acid = 5.7 ml

0.5M EDTA = 10.0 ml

They were combined and volume was made upto 100 ml, autoclaved and stored at room temperature.

3. Ethidium bromide(10mg/ml)

Ethidium bromide = 10.0 mg

Distilled water = 1.0 ml

They were dissolved properly and stored at 4°C. Proper precaution measures were taken as it is carcinogenic and hence handled carefully wearing nitrile gloves.

C. Reagents for Protein estimation

Conc. Sulphuric acid = 10 ml

Catalyst mixture(3g) = Copper sulphate and Potasium sulphate(1:5)

Sodium hydroxide(40%) = 40g NaOH dissolved in distilled water to make the final volume upto 100 ml

Boric acid(4%) = 4g boric acid dissolved in distilled water to make the final volume upto 100 ml

Indicator = Methyl red and bromocresol green

HCl (0.1N for titration)

D. Reagents for amylose test

NaoH(1N)	=	40g NaOH dissolved in distilled water to make the final volume upto 1000 ml
Iodine reagent	=	1g iodine and 10g potassium iodide dissolved in distilled water to make the final volume upto 500 ml

Standard amylose solution:

For preparation of standard amylase solution 0.2g, 0.4g, 0.6g, 0.8g and 1.0g of rice powder samples were weighed in flask. 1ml of 95% ethanol was added followed by addition of 9ml of 1N NaOH. The mixture was boiled at 85 °C for 10-15 minutes to gelatinize the starch. The mixture was cooled to room temperature for 1 hour and distilled water was added to it to make the volume to 100ml and mixed well.

Amylose standards for reference:

For this 1, 2, 3, 4 and 5ml of standard amylose was taken in flasks and one flask was taken without standard amylose as blank. 1ml of 0.1N of HCl and 1ml of iodine reagent were mixed and volume was made 50ml, even in the blank. The mixture was kept at room temperature for 20 minutes and absorbance was taken at 620nm. The standards including blanks corresponded to 0%, 4%, 8%, 12%, 16%, and 20%. The standard curve was prepared using the absorbance readings.

3.2 Methods applied:

3.2.1. Methodology of work

Marker assisted backcross breeding (MABB) method was followed for introgression of blast resistance genes from donor lines into the genetic background of rice variety K 343. In order to carry out the present study, crossing scheme as described in Figure 3.1 was followed.

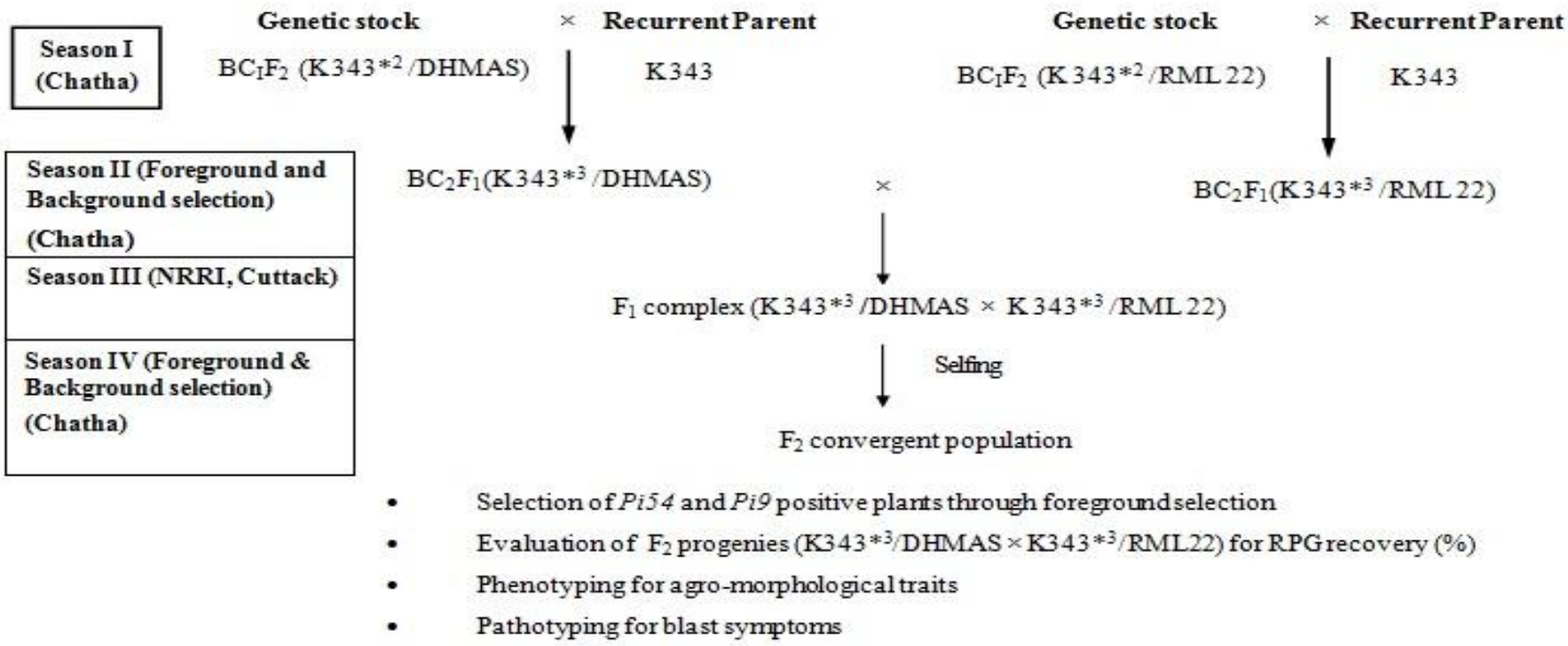


Figure 3.1: Crossing scheme for development of BC_2F_1 and F_2 convergent population

3.2.1.1 Development of BC_2F_1 and F_2 convergent populations

- **BC_2F_1 population**

Staggered sowing of recurrent parent and genetic stocks was done in season I in greenhouse (*Kharif* 2016) to achieve synchronism in flowering of male and female genotypes. For developing BC_2F_1 seeds, the recurrent parent (K 343) was used as a male parent and crossed with BC_1F_1 genetic stocks (K 343*^{2} /DHMAS) and K 343*^{2} /RML 22) which were used as female plants. For achieving this, plants in the identified BC_1F_1 genetic stocks, with higher recovery of recurrent parent genome (Hangloo, 2018) were emasculated to avoid self fertilization. Emasculation was carried out in the morning hours well before the anthesis. Panicles which had partially emerged from flag leaves and in which anthers had not dehisced were selected for emasculation. For emasculation, the spikelets from top and base of each panicle were removed and the spikelets from middle portion of the panicle were utilized. They were emasculated by first cutting the glumes of each spikelet slightly above the mid with sharp scissors and then carefully picking up the all the six anthers from each spikelet with pointed forceps. The emasculated panicles were covered with glassine bags and pollinated the next day afternoon by putting the pollen dust from the male parent. After dusting of pollens the emsculated panicles were covered with glassine bags to avoid chance cross pollination. After 7 to 10 days, seed development was noticed and seeds were harvested after 30-35 days when they had properly matured and designated as BC_2F_1 seeds. The harvested BC_2F_1 seeds were stored at room temperature during off season. They were sown in the pots during *Kharif* 2017 and 20 days old seedlings were transplanted. Leaves were collected for DNA isolation from BC_2F_1 plants and foreground selection was carried out with the help of marker RM 206 for *Pi54* gene in the stock K 343*^{3} /DHMAS and with marker AP5930 for *Pi9* gene in stock K 343*^{3} /RML 22. The plants that were confirmed positive for target genes were carried forward for background screening with polymorphic SSR markers for both the stocks (as described in Table 3.2 and 3.3).

- **F_1 complex seeds and F_1 complex population**

The background genotyping data of target gene positive plants was analysed using GGT 2.0 software and plants were identified which had higher recurrent parent genome recovery. Such plants in both the stocks were intercrossed to develop F_1 complex seeds during *Kharif* 2017 at SKUAST-J, Chatha. The mature F_1 seeds were

harvested. They were sown during off season i.e. January-May, 2018 at NRRI, Cuttack, Odisha to raise F_1 population (61 plants) and produce F_2 convergent seeds via selfing (K343*^{3}/DHMAS × K 343*^{3}/RML 22).

- **F_2 convergent population**

The F_2 convergent population (K 343*^{3}/DHMAS×K 343*^{3}/RML 22) comprising of 61 plant progeny rows (4000 plants) was raised during *Kharif* 2018. The population was screened to identify F_2 plants having the target genes *Pi54* and *Pi9* genes using respective foreground SSR markers through multiplex PCR. The F_2 plants confirmed pyramided with both the genes were subjected to background selection using identified 101 polymorphic SSRs for analysing recovery of recurrent parent genome using GGT 2.0 software (Van Berloo, 1999).

3.2.2 Laboratory protocols

3.2.2.1 Isolation of genomic DNA

Genomic DNA was isolated following Doyle and Doyle (1990) method, with slight modifications. The genotypes were grown in pots and at Experimental Farm of School of Biotechnology, SKUAST -J, Chatha for 3 weeks. About 6-8cm, young and actively growing fresh leaves were harvested for genomic DNA extraction. About 5gm of the plant tissue was taken for each genotype and grinded in liquid nitrogen by using pestle and mortar to obtain fine powder. It was followed by putting this fine powder into 2 ml eppendorf tube which contained 800µl of extraction buffer (CTAB buffer). The eppendorf tubes were incubated in a water bath at 65˚C for 60 min. and contents of the tubes were mixed by intermittently inverting them after every10 min. An equal volume of Chloroform: Isoamylalcohol (24:1) was added in the tube and slowly mixed by inverting the tubes for 5 min. The samples were then transferred to centrifuge tubes and centrifuged at 8,000 rpm for 10 min. The supernatant (upper phase) was transferred into fresh tubes and again treated with Chloroform: Isoamylalcohol (24:1), mixed slowly for 10 min and centrifugation was done at 10,000 rpm for 10 min. To precipitate the DNA, an equal volume of chilled Isopropanol was added to the supernatent and stored at 4 ^{0}C for 1-2 hrs. Centrifugation was done at 10,000 rpm for 15 min at 4^{0}C. The supernatant was discarded and the pellets were washed with 70 percent ethanol, centrifuged at 10,000 rpm for 5 min. and the pellets air dried. Then, 200µl of 1x TE buffer was added to dissolve the pellet and stored at 4˚C.

DNA purification

RNase treatment was given to sample by adding 2µl of RNase (10mg/ml) to

the samples (1ml T.E/DNA mixture) and incubated at 37 ^{0}C for 1hr. in water bath. An equal volume of Phenol: Chloroform: Isoamylalcohol (25:24:1) was added and gently mixed for 10 min and centrifuged at 12,000 rpm for 10 min. The supernatant was collected in another tube and again equal volume of C: I (Chloroform: Isoamylalcohol) (24:1) was added. The tubes containing supernatant were centrifuged for 10 min at 10,000 rpm and then an equal volume of ice chilled pure ethanol was added and it was kept in refrigerator for 10 min. It was centrifuged at 7000 rpm for 5 min for pelleting the DNA. The DNA pellet was washed with 70 percent ethanol, centrifuged at 7000 rpm for 5 min, air dried, dissolved in 100µl of 1xTE (Tris-Cl, EDTA) buffer and stored at 4^{0}C for further use.

3.2.2.2. Quantification and quality analysis of genomic DNA

a. Spectrophotometric quantification and purity estimation of genomic DNA

Precisely 10µl DNA sample was added to 490 µl TE, and mixed by inverting the tube. The absorbance was read in spectrophotometer by setting the blank against TE buffer. The absorbance was taken at 260nm and 280nm. The ratio of A_{260}/A_{280} provides an estimate of the purity of nucleic acids. Pure preparation of DNA has ratio between 1.8 and 2.0. The value less than 1.8 indicates the presence of protein contaminants and greater value than 2 indicates the presence of RNA. DNA samples were diluted using sterilized Milli Q water, to have final concentration of 50 ng/µl.

DNA concentration was calculated by using the following formula:

$$\text{Concentration of DNA (µl/ml)} = \frac{A_{260} \times 50 \times \text{Dilution factor}}{1000}$$

$$\text{Dilution factor} = \frac{\text{Total volume of sample in cuevette}}{\text{Volume of the DNA taken from stock}}$$

The DNA samples were stored at -20 °C. The formula used for dilution "D" of DNA sample is as under:

D = (Required concentration/Actual concentration) x Final volume

b. Agarose gel electrophoresis

Agarose gel electrophoresis is a standard method used to check the quality of DNA fragments. For resolving the DNA fragments on the gel, the DNA samples were loaded on to the wells of 0.8 percent agarose gel. For this, 3µl of DNA of each genotype was mixed with 2µl of loading dye (0.25% w/v bromophenol blue, 50% glycerol in sterile water). For preparation of agarose gel, 0.8 g of agarose was weighed

and put in 100 ml 1XTBE (Tris, Borate EDTA, 1x) buffer and heated in a microwave for 3 minutes for dissolving agarose. It was then cooled for few minutes, followed by addition of 6 µl of ethidium bromide for visualization of DNA bands and stirred for some time. The gel was poured into the casting tray with combs in it and allowed to polymerize at room temperature for 20-25 min. Marker DNA (ladder) of known band size was also loaded for precise quantitative estimation of DNA bands in gels. The electrophoresis was carried out at 80V for 1 hour. DNA samples were observed under photo gel documentation system (Vilber, E-Box CX5, Germany). The intensity of fluorescence of each sample was compared with that of a standard marker and then DNA concentration of each sample was ascertained. The quality of DNA samples were judged based on whether DNA formed a single high molecular weight band (good quality) or a smear (degraded/poor quality).

3.2.2.3 PCR amplication

a. Components used for PCR Reaction

DNA amplification was carried out in polymerase chain reaction (PCR) tubes containing 10 µL reaction mixture. The reaction mixture contained 1 µl of template DNA (50ng/µl), 2.5 mM/ µl of each dNTP (dTTPs, dGTPs, dCTPs, dATPs), 0.5 µl of each forward and reverse primers, 5 U of Taq polymerase (D1806- Sigma Aldrich, USA), 2.2 µL of 10X PCR buffer with $MgCl_2$, The quantity of these components used in a reaction is given in Table 3.4.

Table 3.4: Reagents with their concentration and quantity used for single PCR reaction

S.No.	Reagents	Stock concentration in PCR reaction	Quantity
1.	Template DNA	50 ng/ µl	1.0 µl
2.	dNTPs	2.5 mM / µl	0.3 µl
3.	Primer	10 pmole	1.0 µl
4.	PCR Buffer with $MgCl_2$	10 X buffer & 15mM ($MgCl_2$)	2.2 µl
5.	*Taq* polymerase	5 U	0.2µl
6.	Sterile water		5.3 µl
	Total		**10 µl**

b . PCR amplification programme

PCR tubes containing master mix and DNA template were thoroughly mixed and subjected to the PCR thermal profile (Table 3.5) in 96 well Universal Gradient Thermal Cycler (Eppendorf AG, Hamburg, Germany). An initial denaturation step (94°C) of 5 min was programmed in the thermo Cycler, followed by a loop of 35 cycles each consisting of denaturation (94°C for 30 sec), annealing (55°C – 58°C for 30 sec depending on the marker used) and extension (72°C for 30 sec). The final extension was performed at 72°C for 7 min. The PCR products were then stored at 4°C.

Table 3.5: Thermal profile used for DNA amplification

Steps	Cycles	Temperature	Duration
Initial denaturation	1	94°C	5 min
Denaturation	35	94°C	30 sec
Annealing		55°C- 58°C	30 sec
Extension		72°C	30 sec
Final Extension	1	72°C	7 min

The same reaction mixture without genomic DNA was run for each reaction to serve as a negative control.

c. SSR-PCR banding Profile

PCR products were resolved on 3 percent agarose gel. For this 3 g of agarose was added to 100ml 1xTE buffer and this mixture was heated in microwave for 2-3 min to dissolve agarose. It was allowed to cool for some time and then 5µl of ethidium bromide (EtBr) was added for visualization. The mixture was poured into casting tray containing combs and allowed to polymerize at room temperature for 20-25 min. loading dye, Bromophenol blue (5µl) was added to 10µl of PCR product in a PCR tube, mixed gently and then loaded in the well of the gel. It was repeated for all the PCR products obtained with different primer pairs. Ladder DNA (100bp) was also loaded which served as the molecular marker for determining the product size of SSR primers. Electrophoresis was carried out at 100V for 3 hrs. and then viewed under UV light (Vilber, E-Box CX5, Germany). The size of each band was determined in comparison to marker bands; and SSR band profiles obtained for each genotype (plant) were analysed in comparison to the bands of parents involved in the study.

3.2 Genotyping of research material generated

3.3.1 Foreground selection for *Pi54* and *Pi9* genes in BC_2F_1 and F_2 convergent population

The foreground selection was done for *Pi54* and *Pi9* genes separately on BC_2F_1 and plants of F_2 convergent population for both backcross generations i.e. K343*[3]/DHMAS & K 343*[3]/RML22 and K343*[3]/DHMAS × K 343*[3]/RML22, respectively using closely linked markers have been found to co segregate with target genes (Hangloo, 2018) to identify the target gene positive plants.

3.3.2 Background selection of *Pi54* and *Pi9* positive BC_2F_1 plants and F_2 gene positive pyramided plants using polymorphic SSR markers

The SSR markers that were found polymorphic between donor and recipient parents for both the sets of parents were used for screening of BC_2F_1 and F_2 plants found positive for target genes through foreground selection in both backcross generations (K 343*[3]/DHMAS and K343*[3]/RML 22) and (K343*[3]/DHMAS × K 343*[3]/RML22). It was done to assess the recovery of recurrent parent genome and to select only those plants having maximum recovery of recurrent parent genome.

3.4. Evaluation of BC_2F_1 and F_2 gene positive pyramided plants to identify superior plants

3.4.1 Evaluation of recurrent parent genome recovery in BC_2F_1 and F_2 gene positive pyramided plants using GGT 2.0 software

The SSR bands for all the plants in BC_2F_1 and F_2 convergent populations were counted and scored manually as 'A' for their resemblance with the one parent, 'B' for its resemblance with the other parent, 'H'' if both the bands were present i.e. resembled with both the parents and '-' if no band was present. The sizes of the bands were estimated by comparing them with 100bp standard marker along with the both the parents. The graphical representation of molecular marker data was done using computer programme GGT 2.0 (an acronym for Graphical GenoTypes) developed by Van Berloo (1999) at Wageningen University, The Netherlands. GGT 2.0 software is able to graphically represent chromosome wise and overall recovery of recurrent parent genome and also gives numerical representation of recurrent parent genome recovery (%) of each plant genotyped.

3.4.2 Phenotyping for agro-morphological traits in BC_2F_1 and F_2 gene positive pyramided plants

The BC_2F_1 plants and F_2 convergent population along with parents K 343,

DHMAS and RML 22 were evaluated at Experimental Research Farm and Greenhouse at School of Biotechnology, SKUAST-Jammu during *Kharif* seasons of 2017 and 2018, respectively. The 25 days old selected plants were transplanted with spacing of 15 × 20 cm in augmented-II design in the field. Observations on single plants were recorded as per the DUS guidelines of DRR, Hyderabad (Rani *et al*., 2006).

3.4.2.1 Observations recorded

Data on different traits were recorded as under:

a. Morphological traits:

- **Plant height (cm):** Plant height of the main tiller was measured from the soil surface to the tip of the panicle (excluding awns).

b. Phenological traits

- **Days to flowering:** Number of days from the date of seeding to 50 percent flowering stage were recorded.
- **Days to maturity:** Number of days from the date of seeding to the stage when plants achieved stable maturity were recorded.
- **Duration of grain filling**: Difference of days to maturity and days to 50 percent flowering was calculated.

c. Yield and its components:

- **Panicle length (cm):** Length of the panicle was measured from the base of the main rachis to the tip of the top most grain of the panicle, excluding awns, if any.
- **Number of effective tillers per plant:** It was recorded by counting number of tillers that possessed panicles per hill.
- **Grain length (mm):** The length of dehusked grains from random samples of the bulk produce of each genotype was recorded using electronic Varnier Caliper.
- **Grain breadth (mm):** The breadth of dehusked grains from random samples of the bulk produce of each entry was recorded using electronic Varnier Caliper.
- **Yield per plant (g):** Panicles harvested from each hill were threshed with hand, grains cleaned, dried and weighed on a sensitive electronic balance.
- **1000 grain weight (g):** The weight of the thousand grains was calculated in grams (g) by using electronic weighing balance

d. Quality traits

- **Amylose content (%)**

The amylose test was estimated by using Juliano method (Juliano *et al*., 1981). For this 0.1 g of rice powder was weighed in flask. 1ml of 95% ethanol was added and then 9 ml of 1N NaOH was added to it. The mixture was boiled at 85°C for 10-15 minutes to gelatinize the starch. Then the mixture was cooled at Room temperature for 1 hr and distilled water was added to it to make the volume to 100 ml and mixed well. 2.5 ml of the above extract was taken and distilled water was added to make the volume to 20 ml. 3 drops of phenolphthalein was added and mixed well. 0.1N HCl was added drop wise till the pink color disappear. 1 ml of iodine reagent was added, the volume was made upto 50 ml and kept at room temperature for 10-20 minutes , absorbance was taken at 620 nm. 1 ml of iodine in 49 ml distilled water was taken as blank. Using the absorbance the concentration (%) of the amylose was obtained from the standard curve and categorized into various categories based on the amylose content (%) as shown in Table 3.6.

Table 3.6: Various categories based on amylose content (%)

Category	Amylose content(%)
Waxy	1-2
Very low amylase	2-9
Low	10-20
Intermediate	20-30
High	30-40
Very high	>40

- **Protein content (%)**

The Protein content was determined by using Kjeldahl method (McKenzie and Wallace, 1954). For this 1.0 g finely powdered seed sample was taken in digestion tube. 10 ml of concentrated sulphuric acid and catalyst mixture (Potassium sulphate and copper sulphate) were added to the tube and sample was digested at 418°C for 20 min in digestor until the solution became clear. The tubes were then transferred to the distillation unit and in the steam chamber of distillation unit. 40% NaOH was added to the digested sample in the tube which resulted in the production of ammonia which then reacted with boric acid (present in the titration flask) to form ammonium borate.

The ammonium borate was titrated with 0.1N HCl till bluish green colour changed to pink. An estimation of the percentage of protein based on the nitrogen content was calculated using a factor of 6.25.

3.4.3. Pathotyping of BC_2F_1 and F_2 convergent populations for blast symptoms

The pathotypic screening of the BC_2F_1 plants and F_2 convergent population was done using the PLP-1isolate of *M. oryzae,* which is the predominant biotype in the North Western Himalayan region.

3.4.3.1. Maintaining fungal (PLP-1) culture

The pure culture of PLP-1 strain of *M. oryzae* was obtained from Division of Plant Pathology, SKUAST Jammu. The culture was maintained by subculturing on Oat Meal Agar media (OMA) media in petriplate and slants. The subculturing was done at monthly intervals on OMA slants and maintained at $4^{\circ}C$ for further use (Plate 3.7).

3.4.3.2. Inoculation of plants

All BC_2F_1 and F_2 convergent population along with parents were inoculated with PLP-1 using spray as standardized by Bonman *et al*. (1986) under greenhouse at School of Biotechnology. The seedlings were inoculated with conidial suspension (1×10^5 spores/ml) of *Magnaporthe oryzae* at the three to four leaf stage as described by Sharma *et al*. (2005b). The inoculated plants were then placed in dark at high relative humidity (> 90%) for 24 h, and subsequently transferred to a polyhouse, under a regime of 16 h light/8 h dark at 80 per cent relative humidity. Day and night temperatures were maintained at 35$\pm$ 2°C and 21$\pm$ 2°C, respectively.

3.4.3.3. Recording of observations for symptoms developed

Disease reactions of inoculated plants were recorded on a scale of 0–5 (Bonman *et al*., 1986), 6–7 days after inoculation. The plants exhibiting reactions that scored 0-2 were considered resistant while those showing reactions that scored 3-5 were categorized as susceptible (Table No. 3.7)

Table 3.7: Pathotyping for disease scoring (Bonman *et al*., 1986)

S.No.	Disease reaction	Score
1.	Immune/ highly resistant	0
2.	Resistant	1

Plate 3.7: *M. oryzae* (PLP-1) culture on oat meal agar media (OMA) on petriplate and slants

3.	Moderately resistant	2
4.	Susceptible	3
5.	Highly susceptible	4-5

3.5 Statistical analysis

All the data recorded for various agro-morphological parameters were subjected to the following statistical analysis:

3.5.1 Analysis of Variance (ANOVA) for augmented design-II

To test the significance of variations among different genotypes evaluated in the study, data with respect to blocks and treatments (including checks and test genotypes) were subjected to analysis of variance as per augmented design-II (Federer, 1956) to obtain adjusted trait values for checks as well as test genotypes. Single plant data was analyzed for descriptive statistical analysis with R software.To obtain the estimate of variance, following ANOVA was used (Table 3.8).

Table 3.8: Analysis of variance for single plant data in BC_2F_1 and F_2 convergent population

Source of variance	**d.f.**	**S.S.**	**M.S.**	**F ratio**
Test entries	b-1	SSB	MSB	MSB/MSE
Treatments	g-1	SSG	MSG	MSG/MSE
Tests	t-1	SST	MST	MST/MSE
Checks	c-1	SSC	MSC	MSC/MSE
Tests v/s Checks	1	SSTC	MSTC	MSTC/MSE
Error	(b-1) (g-1)	SSE	MSE	

$$SSTotal = \sum_i \sum_j X_{ij}^2 - G^2/bc$$

$$SSB = \frac{1}{c} \sum_j B_j^2 - G^2/bc$$

$$SSG = 1/b \sum_j G_i^2 - G^2/bc$$

$$SST = 1/b\sum_{j} T_i^2 - G^2/bc$$

$$SSC = \frac{1}{b}\sum_{j} C_i^2 - G^2/bc$$

$$MSE = SSE/(b-1)(c-1)$$

The subsequent analyses were performed on the adjusted means for all the genotypes towards each character, which were calculated as follows:

$$Y_i = X_{ij} - r_j$$

where,

Y_i = adjusted mean of the character for i^{th} genotypes in the j^{th} block

X_{ij} = unadjusted mean of the character for i^{th} genotype in the j^{th} block

r_j = block effect for j^{th} block, and is estimated as mentioned below:

$$r_j = \frac{1}{c}(B_j - M)$$

1.. Variances for different pair wise comparison

i. Difference between two check means = 2 MSE/b

ii. Difference between adjusted yield of two genotypes in the same blocks = 2 MSE

iii. Difference between adjusted yield of two genotypes in different blocks = 2 MSE $\left[1+\frac{1}{c}\right]$

iv. Difference between adjusted yield of genotype and check mean = MSE (b+1) (c+1)/bc.

2 Parameters of variability

To test the significance of differences among parameter wise means of single plants in the study, the data on mean values for different characters was analysed as per standard statistical procedure for augemented design II. Different biometrical measures that were used to explain dispersion of variability includes: -

i. Mean

The mean was calculated by dividing the sum of the observations with the number of observations.

Mean of trait = $\Sigma X /n$

where,

ΣX = Sum of x character

n = Number of seed sources/progenies

ii. Range

It was expressed as the difference between the lowest value and the highest value present in the observation for each trait.

iii. Variance

Expressed as the average of squared deviation of all the individual observation from the mean. Mathematically,

$$\text{Variance (var.) or } \sigma^2 = \frac{\sum (x - \bar{x})^2}{N - 1}$$

iv. Standard deviation (σ)

Expressed in terms of square root of variance.

$$\text{SD} = \sqrt{Var} = \sqrt{\sigma^2} = \sigma$$

v. Standard error (SE)

Expressed as the mean difference between sample estimates of mean and the population parameter μ i.e. it is the measure of uncontrolled variation present in a sample. The Standard error of a variable mean was calculated by dividing the estimate of Standard deviation by the root of the number of the observations in the sample. Mathematically

$$\text{Standard error} = \frac{\text{Standard deviation}}{\sqrt{\text{N}}}$$

where,

N = Total number of observations

vi. Critical Difference (CD)

Critical difference was calculated with the help of SE for testing the difference of two means.

CD = SE(d) × 't' tab. at error d.f.

where,

SE (d) = Standard error (difference of two means).

vii. Coefficient of variation (CV)

CV (%) = Standard deviation /Mean x100

Chapter-4

Results

CHAPTER-4

RESULTS

The present study entitled **"Molecular marker assisted pyramiding of *Pi9* and *Pi54* blast resistance genes in rice cultivar K 343"** was conducted to pyramid blast resistance genes *Pi54* and *Pi9* in the genetic background of susceptible temperate rice variety K 343, to validate the introgressed genes in the target background using linked SSR markers and to evaluate the pyramided plants for different traits for identification of superior lines.

The experimental results of the present study are presented under following headings:

4.1 Isolation, quantification and quality analysis of genomic DNA

4.2 Foreground selection for *Pi54* and *Pi9* genes in BC_2F_1 plants using linked markers

4.3 Background selection of *Pi54* and *Pi9* positive BC_2F_1 plants using polymorphic SSR markers

4.4 Evaluation of genetic stocks to identify superior plants

- 4.4.1 Evaluation of recurrent parent genome recovery in gene positive BC_2F_1 plants using GGT 2.0 software
- 4.4.2 Phenotyping for agro-morphological traits in gene positive BC_2F_1 plants
- 4.4.3 Pathotyping of gene positive BC_2F_1 plants for blast symptoms
- 4.4.4 Agronomical and pathological status of maximum recurrent parent genome recovered genetic stocks (K 343*^{3}/DHMAS and K 343*^{3}/RML 22)

4.5 Foreground selection for *Pi54* and *Pi9* genes in F_2 convergent population using linked markers

4.6 Background selection of *Pi54* and *Pi9* gene pyramided plants in F_2 convergent population using polymorphic SSR markers

4.7 Evaluation of pyramided plants of F_2 convergent population to identify superior plants.

- 4.7.1 Evaluation of recurrent parent genome recovery in pyramided plants of F_2 convergent population using GGT 2.0 software
- 4.7.2 Phenotyping for agro-morphological traits in F_2 pyramided plants

4.7.3 Pathotyping of F_2 pyramided plants for blast symptoms

4.7.4 Agronomical and pathological status of maximum recurrent parent genome recovery in F_2 pyramided plants

4.1 Isolation, quantification and quality analysis of genomic DNA

The isolated DNA loaded on 0.8 percent agarose gel, showed clear and discrete bands (Plate 4.1, Plate 4.2 and Plate 4.3), when observed under gel documentation system indicating a good quality DNA which could be used for genotyping purpose. The concentration of gDNA varied from 100ng/µl to 200 ng/µl and Absorbance ($A_{260/280}$) ratio ranged from 1.7 to 2.0 (Table 4.1, Table 4.2 and Table 4.3) which further indicated the purity of DNA. The intensity of florescence and thickness of bands as observed under UV light indicated sufficient concentration of genomic DNA in all the samples which further could be diluted accordingly to carry out genotyping.

Table 4.1: Concentration of genomic DNA and absorbance ratio scores of parents, BC_2F_1 plants involving DHMAS as donor parent

S.No.	Genotypes	Concentration of DNA (ng/ µl)	Absorbance ratio (A260/280)
1.	K 343	156.23	1.78
2.	DHMAS	145.01	1.84
3.	P1 =BC_2F_1(K 343*^{3}/DHMAS)	125.47	1.77
4.	P2 = BC_2F_1(K 343*^{3}/DHMAS)	116.54	1.71
5.	P3=BC_2F_1(K 343*^{3}/DHMAS)	145.70	1.96
6.	P4=BC_2F_1(K 343*^{3}/DHMAS)	119.01	1.70
7.	P5=BC_2F_1(K 343*^{3}/DHMAS)	118.34	1.73
8.	P6=BC_2F_1(K 343*^{3}/DHMAS)	116.54	1.71
9.	P7=BC_2F_1(K 343*^{3}/DHMAS)	145.70	1.96
10.	P8 =BC_2F_1(K 343*^{3}/DHMAS)	119.01	1.70
11.	P9=BC_2F_1(K 343 *^{3}/DHMAS)	118.34	1.73
12.	P10=BC_2F_1(K 343*^{3}/DHMAS)	135.71	1.86
13.	P11=BC_2F_1(K 343*^{3}/DHMAS)	145.98	1.74
14.	P12=BC_2F_1(K 343*^{3}/DHMAS)	130.99	1.70
15.	P13=BC_2F_1(K 343*^{3}/DHMAS)	185.12	1.86
16.	P14=BC_2F_1(K 343*^{3}/DHMAS)	125.55	1.76
17.	P15=BC_2F_1(K 343*^{3}/DHMAS)	134.66	1.79
18.	P16=BC_2F_1(K 343*^{3}/DHMAS)	125.55	1.76

19.	P17=BC_2F_1(K 343*^{3}/DHMAS)	134.66	1.79
20.	P18=BC_2F_1(K 343*^{3}/DHMAS)	115.03	1.71
21.	P19=BC_2F_1(K 343*^{3}/DHMAS)	112.01	1.72
22.	P20=BC_2F_1(K 343*^{3}/DHMAS)	120.78	1.77
23.	P21=BC_2F_1(K 343*^{3}/DHMAS)	130.62	1.82
24.	P22 =BC_2F_1(K 343*^{3}/DHMAS)	138.15	1.83
25.	P23=BC_2F_1(K 343*^{3}/DHMAS)	156.23	1.78
26.	P24=BC_2F_1(K 343*^{3}/DHMAS)	145.01	1.84
27.	P25=BC_2F_1(K 343*^{3}/DHMAS)	125.47	1.77
28.	P26=BC_2F_1(K 343*^{3}/DHMAS)	124.61	1.87
29.	P27=BC_2F_1(K 343*^{3}/DHMAS)	130.62	1.82
30.	P28=BC_2F_1(K 343*^{3}/DHMAS)	138.15	1.83
31	P29=BC_2F_1(K 343*^{3}/DHMAS)	156.31	1.92
32	P30=BC_2F_1(K 343*^{3}/DHMAS)	142.55	1.83
33	P31=BC_2F_1(K 343*^{3}/DHMAS)	124.00	1.70
34	P32=BC_2F_1(K 343*^{3}/DHMAS)	138.54	1.82
35	P33=BC_2F_1(K 343*^{3}/DHMAS)	112.34	1.77
36	P34=BC_2F_1(K 343*^{3}/DHMAS)	124.61	1.87
37	P35=BC_2F_1(K 343*^{3}/DHMAS)	154.23	1.81
38	P36=BC_2F_1(K 343*^{3}/DHMAS)	175.96	1.85
39	P37=BC_2F_1(K 343*^{3}/DHMAS)	145.98	1.74
40	P38=BC_2F_1(K 343*^{3}/DHMAS)	130.99	1.70
41	P39=BC_2F_1(K 343*^{3}/DHMAS)	142.55	1.83
42	P40=BC_2F_1(K 343*^{3}/DHMAS)	170.67	1.97
43	P41=BC_2F_1(K 343*^{3}/DHMAS)	156.23	1.78
44	P42=BC_2F_1(K 343*^{3}/DHMAS)	145.01	1.84

Table 4.2: Concentration of genomic DNA and absorbance ratio scores of parents, BC_2F_1 plants involving RML 22 as donor parent

S.No.	Genotypes	Concentration of DNA (ng/ µl)	Absorbance ratio (A260/280)
1.	K 343	157.01	1.77
2.	RML 22	124.47	1.97
3.	P1=BC_2F_1(K 343*^{3}/RML 22)	112.34	1.77
4.	P2=BC_2F_1(K 343*^{3}/RML 22)	186.71	1.96
5.	P3=BC_2F_1(K 343*^{3}/RML 22)	134.66	1.79
6.	P4=BC_2F_1(K 343*^{3}/RML 22)	115.03	1.71
7.	P5=BC_2F_1(K 343*^{3}/RML 22)	112.01	1.72

8.	P6=BC_2F_1(K 343*[3]/RML 22)	120.78	1.77
9.	P7=BC_2F_1(K 343*[3]/RML 22)	130.62	1.82
10.	P8 = BC_2F_1(K 343*[3]/RML 22)	157.01	1.77
11.	P9=BC_2F_1(K 343*[3]/RML 22)	124.47	1.97
12.	P10=BC_2F_1(K 343*[3]/RML 22)	178.54	1.75
13.	P11=BC_2F_1(K 343*[3]/RML 22)	130.01	1.80
14.	P12=BC_2F_1(K 343*[3]/RML 22)	112.34	1.77
15.	P13=BC_2F_1(K 343*[3]/RML 22)	186.71	1.96
16.	P14=BC_2F_1(K 343*[3]/RML 22)	175.92	1.80
17.	P15=BC_2F_1(K 343*[3]/RML 22)	118.23	1.75
18.	P16=BC_2F_1(K 343*[3]/RML 22)	165.96	1.85
19.	P17=BC_2F_1(K 343*[3]/RML 22)	169.98	1.74
20.	P18=BC_2F_1(K 343*[3]/RML 22)	145.99	1.75
21.	P19=BC_2F_1(K 343*[3]/RML 22)	157.12	1.86
22.	P20=BC_2F_1(K 343*[3]/RML 22)	135.55	2.00
23.	P21=BC_2F_1(K 343*[3]/RML 22)	178.54	1.75
24.	P22=BC_2F_1(K 343*[3]/RML 22)	145.70	1.96
25.	P23=BC_2F_1(K 343*[3]/RML 22)	130.01	1.80
26.	P24=BC_2F_1(K 343*[3]/RML 22)	112.34	1.77
27.	P25=BC_2F_1(K 343*[3]/RML 22)	186.71	1.96
28.	P26=BC_2F_1(K 343*[3]/RML 22)	118.23	1.75
29.	P27=BC_2F_1(K 343*[3]/RML 22)	157.01	1.77
30.	P28=BC_2F_1(K 343*[3]/RML 22)	124.47	1.97
31	P29=BC_2F_1(K 343*[3]/RML 22)	145.99	1.75
32	P30=BC_2F_1(K 343*[3]/RML 22)	157.12	1.86
33	P31=BC_2F_1(K 343*[3]/RML 22)	135.55	2.00
34	P32=BC_2F_1(K 343*[3]/RML 22)	185.66	1.99
35	P33=BC_2F_1(K 343*[3]/RML 22)	140.03	1.72
36	P34=BC_2F_1(K 343*[3]/RML 22)	185.66	1.99
37	P35=BC_2F_1(K 343*[3]/RML 22)	140.03	1.72
38	P36=BC_2F_1(K 343*[3]/RML 22)	130.01	1.80
39	P37=BC_2F_1(K 343*[3]/RML 22)	101.62	1.92
40	P38=BC_2F_1(K 343*[3]/RML 22)	131.61	1.77
41	P39=BC_2F_1(K 343*[3]/RML 22)	190.29	2.00
42	P40=BC_2F_1(K 343*[3]/RML 22)	186.71	1.96
43	P41=BC_2F_1(K 343*[3]/RML 22)	186.71	1.96
44	P42=BC_2F_1(K 343*[3]/RML 22)	175.92	1.80

Table 4.3: Concentration of genomic DNA and Absorbance ratio scores of parents, F_2 pyramided plants

S.No.	Genotypes	Concentration of DNA (ng/ µl)	Absorbance ratio (A260/280)
1.	K 343	124.00	1.70
2.	DHMAS	115.03	1.71
3.	RML 22	138.54	1.82
4.	P1 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	105.65	1.77
5.	P2 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	121.61	1.74
6.	P3 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	138.29	1.85
7.	P4 =F_2(K 343^{*3}/DHMAS ×K 343^{*3}/RML 22)	186.21	1.96
8.	P5 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	175.96	1.85
9.	P6 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	145.98	1.74
10.	P7 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	130.99	1.70
11.	P8 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	185.12	1.86
12.	P9 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	125.55	1.76
13.	P10 =F_2(K 343^{*3}/DHMAS ×K 343^{*3}/RML 22)	134.66	1.79
14.	P11 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	115.03	1.71
15.	P12 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	112.01	1.72
16.	P13 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	120.78	1.77
17.	P14=F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	105.65	1.77
18.	P15=F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	121.61	1.74
19.	P16 =F_2(K 343^{*3}/DHMAS ×K 343^{*3}/RML 22)	138.29	1.85
20.	P17 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	186.21	1.96
21.	P18 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	175.96	1.85
22.	P19 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	145.98	1.74
23.	P20 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	130.99	1.70
24.	P21 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	185.12	1.86
25.	P22 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	125.55	1.76
26.	P23=F_2(K 343^{*3}/DHMAS ×K 343^{*3}/RML 22)	134.66	1.79
27.	P24=F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	115.03	1.71
28.	P25 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	112.01	1.72
29.	P26 =F_2(K 343^{*3}/DHMAS ×K 343^{*3}/RML 22)	186.21	1.96
30.	P27 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	120.78	1.77

31	P28 =F_2(K 343^{*3}/DHMAS×K 343^{*3}/RML 22)	175.96	1.85
32	P29=F_2(K 343^{*3}/DHMAS×K 343^{*3}/RML 22)	130.99	1.70
33	P30 =F_2(K 343^{*3}/DHMAS ×K 343^{*3}/RML 22)	105.65	1.77
34	P31 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	121.61	1.74
35	P32 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	138.29	1.85
36	P33 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	186.21	1.96
37	P34 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	175.96	1.85
38	P35 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	145.98	1.74
39	P36 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	130.99	1.70
40	P37 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	185.12	1.86
41	P38=F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	125.55	1.76
42	P39 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	134.66	1.79
43	P40 =F_2(K 343^{*3}/DHMAS×K 343^{*3}/RML 22)	115.03	1.71
44	P41 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	112.01	1.72
45	P42 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	186.21	1.96
46	P43 =F_2(K 343^{*3}/DHMAS ×K 343^{*3}/RML 22)	120.78	1.77
47	P44 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	130.99	1.70
48	P45 =F_2(K 343^{*3}/DHMAS × K 343^{*3}/RML 22)	130.99	1.70

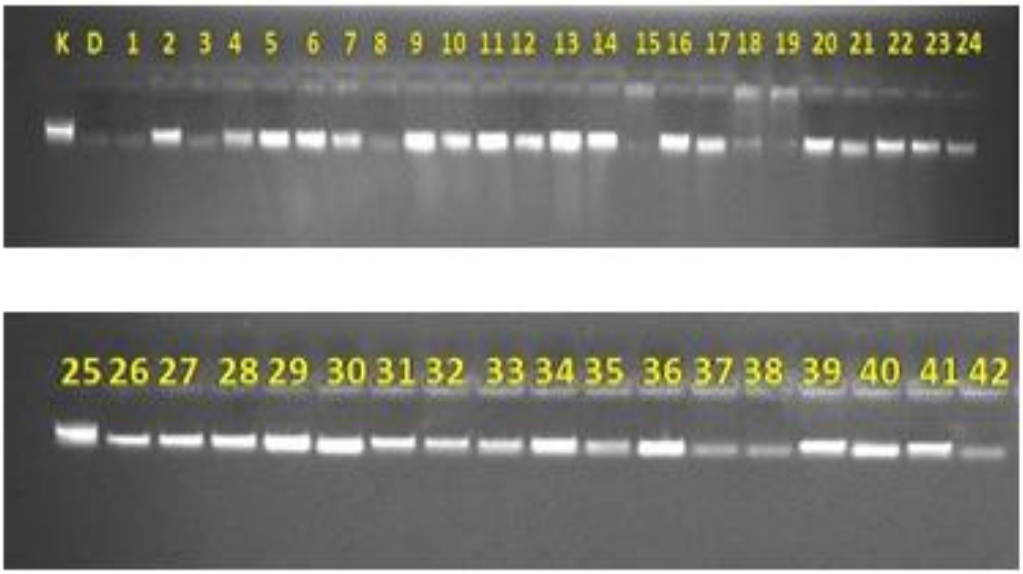


Plate 4.1: Quality of DNA of parents, BC_2F_1 plants as observed on 0.8 percent agarose gel (K=K343, D=DHMAS, 1-42=BC_2F_1 (K343*3/DHMAS) plants

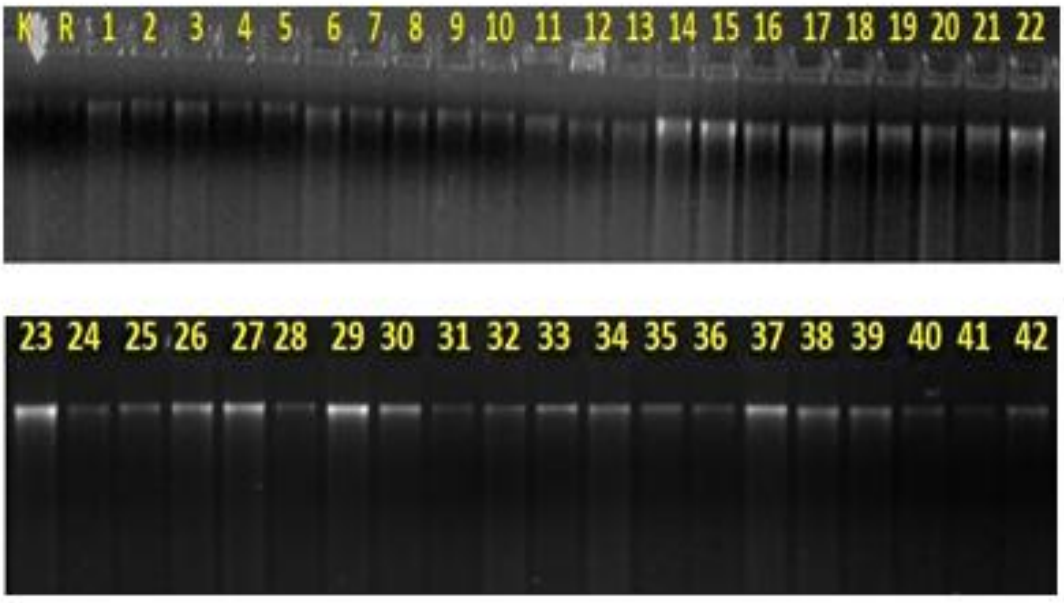


Plate 4.2: Quality of DNA of parents, BC_2F_1 plants as observed on 0.8 percent agarose gel (K=K343, R= RML 22, 1-42 = BC_2F_1 (K 343*3/RML 22) plants

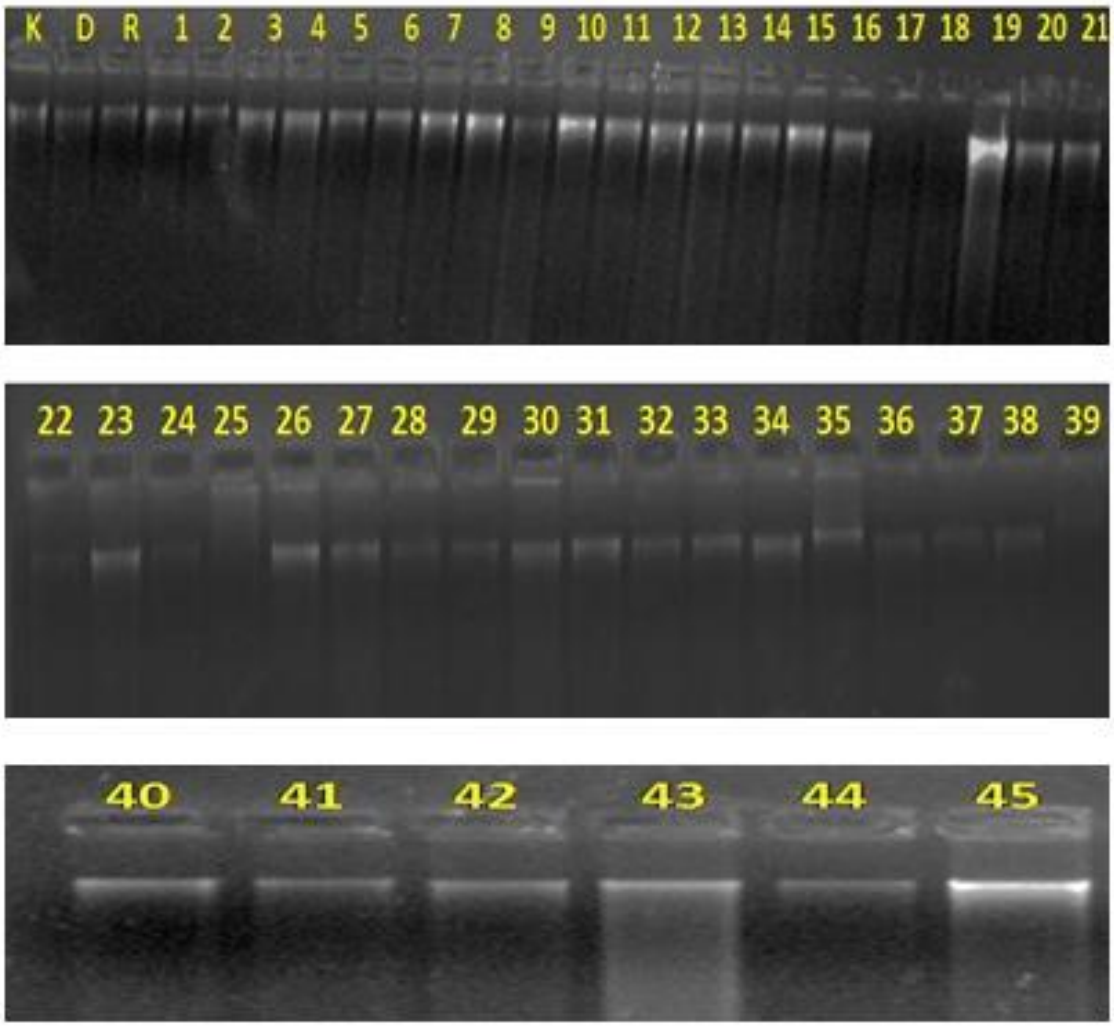


Plate 4.3: Quality of DNA of parents, F_2 plants as observed on 0.8 percent agarose gel (K= K 343, D= DHMAS and R = RML 22, 1-45 = F_2 pyramided plants (K 343*3 /DHMAS x K 343*3/RML 22)

4.2 Foreground selection for *Pi54 and Pi9* genes in BC_2F_1 generation using linked markers (Table 4.4)

4.2.1 Foreground selection for *Pi54* gene in BC_2F_1 population

A total of 42 BC_2F_1 plants were grown and screened for the presence of *Pi54* gene by using closely linked marker RM206 (0.7cM away from *Pi54* locus). Out of the 42 BC_2F_1 plants (K 343*^{3}/DHMAS), 30 plants were found positive for *Pi54* gene with K 343 having amplicon size of 170bp and DHMAS having amplicon size of 150 bp with primer RM 206 (Plate 4.4).

4.2.2 Foreground selection for *Pi9* gene in BC_2F_1 population

A total of 42 BC_2F_1 plants were grown and screened for the presence of *Pi9* gene by using closely linked marker AP5930 (0.05 cM away from *Pi9* locus). Out of the 42 BC_2F_1 plants (K 343*^{3} /RML 22), 30 plants were found positive for *Pi9* gene with K 343 having amplicon size of 160bp and RML 22 having amplicon size of 180 bp with primer AP 5930, (Plate 4.5).

4.3 Background selection of *Pi54* and *Pi9* positive BC_2F_1 plants using polymorphic SSR markers

4.3.1 Background selection of *Pi54* positive BC_2F_1 plants

The background selection for analyzing recovery of recurrent parent genome was done on 30 BC_2F_1 (K 343*^{3}/DHMAS) positive plants for the target resistance gene (*Pi54*). They were screened with 50 polymorphic SSR markers for selecting those positive plants possessing maximum recovery of recurrent parent genome (Plates 4.6 – 4.10).

4.3.2 Background selection of *Pi9* positive BC_2F_1 plants

The background selection for analyzing recovery of recurrent parent genome was done on 30 BC_2F_1 (K 343*^{3}/RML 22) positive plants for the target resistance gene (*Pi9*). They were screened with 51 polymorphic SSR markers for selecting those positive plants possessing maximum recovery of recurrent parent genome (Plate 4.11 – 4.16).

Table 4.4: Selection of plants followed by marker assisted foreground selection for genes *Pi54* and *Pi9*

Generation	Donor gene	No. of plants analyzed	No. of plants positive for target gene (s)
BC_2F_1	*Pi54*	42	30
BC_2F_1	*Pi9*	42	30
F_2 convergent population	*Pi54* & *Pi9*	4000	45

4.4 Evaluation of genetic stocks to identify superior plants

4.4.1. Evaluation of recurrent parent genome recovery in gene (*Pi54*) positive BC_2F_1 plants (K 343*^{3}/DHMAS)

The maximum recovery of recurrent parent genome was calculated using software Graphical GenoTypes (GGT 2.0) (Van Berloo, 1999). A graphical representation of all the individual plants for all the chromosomes of the selected genetic stocks for blast resistance is shown in Figure 4.1. In BC_2F_1 population subjected to background analysis the recovery of recurrent parent genome varied from 29.75 percent (P20) to 86.4 percent (P1).The maximum recovery of recurrent parent genome was observed in plant P1 (86.4%), followed by P17 (83.65%), P3 (83.40%) and P25 (79.15%) (Figure 4.1 and Table 4.5). In the Figure 4.1 the red coloured regions represent the genomic regions of the recipient parent and the maximum recovery of recurrent parent genome was observed for chromosome number 1 and 2 while blue coloured regions represent genome of donor parent. Most of the residual segments from donor genome were distributed on chromosomes 3, 4, 5, 6, 8, 9, 10, 11 and 12, while the light green coloured regions indicate heterozygous genome.

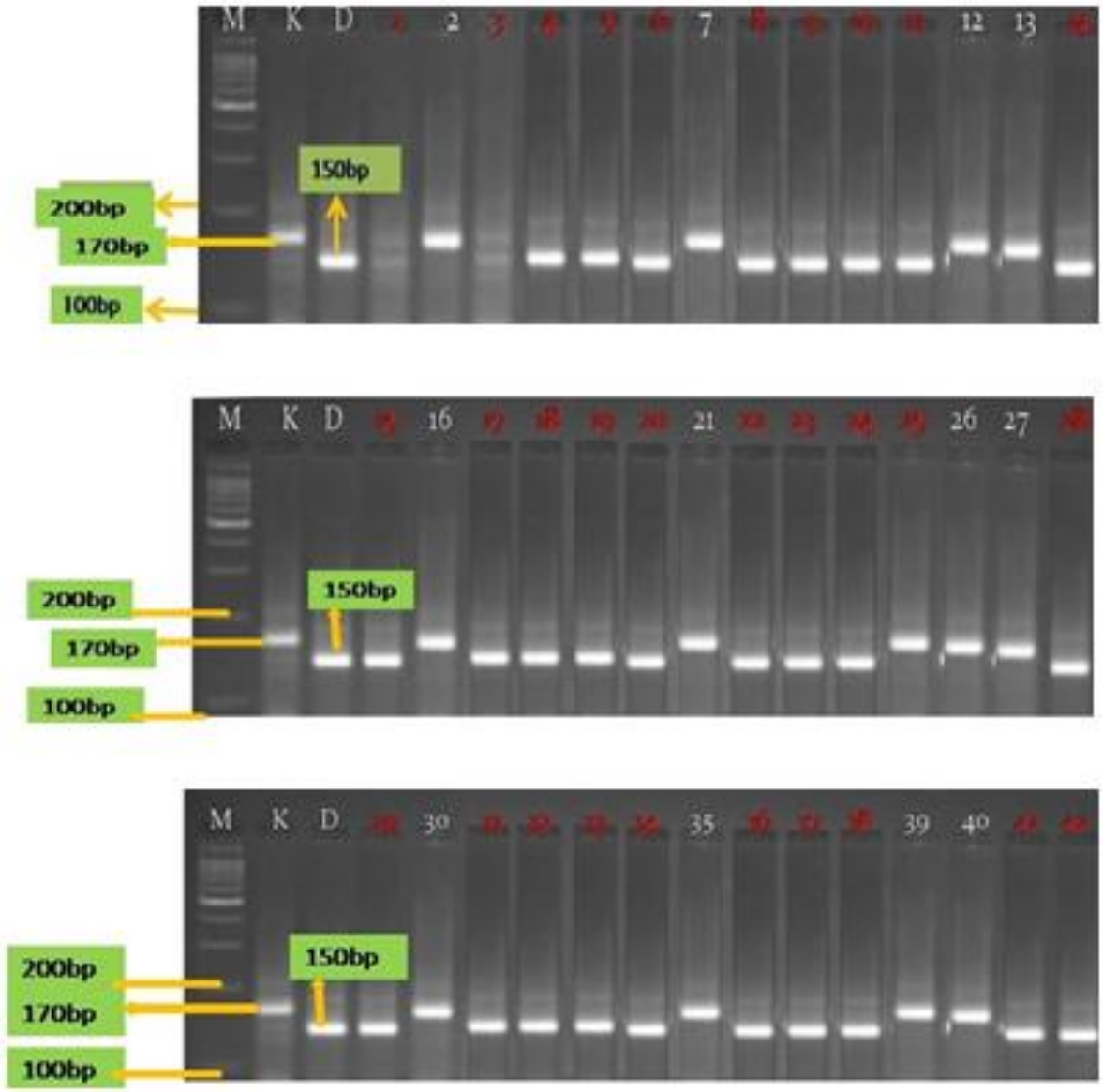


Plate 4.4: Foreground selection of *Pi54* gene in BC_2F_1 generation using RM206 marker; (K = K 343; D =DHMAS; 1-42=BC_2F_1 plants; RED colour shows *Pi54* positive plants)

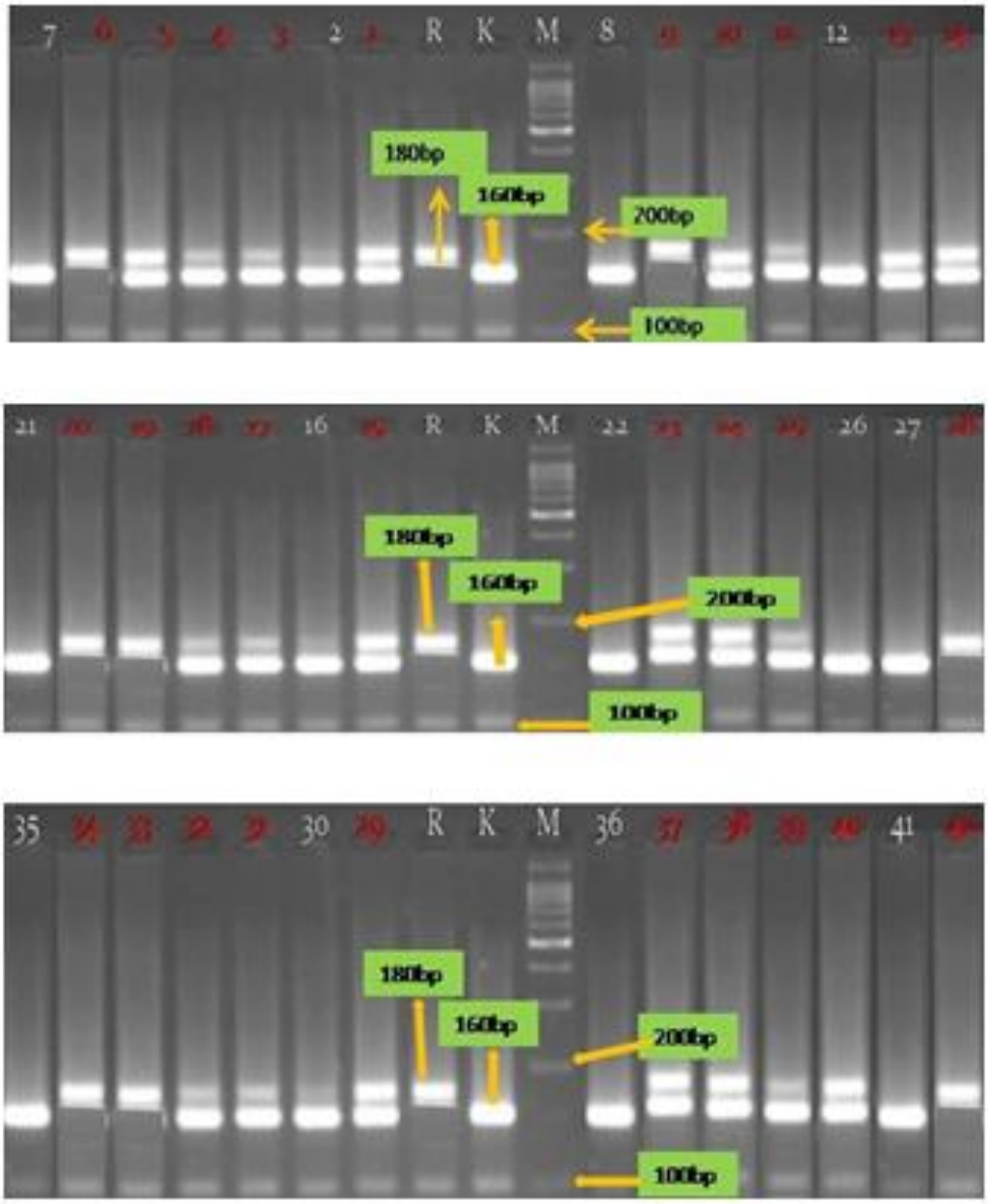


Plate 4.5: Foreground selection of *Pi9* gene in BC_2F_1 generation using AP5930 marker (K = K 343; R =RML 22; 1-42= BC_2F_1 plants; RED colour shows *Pi9* plants)

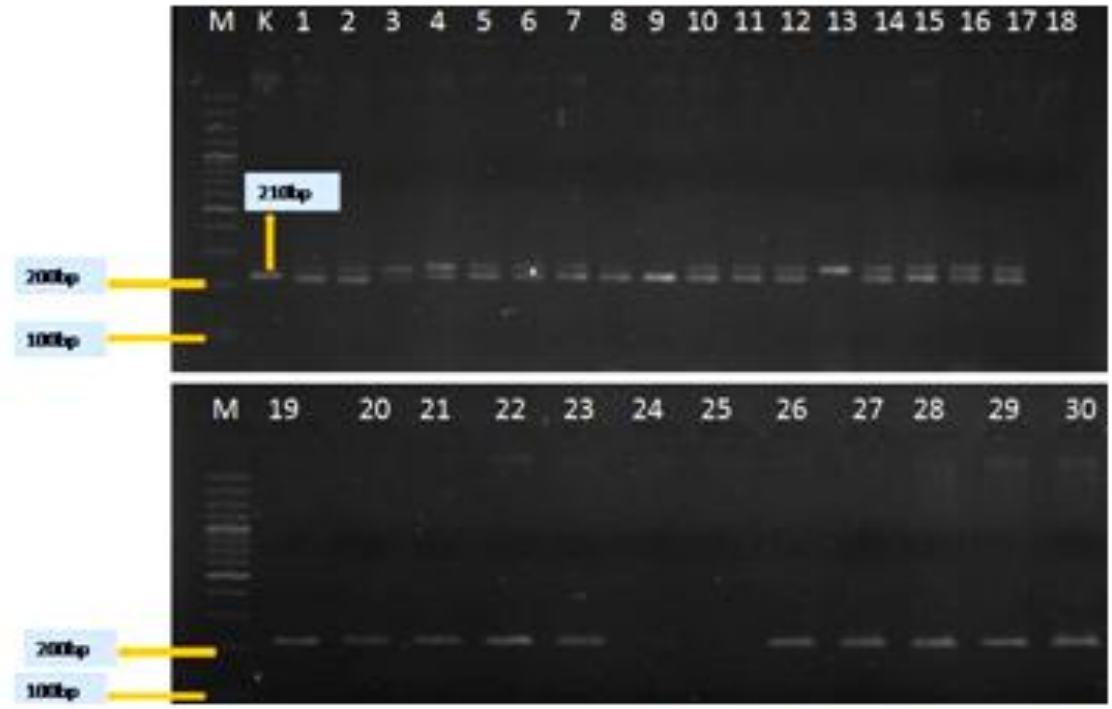


Plate 4.6: Band amplification pattern of SSR marker RM162 (K= K 343; 1 to 30 = BC_2F_1 plants (K343*^{3}/DHMAS)

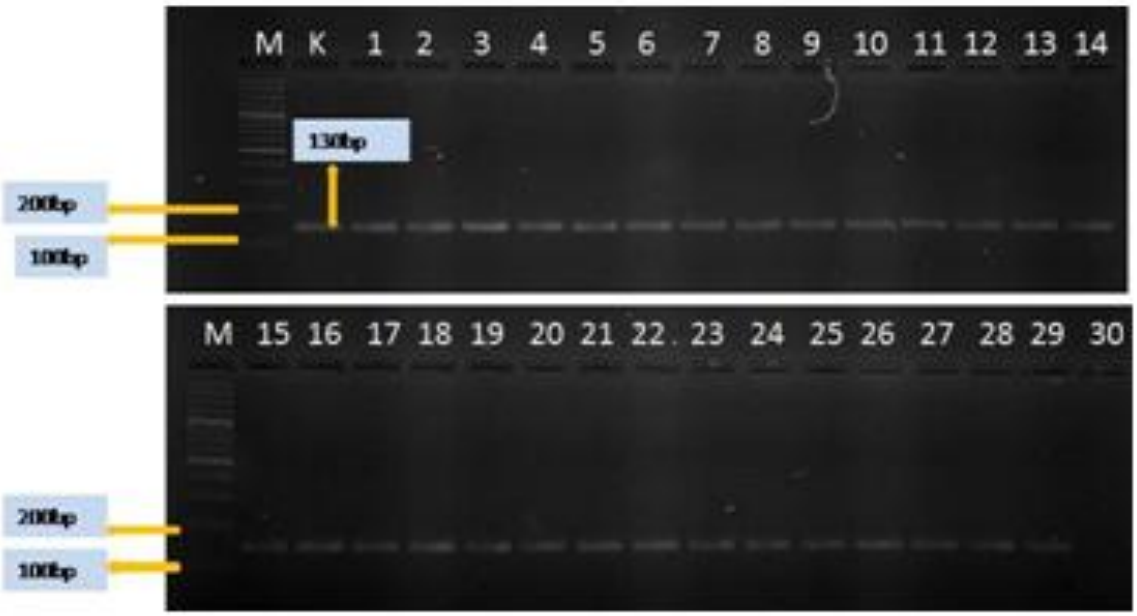


Plate 4.7: Band amplification pattern of SSR marker RM110 (K= K 343; 1 to 30 = BC_2F_1 plants (K343*^{3}/DHMAS)

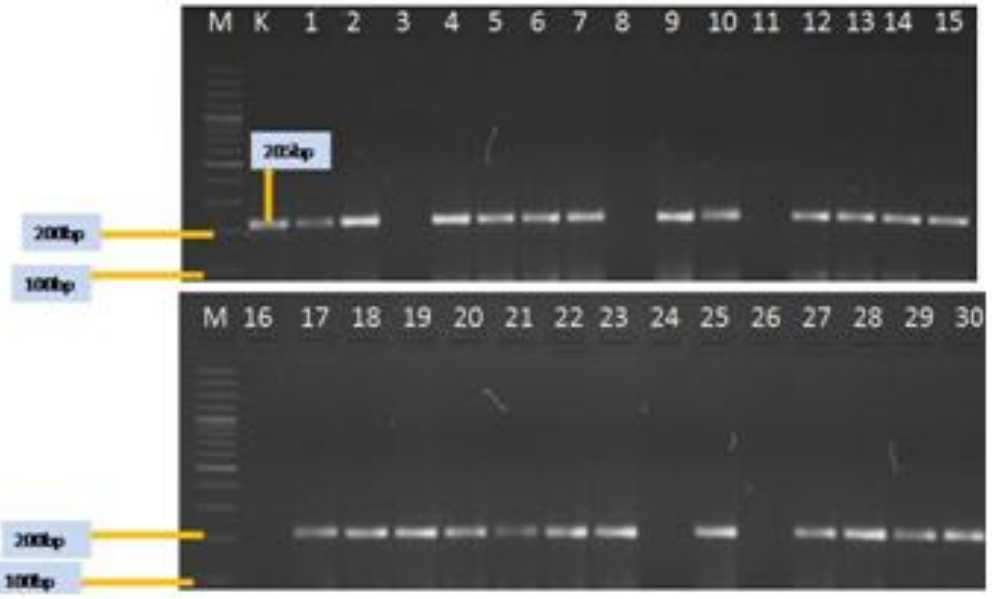


Plate 4.8: Band amplification pattern of SSR marker RM144 (K= K 343; 1 to 30 = BC_2F_1 plants (K343*3/DHMAS)

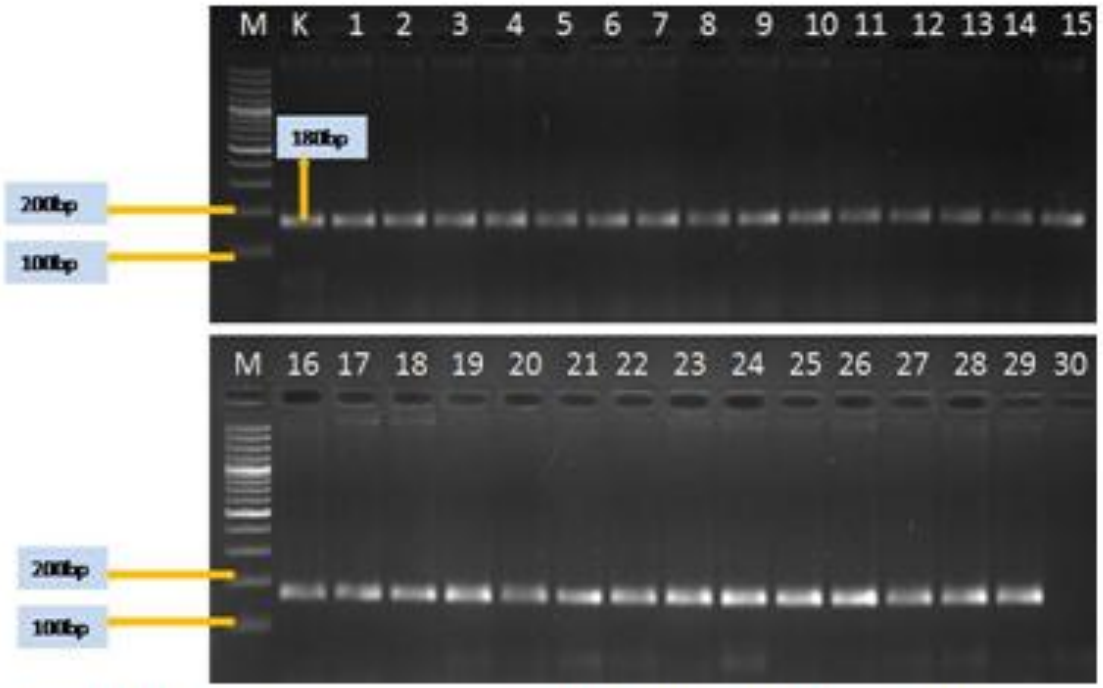


Plate 4.9: Band amplification pattern of SSR marker RM274 (K= K 343; 1 to 30 = BC_2F_1 plant (K343*3/DHMAS)

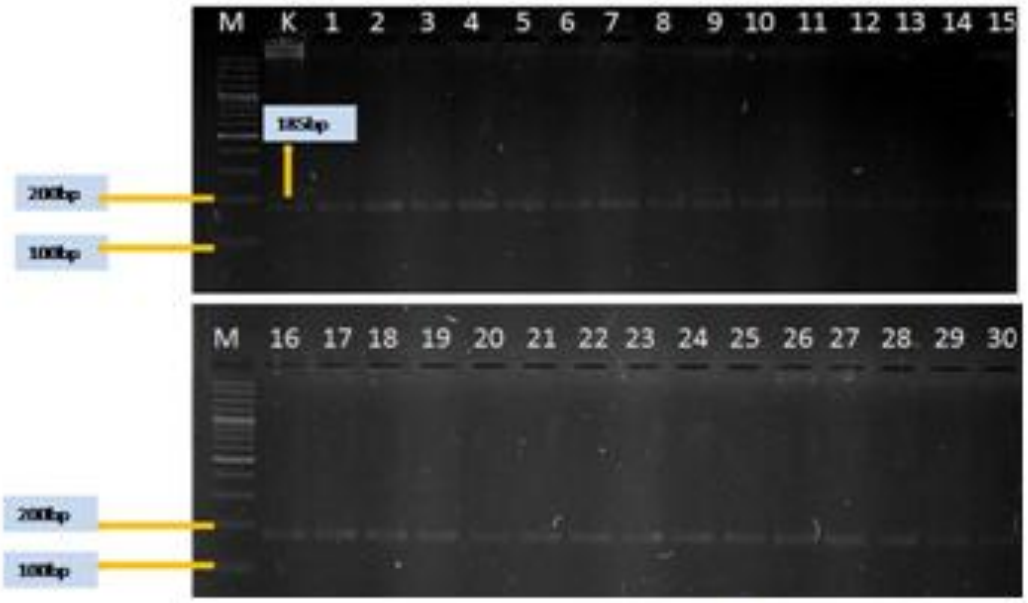


Plate 4.10: Band amplification pattern of SSR marker RM304 (K= K 343; 1 to 30 = BC_2F_1 plants (K343*^{3}/DHMAS)

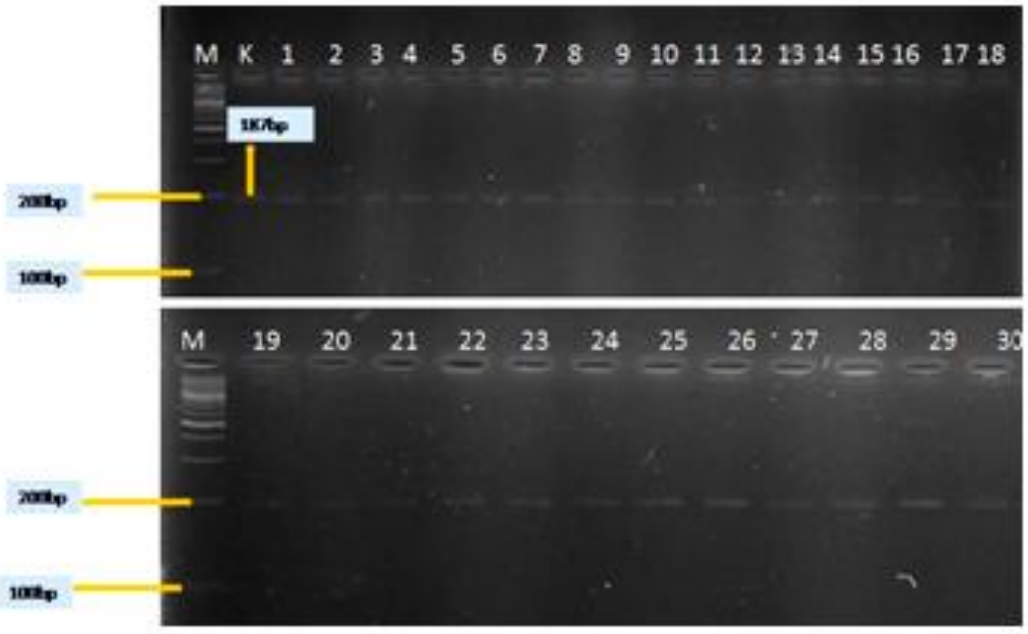


Plate 4.11: Band amplification pattern of SSR marker RM334 (K= K 343; 1 to 30 = BC_2F_1 plants (K343*^{3}/DHMAS)

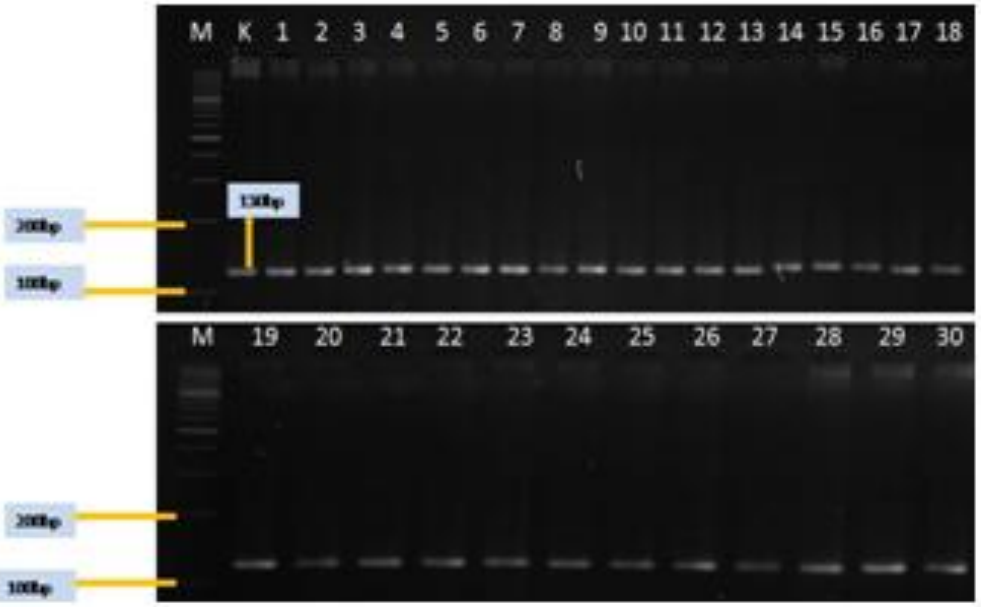


Plate 4.12: Band amplification pattern of SSR marker RM408 (K= K 343; 1 to 30 = BC_2F_1 plants (K343*²/RML 22)

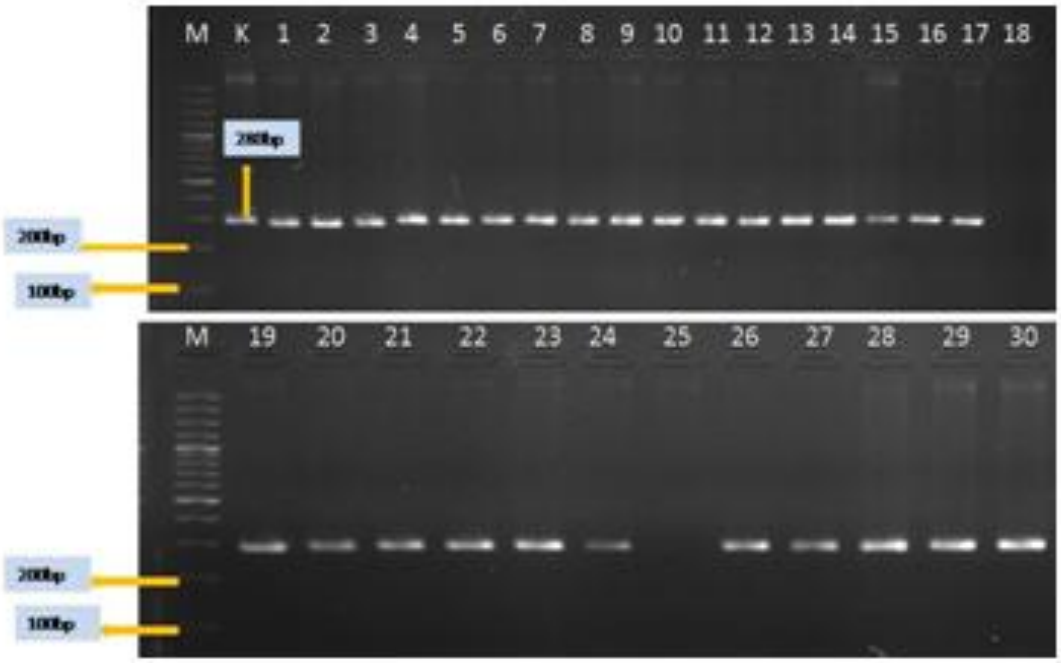


Plate 4.13: Band amplification pattern of SSR marker RM517 (K= K 343; 1 to 30 = BC_2F_1 plants (K343*²/RML 22)

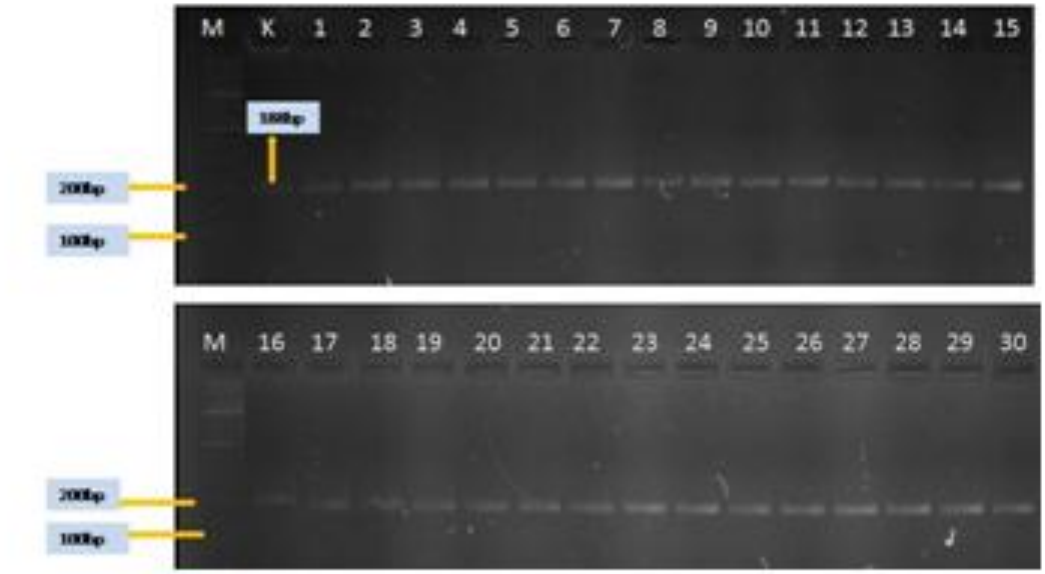


Plate 4.14: Band amplification pattern of SSR marker RM583 (K= K 343; 1 to 30 = BC_2F_1 plants (K343*²/RML 22)

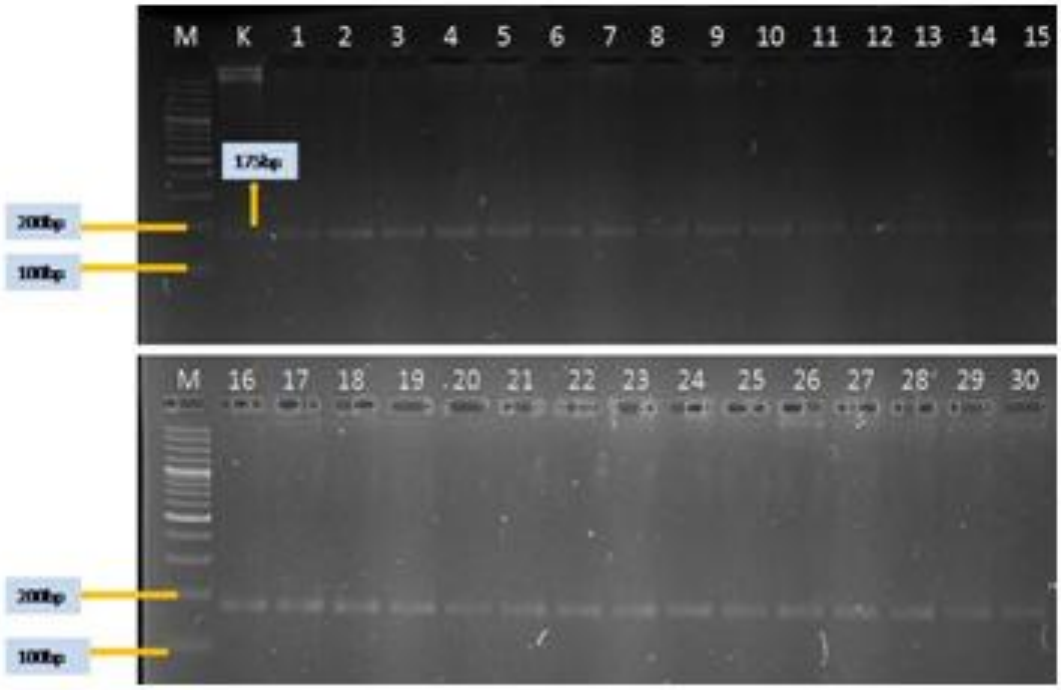


Plate 4.15: Band amplification pattern of SSR marker RM804 (K= K 343; 1 to 30 = BC_2F_1 plants (K343*²/RML 22)

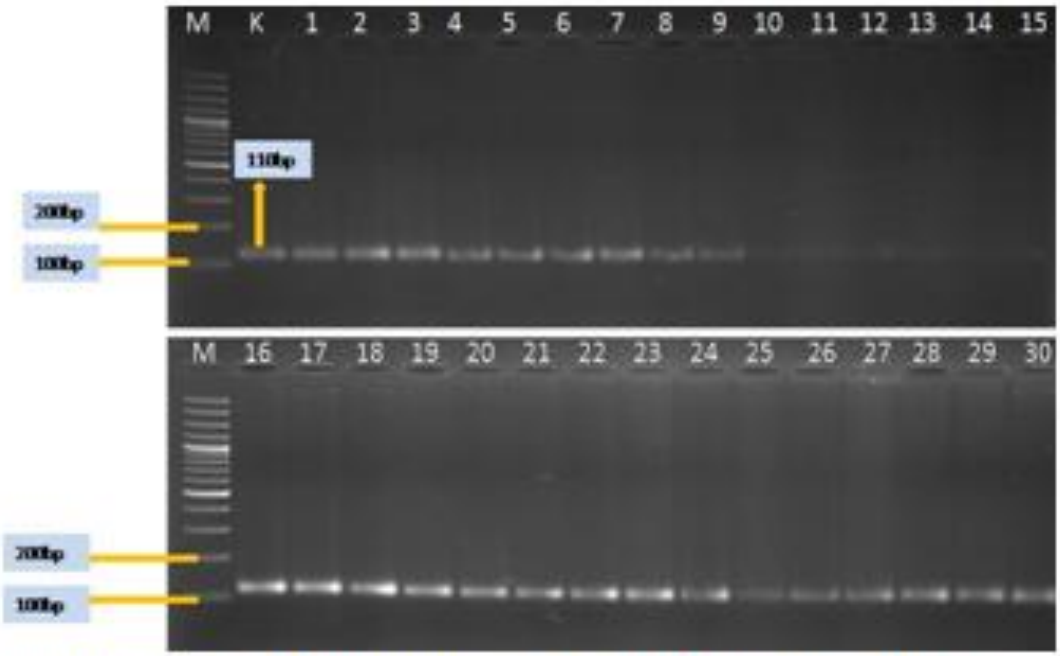


Plate 4.16: Band amplification pattern of SSR marker RM1112 (K= K 343; 1 to 30 = BC_2F_1 plants (K343*²/RML 22)

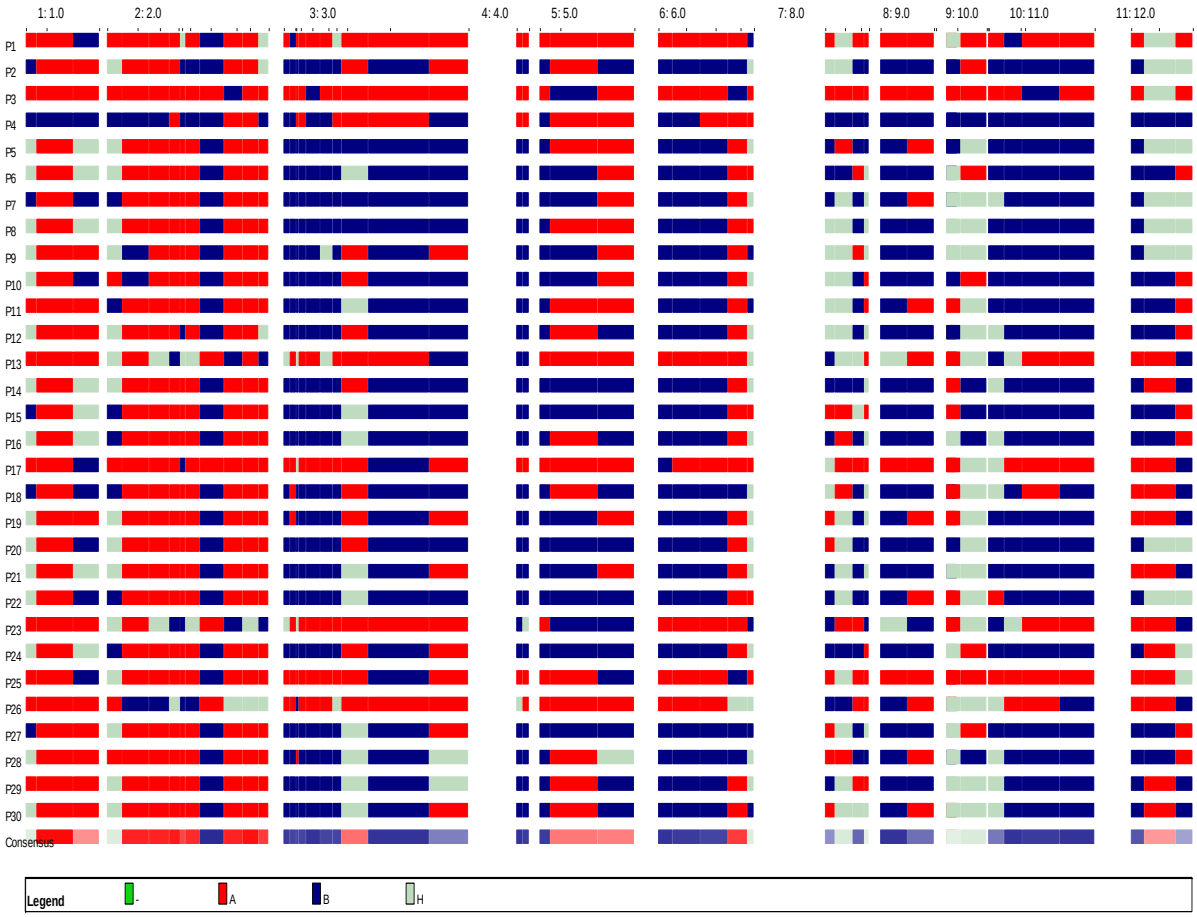


Figure 4.1: Genome introgression profile of 30 BC_2F_1 (K 343*3/DHMAS) plants using software GGT 2.0 (Van Berloo, 1999)

Table 4.5: Recurrent parent genome recovery in BC_2F_1 population (K 343*^{3}/DHMAS)

Plant	A% (Recurrent parent genome)	B% (Donor parent genome)
P1	86.40	13.60
P2	36.95	63.05
P3	83.40	16.60
P4	33.10	66.90
P5	40.80	59.20
P6	35.60	64.40
P7	33.75	66.25
P8	38.30	61.80
P9	40.00	59.90
P10	32.20	67.80
P11	43.30	56.70
P12	35.90	64.20
P13	75.30	24.70
P14	31.20	68.80
P15	30.25	69.75
P16	33.65	66.35
P17	83.65	16.35
P18	42.10	57.90
P19	47.95	52.05
P20	29.75	70.25
P21	40.85	59.25
P22	32.40	67.60
P23	68.15	31.85
P24	36.80	63.10
P25	79.15	20.95
P26	72.40	27.60
P27	34.10	66.00
P28	43.40	56.50
P29	42.05	57.95
P30	45.85	54.65

4.4.2 Evaluation of recurrent parent genome recovery in gene (*Pi9*) positive BC_2F_1 plants (K 343*^{3}/RML 22)

A graphical representation of all the individual plants for all the chromosomes of the selected genetic stocks for blast resistance is shown in Figure 4.2. The statistical data showed that in BC_2F_1 population the recurrent parent genome recovery ranged between 29.8 percent (P17) to 93.25 percent (P28) (Table 4.6). In this population, plant P28 showed maximum recovery of recipient genome (93.25%), followed by plants P3 (86.4%), P11 (85.80%) and P1 (84.85%).

In the Figure 4.2 the red coloured regions represent the homozygous regions of the recipient genome and the maximum recovery of recurrent parent genome was observed for chromosomes 1, 2, 5 and 10. The blue coloured regions

represent introgression from donor parent genome. Most of the residual segments from donor genome content were distributed on chromosomes 3, 6, 8, 11 while the light green coloured region indicated heterozygous regions.

Table 4.6: Recurrent parent genome recovery in BC_2F_1 population (K 343*^{3}/RML 22)

Genotypes	A% (Recurrent parent genome)	B%(Donor parent genome)
P1	84.85	15.15
P2	55.80	44.10
P3	86.40	13.50
P4	80.80	19.20
P5	70.50	29.50
P6	59.05	40.95
P7	70.05	29.95
P8	79.50	20.50
P9	70.50	29.50
P10	69.20	30.70
P11	85.80	14.20
P12	63.90	36.10
P13	74.35	25.65
P14	68.55	31.45
P15	61.60	38.40
P16	65.30	34.70
P17	29.80	70.20
P18	33.50	66.50
P19	60.95	39.05
P20	73.30	26.70
P21	56.75	43.25
P22	64.40	35.60
P23	58.75	41.25
P24	63.60	36.40
P25	71.70	28.20
P26	65.50	34.50
P27	65.40	34.60
P28	93.25	6.75
P29	74.65	25.35
P30	64.35	35.65

4.4.2 Phenotyping for agro-morphological traits in gene positive BC_2F_1 plants

Both BC_2F_1 populations i.e. K 343*^{3}/DHMAS) and (K 343*^{3}/RML 22, along with the respective parents i.e. K 343, DHMAS and RML 22 were evaluated for

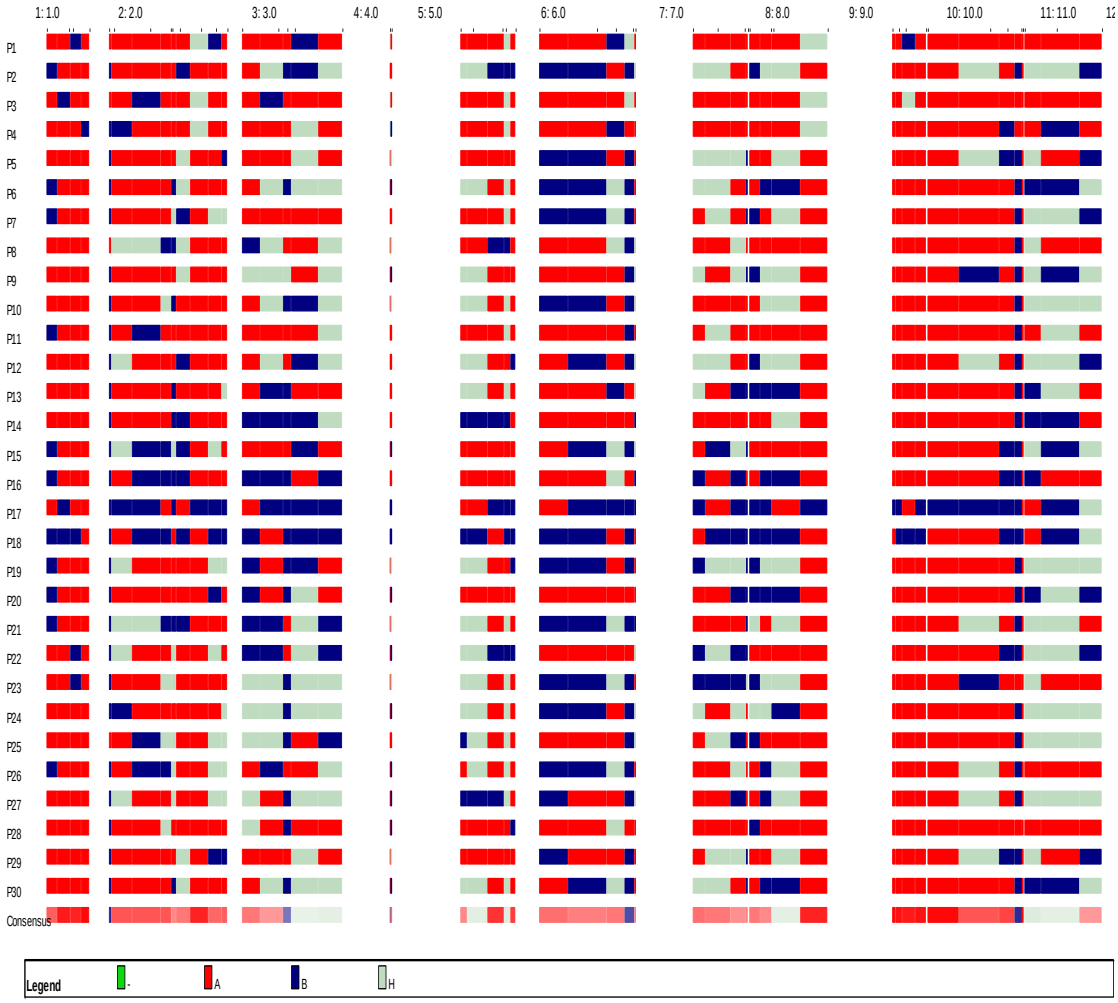


Figure 4.2: Genome introgression profile of 30 BC_2F_1 (K 343*3/ RML 22) plants using software Graphical GenoTypes (GGT 2.0) (Van Berloo, 1999)

agronomic traits using augmented design II. Single plant data were recorded for all plants found homozygous for the target genes for the traits such as plant height (PH), days to 50 percent flowering (DTF), days to maturity (DTM), duration of grain filling (DGF), panicle length (PL), number of effective tillers (EF), grain yield per plant (GPP), and thousand grain weight (TGW). Further, the lines were also analyzed for the grain dimension parameters *viz*., grain length (GL) and grain breadth (GB)

4.4.2.1 Analysis of Variance of genotypes BC_2F_1 (K 343*^{3}/DHMAS)

The analysis of variance (ANOVA) for grain yield and its component traits (Table 4.7) indicated that the BC_2F_1 plants (K 343*^{3}/DHMAS) showed significant variations for plant height, panicle length, effective tillers per plant and grain length. However checks i.e. parents showed significant variations except days to maturity, duration of grain filling and 1000- grain weight.

The mean values observed for various traits in parent K 343 as depiceted in Table 4.8 are plant height (130.02cm), days to 50 per cent flowering (93), days to maturity (128), duration of grain filling (35), panicle length (23.5 cm), number of effective tillers per plant (10), grain length (6.25 mm), grain breadth (2.42 mm), yield per plant (24.60 g), and 1000- grain weight (25.02g).

The mean values observed for various traits in parent DHMAS are plant height (127.50 cm), days to 50 per cent flowering (87), days to maturity (120), duration of grain filling (33), panicle length (19 cm), number of effective tillers per plant (9), grain length (5.21mm), grain breadth (2.21), yield per plant (24.5g) and 1000- grain weight (23g) (Table 4.8)

4.4.2.2 Observations recorded for the morphological traits in the BC_2F_1 population

(K 343*^{3}/DHMAS) are as follows (Table 4.8) :

i. Plant height (cm)

The plant height ranged from 120.20 cm - 131.90 cm. The maximum plant height was recorded in P8 (131.90cm) followed by P23 (131.30cm), P20 (130.3cm) and P22 (130.10cm) where as P4 recorded a minimum plant height i.e. 120.20 cm. The average plant height for the population was observed as 126.66 cm.

ii. Days to 50 percent flowering

Values for days to 50 percent flowering ranged between 88-94 with an average value of 92.43 days. The plant P10 took maximum number of days 94 for reaching 50 percent flowering, where as the minimum number of days to 50 per cent flowering were recorded in P2 (88 days).

iii. Days to maturity

Days to maturity in the BC_2F_1 plants ranged from 128 to 131 days with an average of 128.3 days. Plant P10 took maximum days to mature (131 days) followed by P2 (130 days) whereas most of genotypes took about 128 days for achieving maturity.

iv. Duration of grain filling

Duration of grain filling ranged from 35-39 days with an average 35.80 days. Plants P21, P22, P23, P24 and P25 took maximum duration for grain filling (39 days). However, most of the remaining plants except P9 (37 days) took minimum duration of grain filling (35 days).

v. Panicle length (cm)

The panicle length varied from 18.6 to 23.5cm with an average value of 21.17 cm. The maximum value for panicle length was recorded in case of plants P1 and P20 (23.50cm) followed by P14 (23.3cm), P15 (22.9cm) and P29 (22.7cm) where as minimum panicle length was observed in P22 (18.60cm)

vi. Number of effective tillers per plant

The number of effective tillers per plant ranged between 8- 10 with an average of 9.13. The maximum number of effective tillers per plant were recorded in plants P1, P3 P7, P10, P13, P14, P15 and P21 (10) where as minimum number (8) was recorded in case of plants P5, P16, P22, P27 and P29.

vii. Grain length

The grain quality attribute like grain length showed an average value of 5.77 mm with a range varying from 5.11-6.91mm. The maximum value for grain length was recorded in P6 (6.91mm) followed by P15 (6.33mm), P23 (6.29mm), P12 (6.20) whereas the minimum grain length was observed in P8 (5.11mm).

viii. Grain breadth

The grain breadth showed an average value of 2.53mm with a range varying

from 2.30- 2.72mm. In case of grain breadth, the maximum breadth of grains was recorded in P23 (2.72 mm) followed by P3 (2.68mm) and P15 (2.67mm) whereas the minimum grain breadth was recorded in P2 (2.22 mm).

ix. Grain yield per plant (g)

The grain yield per plant varied from 22.40 g to 29.10 g. The maximum grain yield was recorded in case of plants P8 and P5 (29.1g) while minimum grain yield (22.40 g) was observed in P2.The average grain yield per plant was recorded as 26.10 g.

x. 1000- grain weight (g)

The 1000-grain weight ranged from 21.70 to 29.20 g with average of 26.10 g. The highest 1000- grain weight was observed in plants P8 and P21 (29.20g) while as minimum 1000- grain weight was observed in P2 (21.70 g).

Table 4.7: Analysis of Variance of genotypes BC_2F_1 ((K 343*^{3}/DHMAS) for yield and yield contributing traits

Source of variation	DF	Plant height (cm)	Days to 50% Flowering	Days to maturity	Duration of grain filling	Panicle length (cm)	Number of Effective tillers per plant	Grain length (mm)	Grain breadth (mm)	Grain yield /plant (g)	1000 grain weight (g)
Mean sum squares											
Block	2	0.17	2.16	3.50	10.66	2.85*	0.00	0.001	0.0004	1.68	2.13
Treatments	31	46.90*	3.65	4.37	1.89	1.65*	0.45*	0.21*	0.018	2.20	2.75
Tests	29	48.79*	1.45	0.93	1.88	1.41*	0.41*	0.17*	0.007	1.38	2.14
Checks	1	13.05*	32.66*	54.00	2.66	9.15*	1.50*	1.46*	0.12*	9.15*	5.41
Tests v/s checks	1	25.80*	38.27*	54.45	1.42	1.05*	0.67*	0.02*	0.22*	18.7*	17.75
Error	2	0.11	1.16	3.50	0.66	0.001	0.00	0.001	0.005	0.32	1.35

* - Significant at 5%

p-Value < 0.05 - Significant at 5%, p-Value < 0.01 - Significant at 1%

Table 4.8: Mean performance of genotypes BC_2F_1 (K 343*^{3}/DHMAS) for yield and yield contributing traits

Test/check	RPG (%)	Plant height(cm)	Days to 50% Flowering	Days to maturity	Duration of grain filling	Panicle length (cm)	No. of effective tillers/ plant	Grain length (mm)	Grain breadth (mm)	Grain yield /plant (g)	1000 grain weight (g)
P1	86.40	122.23	93	128	35	23.50	10	6.25	2.42	25.02	24.60
P2	36.95	125.71	88	130	33	21.10	9	5.22	2.22	22.40	21.70
P3	83.40	125.14	93	128	35	21.10	10	6.05	2.68	28.40	24.50
P4	33.10	120.20	93	128	35	20.80	9	6.01	2.57	25.50	25.20
P5	40.80	130.00	93	128	35	21.51	8	5.22	2.53	29.10	24.30
P6	35.60	125.00	93	128	35	20.70	9	6.91	2.60	25.23	25.00
P7	33.75	125.21	93	128	35	20.31	10	5.21	2.30	26.90	23.60
P8	38.30	131.90	93	128	35	20.00	9	5.11	2.52	29.10	29.20
P9	40.00	125.31	93	128	35	21.20	9	5.21	2.45	25.50	25.00
P10	32.20	127.87	94	131	37	20.21	10	5.44	2.45	25.80	26.30
P11	43.30	126.21	93	128	35	20.90	9	5.70	2.48	25.30	27.40
P12	35.90	126.56	93	128	35	21.20	9	6.20	2.54	25.70	25.34
P13	75.30	125.21	93	128	35	22.13	10	6.20	2.52	26.00	28.20
P14	31.20	126.62	93	128	35	23.30	10	5.92	2.63	26.20	27.40
P15	30.25	121.71	93	128	35	22.90	10	6.33	2.67	26.00	28.10
P16	33.65	121.51	93	128	35	21.70	8	6.19	2.60	25.61	25.40
P17	83.65	125.52	93	128	35	21.20	9	6.11	2.45	25.43	28.30
P18	42.10	126.26	93	128	35	21.00	10	5.22	2.47	26.13	27.50
P19	47.95	121.21	93	128	35	21.60	9	5.19	2.52	26.21	28.50
P20	29.75	130.30	93	128	35	23.50	9	5.61	2.61	25.60	24.30
P21	40.85	126.20	89	128	39	19.80	10	5.30	2.50	25.1	29.20
P22	32.40	130.10	89	128	39	18.60	8	5.91	2.52	27.00	27.60
P23	68.15	131.30	89	128	39	19.30	9	6.29	2.72	24.20	26.20
P24	36.80	128.70	89	128	39	22.60	9	5.90	2.61	27.10	25.20
P25	79.15	126.10	89	128	39	21.60	9	5.90	2.60	25.30	25.50
P26	72.4	124.20	93	128	35	21.20	9	5.88	2.51	26.00	26.20
P27	34.1	123.60	93	128	35	22.30	8	5.72	2.53	26.10	25.40
P28	43.4	125.10	93	128	35	20.90	9	6.02	2.60	26.00	24.10
P29	42.05	124.50	93	128	35	22.70	8	5.81	2.41	25.41	25.40
P30	45.85	121.20	93	128	35	20.20	9	5.88	2.54	25.43	26.30
K 343 (C)	100.00	130.02	93	128	35	23.50	10	6.25	2.42	24.60	25.02
DHMAS (C)	0.00	127.50	87	120	33	19.00	9	5.21	2.21	24.50	23.00
Mean		126.60	92.43	128.30	35.80	21.10	9.10	5.70	2.53	26.10	26.10
CV (%)		3.55	2.00	1.30	2.55	1.80	4.00	2.10	3.00	2.25	5.15
SE(m)		0.50	0.10	0.20	0.10	0.60	0.22	0.40	0.04	0.10	0.10
CD (5%)		4.00	6.50	3.10	4.50	3.60	1.50	1.30	0.50	7.70	7.55

4.4.2.3 Analysis of Variance of genotypes BC_2F_1 (K 343*^{3} /RML 22)

The analysis of variance (ANOVA) for yield and its component traits (Table 4.9) indicated that the BC_2F_1 plants (K 343*^{3}/RML 22) showed highly significant variations for plant height, number of effective tillers per plant and grain length.The check genotypes showed significant variations except days to maturity, duratio of grain filling and 1000 grain weight. The mean values observed for various traits in parent K 343 as depicetd in Table 4.10 are plant height (130.0 cm), days to 50 per cent flowering (93), days to maturity (128), duration of grain filling(35), panicle length (23.50 cm), number of effective tillers per plant (10), grain length (6.25), grain breadth (2.42mm), yield per plant (24.60), and 1000- grain weight (25.02g).

The mean values observed for various traits in parent RML 22 are plant height (126.5cm), days to 50 per cent flowering (86), days to maturity (120), duration of grain filling(34), panicle length (18cm), number of effective tillers per plant (8), grain length (5.11mm), grain breadth (2.01), yield per plant(22.51g), and 1000- grain weight (24g) (Table 4.10).

4.4.2.4 Observations recorded for the morphological traits on the BC_2F_1 population (K 343*^{3}/RML 22) are as follows (4.10):

i. Plant height

The range for plant height in the BC_2F_1 population was between 121.21- 133.10 cm with an average of 128.77cm. The maximum value was recorded in plants P2, P3 and P5 (133.10 cm), followed by P21 (132.10 cm) and P23 (131.70) where as P29 recorded a minimum plant height i.e. 121.21cm.

ii. Days to 50 percent flowering

Duration of days to 50 % flowering in BC_2F_1 plants ranged between 89-94 with an average value of 92.66 days. Th plants which took maximum days to flowering were P11, P12, P13, P14, P15 and P16 (94 days) where as the minimum number of days to 50 percent flowering were recorded in plants P17, P18, P19, P20 (89 days).

iii. Days to maturity

Days to maturity in the BC_2F_1 ranged from and 128-131 with an average value of 128.6 days. Plants P11, P12, P13, P14, P15 and P16 took maximum days to mature (131days) whereas the remaining all the plants matured in 128 days.

iv. Duration of grain filling

Duration of grain filling in the BC_2F_1 ranged from 35-39 with an average value of 35.93 days. Plants P17, P18, P19, P20 took maximum duration of grain filling (39days) whereas msot other plants took minimum of 35 days for grain filling.

iv. Panicle length

The panicle length had a range varying from 18.90 -26.20cm with an average value of 22.12 cm. The maximum value of panicle length was recorded in P3 (26.20cm) followed by P12 (25cm) and P13 (24.4cm) whereas the minimum value was recorded in P16 (18.90cm).

v. Number of effective tillers per plant

The number of effective tillers per plant ranged between 7-9 with an average of 8.75. Most of the genotypes had effective tillers between 8-9, while the plants P18 and P26 had minimum number of effective tillers per plant (7).

vi. Grain length

The grain quality attributes like grain length showed an average value of 5.45mm with a range varying from 5.01-5.99mm. The maximum value for grain length was recorded in P13(5.99mm) followed by P17(5.95mm) and P4(5.92mm) whereas the minimum grain length was observed in P16(5.01mm).

vii. Grain breadth

The grain quality attributes like grain breadth showed an average value of 2.50 mm with a range varying from 2.18-2.70 mm. The maximum breadth of grains was recorded in P13 (2.70 mm) followed by P21(2.68mm), P5(2.67mm) and P15(2.65mm) whereas the minimum grain breadth was recorded in P14 (2.18 mm).

viii. Grain yield per plant

The average grain yield per plant was recorded as 25.60g with the range varying from 24.00 g- 27.00 g. Maximum grain yield was recorded in P12 (27.00 g) followed by P25 (26.90 g), P11 (26.50 g) and P13 (26.30 g) whereas a minimum grain yield of 24.00 g was recorded in P30.

ix. 1000- grain weight

The mean value of 1000- grain weight recorded was 25.91 g with range between 23.60 to -28.50 g. Highest value of 1000- grain weight was observed in P9 (28.50 g), followed by P15 (28.10 g) and P17 (28.00 g) whereas the lowest 1000- grain weight was recorded in P25 (23.60g).

Table 4.9 Analysis of Variance of genotypes BC_2F_1 (K 343*[3]/RML 22) for yield and yield contributing traits

Source of variation	DF	Plant height (cm)	Days to 50% flowering	Days to maturity	Duration of grain filling	Panicle length (cm)	Number of Effective tillers per plant	Grain length (mm)	Grain breadth (mm)	Grain yield /plant (g)	1000 grain weight (g)
Mean sum squares											
Blocks	2	0.07	2.67	0.00	2.66	4.80	0.000	0.03	0.003	1.35	2.10
Treatments	31	7.91*	6.28	7.32	0.561	3.47	0.20*	0.073*	0.035	1.65	1.54
Tests	29	7.87*	2.11	0.87	0.355	2.86	0.14*	0.07*	0.019	0.54	0.98
Checks	1	11.76*	60.16*	96.00	4.16	8.16*	1.50*	0.20*	0.170*	12.24*	6.82
Tests v/s checks	1	5.08*	73.47*	105.80	2.93	16.56*	0.55*	0.16*	0.374*	23.42*	12.55
Error	2	0.01	0.66	0.00	0.66	0.41	0.00	0.003	0.005	0.37	0.22

* - Significant at 5%

p-Value < 0.05 - Significant at 5%, p-Value < 0.01 - Significant at 1%

Table 4.10: Mean performance of genotypes BC_2F_1 (K 343*[3]/RML 22) for yield and yield contributing traits

Genotypes	RPG (%)	Plant height(cm)	Days to 50% Flowering	Days to maturity	Duration of grain filling	Panicle length (cm)	No. of effective tillers/ plant	Grain length (mm)	Grain breadth (mm)	Grain yield /plant (g)	1000 grain weight (g)
P1	84.85	129.20	93	128	35	20.90	9	5.70	2.48	25.30	27.40
P2	55.8	133.10	93	128	35	21.20	9	5.31	2.54	25.70	25.10
P3	86.4	133.10	93	128	35	26.20	9	5.21	2.52	26.00	25.50
P4	80.8	125.30	93	128	35	23.30	8	5.92	2.63	26.20	27.40
P5	70.5	133.10	93	128	35	22.90	9	5.44	2.67	26.00	25.20
P6	59.05	129.30	93	128	35	21.70	8	5.64	2.60	26.10	26.60
P7	70.05	130.30	93	128	35	21.20	9	5.55	2.45	26.21	26.30
P8	79.5	129.10	93	128	35	21.00	9	5.22	2.47	25.00	27.50
P9	70.5	127.10	93	128	35	21.60	9	5.19	2.52	26.20	28.50
P10	69.2	130.30	93	128	35	23.50	9	5.61	2.61	25.60	25.30
P11	85.8	128.90	94	131	37	22.20	9	5.39	2.53	26.50	26.40
P12	63.9	126.30	94	131	37	25.00	8	5.59	2.37	27.00	26.40
P13	74.35	127.30	94	131	37	24.40	9	5.99	2.70	26.30	26.50
P14	68.55	127.30	94	131	37	19.50	9	5.25	2.18	26.20	25.70
P15	61.6	131.30	94	131	37	22.90	9	5.57	2.65	25.50	28.10
P16	65.3	129.10	94	131	37	18.90	9	5.01	2.36	26.10	25.30
P17	29.8	130.10	89	128	39	21.30	9	5.95	2.43	25.20	28.00
P18	33.5	130.90	89	128	39	21.60	7	5.34	2.58	25.60	26.60
P19	60.95	129.80	89	128	39	22.90	9	5.25	2.45	26.10	25.40
P20	73.3	129.30	89	128	39	22.30	9	5.87	2.65	25.20	25.20
P21	56.75	132.10	93	128	35	21.10	9	5.43	2.68	25.20	25.50

P22	64.4	127.30	93	128	35	20.80	9	5.21	2.57	25.50	25.20
P23	58.75	131.70	93	128	35	24.30	9	5.22	2.53	25.11	24.30
P24	63.6	129.20	93	128	35	20.70	9	5.55	2.60	24.20	25.00
P25	71.7	129.00	93	128	35	23.20	9	5.21	2.30	26.90	23.60
P26	65.5	121.51	93	128	35	21.70	7	5.56	2.60	25.61	25.40
P27	65.4	125.52	93	128	35	21.20	9	5.42	2.45	25.43	26.21
P28	93.25	125.20	93	128	35	21.00	9	5.22	2.47	24.21	24.12
P29	74.65	121.21	93	128	35	21.60	9	5.19	2.21	24.01	25.51
P30	64.35	130.30	93	128	35	23.50	9	5.61	2.21	24.00	24.30
K 343 (C)	100	130.00	93	128	35	23.50	10	6.25	2.42	24.60	25.02
RML 22 (C)	0.00	126.50	86	120	34	18.00	8	5.11	2.01	22.51	24.00
Mean		128.77	92.66	128.60	35.93	22.12	8.75	5.45	2.50	25.60	25.91
CV (%)		6.00	2.00	3.15	2.44	3.25	0.75	1.75	1.25	2.43	2.50
SE(m)		0.50	0.17	0.33	0.17	0.17	0.44	0.12	0.11	0.17	0.18
CD (5%)		5.10	5.20	4.12	4.75	7.50	1.22	0.56	0.60	4.25	5.15

4.4.3 Pathotyping of gene positive BC_2F_1 plants for blast symptoms

4.4.3.1Pathotyping of BC_2F_1 population (K 343*^{3} /DHMAS)

All the BC_2F_1 plants selected through marker assisted selection (foreground and background selection) were screened for blast symptoms both under natural and artificial conditions. All BC_2F_1 plants along with parents were inoculated with PLP-1 strain of *Magnaporthe oryzae* using spray method under standard conditions (Bonman *et al.*, 1986; Sharma *et al*., 2005b) in Greenhouse and Experimental Farm of School of Biotechnology. Disease reactions of inoculated plants were recorded on a scale of 0–5 (Bonman *et al*., 1986) (Table 4.11). All the 30 plants (K 343*^{3}/DHMAS) showed 0-2 score depicting moderately resistant to highly resistant reaction while the recipient parent K 343 showed susceptible reaction with score 3.

Table 4.11: Pathotyping of BC_2F_1 (K 343*^{3}/DHMAS) plants for blast symptoms

S. No.	Genotype	Score	Disease reaction
1	K 343	3	Susceptible
2	DHMAS	0	Highly Resistant
3	P1	0	Highly resistant
4	P2	0	Highly resistant
5	P3	0	Highly resistant
6	P4	2	Moderately resistant
7	P5	2	Moderately resistant
8	P6	2	Moderately resistant
9	P7	1	Resistant
10	P8	2	Moderately resistant
11	P9	2	Moderately resistant
12	P10	2	Moderately resistant
13	P11	2	Moderately Resistant
14	P12	2	Moderately resistant
15	P13	0	Highly resistant
16	P14	2	Moderately resistant
17	P15	2	Moderately resistant
18	P16	1	Resistant
19	P17	0	Highly Resistant
20	P18	2	Moderately resistant
21	P19	2	Moderately resistant
22	P20	2	Moderately Resistant
23	P21	2	Moderately resistant
24	P22	2	Moderately Resistant
25	P23	2	Moderately resistant
26	P24	2	Moderately resistant
27	P25	1	Resistant
28	P26	2	Moderately resistant
29	P27	2	Moderately Resistant
30	P28	2	Moderately Resistant
31	P29	1	Resistant
32	P30	1	Resistant

4.4.3.2 Pathotyping of BC_2F_1 population (K 343*^{3} /RML 22)

All BC_2F_1 plants along with parents were inoculated with PLP-1 strain of *M. oryzae* and disease reactions of inoculated plants were recorded on a scale of 0–5 (Bonman *et al*., 1986) (Table 4.12). All the 30 plants (K 343*^{3}/RML 22) showed 0-2 score depicting moderately resistant to highly resistant reaction while the recipient parent K 343 showed susceptible reaction with the score 3.

Table 4.12: Pathotyping of BC_2F_1 (K 343*^{3}/RML 22) plants for blast symptoms

S. No.	Genotype	Score	Disease reaction
1	K 343	3	Susceptible
2	RML 22	0	Highly Resistant
3	P1	0	Highly Resistant
4	P2	1	Resistant
5	P3	0	Highly Resistant
6	P4	0	Highly Resistant
7	P5	2	Moderately Resistant
8	P6	2	Moderately Resistant
9	P7	2	Moderately Resistant
10	P8	0	Highly Resistant
11	P9	2	Moderately Resistant
12	P10	1	Resistant
13	P11	0	Moderately Resistant
14	P12	2	Moderately Resistant
15	P13	2	Moderately Resistant
16	P14	2	Moderately Resistant
17	P15	2	Moderately Resistant
18	P16	2	Moderately Resistant
19	P17	0	Highly Resistant
20	P18	1	Resistant
21	P19	2	Moderately Resistant
22	P20	2	Moderately Resistant
23	P21	2	Moderately Resistant
24	P22	2	Moderately Resistant
25	P23	2	Moderately Resistant
26	P24	2	Moderately Resistant
27	P25	2	Moderately Resistant
28	P26	2	Moderately Resistant
29	P27	2	Moderately Resistant
30	P28	0	Highly Resistant
31	P29	1	Resistant
32	P30	1	Resistant

4.4.4 Agronomical and pathological status of maximum recurrent parent genome recovery in genetic stocks of BC_2F_1

4.4.4.1 Agronomical and pathological status of maximum recurrent parent genome recovery in genetic stock (K 343*^{3}/DHMAS)

The genetic stocks of K 343*^{3}/DHMAS with maximum recovery of recurrent parent genome (>83%) were compared agronomically and pathologically with the recurrent parent Table 4.13. The maximum recovered recurrent parent genome in plant numbers P1 (86.40%), P3 (83.40) and P17 (83.65) had broader agronomical similarity to the recurrent parent and pathologically related to the donor parent.

4.4.4.2 Agronomical and pathological status of maximum recurrent parent genome recovery in genetic stock (K 343*^{3}/RML 22)

The genetic stocks of K 343*^{3}/RML 22 with maximum recovery of recurrent parent genome were compared agronomically and pathologically with the recurrent parent Table 4.13. The maximum recovered recurrent parent genome in plant numbers P3 (86.40%), P11 (85.80%) and P28 (93.25%) had broader agronomical similarity to the recurrent parent and pathologically related to the donor parent.

Table 4.13: Agronomical and pathological status of genetic stocks K 343*^{3}/DHMAS and K 343*^{3}/RML 22 with maximum RPG recovery

A: K 343*^{3}/DHMAS					
Gene positive plants *Pi54*	**DHMAS**	**K 343**	**P1**	**P3**	**P17**
RPG (%)	0	100	86.40	83.40	83.65
Disease score	0	3	0	0	0
Plant height (cm)	127.50	130.00	122.23	125.10	125.52
Days to 50 percent flowering	87	93	93	93	93
Days to maturity	120	128	128	128	128
Duration of grain filling	33	35	35	35	35
Panicle length (cm)	19.00	23.50	23.50	21.10	21.20
Effective tillers	9	10	10	10	9
Grain length (mm)	5.21	6.25	6.25	6.05	6.11

Grain breadth (mm)	2.21	2.42	2.42	2.68	2.45
Yield per plant (g)	24.50	24.60	25.02	28.40	25.43
1000 grain weight (g)	23.00	25.02	24.60	24.50	28.30
B: K 343*^{3}/RML 22					
Gene positive plants *Pi9*	**RML 22**	**K 343**	**P 3**	**P 11**	**P 28**
RPG (%)	0	100	86.40	85.80	93.25
Disease score	0	3	0	0	0
Plant height (cm)	126.50	129.20	133.10	128.90	125.20
Days to 50 percent flowering	86	93	93	94	93
Days to maturity	120	128	128	131	128
Duration of grain filling	34	35	35	37	35
Panicle length (cm)	18.00	19.50	26.20	22.20	21.00
Effective tillers	8	9	9	9	9
Grain length (mm)	5.10	5.40	5.21	5.39	5.22
Grain breadth (mm)	2.00	2.40	2.52	2.53	2.47
Yield per plant (g)	22.50	26.10	26.00	26.50	24.21
1000 grain weight (g)	24.00	25.80	25.50	26.40	24.12

4.5 Foreground selection for *Pi54 and Pi9* genes in F_2 convergent population using linked markers

Genotyping of 4000 individual F_2 plants (K 343*^{3}/DHMAS× K 343*^{3}/RML 22) along with parents was done. They were screened with SSR markers closely linked to *Pi54* gene i.e. RM206 (0.7 cM away from *Pi54* locus) and SSR markers closely linked *Pi9* gene i.e. AP5930 (0.05 cM away from *Pi9* locus) based on previous study (Hangloo, 2018) for identification of plants having the target resistance genes i.e. both *Pi54* and *Pi9* via multiplex PCR. Plants homogenous and heterogenous for target locus were observed in F_2 convergent population (Plate 4.17- 4.21). In F_2 convergent population out of 4000 plants genotyped for the presence of *Pi54* and *Pi9* genes, 45 plants were observed to be positive for both *Pi9* and *Pi54* genes. They were subjected to background screening i.e. for identification of plants having maximum background recovery (RPG) of recurrent parent genome

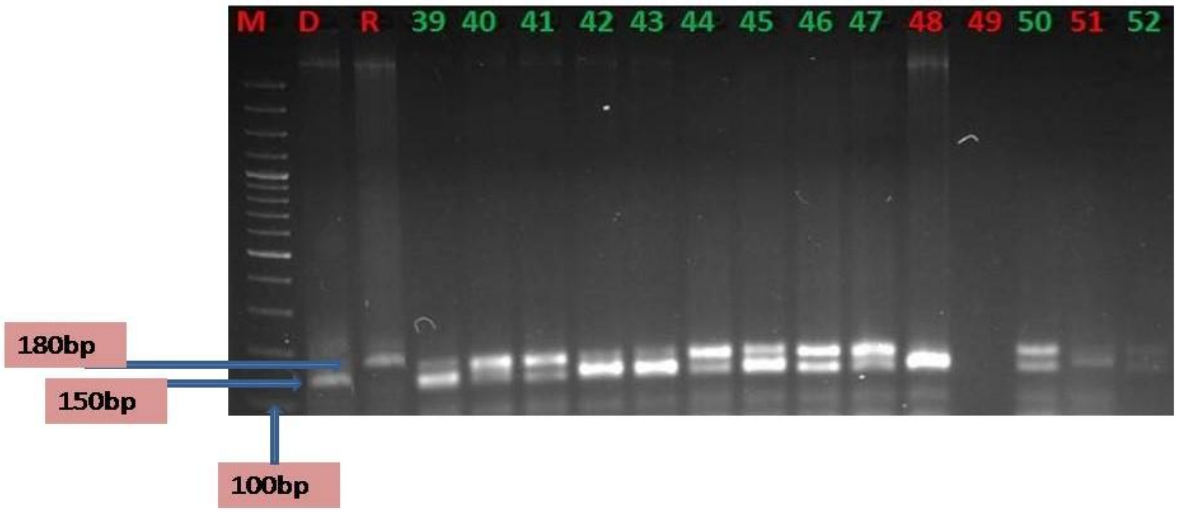


Plate 4.17: Foreground selection for *Pi54* and *Pi9* genes; D=DHMAS and R=RML 22, (39-52) =F_2 Plants of P6 plant progeny; GREEN colour shows (*Pi54* +*Pi9*) positive plants (pyramided)

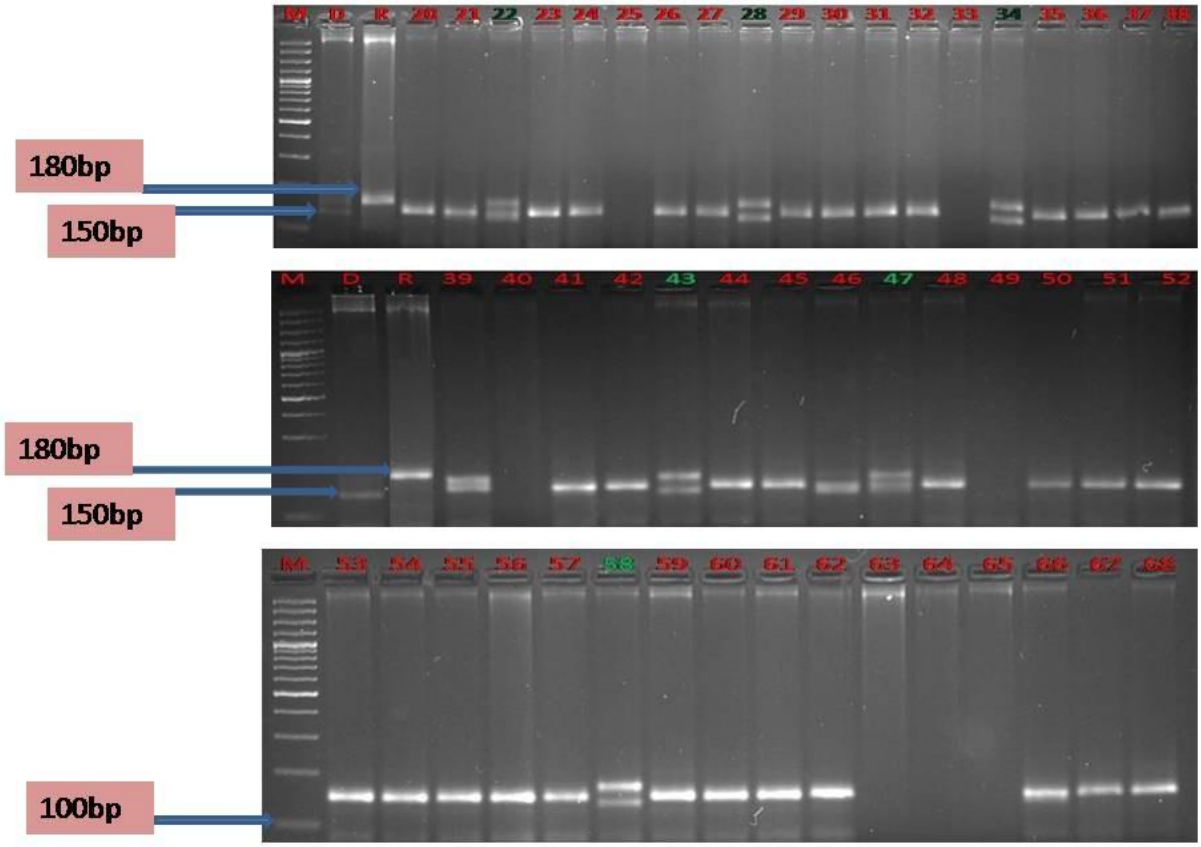


Plate 4.18: Foreground selection for *Pi54* and *Pi9* genes; D=DHMAS and R=RML 22, (20-68) = F_2 Plants of P7 plant progeny ; GREEN colour shows (*Pi54* +*Pi9*) positive plants(pyramided)

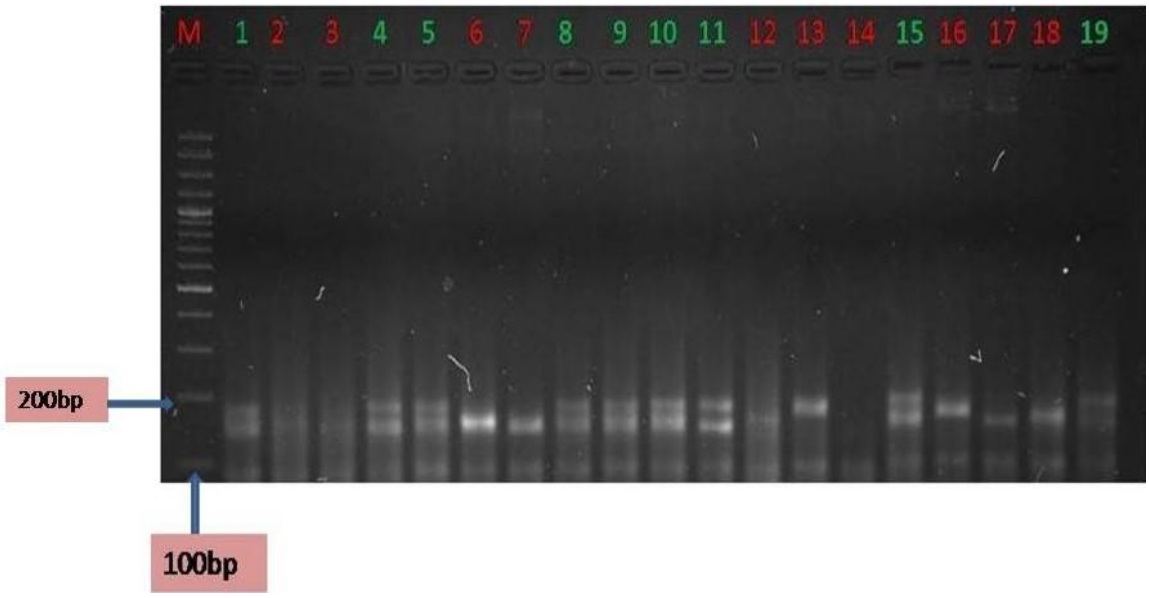


Plate 4.19: Foreground selection for *Pi54* and *Pi9* genes; (1-19)= F_2 Plants of P12 plant progeny; GREEN colour shows (*Pi54* +*Pi9*) positive plants (pyramided)

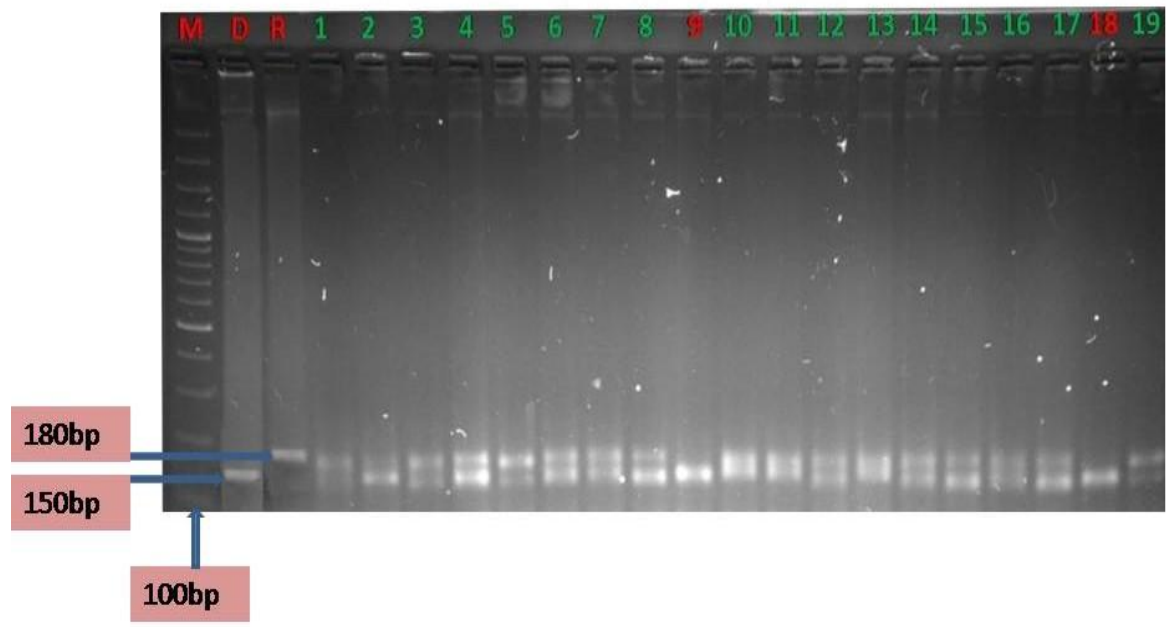


Plate 4.20: Foreground selection for *Pi54* and *Pi9* genes; D=DHMAS and R=RML 22, (1-19)= F_2 Plants of P-16 plant progeny ; GREEN GREEN colour shows (*Pi54* +*Pi9*) positive plants(pyramided)

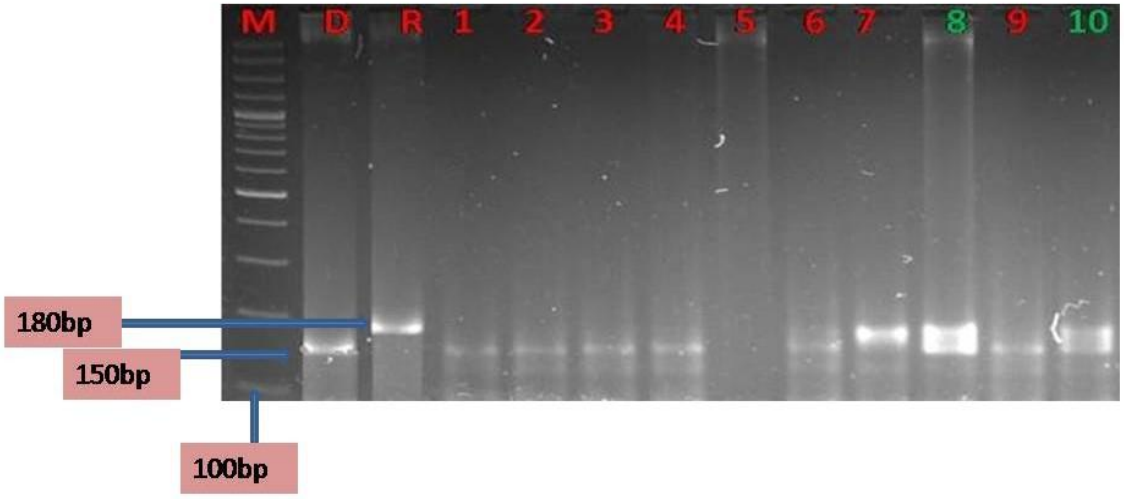


Plate 4.21 Foreground selection for *Pi54* and *Pi9* genes; D=DHMAS and R=RML 22, (1-10)= F_2 Plants of p-18 line GREEN colour shows (*Pi54* +*Pi9*) positive plants(pyramided)

4.6 Background selection of *Pi54 and Pi9* gene positive pyramided plants in F_2 convergent population using polymorphic SSR markers

The background selection for analyzing recovery of recurrent parent genome was done on 45 plants of F_2 covergent population (K 343*^{3}/DHMAS×K 343*^{3}/RML 22) found positive for the target resistance gene (*Pi54* and *Pi9*). They were screened with 101 polymorphic SSR markers (Plates 4.22- 4.35). The amplicon for each plant with individual marker was matched with size of amplicon of recurrent parent. The data with respect to observed amplification products of each marker for 45 plants was subjected to software GGT 2.0 for assessing the status of recovery of recurrent parent genome. In F_2 plants, subjected to background analysis the recovery of recurrent parent genome was found to vary from 45.15 percent (P2) to 95.5 percent (P42).The maximum recovery of recurrent parent was observed in P42 (95.5%) followed by P4 (95.3%), P45 (93.0) and P16 (91.85%) (Table 4.14). Plants with maximum recovery of recipient parent genome can be used as pyramided lines for development of blast resistant commercial varieties.

The SSR banding profile of the markers RM408, RM3, RM112, RM114, RM162, RM168, RM169, RM225, RM234, RM333, RM430, RM440, RM475, RM587 are depicted in (plate 4.22- 4.35) respectively.

4.7 Evaluation of pyramided plants of F_2 convergent population to identify superior plants

4.7.1 Evaluation of recurrent parent genome recovery in F_2 pyramided plants by using GGT 2.0 software

The maximum recovery of recipient parent genome was calculated using software Graphical GenoTypes (GGT 2.0) (Van Berloo, 1999). A graphical representation of all the individual plants for all the chromosomes for blast resistance is shown in Figure 4.3. In F_2 convergent population (K 343*^{3}/DHMAS× K 343*^{3}/RML 22) out of 45 pyramided plants, 8 plants had recurrent parent genome (RPG) recovery more than 90 percent i.e. P4, P5, P6, P8, P16, P37, P42, P45 (RPG recovery 95.3%, 91.1%, 90.1%, 90.45%, 91.85%, 91.60%, 95.5% and 93%, respectively). The recurrent parent genome recovery ranged between 45.15 percent (P2) to 95.5 percent (P42) (Table 4.14).

In the Figure 4.3 GGT graph, the red coloured regions represent the homozygous regions of the recipient genome and the maximum recovery of recurrent

parent genome was observed for chromosomes 2, 8, 9 and 12. The blue coloured regions represent introgression from donor parent genome. Most of the residual segments from donor genome content were distributed on chromosomes 3, 4, 5, 6, 7, 8, 10, 11 and 12 while the light green coloured region indicated heterozygous regions.

In Figures 4.4 - 4.11 chromosome wise recovery of the plants P4, P5, P6, P8, P16, P37, P42, P45 is shown. It is seen clearly that almost all the chromosomes have recovered recepient parent (K 343 genome) except few portions of some chromosomes, which are depicted via different colours i.e. red colored areas shows recovered areas of recurrent parent and blue color shows donor genome areas.

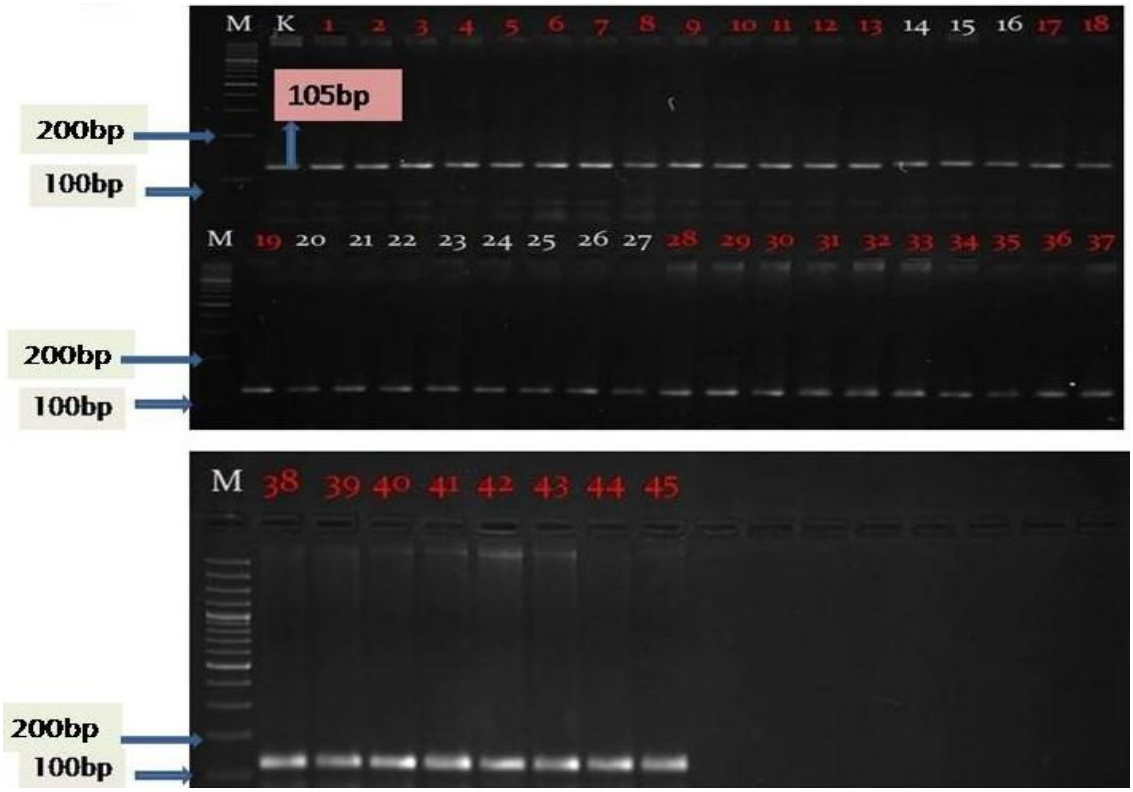


Plate 4.22 Band amplification pattern of SSR marker RM408 (K=K 343; 1-45 = F_2 (pyramided plants); Red colour indicates plants similar to recipient parent (K 343)

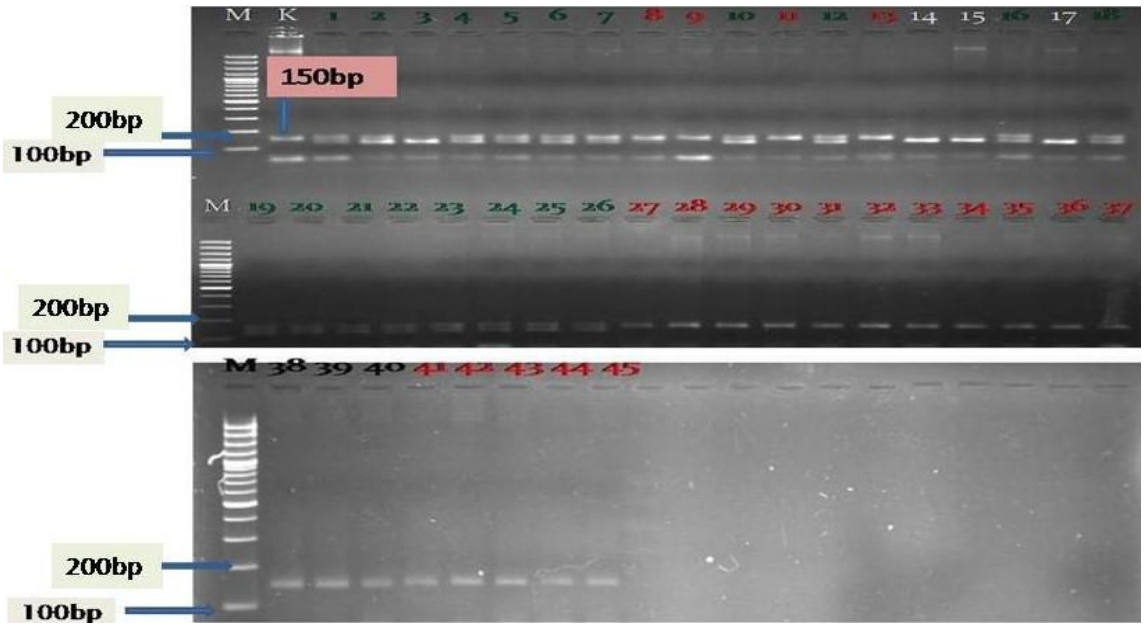


Plate 4.23 Band amplification pattern of SSR marker RM3 (K=K 343; 1-45 = F_2 pyramided plants); Red colour indicates plants similar to recipient parent (K 343)

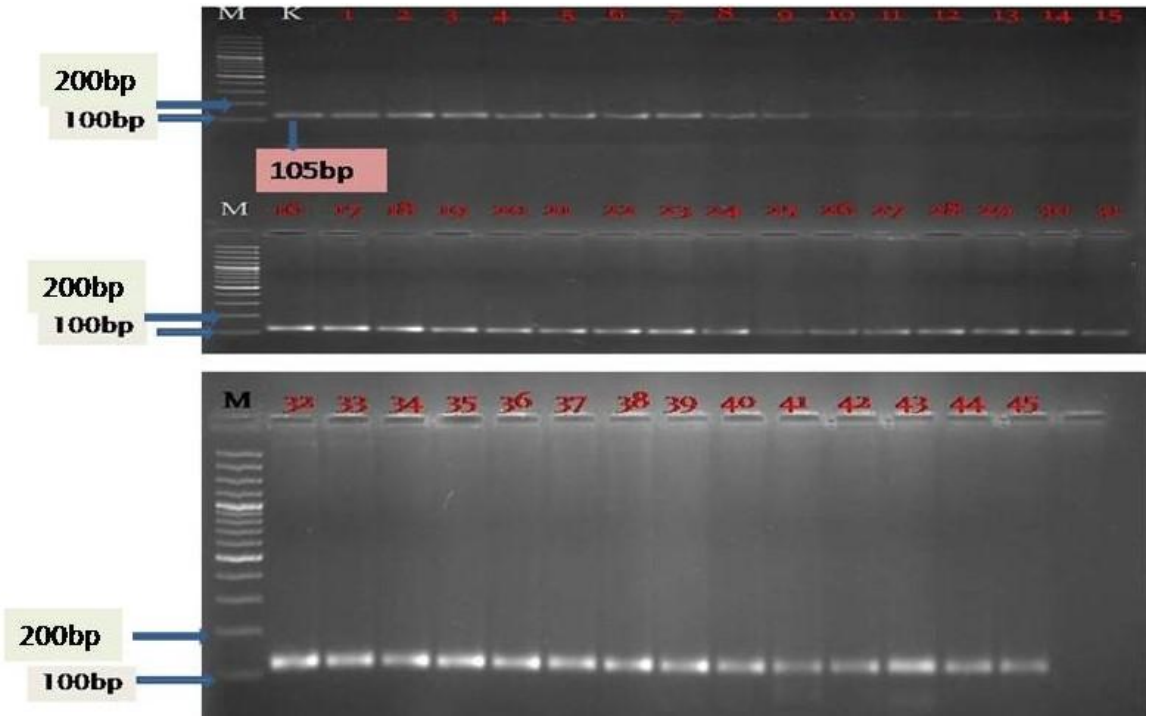


Plate 4.24 Band amplification pattern of SSR marker RM112 (K=K 343; 1-45 = F_2 pyramided plants); Red colour indicates plants similar to recipient parent (K 343)

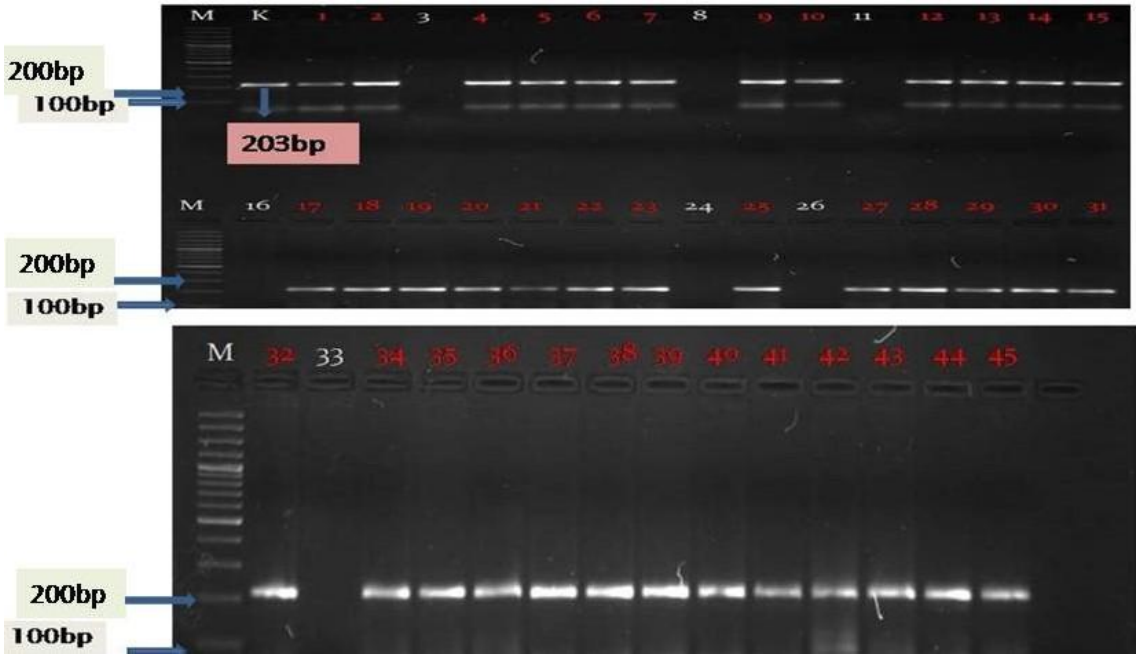


Plate 4.25 Band amplification pattern of SSR marker RM114 (K=K 343; 1-45 = F_2 pyramided plants); Red colour indicates plants similar to recipient parent (K 343)

Plate 4.26 Band amplification pattern of SSR marker RM162 (K=K 343; 1-45 = F_2 pyramided plants); Red colour indicates plants similar to recipient parent (K 343); Green colour depicts hybrid plants.

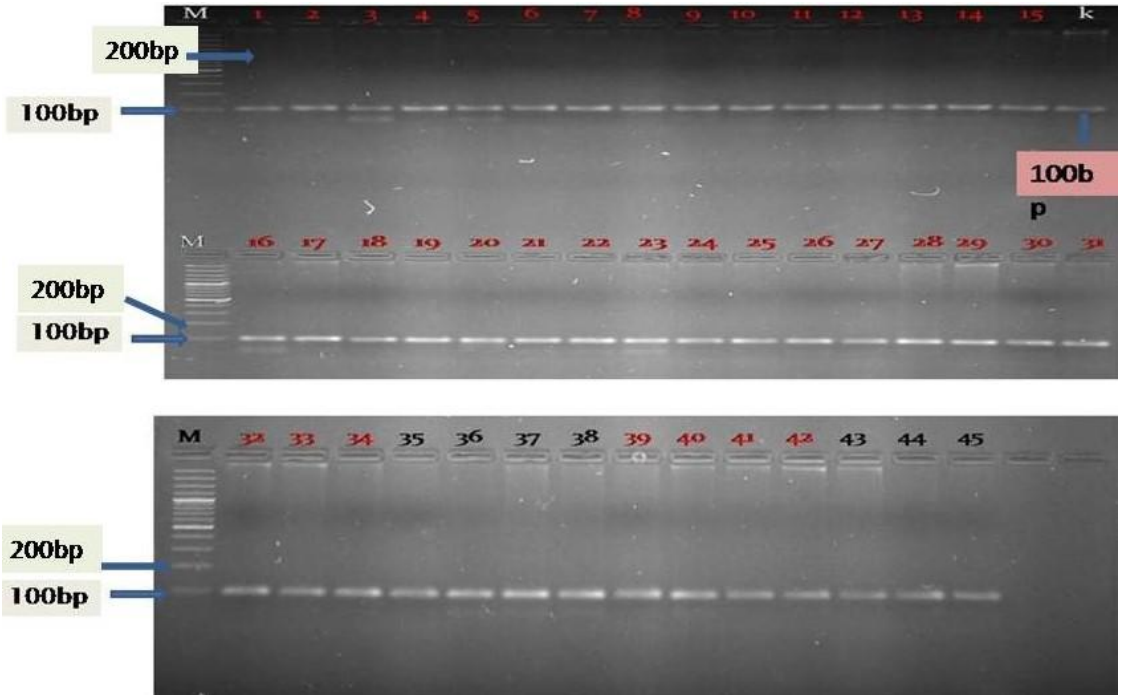


Plate 4.27 Band amplification pattern of SSR marker RM168 (K=K 343; 1-45 = F_2 pyramided plants); Red colour indicates plants similar to recipient parent (K 343)

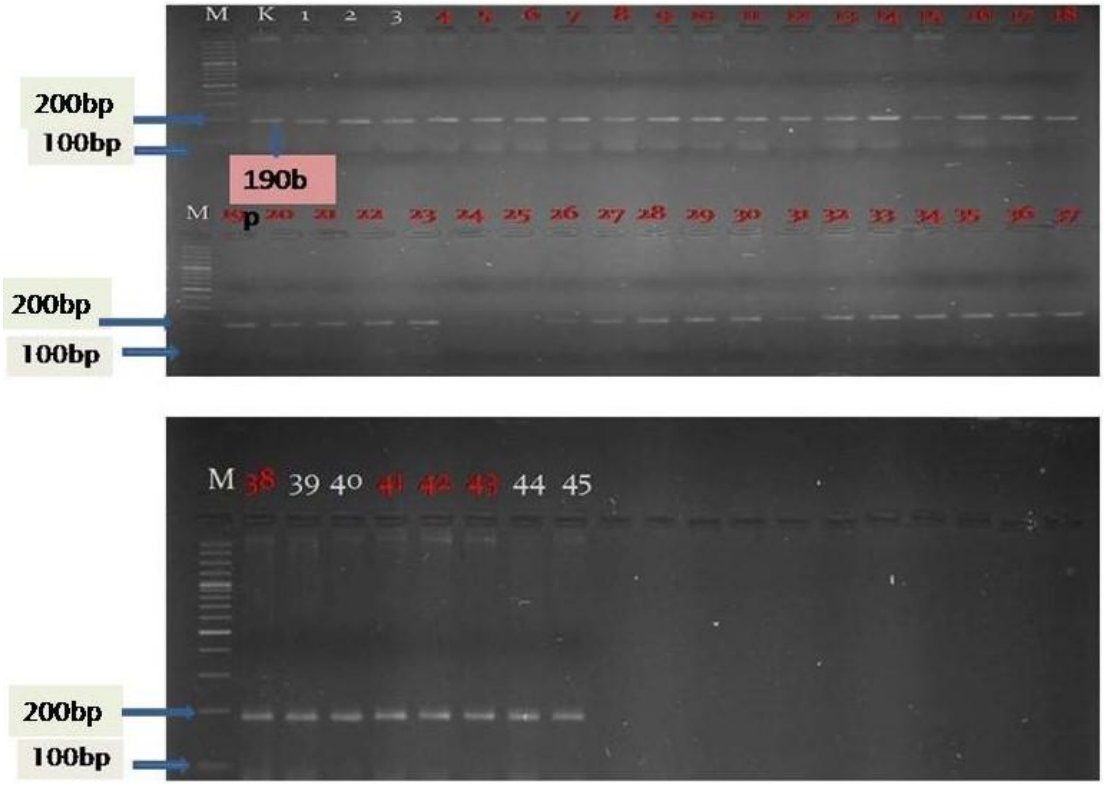


Plate 4.28 Band amplification pattern of SSR marker RM169 (K=K 343; 1-45 = F_2 pyramided plants); Red colour indicates plants similar to recipient parent (K 343)

Plate 4.29 Band amplification pattern of SSR marker RM225 (K=K 343; 1-45 = F_2 pyramided plants); Red colour indicates plants similar to recipient parent (K 343)

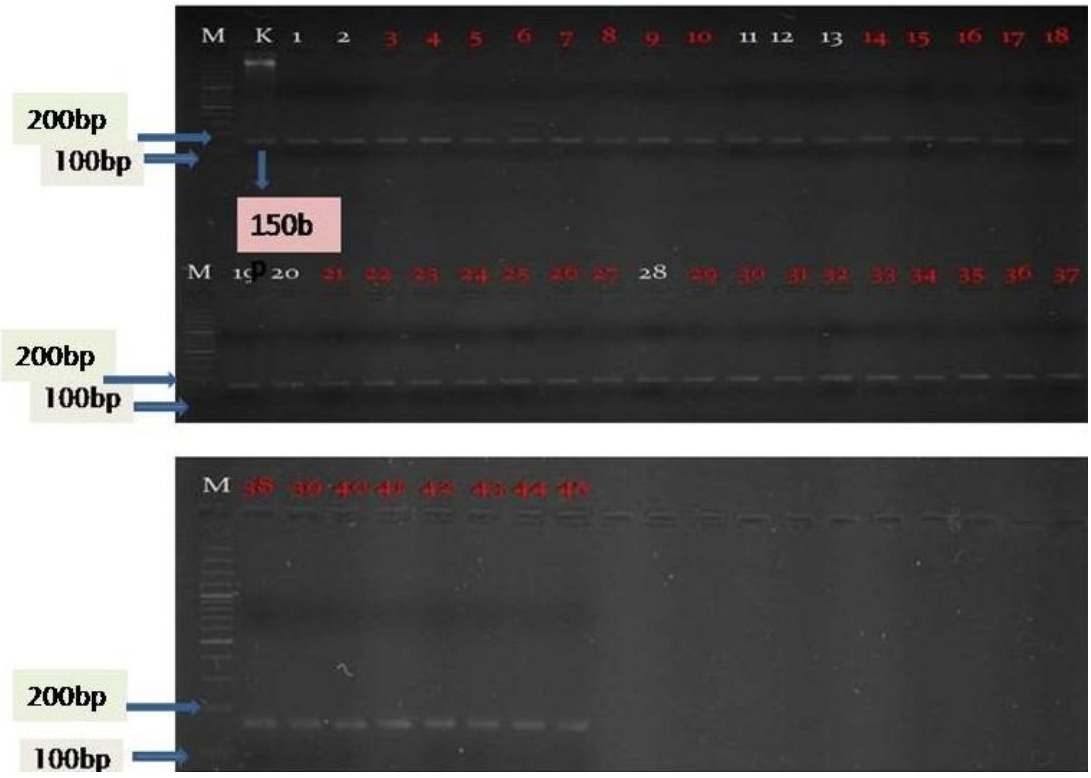


Plate 4.30 Band amplification pattern of SSR marker RM234 (K=K 343; 1-45 = F_2 pyramided plants); Red colour indicates plants similar to recipient parent (K 343)

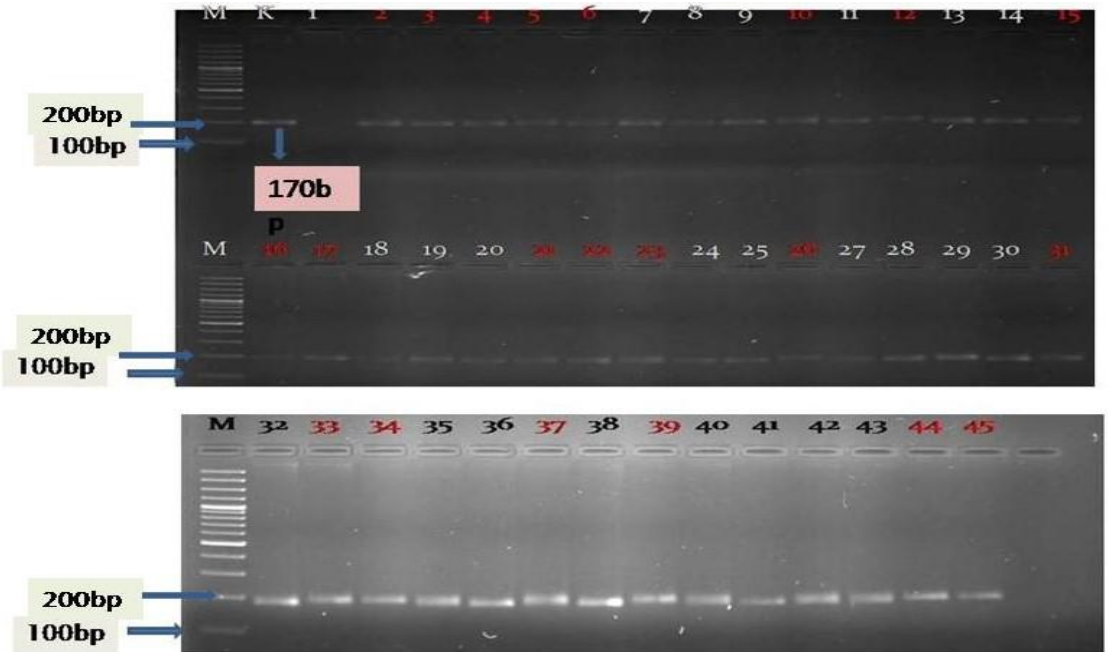


Plate 4.31 Band amplification pattern of SSR marker RM333 (K=K 343; 1-45 = F_2 pyramided plants); Red colour indicates plants similar to recipient parent (K 343)

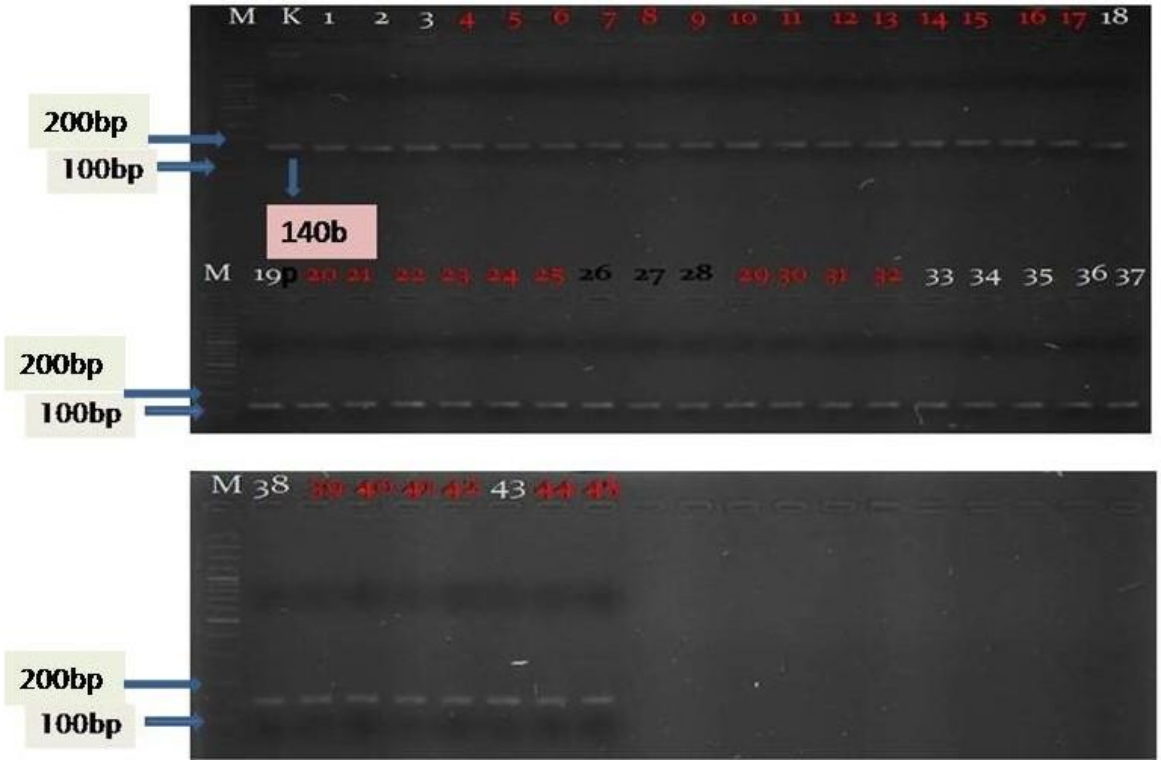


Plate 4.32 Band amplification pattern of SSR marker RM430 (K=K 343; 1-45 = F_2 pyramided plants); Red colour indicates plants similar to recipient parent (K 343)

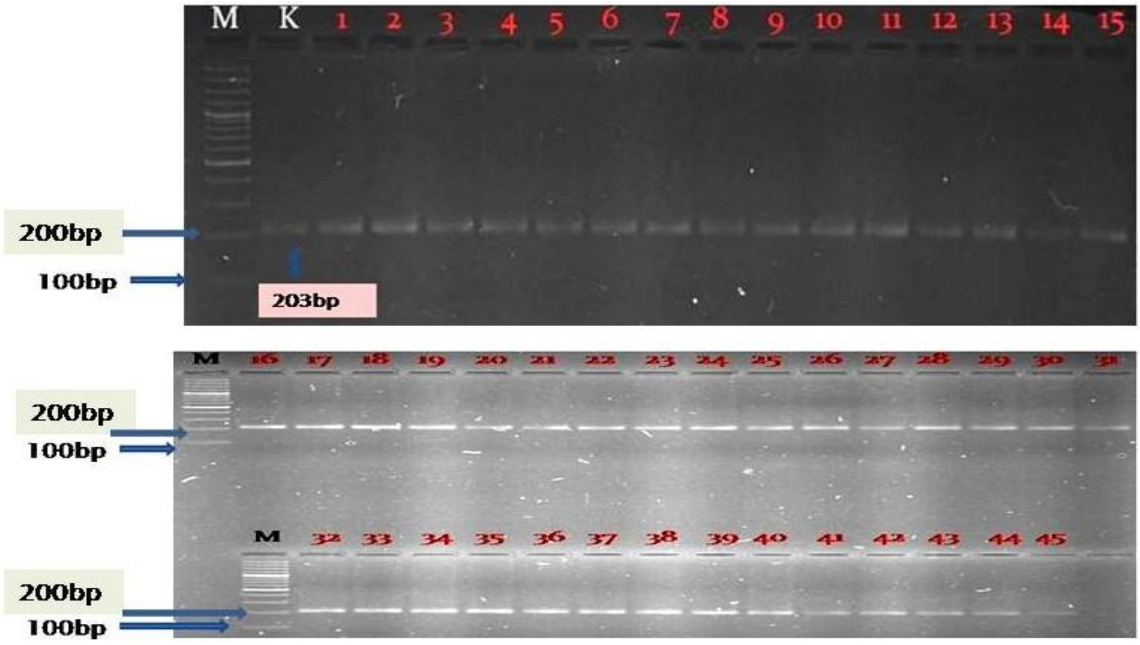


Plate 4.33 Band amplification pattern of SSR marker RM440 (K=K 343; 1-45 = F_2 pyramided plants); Red colour indicates plants similar to recipient parent (K 343)

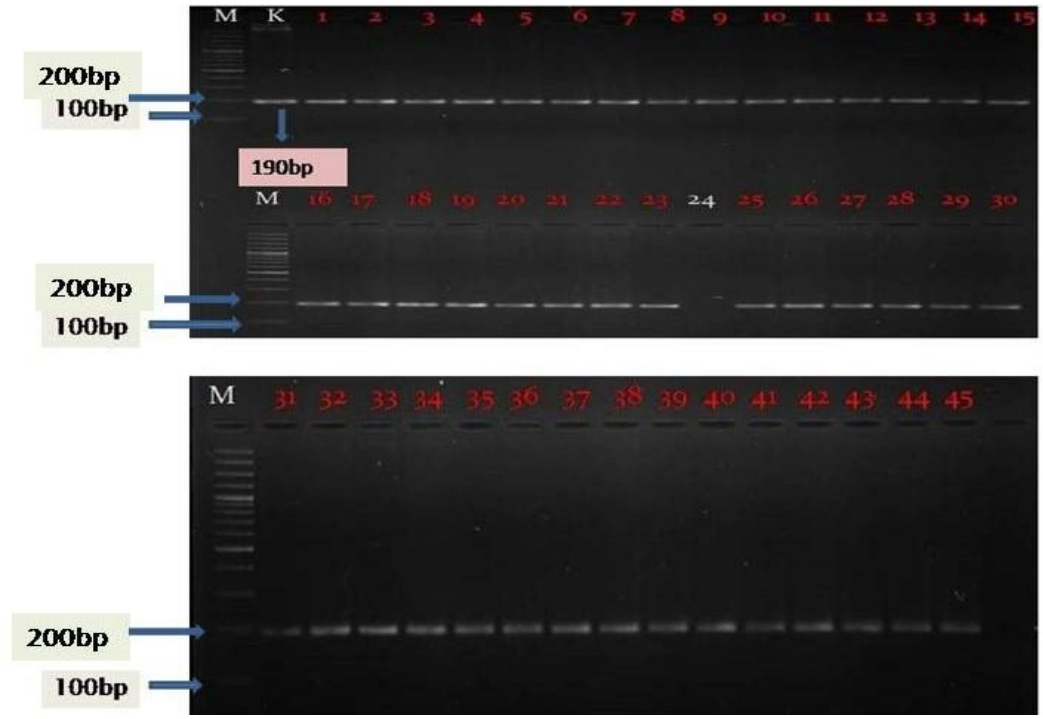


Plate 4.34 Band amplification pattern of SSR marker RM475 (K=K 343; 1-45 = F_2 pyramided plants); Red colour indicates plants similar to recipient parent (K 343)

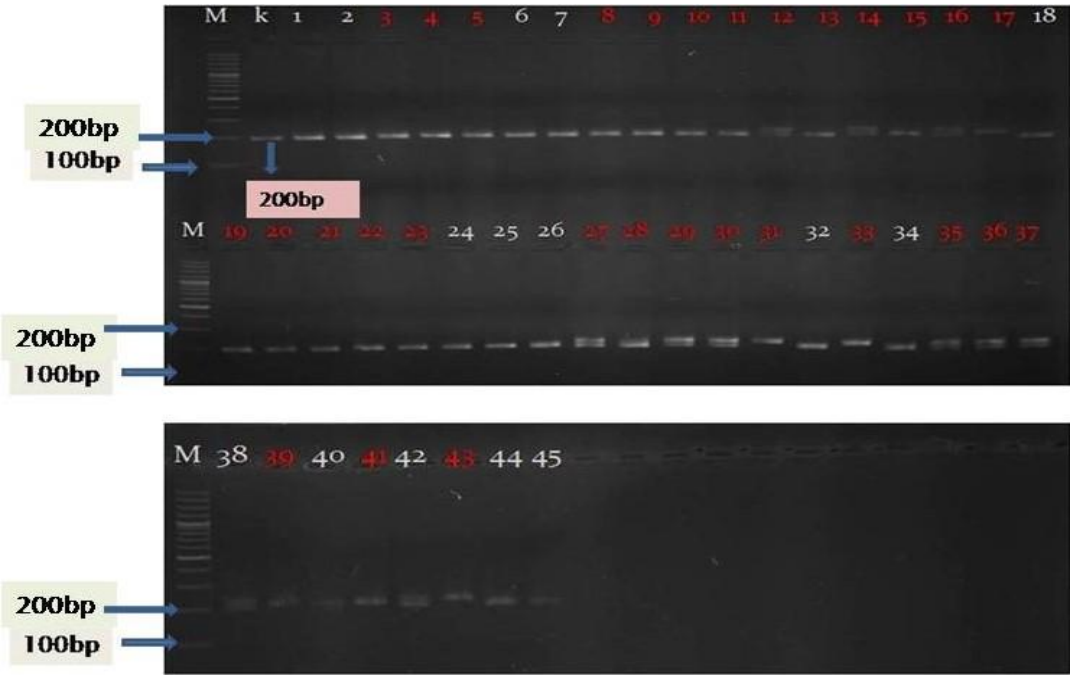
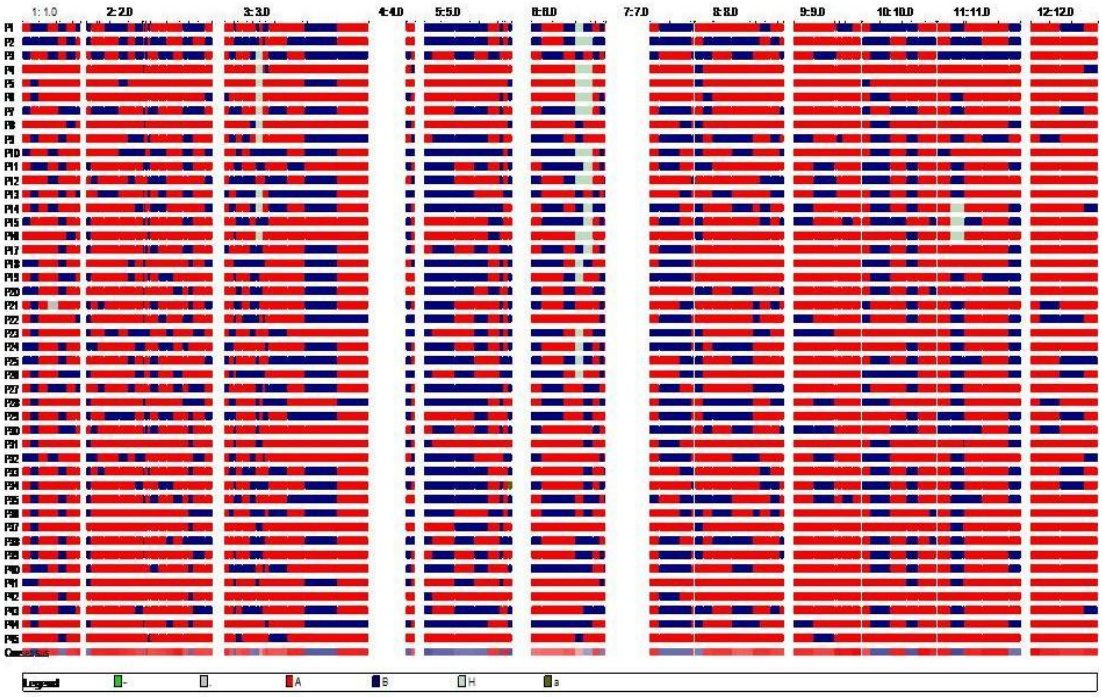


Plate 4.35 Band amplification pattern of SSR marker RM587 (K=K 343; 1-45 = F_2 pyramided plants); Red colour indicates plants similar to recipient parent (K 343)



A: Recurrent Parent, B: Donor parent. H: Hybrid

Figure 4.3 Genome introgression profile of 45 F_2 (K 343*^{3}/DHMAS × K 343*^{3}/ RML 22) pyramided plants using software Graphical GenoTypes (GGT 2.0) (Van Berloo, 1999)

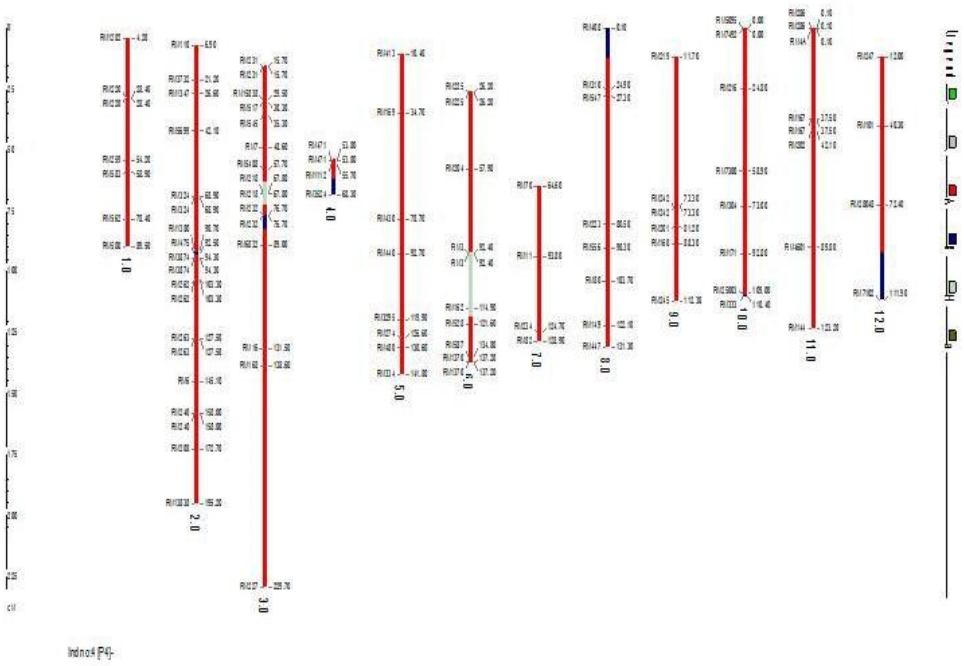


Figure 4.4 Chromosome wise recurrent parent genome recovery of plant P4; Red colour depicts recipient parent and Blue colour depicts donor parent

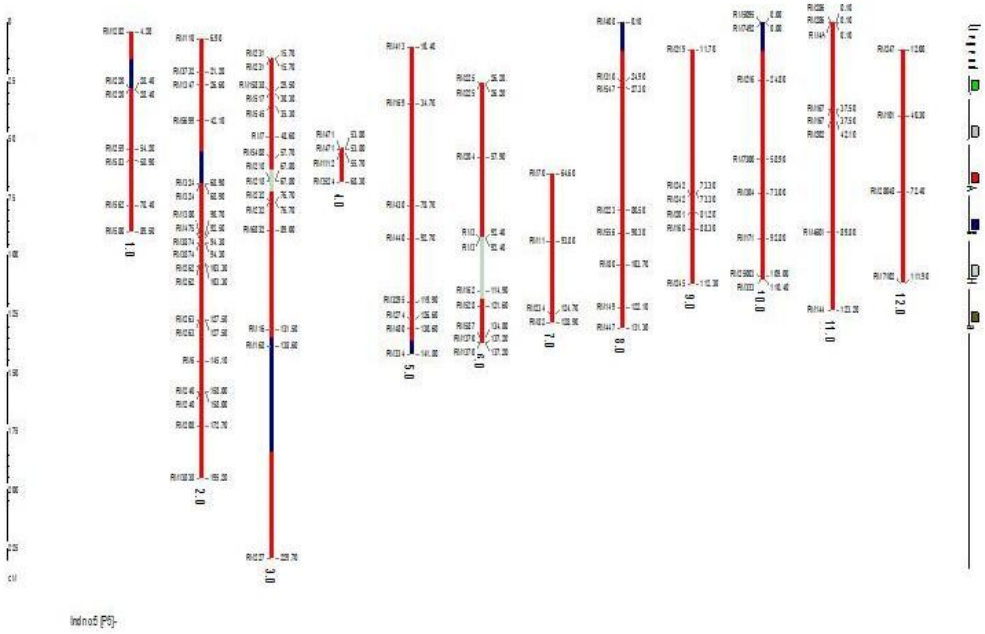


Figure 4.5 Chromosome wise recurrent parent genome recovery of plant P5; Red colour depicts recipient parent and Blue colour depicts donor parent

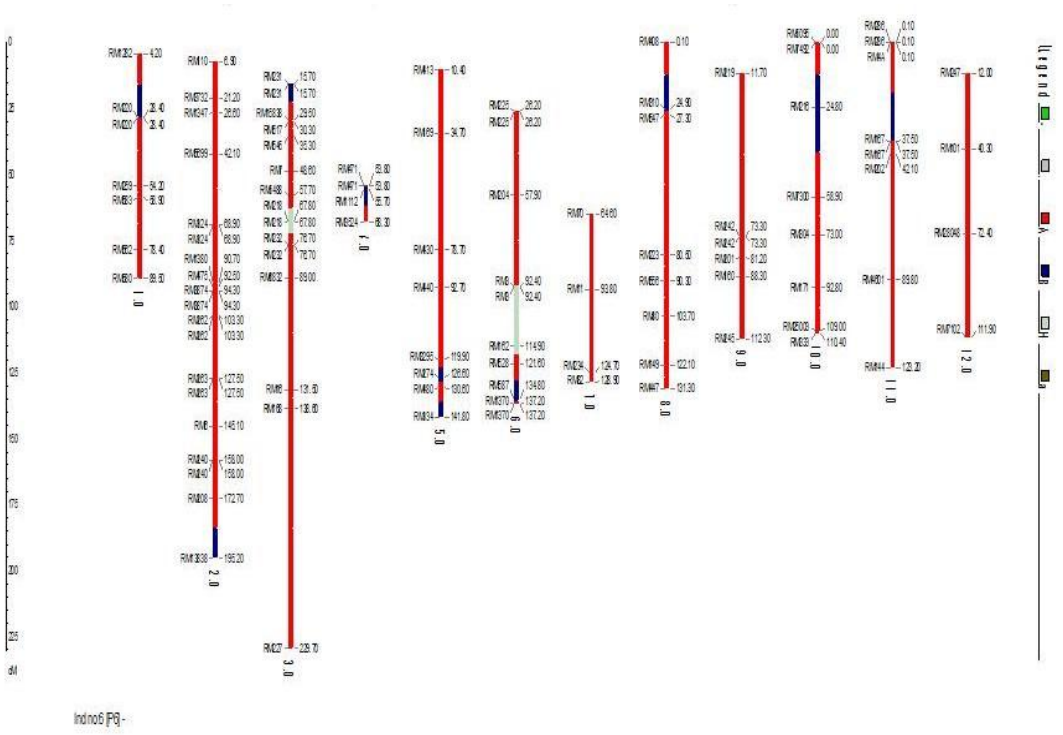


Figure 4.6 Chromosome wise recurrent parent genome recovery of plant P6; Red colour depicts recipient parent and Blue colour depicts donor parent

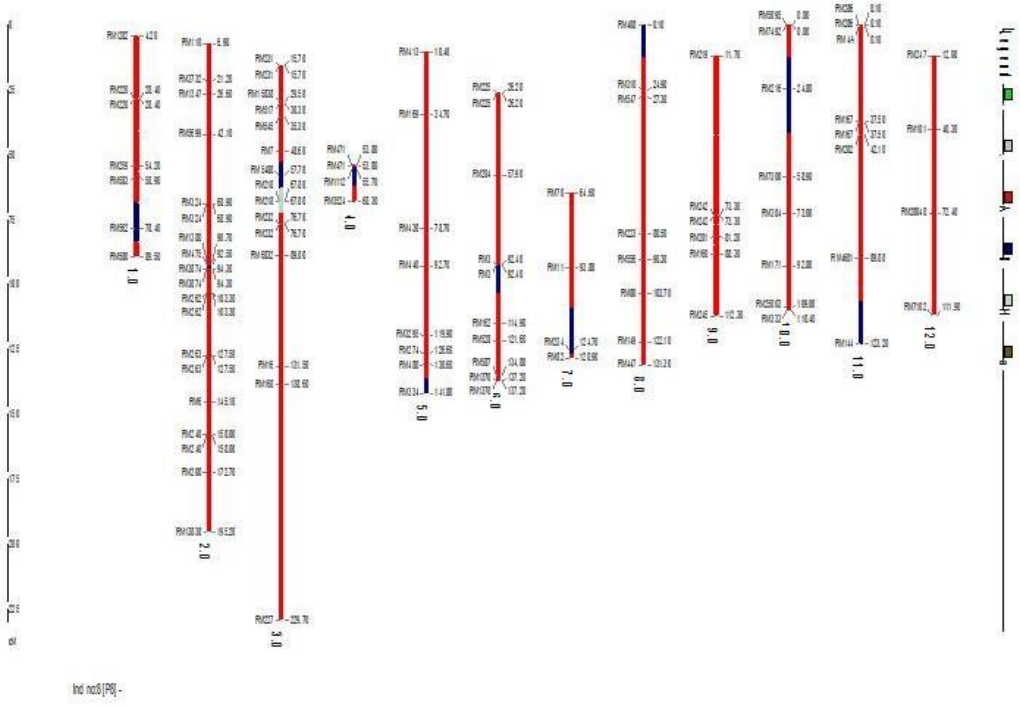


Figure 4.7 Chromosome wise recurrent parent genome recovery of plant P8; Red colour depicts recipient parent and Blue colour depicts donor parent

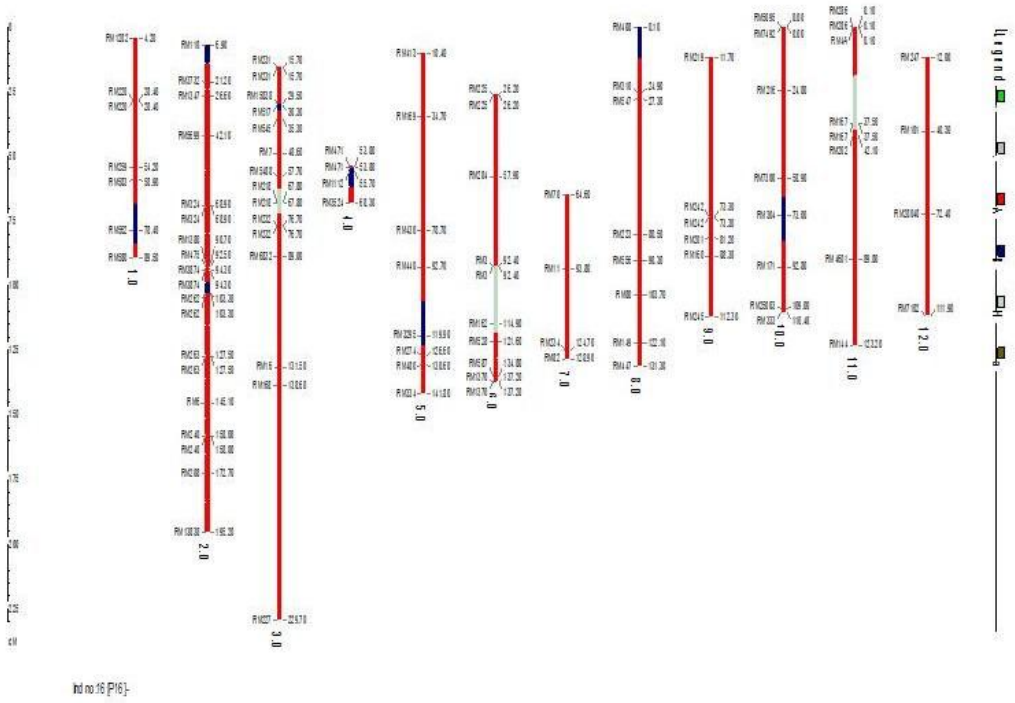


Figure 4.8 Chromosome wise recurrent parent genome recovery of plant P16; Red colour depicts recipient parent and Blue colour depicts donor parent

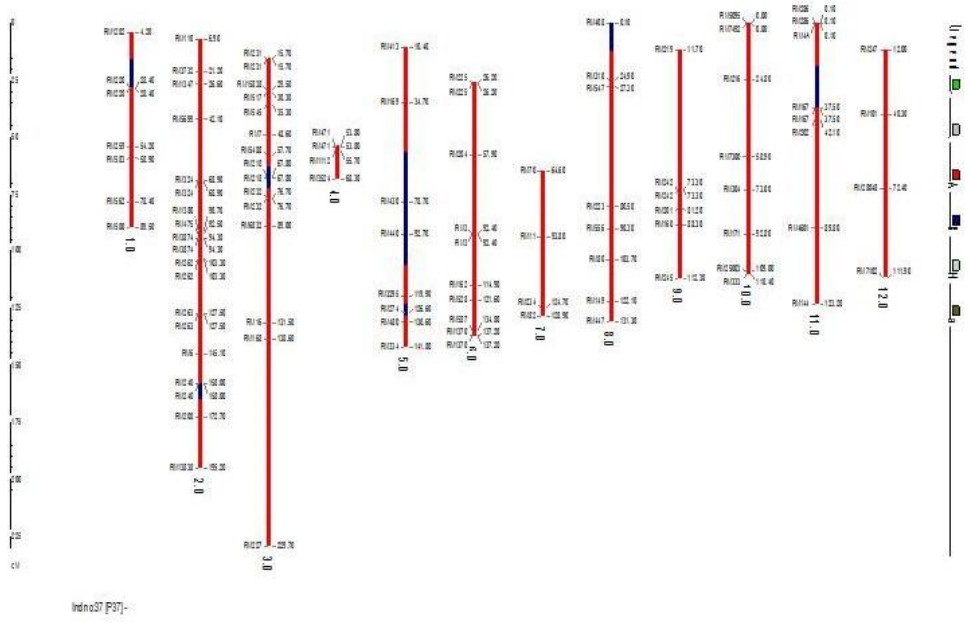


Figure 4.9 Chromosome wise recurrent parent genome recovery of plant P37; Red colour depicts recipient parent and Blue colour depicts donor parent

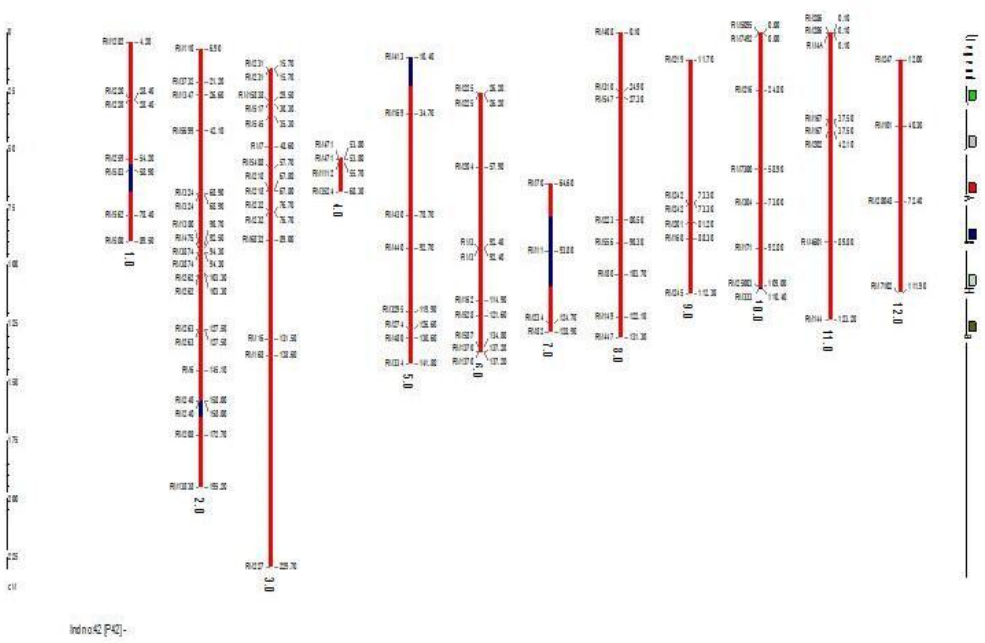


Figure 4.10 Chromosome wise recurrent parent genome recovery of plant P42; Red colour depicts recipient parent and Blue colour depicts donor parent

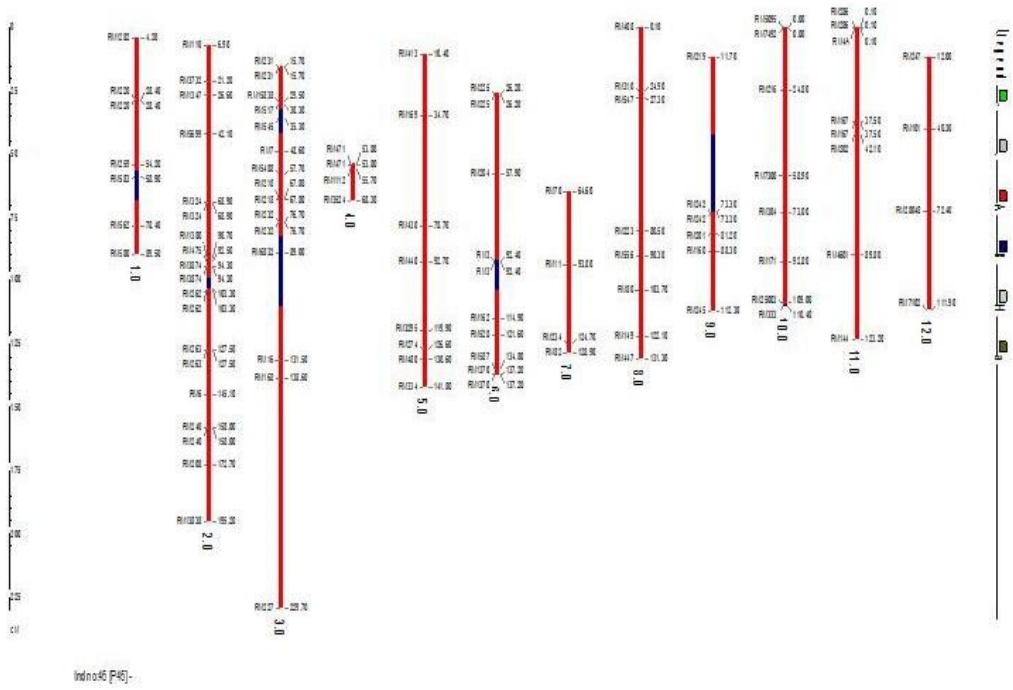


Figure 4.11 Chromosome wise recurrent parent genome recovery of plant; Red colour depicts recipient parent and Blue colour depicts donor parent

Table 4.14: Recurrent parent genome recovery in pyramided plants of F_2 convergent population (K 343*^{3}/DHMAS × K 343*^{3}/RML 22)

Plants/genotypes	A% (Recurrent parent genome)	B% (Donor parent genome
P1	56.00	43.90
P2	45.15	54.95
P3	49.05	50.95
P4	95.30	4.70
P5	91.10	8.90
P6	90.10	9.90
P7	60.40	39.60
P8	90.45	9.55
P9	52.25	47.75
P10	56.45	43.55
P11	66.05	34.05
P12	66.45	33.55
P13	60.35	39.65
P14	56.15	43.85
P15	69.10	30.92
P16	91.85	8.15
P17	72.75	27.45
P18	65.70	34.2
P19	66.50	33.50
P20	69.30	30.70
P21	72.00	26.90
P22	63.40	36.60
P23	64.90	35.10
P24	64.80	35.20
P25	57.70	42.30
P26	62.30	37.70
P27	60.40	39.60
P28	57.60	42.40
P29	59.30	40.70
P30	63.00	37.00
P31	89.00	11.00
P32	67.20	32.80
P33	67.30	32.70
P34	64.90	34.70
P35	55.90	44.10
P36	65.60	34.40
P37	91.60	8.40
P38	66.10	33.90
P39	76.50	23.50
P40	65.60	34.40
P41	87.40	12.60
P42	95.50	4.50
P43	65.70	34.30
P44	59.50	40.50
P45	93.00	7.00

Where,

A: Recurrent parent

B: Donor parent

4.7.2 Phenotyping for agro-morphological traits in of F_2 pyramided plants

F_2 population comprising of 45 plants along with parents i.e. K 343, DHMAS and RML 22 were evaluated for agronomic traits with augmented design-II. Single plant data were recorded and evaluated for agro-morphological traits such as plant height (PH), days to 50 percent flowering (DTF), days to maturity (DTM), duration of grain filling (DGF), panicle length (PL),number of effective tillers per plant (EF), grain yield per plant (GPP) and thousand grain weight (TGW). Individual plants were also analyzed for the grain dimension parameters *viz*., grain length (GL) and grain breadth (GB).

4.7.2.1 Analysis of Variance of gene positive F_2 pyramided plants

The analysis of variance (ANOVA) for yield and its component traits (Table 4.15.) indicated that the F_2 plants (K 343*^{3}/DHMAS/ K 343*^{3}/RML 22) showed highly significant variations for plant height, grain length and grain breadth while significant variation was observed for number of effective tillers per plant. Similarily, checks also depicted highly significant variation for most of the traits except days to 50% flowering, panicle length and 1000 grain weight.

The mean values observed for various traits in parent K 343 as depicted in Table 4.16 are plant height (130.40cm), days to 50 per cent flowering (93), days to maturity (128), duration of grain filling(35), panicle length (23.10cm), number of effective tillers per plant (10), grain length (5.87mm), grain breadth (2.52mm), yield per plant(27.20 g) and 1000- grain weight (28.00g). The mean values observed for various traits in parent DHMAS included plant height (129.2cm), days to 50 per cent flowering (91), days to maturity (125) duration of grain filling(34), panicle length (19.50cm), number of effective tillers per plant (9), grain length (5.44mm), grain breadth (2.47), yield per plant(25.80g), and 1000- grain weight (26.10g). Similarilly, the observed mean values for the parent RML 22 are plant height (127.5cm), days to 50 per cent flowering (87), days to maturity (120), duration of grain filling (33), panicle length (19.00cm), number of effective tillers per plant (9), grain length (5.21mm), grain breadth (2.21), yield per plant(23.00g), and 1000- grain weight (24.50g).

4.7.2.2 Observations recorded on F_2 plants (Table 4.16)

i. Plant height (cm)

The plant height ranged from 126.30- 139.10 cm. The maximum plant height was recorded in plants P4, P14, P24 and P39 (139.10 cm) followed by plants P2, P3, P5, P16 and P41 and (133.10cm) where as P22 recorded a minimum plant height i.e. 126.3cm. The average plant height for the population was observed as130.97 cm.

ii. Days to 50 percent flowering

Values for days to 50 percent flowering ranged between 89-94 with an average value of 91.16 days. The plants P18, P19, P20, P21, P22, P23, P24, P25 and P26 took maximum number of 94days for reaching 50 percent flowering where as where as the minimum number of days to 50 per cent flowering were recorded in large number of plants from P27 to P45 (89 days).

iii. Days to maturity

Days to maturity in the F_2 plants ranged from 128 to 131 days with an average of 129.61 days. Plants such P18, P19, P20, P21, P22, P23, P24, P25 and P26 took maximum days to mature (131 days) whereas remaining plants matured in 128 days.

iv. Duration of grain filling

Duration of grain filling ranged from 35-39 days with an average 36.45 days. Large number of plants matured in about 39 days (P27 to P45) whereas plants P8 to P17 took minimum duration of grain filling (35 days).

v. Panicle length (cm)

The panicle length varied from 18.6 to 26.50 cm with an average value of 21.80 cm. The maximum value for panicle length was recorded in case of P18 (26.5cm) followed by P3 (26.2cm), P22(25.00mcm) and P23(24.40 cm) where as minimum panicle length was observed in P42 (18.60cm).

vi. Number of effective tillers per plant

The number of effective tillers per plant ranged between 8- 13 with an average of 9.78. The maximum number of effective tillers per plant were recorded in P36 (13) followed by P24 (12), and minimum number of effective tillers was observed in P6 and P42.

vii. Grain length

The grain quality attribute like grain length showed an average value of 5.76 mm with a range varying from 5.00-6.90mm. The maximum value for grain length was recorded in P14 (6.90 mm) followed by P35 (6.30 mm), P5 (6.30mm), P34 (6.30) whereas the minimum grain length was observed in P26 (5.00 mm).

viii. Grain breadth

The grain breadth showed an average value of 2.49 mm with a range varying from 2.10- 2.70mm. In case of grain breadth, the maximum breadth of grains was recorded in plants P43 (2.70mm) and P23 (2.70mm) whereas the minimum grain breadth was recorded in P24 (2.10 mm).

ix. Grain yield per plant (g)

The grain yield per plant varied from 25.20 g to 29.10 g. The maximum grain yield was recorded in case of plants P8, P16, P29, P32 and P36 (29.10g) while minimum grain yield per plant (25.20g) was observed in plants P20, P25 and P40. The average grain yield per plant was recorded as 27.11g. (Table 4.18)

x. 1000- Grain weight (g)

The 1000-grain weight ranged from 23.60 g to 32.00 g with average of 27.44 g. The highest 1000- grain weight was observed in P30 (32.00 g) followed by P31 (30.40g), P30 (30.20g) and P28 (30.10g) and minimum 1000- grain weight was observed in P15 (23.60 g).

Table 4.15: Analysis of Variance of F_2 plants (K 343*^{3}/DHMAS/ K 343*^{3}/RML 22) for yield and yield contributing traits

Source of variation	DF	Plant height (cm)	Days to 50% flowering	Days to maturity	Duration of grain filling	Panicle length (cm)	Number of effective tillers per plant	Grain length (mm)	Grain breadth (mm)	Grain yield /plant (g)	1000 grain weight (g)
Mean sum squares											
Blocks	4	5.43**	36.24*	16.54	26.90**	3.85	1.32	0.33**	0.33**	0.28	19.94
Treatments	47	8.54**	3.35	7.46	4.04	4.29	1.21*	0.21**	0.21**	4.10	3.39
Test entries	45	6.96**	2.31	1.24	2.02	4.22**	1.16*	0.16**	0.16**	1.96	2.45
Checks	2	44.26**	26.88	147.57**	49.48**	5.98	2.54*	1.37**	1.37**	52.42**	24.57
Error	8	0.28	3.73	2.90	1.43	0.34	0.20	0.01	0.01	0.39	2.97

* - Significant at 5% ; **- Significant at 1%

p-Value < 0.05 - Significant at 5%, p-Value < 0.01 - Significant at 1%

Table 4.16: Mean performance of F_2 plants (K 343*[3]/DHMAS/ K 343*[3]/RML 22) **for yield and yield contributing traits**

Genotypes	Plant height(cm)	Days to 50% Flowering	Days to maturity	Duration of grain filling	Panicle length (cm)	No. of effective tillers / plant	Grain length (mm)	Grain breadth (mm)	Grain yield /plant (g)	1000 grain weight (g)
P1	**130.30**	**93**	**128**	**35**	**20.90**	**12**	**5.70**	**2.40**	**25.30**	**27.40**
P2	133.10	93	128	35	21.20	9	6.20	2.50	25.70	30.20
P3	133.10	93	128	35	26.20	10	6.20	2.50	26.00	28.20
P4	139.10	93	128	35	23.30	10	5.90	2.60	26.20	27.40
P5	133.10	93	128	35	22.90	10	6.30	2.60	26.00	28.10
P6	130.70	93	128	35	21.70	8	6.10	2.60	28.10	28.40
P7	130.30	93	128	35	21.20	9	6.10	2.40	28.30	28.30
P8	131.30	93	128	35	21.00	10	5.20	2.40	29.10	27.50
P9	130.70	93	128	35	21.60	12	5.10	2.50	28.10	28.50
P10	130.30	93	128	35	23.50	10	5.60	2.60	25.60	29.10
P11	**132.10**	**93**	**128**	**35**	**21.10**	**12**	**6.00**	**2.60**	**28.40**	**25.50**
P12	132.40	93	128	35	20.80	9	6.00	2.50	25.50	25.20
P13	131.70	93	128	35	24.30	10	5.20	2.50	29.10	24.30
P14	139.10	93	128	35	20.70	10	6.90	2.60	26.40	25.00
P15	130.00	93	128	35	24.30	10	5.20	2.30	26.90	23.60
P16	133.10	93	128	35	20.00	9	5.10	2.50	29.10	29.20
P17	130.70	93	128	35	21.20	9	5.20	2.40	25.50	25.00
P18	132.80	94	131	37	26.50	10	5.40	2.40	25.80	26.30
P19	129.80	94	131	37	19.80	10	5.30	2.40	28.8	27.80
P20	130.60	94	131	37	23.50	10	5.20	2.40	25.20	27.50
P21	**128.90**	**94**	**131**	**37**	**22.20**	**9**	**5.30**	**2.50**	**26.50**	**29.00**
P22	**126.30**	**94**	**131**	**37**	**25.00**	**10**	**5.50**	**2.30**	**27.00**	**26.40**

P23	130.90	94	131	37	24.40	9	5.90	2.70	26.30	26.50
P24	139.10	94	131	37	19.50	12	5.20	2.10	26.20	25.70
P25	131.30	94	131	37	22.90	9	5.50	2.60	25.20	28.10
P26	129.10	94	131	37	18.90	10	5.00	2.30	29.00	29.40
P27	130.10	89	128	39	21.30	10	5.90	2.40	28.10	28.00
P28	130.90	89	128	39	21.60	8	6.00	2.50	25.60	30.10
P29	129.80	89	128	39	22.90	10	6.10	2.40	29.10	32.00
P30	129.30	89	128	39	22.30	10	5.80	2.60	28.50	30.20
P31	**130.80**	**89**	**128**	**39**	**19.60**	**9**	**6.10**	**2.60**	**26.20**	**30.40**
P32	132.80	89	128	39	22.00	10	5.80	2.40	29.10	28.60
P33	130.60	89	128	39	18.90	9	6.00	2.40	25.60	29.40
P34	130.30	89	128	39	19.30	10	6.30	2.50	28.00	27.30
P35	130.70	89	128	39	21.40	10	6.30	2.40	27.50	30.20
P36	131.30	89	128	39	22.90	13	6.10	2.50	29.10	27.60
P37	129.90	89	128	39	20.60	12	6.00	2.60	27.20	28.10
P38	130.90	89	128	39	20.00	12	6.00	2.60	28.10	29.40
P39	139.10	89	128	39	22.70	10	6.10	2.40	26.20	29.00
P40	130.30	89	128	39	21.30	10	6.20	2.40	25.20	30.10
P41	133.10	89	128	39	19.80	10	5.30	2.50	28.30	29.20
P42	130.10	89	128	39	18.60	8	5.90	2.50	27.00	27.60
P43	**131.30**	**89**	**128**	**39**	**19.30**	**9**	**6.20**	**2.70**	**26.40**	**28.70**
P44	128.70	89	128	39	22.60	9	5.90	2.60	27.10	25.20
P45	131.30	89	128	39	21.60	10	5.90	2.60	28.20	25.50
K 343 (C)	130.40	93	128	35	23.10	10	5.87	2.52	27.20	28.00
DHMAS (C)	129.20	91	125	34	19.50	9	5.44	2.47	25.80	26.10
RML 22 (C)	127.50	87	120	33	19.00	9	5.21	2.21	23.00	24.50

Mean	130.97	91.16	129.61	36.45	21.8	9.78	5.76	2.49	27.11	27.44
CD(checks)	0.78	2.818	2.48	1.75	0.85	0.65	0.17	0.09	0.91	2.51
CD(tests)	1.55	5.63	4.97	3.49	1.71	1.30	0.33	0.19	1.89	5.03
CV	0.40	2.11	1.32	3.23	2.69	4.50	1.96	2.51	2.30	6.18
SE(m)	0.08	0.28	0.25	0.17	0.08	0.06	0.02	0.01	0.09	0.25

4.7.3 Pathotyping of F_2 population (K 343*^{3} /DHMAS× K 343*^{3} /RML 22)

All the 45 F_2 plants selected through MAS (foreground and background selection) were screened for blast symptoms both under natural and artificial conditions (Plate 4.36). They were inoculated with PLP-1 strain using spray method under standard conditions (Bonman *et al.,* 1986, Sharma, 2005b). Disease reactions of inoculated plants were recorded on a scale of 0–5 (Bonman *et al*., 1986). All the 45 plants showed 0-1 score depicting highly resistant to resistant reaction while the recipient parent K 343 showed susceptible reaction. (Table 4.17 and Plates 4.36)

Table 4.17: Pathotyping of F_2 (K 343*^{3}/DHMAS/ K 343*^{3}/RML 22) plants for blast symptoms

S. No.	Genotype	Score	Disease reaction
1	K 343	3	Susceptible
2	RML 22	0	Highly resistant
3	DHMAS	0	Highly resistant
4	P1	0	Highly resistant
5	P2	1	Resistant
6	P3	0	Highly resistant
7	P4	0	Highly resistant
8	P5	0	Highly resistant
9	P6	0	Highly resistant
10	P7	0	Highly resistant
11	P8	0	Highly resistant
12	P9	1	Resistant
13	P10	0	Highly resistant
14	P11	0	Highly resistant
15	P12	0	Highly resistant
16	P13	0	Highly resistant
17	P14	1	Resistant
18	P15	0	Highly resistant
19	P16	0	Highly resistant
20	P17	0	Highly resistant
21	P18	0	Highly resistant
22	P19	1	Resistant
23	P20	0	Highly resistant

24	P21	0	Highly resistant
25	P22	0	Highly resistant
26	P23	0	Highly Resistant
27	P24	0	Highly resistant
28	P25	0	Highly resistant
29	P26	0	Highly resistant
30	P27	1	Resistant
31	P28	0	Highly resistant
32	P29	0	Highly resistant
33	P30	0	Highly Resistant
34	P31	0	Highly Resistant
35	P32	0	Highly Resistant
36	P33	0	Highly Reistant
37	P34	1	Resistant
38	P35	0	Highly Resistant
39	P36	0	Highly Resistant
40	P37	0	Highly Resistant
41	P38	0	Highly Resistant
42	P39	0	Highly Resistant
43	P40	1	Resistant
44	P41	0	Highly Resistant
45	P42	0	Highly Resistant

4.7.4 Agronomical and pathological status of F_2 plants (K 343*^{3}/DHMAS × K 343*^{3}/RML 22) with maximum recurrent parent genome recovery

The 45 pyramided F_2 plants of **(**K 343*^{3}/DHMAS×K 343*^{3}/RML 22) with maximum recovery of recurrent parent genome were compared agronomically and pathologically with the recurrent parent Table 4.18. The maximum recovered recurrent parent genome in plant numbers P4, P5, P6, P8, P16, P37, P42 and P45 had broader agronomical similarity to the recurrent parent and pathologically related to the donor parent. All the plants showed highly resistant reaction to blast disease.

DHMAS (P)
Resistant

RML 22 (P)
Resistant

F_2 Pyramided plants
Highly resistant

Blast infected leaves of susceptible parerent K 343

Plate 4.36: Typical symptoms of blast as seen after 30 days of inoculation in experimental field

Table 4.18: Agronomical and pathological status of pyramided plants (K 343*^{3}/DHMAS / K 343*^{3}/RML 22) with maximum RPG recovery

Gene positive plants (*Pi54+Pi9*)	**DHMAS**	**RML 22**	**K 343**	**P4**	**P5**	**P6**	P8	P16	P37	P42	P45
RPG (%)	0	0	100	95.30	91.10	90.10	90.45	91.85	91.60	95.50	93.00
Disease score	0	0	3	0	0	0	0	0	0	0	0
Plant height (cm)	129.20	127.50	130.4	139.10	133.10	130.70	131.30	133.1	129.9	130.10	131.30
Days to 50 percent flowering	91	87	93	93	93	93	93	93	89	89	89
Days to maturity	125	120	128	128	128	128	128	128	128	128	128
Duration of grain filling	34	33	35	35	35	35	35	35	39	39	39
Panicle length (cm)	19.50	19.00	23.10	23.30	22.90	21.70	21.00	20	20.60	18.60	21.60
Effective tillers	9	9	10	10	10	8	10	9	12	8	10
Grain length (mm)	5.44	5.21	5.87	5.90	6.30	6.10	5.20	5.1	6.00	5.90	5.90
Grain breadth (mm)	2.47	2.21	2.52	2.60	2.60	2.60	2.40	2.5	2.60	2.50	2.60
Yield per plant (g)	25.80	23.00	27.20	26.20	26.00	28.10	29.10	29.1	27.20	27.00	28.20
1000 grain weight (g)	26.10	24.50	28.00	27.40	28.10	28.40	27.50	29.2	28.10	27.60	25.50

4.7.5 Quality trait analysis of gene positive plants of F_2 convergent population

4.7.5.1 Amylose content (%)

All the 08 pyramided F_2 plants found positive for both the genes *Pi9* and *Pi54* with high RPG (%) along with recepient parent K 343 were analysed for amylose and protein content (%). Plant P37 showed the maximum amylose content of 19.83 percent followed by P4 (17.45%), P42 (16.32%) and P8 (15.01%) whereas the minimum value of 11.27 percent was observed in P5 (Table 4.19).

Table 4.19: Amylose content of pyramided plants (K 343*^{3}/DHMAS / K 343*^{3}/RML 22) with maximum RPG recovery

Samples	Amylose content (%)
K 343	11.79 (low amylose content)
P4	17.45 (low amylose content)
P5	11.27 (low amylose content)
P6	12.97 (low amylose content)
P8	15.01 (low amylose content)
P16	12.53 (low amylose content)
P37	19.83 (low amylose content)
P42	16.32 (low amylase content)
P45	13.48 (low amylose content)

4.7.5.2 Protein content (%)

The plant P42 showed the maximum protein content of 7.5 percent followed by P4 (7.30%), P45 (7.20%), P6 (7.00%) and the minimum value of 5.50 was observed in P37 (Table 4.20)

Table 4.20: Protein content of pyramided plants (K 343*^{3}/DHMAS / K 343*^{3}/RML 22) with maximum RPG recovery

Samples	Protein content (%)
K 343	7.00
P4	7.30
P5	5.20
P6	7.00
P8	6.10
P16	5.80
P37	5.50
P42	7.50
P45	7.20

Chapter-5

Discussion

CHAPTER-5

DISCUSSION

Rice is an important staple food crop, which forms a major part of the human diet and a good source of carbohydrate for over half of the world's human population. Asia alone produces 90 percent of the world's produced rice. In Jammu and Kashmir, rice plays an important role as it is a staple food for the majority of population. In order to meet the demand of the ever growing population, there is a constant need to increase the production. So, one of the important strategies is to develop the rice varieties for major biotic and abiotic stresses. Rice blast caused by the fungus *M. oryzae* is one of the most disastrous disease which occurs to rice and is a major threat to rice production leading to significant yield losses (Li *et al.*, 2007; Khush and Jena, 2009; Skamnioti and Gurr, 2009 and Helliwell *et al*., 2013; Kulkarni and Peshwe, 2019).

Rice blast is considered as the major disease of rice because of its wide distribution and extent of destruction under favorable conditions. In Jammu and Kashmir, it is the most devastating disease in hill and temperate ecologies where rice is grown in hundred percent irrigated and cool night ecology of *Kharif* season (Ali *et al*., 2009) which aids in blast build up and subsequently widely occurring blast epidemics in rice in the Union territory. Most of the rice varieties cultivated in hills of Jammu and Kashmir shows variable reaction from moderately resistant to highly susceptible response depending on prevailing weather conditions (Anwar *et al*., 2003; Anwar *et al*., 2009; Anwar *et al*., 2011). A major challenge before plant breeders is to accumulate genes for resistances to pests and diseases (Stam *et al*., 2014; Wiesner-Hanks and Nelson, 2016) to develop resistant varieties. Although chemical control of the disease is feasible, it remains economically impractical for resource poor farmers and is also environmentally undesirable. Deployment of single resistance gene, by transgenics as well as marker-assisted backcross breeding has been the most commonly followed approach for the management of various plant diseases including rice blast. However, this approach has failed to deliver durable resistance as these R genes are continuously subjected to pressure imposed by fast evolving pathogen effector genes and leading to the breakdown of resistance response (Sprague *et al*., 2006; Singh *et al.*, 2014). Therefore, stacking of multiple genes and their alleles has

been widely used to overcome this limitation. Pradhan *et al.* (2015) reported that pyramiding of multi-resistance genes into single line often confers wider spectrum of resistance and durability. Pyramiding entails stacking multiple genes leading to simultaneous expression of more than one gene in a variety to develop durable resistance expression. The use of DNA markers, which permits gene identification of progeny at each generation, increases the speed of pyramiding process (Joshi and Nayak, 2008). Gene pyramiding holds greater prospects to attain durable resistance against biotic and abiotic stresses in crops; and is gaining considerable importance as it would improve the efficiency of plant breeding leading to the development of genetic stocks and precise development of broad spectrum resistance capabilities.

The susceptibility of the elite rice variety, K 343, to blast disease is a major factor offsetting its overwhelming performance. The marker assisted backcross breeding approach coupled with phenotypic selection helped in improving the elite genotype. The polymorphic markers between the parents play a crucial role in any marker assisted backcross breeding programme. In the present study, selection and use of SSR markers that were evenly distributed on chromosomes was able to detect the percentage of recurrent parent genome recovered in any particular backcross progeny and helped in reducing the donor genome and the number of generations essential to develop the lines with target genes.

Marker assisted foreground selection has been used to track the genes (*Pi54* and *Pi9*) in subsequent backcross and convergent populations. Closely linked SSR markers validated in previous study (Hangloo, 2018) have been used for foreground selection. A total of 101 genome wide polymorphic SSR markers have been used for background screening of gene positive plants for determining the plants with maximum recurrent parent genome recovery (RPG).

The results of the study are discussed in detail in this chapter to draw inferences about significance of the findings and their implications in crop improvement. Genomic DNA isolation, purification and quantification carried out by following standard techniques led to availability of good quality DNA which could be used for carrying out genotyping studies. However, looking at the variations in the quality and quantity of extracted genomic DNA samples, slight changes in methodology for extraction and purification were made to further improve the quality and quantity of extracted genomic DNA. Similar, modifications in rice genomic DNA isolation protocols have been made earlier by Fjellstrom *et al*. (2006), Xu *et al.* (2008)

and Kumar *et al*. (2010).

Foreground selection in BC_2F_1 stocks

BC_1F_1 plants were backcrossed with the recurrent parents K 343 to develop BC_2F_1 population. Backcrossing is done to further increase the recovery of recurrent parent genome in upcoming generations while the foreground selection is done to track a particular trait like disease resistance using marker assisted selection (MAS). In foreground selection homozygous plants for target genes were selected as they do not segregate during crossing over in the process of recombination and hence are stable. BC_2F_1 population was subjected to foreground selection to track the presence of *Pi54* or *Pi9* genes and to ensure that they are not lost during the process of recombination.

Foreground selection of *Pi54* and *Pi9* in BC_2F_1 populations using SSR markers RM206 and AP5930, respectively led to the identification of 30 gene positive plants in each of the two genetic stocks (K 343*^{3}/DHMAS and K 343*^{3}/RML 22). The BC_2F_1 plants confirmed positive for the gene *Pi54* or *Pi9* were subjected to background selection to identify the plants with maximum percentage of recurrent parent genome. Foreground selection is often followed by recombinant selection process to select for recurrent parent alleles at markers flanking target regions with the aim of reducing linkage drag. Similar studies have carried out earlier by Singh *et al*. (2012a); Patroti *et al*. (2019) and Sagar *et al*. (2020)

Background selection in BC_2F_1 stocks

Background selection is the process of using markers to minimize the length of the donor segment around a target locus to accelerate the recovery of recurrent parent genome during backcrossing. Background selection in target gene (*Pi54* or *Pi9*) positive plants in each of the genetic stock (K 343*^{3}/DHMAS and K 343*^{3}/RML 22) led to estimation of percent recurrent parent genome recovery using genome wide polymorphic SSR markers. Genotypic data when analyzed using GGT 2.0 software (Van Berloo, 1999) identified 3 plants (P1=86.4%, P17= 83.65% and P3= 83.40) which had recurrent parent genome recovery more than 83 percent in the genetic stock K 343*^{3}/ DHMAS with chromosomes 1 and 2 showing more than 90 percent recovery in most of the plants. While in the genetic stock K 343*^{3}/ RML 22 three plants had recurrent parent genome recovery more than 85 percent. They were identified as P3 (86.4%), P11 (85.8%) and P28 (93.25%) with chromosomes 1, 2 and 10 showing 85-90 percent of recovery in most of the plants in the stock population. Such plants in

both the genetic stocks could be identified as potential genetic stocks for rice blast resistance; along with superior agronomical traits and reaction to predominant races of blast fungus *M. oryzae*. Thus marker assisted background selection is a potential tool to identify the plants among the large population having more than average recurrent parent genome recovery and thus accelerates the pace of selection and development of varieties in comparison to conventional breeding approaches of selection. Integration of foreground, background and /or phenotypic selection to achieve high recovery of recurrent parent genome and phenome has been practiced in various studies (Neeraja *et al.,* 2005; Sundaram *et al.,* 2008; Gopala Krishnan *et al.,* 2008; Singh *et al.,* 2012a; Divya *et al.,* 2014; Miah *et al.,* 2014; Patroti *et al*., 2019; Sagar *et al*., 2020.

Analysis of variance for morphological/agronomical traits in both BC_2F_1 populations exhibited non-significant variations for most of the agro-morphological traits except for plant height, number of effective tillers and grain length which gave indication about uniformity of traits in genetic stocks. A closer look at agronomical traits recorded in the study depicted that genetic stocks identified as positive for target genes and having more than 80 percent recovery of recurrent parent genome in both BC_2F_1 populations had broader similarity with recurrent parent genome i.e. K 343.

Screening of the backcross populations (K 343*^{3}/ DHMAS and (K 343*^{3}/ RML 22) with PLP-1 depicted variable reactions under controlled conditions ranging from resistant to highly resistant reaction which indicates the nature of strong resistance provided individually by *Pi54* and *Pi9* genes to the prevalent predomination strain of blast fungus. It revalidates the findings of Sharma *et al.* (2005a) and Rathour *et al.* (2008). These genetic stocks served as potential individual plants for inter-stock crossing to produce F_1 complex and subsequently F_2 convergent population for identifying pyramided plants for the target genes i.e. *Pi54* and *Pi9*.

Foreground selection in F_2 convergent population

Phenotypic identification of rice blast resistance genes is difficult as their expression is variable in different environments and locations. Additionally, it is difficult to pyramid multiple R genes through conventional breeding strategy in case when the resistance reactions of one R gene could be masked by other R genes (Koide *et al*. 2010). The both disadvantages can be overcome through MAS for its efficiency and effectiveness (Mi *et al*., 2018). Based on the MAS approach, several advanced breeding lines/varieties for blast resistance have been developed successfully (Khanna et *al*., 2015; Ellur *et al*., 2016). In the present study, the BC_2F_1 plants in the genetic

stock K 343*^{3}/ DHMAS confirmed positive for *Pi54* gene and having more than 83 percent recovery of recurrent parent genome (P1, P17, P3) and in genetic stock K 343*^{3}/ RML 22 confirmed positive for the gene *Pi9* gene with more than 85 percent recovery of recurrent parent genome (P3, P11 and P28) were intercrossed to generate F_1complex population, which normally is not suitable for selection process as most of the loci are in heterozygous state and have to undergo segregation in next generation i.e. F_2 convergent population which is expected to have more number of homozygous loci and therefore considered suitable for selection including foreground selection, background selection, phenotyping and pathotyping.

Foreground selection for *Pi54* and *Pi9* gene in F_2 convergent population (K 343*^{3}/DHMAS X K 343*^{3}/RML 22) comprising of 4000 plants with SSR markers RM206 and AP5930 via multiplex PCR could identify 45 plants having both the genes *Pi9* and *Pi54* i.e. pyramided plants which were further subjected to background screening. This probability of getting two pyramided genes in same stock is similar to the theoretically calculated ratio keeping in view the distance of markers from both the genes and population size. The screening for both the gene combinations in F_2 plants involved large quantum of work. Foreground selection for target genes in every generation is an essential step to ensure that target gene(s) are not lost in the process of recombination due to crossing over as well as under selection pressure. It also sometimes serves as substitute to artificial inoculations for selection of resistant plants, specifically when target gene under transfer imparts resistance to a particular disease. This type of selection permits the direct selection of genes that control the disease resistance phenotype providing an alternative to overcome the limitations of conventional breeding (Jena and Mackill, 2008; Hospital, 2009; Mi *et al*., 2018)

Khan *et al*. (2018) used foreground selection using markers for transfer of target genes, which is more practical and economical but precise and accurate transfer of target genes relies mainly on the gene based markers. In other studies closely linked markers were used for foreground selection of genes (Septiningsih *et al*., 2009; Ramkumar *et al*., 2011; Madhavi *et al*., 2016; Usatov *et al*., 2016).

Background selection in F_2 convergent population

Background selection for the estimation of percent genome recovery was carried out on identified 45 target genes pyramided lines using 101 genome wide polymorphic SSR markers. Genotypic data were subjected to analysis by GGT 2.0 software (Van Berloo, 1999). Analysis of graphical representation and statistical data

of whole genome led to identification of 8 plants in the F_2 convergent population i,e. P4 (95.30), P5 (91.10), P6 (90.10), P8 (90.45), P16 (91.85%), P37 (91.60%), P42 (95.50%), and P45 (93.00 %) which had recurrent parent (K 343) genome recovery more than 90% with chromosome 1, 2, 3, 9, 11 and 12 showing more than 95 percent recovery in most of the plants in the population. Thus, marker assisted background selection is a potential tool to identify the plants among the large population having more than average recovery of recurrent parent genome and thus accelerates the pace of selection and development of varieties in comparison to conventional breeding approaches of selection. The extent of recurrent parent genome recovery in the identified F_2 plants was up to 95.50 percent. Such a high recovery can be attributed to efficient use of markers employed from BC_1F_1 to F_2 convergent populations. This technique has enhanced the accuracy of selecting individual plants with high RPG. Similar results have been found by Sagar Krishnamurthy *et al*. (2017) who obtained RPG recovery of 90.27 percent in *Pi2* introgressed lines in the genetic background of BPT-5204 (Krishnamurthy *et al*., 2017). Khan *et al.* (2018) introgressed *Pi54*, *Pi1*, and *Pita* genes in the genetic background of landrace Mushk bhudji with a maximum RPG recovery up to 92 percent.

Analysis of variance for morphological/agronomical traits in F_2 convergent population exhibited highly significant variations for plant height, grain length and grain breadth while significant variation was observed for number of effective tillers per plant. The identified 8 pyramided plants with high RPG recovery were critically examined for agro-morphological traits as well as disease reaction. Screening of F_2 convergent population with PLP-1 strains of *M. oryzae* under controlled conditions depicted variable reaction response of the individual plants ranging from resistant to highly resistant. However, plants found positive for both the genes i.e. pyramided plants having more than 90 percent recurrent parent genome recovery showed highly resistant reaction response. It depicted the effectiveness of *Pi9* and *Pi54* genes to together counter the predominated fungal strains of *M. oryzare.* The strong nature and pyramiding effect of both the *Pi9* and *Pi54* genes has been validated through the present study.

A critical examination of agro-morphological traits recorded in the present study on 8 pyramided lines (for target genes with more than 90 percent recovery of recurrent parent genome) with parents (K 343, DHMAS and RML 22) depicted broader similarity with recurrent parent i,e. K 343. However, they were in contrast to

recurrent parent with respect to disease reaction. These identified lines also had yield attributes either at par or better over the recurrent parent. The identified lines showed low amylose and moderate protein content. In contrast to these results some reports indicated that pyramiding R genes into a single line may have fitness costs (Deng *et al*., 2017) such as lower yield. Earlier studies have reported that the yield mainly depends on the productive tillers and number of filled grains per panicle (Deshmukh *et al*., 2010). The increase in productivity leads to an increase in panicle number and thereby higher grain yield per plant (Efisue *et al*., 2014). The result strongly support that phenotypic selection practice was efficient which resulted in the identification and improvement of pyramided plants.

Stringent phenotypic selection is the key in any backcross breeding programme, i.e. selection of plants in every generation which resemble the recurrent parent along with genotypic information was performed successfully in every generation of backcrossing and selfing in the present study. Pyramided plants when compared to non-pyramided plants or plants with single resistance gene (*Pi54* or *Pi9*) of previous generations have shown more effective and enhanced response to disease reactions i.e. highly resistant response. Similar results has been reported by Orasen *et al*. (2020) depicting that the pyramided lines showed almost complete resistance to blast both under artificial selection and natural infection in the field. The grain samples of identified pyramided plants were also analyzed for quality attributes i.e. amylose and protein content. They showed low amylose content (ranging from 11.27 to 19.83 %) and moderate protein content (5.20 to 7.30 %).

Thus, 8 lines namely P4, P5, P6, P8, P16, P37, P42 and P45 have been identified as pyramided plants for blast resistance in the genetic background of temperate rice variety K 343. All the pyramided plants developed through the present study showed broader spectrum of blast resistance as compared to recurrent parent under both artificial and field conditions. The results confirmed the accuracy of marker assisted selection (MAS) for the two genes *Pi54* and *Pi9* using the corresponding markers RM206 and AP5930, respectively. These pyramided plants can be further used as genetic stocks for identification of blast resistance varieties or can be used as donors of genes *Pi9* and *Pi54* in breeding programmes for blast resistance. Thus, these improved pyramided plants having blast resistance are the better choice than the recurrent parent K 343. In earlier studies the backcross derived lines have shown a high level of resistance with best agro-morphological performance (Steele *et*

al., 2006; Tanweer *et al*., 2015). Similar work of gene pyramiding in rice has been done by Kumari *et al*. (2017). They performed stacking of two blast resistance genes *Pi54* and *Pi54*^{rh} in rice against *M. oryzae* via co-transformation. The results in the present study have been revalidated by Patroti *et al*. (2019). They introgressed three major blast resistance genes viz *Pi1*, *Pi2*, and *Pi54* through marker assisted selection. Orasen *et al*. (2020) have done similar work. They pyramided *Pib, Piz, Pik, Pita2* and *Piz-t* genes into temperate *Japonica* materials. Two lines SJKK and SJKT-2 were produced, that have each four pyramided genes. They were fully resistant to blast when tested in field and green house. On similar pattern Chen et *al*. (2020) developed photo-thermo sensitive genic male sterile (PTGMS) rice with resistance to both rice blast and brown plant hopper (BPH). Four broad spectrum blast resistance genes *Pi9*, *Pi47*, *Pi48* and *Pi49* and two BPH resistance genes *Bph14* and *Bph15* have been introgressed into a PTGMS line C815S through backcrossing and gene pyramiding coupled with molecular marker assisted selection (MAS).

MAS along with pyramiding facilitates the introgression of major multiple and desirable genes into a single genotype to obtain the desired trait, which not only is a sustainable and economical way to enhance yield and increase resistance but also enhances the level and spectrum of the resistance in rice against blast. Modern molecular methods, hence, efficiently supplement the conventional breeding methods to identify the novel resistance genes and their introgression into elite genetic backgrounds to improve the modern cultivars.

Chapter-6

Summary and Conclusion

CHAPTER-6

SUMMARY AND CONCLUSIONS

In order to meet the needs of ever-growing population, developing rice varieties for resistance to biotic and abiotic stresses constitutes one of the major research strategies. Rice blast caused by *Magnaporthe oryzae*, a filamentous fungus, poses a serious threat to the world food security and causes significant yield losses. Despite almost 100 years of dedicated efforts into the study of its genetics, rice blast continues to be the most destructive disease of rice. Therefore, the most effective way of management of this pathogen is deployment of host-plant resistance, which proved to be the most economical and environment friendly option for managing the disease. The present investigation entitled "Molecular marker assisted pyramiding of *Pi9* and *Pi54* blast resistance genes in rice cultivar K 343" was carried out at School of Biotechnology, SKUAST-Jammu from 2016-2019 to pyramid blast resistance genes in the genetic background of rice variety K 343; to validate the introgressed genes in the target background using SSR markers; and to evaluate the pyramided lines for different traits to identify superior genotypes. K 343 was used as recipient parent which is an elite *Indica* rice cultivar while DHMAs and RML 22 were used as donor parents for *Pi54* and *Pi9* genes, respectively. For *Pi54* gene closely linked SSR marker RM 206 (0.7 cM from locus) was selected for foreground selection based on previous initial studies. Similarly, for *Pi9* gene closely linked SSR markers AP5930 (0.05 cM from locus) was selected for foreground selection. A total of 101 genome wide polymorphic SSR markers, 50 for the parental pair K 343 and DHMAS and 51 for the parental pair K 343 and RML 22 were selected and used for background selection to find out the percent recovery of recurrent parent genome using GGT 2.0 software. The BC_2F_1 and F_2 convergent population were evaluated for superior agronomic traits as per DUS guidelines (Rani *et al*., 2006) and data were analysed using R-software for augumented design II. Reaction to blast disease (Bonman *et al*., 1986) was studied under both field and green house conditions. For screening in green house, pure conidial suspension of PLP-1 strain of *M. oryzae* was sprayed under controlled conditions.

Foreground selection of *Pi54* gene in BC_2F_1 (K 343*3/DHMAS) population with marker RM206 and *Pi9* gene in BC_2F_1 (K 343*3/RML 22) population with

marker AP5930 identified 30 target gene positive plants in each of the two stocks. Background selection for analysis of recurrent parent genome in target gene positive plant using genome wide polymorphic SSR markers led to identification of three plants (P1, P3, P17) in BC_2F_1 (K 343*^{3}/DHMAS) population and three plants (P3, P11, P28) in BC_2F_1 (K 343*^{3}/RML 22) which had recurrent parent genome recovery more than 83 percent. These plants exhibited broader similarity with recurrent parent with respect to agro-morphological traits. Screening of BC_2F_1 population with PLP-1 strain of rice blast fungus *M. oryzae* under controlled conditions both in field and green house, depicted that plants identified for the target genes in both the stocks (K 343*^{3}/DHMAS & K 343*^{3}/ RML 22) and having high recovery of recurrent parent genome (RPG) showed resistant to highly resistant reaction. It indicates the effectiveness of resistance provided by *Pi54* or *Pi9* genes to the fungal isolate (PLP-1).

Simultaneous foreground selection of *Pi54* and *Pi9* genes done via multiplex PCR in F_2 convergent population comprising of 4000 plants with marker RM206 and AP5930 depicted 45 plants positive for both *Pi54* and *Pi9* genes. Background selection of gene positive plants for analysis of percent recovery of recurrent parent genome using SSR markers led to the identification of 8 pyramided plants (P4, P5, P6, P8, P16, P37, P42 and P45) which had recurrent parent (K 343) genome recovery more than 90 percent. Agronomical traits recorded in the study depicted that the identified pyramided plants had broader similarity with recurrent parent genome. Screening of F_2 convergent population (K 343*^{3}/DHMAS X K 343*^{3}/RML 22) with PLP-1 strain of rice blast fungus *Magnaporthe oryzae* under controlled conditions depicted that genetic stocks identified as positive for target genes and having more than 90 percent recovery of recurrent parent genome (RPG) in F_2 convergent population showed highly resistant reaction. It indicates the strong resistance nature of *Pi54* and *Pi9* genes together to the predominant fungal strain of *Magnaporthe oryzae.* The identified lines showed low amylose and moderate protein content.

Salient conclusions drawn from the study are:

- A total of 8 lines namely P4, P5, P6, P8, P16, P37, P42 and P45 have been identified as pyramided plants for blast resistance in the genetic background of temperate rice variety K 343.
- The 8 identified lines had 90.00 to 95.50 percent recovery of recurrent parent

genome which reflected in terms of agro-morphological and quality attributes

- All the pyramided plants developed through the present study showed broader spectrum of blast resistance as compared to recurrent parent under both artificial and field conditions.
- These improved pyramided plants having blast resistance are the better choice than the recurrent parent K 343.
- These pyramided plants can be further used as genetic stocks for identification of blast resistance varieties or can be used as donors of genes *Pi9* and *Pi54* in breeding programmes for blast resistance genes.
- The results confirmed the accuracy of marker assisted selection (MAS) for the two genes *Pi54* and *Pi9* using the corresponding markers RM206 and AP5930, respectively.
- Markers assisted pyramiding of genes facilitates the introgression of major multiple, desirable genes into a single genotype to obtain the desired trait, which not only is a sustainable and economical way to enhance yield and increase resistance but also enhances the level and spectrum of the resistance in rice against blast.
- Modern molecular techniques efficiently supplement the conventional breeding methods to identify the novel resistance genes and their introgression into elite genetic backgrounds to improve the modern cultivars.

References

REFERENCES

Abdullah, A. B., Ito, S., and Adhana, K. 2006. *Estimate of Rice Consumption in Asian Countries and the World Towards 2050.* Paper presented at the In proceedings for workshop and conference on rice in the world at stake 2 Chiang Mai.

Abe, S. 2004. Breeding of a Blast Resistant Multiline Variety of Rice, Sasanishiki BL. *Japan Agricultural Research Quarterly: JARQ*, **38**(3): 149-154.

Agrios, G. 2005. *Plant Pathology* (5th ed.). San Diego, CA, USA: Elsevier.

Akos, I. S., Yusop, M. R., Ismail, M. R., Ramlee, S. I., Shamsudin, N. A. A., Ramli, B. R., Haliru, B. S., Ismai'la, M., and Chukwu, S. C. 2019. A review on gene pyramiding of agronomic, biotic and abiotic tr aits in rice variety development. *International Journal of Applied Biology.*

Ali, A., Teli, M. A., Bhat, G. N., Parray, G. A., and Wani. 2009. Status of rice blast (Pyricularia grisea), cultivar reaction and races of its causal fungus in temperate agro-ecosystem of Kashmir, India. *SAARC Journal of Agriculture*, **7**(2): 25-37.

Anonymous. 2018. Digest of Statistics 2016-17. Directorate of Economics & Statistics, Govt. of Jammu & Kashmir.

Anonymous. 2019. Pocket Book of Agricultural Statistics. Directorate of Economics and Statistics, Ministry of Agriculture and Farmers Welfare (pp. 63-64). New Delhi.

Anwar, A., Ahmad, N., Zarger, M., Sanghera, G. S., Rather, M., Bhat, G. N., Parrey, G. A., and Salroo, M. Y. 2003. Evaluation of elite rice genotypes against blast disease (Magnaporthe *grisea*) under epiphytotic conditions in Kashmir. *Plant Disease Research,* **18**(10): 77-79.

Anwar, A., Teli, M. A., Bhat, G. N., Parray, G. A. and Wani, S. A. 2011.Characterization of physiological races of *Pyricularia grisea* in temperate agro-ecosystem of Kashmir, India. *Indian Phytopathology,* **64**(1): 51-56.

Anwar, A., Teli, M.A., Bhat, G.N., Parray, G.A. and Wani. 2009. Status of rice blast

(*Pyricularia grisea*) cultivar reaction and races of its causal fungus in temperate agro-ecosystem of Kashmir, India. *SAARC Journal of Agriculture*, **7**(2):25-37

Ashikawa, I., Hayashi, N., Yamane, H., Kanamori, H., Wu, J., Matsumoto, T., Ono, K., and Yano, M. 2008. Two adjacent nucleotide-binding site-leucine-rich repeat class genes are required to confer Pikm-specific rice blast resistance. *Genetics*, **180**(4): 2267-2276.

Ashkani, S., Rafii, M. Y., Shabanimofrad, M., Ghasemzadeh, A., Ravanfar, S. A., and Latif, M. A. 2016. Molecular progress on the mapping and cloning of functional genes for blast disease in rice (Oryza sativa L.): current status and future considerations. *Critical Reviews in Biotechnology*, **36**(2): 353-367.

Balachiranjeevi, C. H., Bhaskar, N. S., Abhilash, V., Akanksha, S., Viraktamath, B. C., Madhav, M. S., Hariprasad, A. S., Laha, G. S., Prasad, M. S., Balachandran, S. M., Neeraja, C. N., Satendra Kumar, M., Senguttuvel, P., Kemparaju, K. B., Bhadana, V. P., Ram, T., Harika, G., Mahadeva Swamy, H. K., Hajira, S. K., Yugander, A., Pranathi, K., Anila, M., Rekha, G., Kousik, M. B. V. N., Dilip Kumar, T., Swapnil, R. K., Giri, A., and Sundaram, R. M. 2015. Marker-assisted introgression of bacterial blight and blast resistance into DRR17B, an elite, fine-grain type maintainer line of rice. *Molecular Breeding*, **35**(7).

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

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

Barman, S. R., and Chattoo, B. B. 2004. Identification of a major blast resistance gene in the rice cultivar Tetep. *Plant Breeding*, **123**:300– 302.

Bonman, J. M. 1986. Physiologic Specialization of Pyricularia oryzaein the Philippines. *Plant Disease*, **70**(8): 767.

Bonman, J. M. 1992. Durable resistance to rice blast disease-environmental influences. *Euphytica*, **63**(1-2): 115-123.

Bryan, G. T., Wu, K. S., 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 resistance gene *Pi-ta*. *Plant Cell,* **12**(11): 2033-2046.

Bussaban, B., Lumyong, S., Lumyong, P., Seelanan, T., Park, D., and Mckenzie, *et al*. 2005. Molecular and morphological characterization of *Pyricularia* and allies genera. *Mycologia,* **97** (2005): 1002-1011.

Chamberlain, J. S., Gibbs, R. A., Ranier, J. E., Nguyen, P. N., and Caskey, C. T. 1988. Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. *Nucleic Acids Research*, **16**(23): 11141-11156.

Chamberlain, J. S., Gibbs, R. A., Ranier, J. E., Nguyen, P. N., and Caskey, C. T. 1989. Multiplex PCR for the diagnosis of Duchenne muscular dystrophy,p.272–281.In: Gelfand, D.H., Innis, M.A., Shinsky, J.J., and White, T.J. (ed.), *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, Calif.

Chen, Q., Zeng, G., Hao, M., Jiang, H., and Xiao, Y. 2020. Improvement of rice blast and brown planthopper resistance of PTGMS line C815S in two-line hybrid rice through marker-assisted selection. *Molecular Breeding*, **40**: 21. https://doi.org/10.1007/s11032-020-1098-9

Chen, X. W., Li, S. G., Xu, J. C., Zhai, W. X., Ling, Z. Z., Ma, B. T., Wang, Y. P., Wang, W. M., Cao, G., Ma, Y. Q., Shang, J. J., Zhao, X. F., Zhou, K. D. and Zhu, L. H. 2004. Identification of Two Blast Resistance Genes in a Rice Variety, Digu. *Journal of Phytopathology,* **152**(2): 77-85.

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

Dar, M. S., and Iqbal, P. F. 2017. Perpetuation of rice blast pathogen (*Magnaporthe grisea*) under temperate conditions of Kashmir, India. *Annals of Plant Sciences*, **6**(12): 1804.

De Jong, J. C., McCormack, B. J., Smirnoff, N., and Talbot, N. J. 1997. Glycerol generates turgor in rice blast. *Nature,* **389**(6648): 244-244.

Dean, R., Van Kan, J. A., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., and Foster, G. D. 2012. The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology,* **13**(4): 414-430.

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

Deng, Y., Zhai, K., Xie, Z., Yang, D., Zhu, X., Liu, J., Wang, X., Qin, P., Yang, Y., Zhang, G., Li, Q., Zhang, J., Wu, S., Milazzo, J., Mao, B., Wang, E., Xie, H., Tharreau, D., and He, Z. 2017. Epigenetic regulation of antagonistic receptors confers rice blast resistance with yield balance. *Science,* **355**(6328): 962-965.

Deshmukh, R., Singh, A., Jain, N., Anand, S., Gacche, R., Singh, A., Gaikwad, K., Sharma, T., Mohapatra, T. and Singh N. 2010. Identification of candidate genes for grain number in rice (Oryza sativa L.). *Functional and Integrative Genomics,***10**:339–347.

Divya, B., Robin, S., Rabindran, R., Senthil, N., Raveendran, M., and Joel, A. J. 2014. Erratum to: Marker assisted backcross breeding approach to improve blast resistance in Indian rice (*Oryza sativa*) variety ADT43. *Euphytica,* **200**(1): 79-79.

Dodds, P. N., Lawrence, G. J., Catanzariti, A. M., Teh, T., Wang, C. I., Ayliffe, M. A., Kobe, B., and Ellis, J. G. 2006. Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. *Proceedings of the National Academy of Sciences of the United States of America,* **103**(23): 8888-8893.

Doyle, J. J., and Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. *Focus,* **12**:13-15. Falconer, D. S. and Mackay, T. F. C. 1996. *Introduction to Quantitative Genetics*, **4th** edition, London, Prentice Hall.

Ebbole, D. J. 2007. *Magnaporthe* as a model for understanding host-pathogen interactions. *Annual Review of Phytopathology,* **45**: 437-456.

Efisue, A. A., Umunna, B. C., and Orluchukwu, J. A. 2014. Effects of yield components on yield potential of some low land rice (*Oryza sativa* L.) in coastal region of south Nigeria. *Journal of Plant Breeding and Crop Sciences*, **6**:119–127.

Elert, E. 2014. Rice by the numbers: A good grain. *Nature,* **514**: 50-51 Fan, X. R., Xie, D., Chen, J. G., Lu, H. Y., Xu, Y. L., Ma, C., and Xu, G. H. 2014. Overexpression of OsPTR6 in rice increased plant growth at different nitrogen supplies but decreased nitrogen use efficiency at high ammonium supply. *Plant Science* **227**: 1–11

Ellur, R. K., Khanna, A., Yadav, A., Pathania, S., Rajashekara, H., Singh, V. K., Gopala Krishnan, S., Bhowmick, P. K., Nagarajan, M., Vinod, K. K., Prakash, G., Mondal, K. K., Singh, N. K., Vinod Prabhu, K., and Singh, A. K. 2016. Improvement of Basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding. *Plant Science,* **242**: 330-341.

FAO. 2019. Food Outlook - Biannual Report on Global Food Markets. Rome. Licence: CC BY-NC-SA 3.0 IGO.

Federer, W. T. 1956. *Augmented designs* (2nd Ed), 208. Hawaiian Planters. Record FL, USA pp: 223.

Fernandez, J., and Wilson, R. A. 2012. Why no feeding frenzy? Mechanisms of nutrient acquisition and utilization during infection by the rice blast fungus *Magnaporthe oryzae*. *Molecular Plant-Microbe Interactions,* **25**(10): 1286-1293.

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. *Molecular Breeding,* **17**(2): 149-157.

Fukuoka, S., Mizobuchi, R., Saka, N., Suprun, I., Matsumoto, T., Okuno, K., and Yano, M. 2012. A multiple gene complex on rice chromosome 4 is involved in durable resistance to rice blast. *Theoretical and Applied Genetics,* **125**(3): 551-559.

Fukuoka, S., and Okuno, K. 2001. QTL analysis and mapping of *pi21*, a recessive gene for field resistance to rice blast in Japanese upland rice. *Theoretical and Applied Genetics,* **103**(2-3): 185-190.

Fukuoka, S., Saka, N., Koga, H., Ono, K., Shimizu, T., Ebana, K., Hayashi, N., Takahashi, A., Hirochika, H., Okuno, K., and Yano, M. 2009. Loss of function of a proline-containing protein confers durable disease resistance in rice. *Science,* **325**(5943): 998-1001.

Fukuoka, S., Saka, N., Mizukami, Y., Koga, H., Yamanouchi, U., Yoshioka, Y., Hayashi, N., Ebana, K., Mizobuchi, R., and Yano, M. 2015. Gene pyramiding enhances durable blast disease resistance in rice. *Scientific Reports,* **5**: 7773.

Fukuoka, S., Yamamoto, S.-I., Mizobuchi, R., Yamanouchi, U., Ono, K., Kitazawa, N., Yasuda, N., Fujita, Y., Thi Thanh Nguyen, T., Koizumi, S., Sugimoto, K., Matsumoto, T., and Yano, M. 2014. Multiple functional polymorphisms in a single disease resistance gene in rice enhance durable resistance to blast. *Scientific Reports,* **4**: 1-7. https://doi.org/10.1038/srep04550

Goff, S.A., Ricke, D., Lan, T.H., Presting, G., Wang, R.L., Dunn, M., Glazebrook, J., Sessions, A., Oeller, P., Varma, H., Hadley, D., Hutchison, D., Martin, C., Katagiri, F., Lange, B.M., Moughamer, T., Xia, Y., Budworth, P., Zhong, J.P., Miguel, T., Paszkowski, U., Zhang, S.P., Colbert, M., Sun, W.L., Chen, L.L., Cooper, B., Park, S., Wood, T.C., Mao, L., Quail, P., Wing, R., Dean, R., Yu, Y., Zharkikh, A., Shen, R., Sahasrabudhe, S., Thomas, A., Cannings, R., Gutin, A., Pruss, D., Reid, J., Tavtigian, S., Mitchell, J., Eldredge, G., Scholl, T., Miller, R.M., Bhatnagar, S., Adey, N., Rubano, T., Tusneem, N., Robinson, R., Feldhaus, J., Macalma. T., Oliphant, A. and Briggs, S. 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). *Science*, **296**: 92–100.

Gowda, M., Roy-Barman, S., and Chattoo, B. B. 2006. Molecular mapping of a novel blast resistance gene *Pi38* in rice using SSLP and AFLP markers. *Plant Breeding* **125**:596–599.

Guerber, C., and TeBeest, D. O. 2006. Infection of rice seed grown in Arkansas by *Pyricularia grisea* and transmission tosSeedlings in the field. *Plant Disease,* **90**(2): 170-176.

Gupta, P. K., and Rastogi, S. 2004. Molecular markers from the transcribed/expressed region of the genome in higher plants. *Functional & Integrative Genomics*, **4**(3): 139-162.

Hamer, J. E., Howard, R. J., Chumley, F. G., and Valent, B. 1988. A mechanism for surface attachment in spores of a plant pathogenic fungus. *Science*, **239**(4837): 288-290.

Hangloo, S. 2018. Generation of genetic stocks for blast resistance in susceptible temperate variety of rice (*Oryza Sativa* L.) using marker assisted selection. *Ph.D. thesis*. Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu.

Hari, Y., Srinivasarao, K., Ramesha, M.S., Viraktamath, B.C., Laha, G.S., Balachandran, S.M., Prasad, M.S., Reddy, C.S., Prasad, A.S.H., Nataraj, Kumar, P., Sujatha, K., and Sundaram, R.M. 2008. Improvement of maintainer line (IR58025B) for bacterial blight (BB) and blast resistance through marker-assisted selection. *National symposium on new biology in agriculture*, Punjab University Chandigarh, **p**-80.

Hayashi, K., Hashimoto, N., Daigen, M., and Ashikawa, I. 2004. Development of PCR-based SNP markers for rice blast resistance genes at the *Piz* locus. *Theoretical and Applied Genetics*, **108**(7): 1212-1220.

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

He, X., Liu, X., Wang, L., Wang, L., Lin, F., Cheng, Y., Chen, Z., Liao, Y., and Pan, Q. 2012. Identification of the novel recessive gene pi55(t) conferring resistance to Magnaporthe oryzae. *Science China Life Sciences*, **55**(2): 141-149.

Helliwell, E. E., Wang, Q., and Yang, Y. 2013. Transgenic rice with inducible ethylene production exhibits broad-spectrum disease resistance to the fungal pathogens *Magnaporthe oryzae* and *Rhizoctonia solani*. *Plant Biotechnology Journal*, **11**(1): 33-42.

Hittalmani, S., Parco, A., Mew, T. V., Zeigler, R. S., and Huang, N. 2000. Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. *Theoretical and Applied Genetics,* **100**(7): 1121-1128.

Horo, J. T., Fujii, T., Yamashita, Y., McGoey, S., and Koizumi, S. 2016. Rice blast control efficacy of three genes (*Pib, pi21 and Pb1*) conferring complete and partial resistance. *Japan Agricultural Research Quarterly: JARQ,* **50**(3): 209–217.

Hospital, F. 2009. Challenges for effective marker-assisted selection in plants. *Genetica,* **136**(2): 303-310.

Hosseyni-Moghaddam, M. and Soltani, J. 2013. An investigation on the effects of photoperiod, aging and culture media onvegetative growth and sporulation of rice blast pathogen *Pyricularia oryzae*. *Progress in Biological Sciences,* **3**(2): 135-143.

Howard, R. J., Bourett, T. M. and Ferrari, M. A. 1991. Infection by *Magnaporthe:* An *in vitro* analysis, In: Electron microscopy of plant pathogens (eds. Mendgen, K., and Lesemann, D. E.), Thomson Press, Springer-Verlag, Berlin, Germany: 251-264.

Hu, J., Li, X., Wu, C., Yang, C., Hua, H., Gao, G., Xiao, J. and He, Y. 2012. Pyramiding and evaluation of the brown planthopper resistance genes *Bph14* and *Bph15* in hybrid rice. *Molecular Breeding,* **29**(1): 61-69.

Hua, L., Wu, J., Chen, C., Wu, W., He, X., Lin, F., Wang, L., Ashikawa, I., Matsumoto, T., Wang, L. and Pan, Q. 2012. The isolation of *Pi1*, an allele at the *Pik* locus which confers broad spectrum resistance to rice blast. *Theoretical and Applied Genetics,* **125**(5): 1047-1055.

Huang, J., Si, W., Deng, Q., Li, P. and Yang, S. 2014. Rapid evolution of avirulence genes in rice blast fungus *Magnaporthe oryzae*. *BMC Genetics,* **15**: 45.

Hulbert, S. H., Webb, C. A., Smith, S. M. and Sun, Q. 2001. Resistance gene complexes: evolution and utilization. *Annual Review of Phytopathology,* **39**: 285-312.

Jain, A. K., Yadava, H. S. and Singh, S. B. 2002. Genetic diversity for morphological characters determining blast resistance in finger millet. *Mysore Journal of Agricultural Sciences,* **36**(2): 135-140.

Jena, K. K. and Mackill, D. J. 2008. Molecular markers and their use in marker assisted selection in rice. *Crop Science,* **48**(4): 1266-1276.

Jeung, J. U., Kim, B. R., Cho, Y. C., Han, S. S., Moon, H. P., Lee, Y. T. and Jena, K. K. 2007. A novel gene, Pi40(t), linked to the DNA markers derived from NBS-LRR motifs confers broad spectrum of blast resistance in rice. *Theoretical and Applied Genetics,* **115**(8): 1163-1177.

Jiang, H., Li, Z., Liu, J., Shen, Z., Gao, G., Zhang, Q. and He, Y. 2019. Development and evaluation of improved lines with broad-spectrum resistance to rice blast using nine resistance genes. *Rice (N Y),* **12**(1): 29. https://doi.org/10.1186/s12284-019-0292-z

Jiang, N., Li, Z., Wu, J., Wang, Y., Wu, L., Wang, S., Wang, D., Wen, T., Liang, Y., Sun, P., Liu, J., Dai, L., Wang, Z., Wang, C., Luo, M., Liu, X. and Wang, G. L. 2012. Molecular mapping of the *Pi2/9* allelic gene *Pi2-2* conferring broad-spectrum resistance to *Magnaporthe oryzae* in the rice cultivar Jefferson. *Rice (N Y),* **5**(1): 29. http://www.thericejournal.com/content/5/1/29 doi: 10.1186/1939-8433-5-29

Jin, L., Richards, A. and Brown, D. W. 1996. Development of a dual target-PCR for detection and characterization of measles virus in clinical specimens. *Molecular and Cellular Probes,* **10**(3): 191-200.

Johnson, V., Gibbons, G., Moldenhuaer, K., and Jia, Y. 2003. Rice variety improvement using marker assisted selection In: Norman, R. J., and Meullenet, J. F (eds) Rice research studies 2002. Arkansas Agricultural Experiment Station

Joseph, M., Gopalakrishnan, S., Sharma, R. K., Singh, V. P., Singh, A. K., K. Singh, N. and Mohapatra, T. 2004. Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. *Molecular Breeding,* **13**(4): 377-387.

Joshi, R. K. and Nayak, S. 2008. Gene pyramiding-A broad spectrum technique for developing durable stress resistance in crops. *Biotechnology and Molecular Biology Review*.**5**(3): 51-60

Juliano, B. O., Perez, C. M., Blakeney, A. B., Castillo, T., Kongseree, N., Laignelet, B., Lapis, E. T., Murty, V. V. S., Paule, C. M. and Webb, B. D. 1981. International cooperative testing on the amylose content of milled rice. *Starch - Stärke*, **33**(5): 157-162.

Kanzaki, H., Yoshida, K., Saitoh, H., Fujisaki, K., Hirabuchi, A., Alaux, L., Fournier, E., Tharreau, D. and Terauchi, R. 2012. Arms race co-evolution of *Magnaporthe oryzae* AVR-*Pik* and rice *Pik* genes driven by their physical interactions. *The Plant Journal*, **72**: 894–907.

Khan, G. H., Shikari, A. B., Vaishnavi, R., Najeeb, S., Padder, B. A., Bhat, Z. A., Parray, G. A., Bhat, M. A., Kumar, R. and Singh, N. K. 2018. Marker-assisted introgression of three dominant blast resistance genes into an aromatic rice cultivar Mushk Budji. *Scientific Reports*, **8**(1): 4091.

Khanna, A., Sharma, V., Ellur, R. K., Shikari, A. B., Gopala Krishnan, S., Singh, U. D., Prakash, G., Sharma, T. R., Rathour, R., Variar, M., Prashanthi, S. K., Nagarajan, M., Vinod, K. K., Bhowmick, P. K., Singh, N. K., Prabhu, K. V., Singh, B. D. and Singh, A. K. 2015. Development and evaluation of near-isogenic lines for major blast resistance gene(s) in Basmati rice. *Theoretical and Applied Genetics*, **128**(7): 1243-1259.

Khush, G. S. and Jena, K. K. 2009. Current status and future prospects for research on blast resistance in rice (*Oryza sativa* L.) In : Wang, G.L., valent, B. *Advances in genetics, genomics, & control of rice blast disease* .New York, **1**-10.

Kingsolver, C. H. 1984. Rice blast epidemiology: *Bulletin of the Pennsylvania Agricultural Experiment Station*; No.853; Pennsylvania State College, Agricultural Experiment Station: State College, PA, USA, 1984; pp. 29–40.

Koide, Y., Kawasaki, A., and Telebanco-Yanoria, M. J *et al*. 2010. Development of pyramided lines with two resistance genes, *Pish* and *Pib*, for blast disease (*Magnaporthe oryzae* B. Couch) in rice (*Oryza sativa* L.). *Plant Breeding* **129**: 670–5.

Kouzai, Y., Kaku, H., Shibuya, N., Minami, E., and Nishizawa, Y. 2013. Expression of the chimeric receptor between the chitin elicitor receptor CEBiP and the receptor–like protein kinase Pi–d2 leads to enhanced responses to the chitin elicitor and disease resistance against *Magnaporthe oryzae* in rice. *Plant Molecular. Biology*, **81**: 287–295.

Kulkarni, K. and Peshwe, S. 2019. Screening, isolation and molecular identification of rice pathogen *Magnaporthe Oryzae*. *International Journal of Advanced Research,* **7(3)**: 428-433.

Kumar, A. and Singh, R. A. 1994. Host range of some isolates of *Magnaporthe grisea* and their grouping into forma specialis. *Indian Phytopathology*, **2**:10-12.

Kumar, P., Pathania, S., Katoch, P., Sharma, T.R., Plaha, P. and Rathour, R. 2010. Genetic and physical mapping of blast resistance gene *Pi-42*(t) on the short arm of rice chromosome 12. *Molecular Breed*ing, **25**:217–228.

Kumar, M. K. P., Gowda, D. K. S., Moudgal, R., Kumar, N. K., Gowda, K. T. P., and Vishwanath, K. 2013. Impact on fungicides on rice production in India. In: Nita, M. (Ed.). *Fungicides-showcases of integrated plant disease management from around the world*. ISBN: 978-953-51-1130-6, InTech.

Kumari, M., Rai, A. K., Devanna, B. N., Singh, P. K., Kapoor, R., Rajashekara, H., Prakash, G., Sharma, V. and Sharma, T. R. 2017. Co-transformation mediated stacking of blast resistance genes *Pi54* and *Pi54rh* in rice provides broad spectrum resistance against *Magnaporthe oryzae*. *Plant Cell Report*, **36**(11): 1747-1755.

Kwon, J. O. and Lee, S. G. 2002. Real-time micro-weather factors of growing field to the epidemics of rice blast. *Research in Plant Disease*, **8**(4): 199-206.

Lamari, L. 2009. Assess 2.0: Image analysis software for plant disease quantification. APS Press: St. Paul, MN, USA: The American Phytophatological Society.

Latif, M. A., Rahman, M. M., Kabir, M. S., Ali. M. A., Islam, M. T., and Rafii, M. Y. 2011. Genetic diversity analyzed by quantitative traits among rice (*Oryza sativa* L.) genotypes resistant to blast disease. *African Journal of Microbiology Research,* **5**(25): 4383–4391.

Lee, S. K., Song, M. Y., Seo, Y. S., Kim, H. K., Ko, S., Cao, P. J., Suh, J. P., Yi, G., Roh, J. H., Lee, S., An, G., Hahn, T. R., Wang, G. L., Ronald, P. and Jeon, J. S. 2009. Rice *Pi5*-mediated resistance to *Magnaporthe oryzae* requires the presence of two coiled-coil-nucleotide-binding-leucine-rich repeat genes. *Genetics,* **181**(4): 1627-1638.

Li, W., Wang, B., Wu, J., Lu, G., Hu, Y., Zhang, X., Zhang, Z., Zhao, Q., Feng, Q., Zhang, H., Wang, Z., Wang, G., Han, B., Wang, Z. and Zhou, B. 2009. The *Magnaporthe oryzae* avirulence gene AvrPiz-t encodes a predicted secreted protein that triggers the immunity in rice mediated by the blast resistance gene *Piz-t. Molecular Plant-Microbe Interactions,* **22**(4): 411-420.

Li, W., Zhu, Z., Chern, M., Yin, J., Yang, C., Ran, L., Cheng, M., He, M., Wang, K., Wang, J., Zhou, X., Zhu, X., Chen, Z., Wang, J., Zhao, W., Ma, B., Qin, P., Chen, W., Wang, Y., Liu, J., Wang, W., Wu, X., Li, P., Wang, J., Zhu, L., Li, S. and Chen, X. 2017. A Natural allele of a transcription factor in rice confers broad-spectrum blast resistance. *Cell,* **170**(1): 114-126 e115.

Li, Y. B., Wu, C. J., Jiang, G. H., Wang, L. Q. and He, Y. Q. 2007. Dynamic analyses of rice blast resistance for the assessment of genetic and environmental effects. *Plant Breeding,* **126**(5): 541-547.

Liang, Z., Wang, L. and Pan, Q. 2016. A new recessive gene conferring resistance against rice blast. *Rice*, **9**: 47. doi 10.1186/s12284-016-0120-7

Lin, F., Chen, S., Que, Z., Wang, L., Liu, X. and Pan, Q. 2007. The blast resistance gene *Pi37* encodes a nucleotide binding site leucine-rich repeat protein and is a member of a resistance gene cluster on rice chromosome 1. *Genetics,* **177**(3): 1871-1880.

Liu, B., Zhang, S., Zhu, X., Yang, Q., Wu, S., Mei, M., Mauleon, R., Leach, J., Mew, T. and Leung, H. 2004. Candidate defense genes as predictors of quantitative blast resistance in rice. *Molecular Plant-Microbe Interactions*, **17**(10): 1146-1152.

Liu, Y., Liu, B., Zhu, X., Yang, J., Bordeos, A., Wang, G., Leach, J. E. and Leung, H. 2013. Fine-mapping and molecular marker development for *Pi56(t)*, a NBS-LRR gene conferring broad-spectrum resistance to *Magnaporthe oryzae* in rice. *Theoretical and Applied Genetics,* **126**(4): 985-998.

Lopez, A. L. C., and Cumagun, C. J. R. 2018. Genetic structure of *Magnaporthe oryzae* populations in three island groups in the Philippines. *European Journal of Plant Pathology*, **153:** 101–118.

Mackill, D. J. and Bonman, J. M. 1992. Inheritance of Blast Resistance in Near-Isogenic Lines of Rice. *Phytopathology*, **82**: 746-749.

Madhavi,, K. R., Rambabu, R., Kumar, A. V., Kumar, S. V., Aruna, J., Ramesh, S., Sundaram R. M., Laha, G. S, Madhav, M. S., Ravindrababu, V. and Prasad, M. S. 2016. Marker-assisted introgression of blast (*Pi-2* and *Pi-54*) genes in to the genetic background of elite, bacterial blight resistant indica rice variety, improved Samba Mahsuri. *Euphytica*. **212**: 331-342.

Manandhar, H.K., Shrestha, K., and Amatya, P. 1992. Seed-borne diseases. In: Mathur, S.B., Amatya, P., Shrestha, K. andManandhar, H.K., (Eds). Plant diseases, seed production and seed health testing in Nepal. *DGISP*, Denmark, pp 59–74.

Manandhar, H. K., Lyngs Jorgensen, H. J., Mathur, S. B. and Smedegaard-Petersen, V. 1998. Suppression of Rice Blast by Preinoculation with Avirulent Pyricularia oryzae and the Nonrice Pathogen Bipolaris sorokiniana. *Phytopathology*, **88**(7): 735-739.

McKenzie, H. A and Wallace, H.S. 1954. *Autralian Journal of Chemistry, **7**: 55*

Mi, J., Yang, D., Chen, Y., Jiang, J., Mou, H., Huang, J., Ouyang, Y. and Mou, T. 2018. Accelerated molecular breeding of a novel PTGMS line with broad-spectrum resistance to rice blast and bacterial blight in two-line hybrid rice. *Rice*, **11**:11. https://doi.org/10.1186/s12284-018-0203-8

Miah, G., Rafii, M. Y., Ismail, M. R., Puteh, A. B., Rahim, H. A., Islam Kh, N. and Latif, M. A. 2013. A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *International Journal of Molecular Sciences*, **14**(11): 22499-22528.

Miah, G., Rafii, M.Y., Ismail, M.R., Puteh, A.B., Rahim, H.A. and Latif, M.A. 2014. Improvement of MR219-rice variety for blast resistance through marker-assisted backcross breeding. *Molecular Breeding*, **27**:129–135.

Mohapatra, T., Nayak, A. K., Raja, R., and Mohammad, S. 2013. *Vision 2050.* Paper presented at the Central Rice Research Institute, ICAR, Cuttack.

Najeeb, S., Parray, G. A., Shikari, A. B., Bhat, Z. A., Bhat, A. M., Iqbal, M. A., Bhat, S. J., Shah, A. H., Manzar, A., Ahangar, M. A. and Wani, A. S. 2016. Revival of endangered high valued mountain rices of Kashmir Himalayas through genetic purification and *in-situ* Conservation. *International Journal of Agriculture Sciences*, ISSN: 0975-3710 & E-ISSN: 0975-9107, **8**(52): 2453-2458.

Narayanan, N. N., Baisakh, N., Vera Cruz, C. M., Gnanamanickam, S. S., Datta, K. and Datta, S. K. 2002. Molecular breeding for the development of blast and bacterial blight esistance in rice cv. IR50. *Crop Science,* **42**(6): 2072-2079.

Neeraja, C.N., Hariprasad, A.S., Malathi, S. and Siddiq, E.A. 2005. Characterization of tall landraces of rice (*Oryza sativa* L.) using gene-derived simple sequence repeats. *Current Science,* **88:**149-152.

Orasen, G., Greco, R., Puja, E., Pozzi, C. and Stile, M. R. 2020. Blast resistance R genes pyramiding in temperate japonica rice. Euphytica, 216: 40. https://doi.org/10.1007/s10681-020-2575-2

Orbach, M. J., Farrall, L., Sweigard, J. A., Chumley, F. G., and Valent, B. 2000. A telomeric avirulence gene determines efficacy for the rice blast resistance gene *Pi-ta*. *Plant Cell,* **12**(11): 2019-2032.

Ou, S.H. 1985. Rice diseases. 2nd ed., *Commonwealth Mycological Institute,* Kew. Surrey, England.

Padmanabhan, S. Y. 1965. Studies on forecasting outbreaks of blast disease of rice. *Proceedings. Indian academy of Sciences,* **(62):** 117–129.

Panda, G., Sahu, C., Yadav, M. K., Aravindan, S., Umakanta, N., Raghu, S., Prabhukarthikeyan, S. R., Lenka, S., Tiwari, J. K., Kar, S. and Jena, M. 2017. Morphological and molecular characterization of *Magnaporthe* oryzae from Chhattisgarh. *ORYZA- An International Journal on Rice,* **54**: 330-336.

Parinthawong, N., and Suksiri, W. 2020. Investigation of blast resistance genes in a broad-spectrum resistant indigenous rice, Yang Mawng variety. *International Journal of Agricultural Technology,* **16**(1): 155-162.

Patroti, P., Vishalakshi, B., Umakanth, B., Suresh, J., Senguttuvel, P. and Madhav, M. S. 2019. Marker-assisted pyramiding of major blast resistance genes in Swarna-Sub1, an elite rice variety (Oryza sativa L.). *Euphytica,* **215**: 179. https://doi.org/10.1007/s10681-019-2487-1

Pennisi, E. 2010. Armed and dangerous. *Science,* **327**(5967): 804-805.

Phillips, R. L and Vasil, I. K. DNA-Based Markers in Plants. 2001. Eds. 2 ed. Vol. 6): Springer Netherlands.

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

Pradhan, S. K., Nayak, D. K., Mohanty, S., Behera, L., Barik, S. R., Pandit, E., Lenka, S. and Anandan, A. 2015. Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deep water rice variety, Jalmagna. *Rice (N Y),* **8**: 19. doi: 10.1186/s12284-015-0051-8

Qu, S., Liu, G., Zhou, B., Bellizzi, M., Zeng, L., Dai, L., Han, B. and Wang, G. L. 2006. The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site-leucine-rich repeat protein and is a member of a multigene family in rice. *Genetics*, **172**(3): 1901-1914.

Raghu, S., Yadav, M. K., Prabhukarthikeyan, S. R, Baite, M. S, Lenka, S. and Jena, M. 2018. Occurrence, pathogenicity, characterization of *Fusarium fujikuroi* causing rice bakanae disease from Odisha and *in vitro* management. *Oryza,* **55**: 214–23.

Ramkumar, G., Srinivasarao, K., Madhan Mohan, K., Sudarsha, I., Sivaranjani, A.K.P., Gopalakrishna, K., Neeraja, C.N., Balachandran, S.M., Sundaram, R.M., Prasad, M.S., Shobha Rani, N., Rama Prasad, A.M., Viraktamath, B.C. and Madhav, M.S. 2011. Development and validation of functional marker targeting an InDel in the major rice blast disease resistance gene *Pi54* (*Pikh*). *Molecular Breeding,* **27**:129– 135.

Rani, N.S., Rao, L.V.S., and Viraktamath, B. C. 2006. National Guidelines for the conduct of tests for Distinctness, Uniformity and Stability: Rice (*Oryza sativa* L.) – Zero Draft, *Directorate of Rice Research,* Hyderabad Andhra Pradesh,

India, **PP**: 39.

Rathour, R., Chopra, M. and Sharma, T. R. 2008. Development and validation of microsatellite markers linked to the rice blast resistance gene *Pi-z* of Fukunishiki and Zenith. *Euphytica*, **163**(2): 275-282.

Raveloson, H., Ratsimiala Ramonta, I., Tharreau, D. and Sester, M. 2018. Long-term survival of blast pathogen in infected rice residues as major source of primary inoculum in high altitude upland ecology. *Plant Pathology*, **67**(3): 610-618.

Ray, S., Singh, P. K., Gupta, D. K., Mahato, A. K., Sarkar, C., Rathour, R., Singh, N. K. and Sharma, T. R. 2016. Analysis of *Magnaporthe oryzae* genome reveals a fungal rffector, which is able to induce resistance response in transgenic rice line containing resistance gene, *Pi54*. *Frontiers in Plant Science,* **7**: 1140. doi: 10.3389/fpls.2016.01140.

Ribot, C., Hirsch, J., Balzergue, S., Tharreau, D., Notteghem, J. L., Lebrun, M. H. and Morel, J. B. 2008. Susceptibility of rice to the blast fungus, Magnaporthe grisea. *Journal of Plant Physiology*, **165**(1): 114-124.

Rithidech, K. N., Dunn, J. J., and Gordon, C. R. 1997. Combining multiplex and touchdown PCR to screen murine microsatellite polymorphisms. *Biotechniques*, **23**: 36-45.

Sagar Krishna Murthy, P., Deshmukh, D. B., Yashvanth Kumar, K. J., Patil, S., Jakkeral, S., Nemappa, G. H., Singh, U. D., Variar, M., Rathour, R., Subbaiyan, G., Singh, A. K. and Sharma, T. R. 2017. Introgression of *Pi2* and *Pi5* Genes for blast (*Magnaporthe oryzae*) resistance in rice and field evaluation of introgression lines for resistance and yield traits. *Journal of Phytopathology,* **165**(6): 397-405.

Sagar, V., Dhawan, G., Gopala Krishnan, S., Vinod, K. K., Ellur, R. K., Mondal, K. K., Rathour, R., Prakash, G., Nagarajan, M., Bhowmick, P. K., Bollinedi, H. and Singh, A. K. 2020. Marker assisted introgression of genes governing resistance to bacterial blight and blast diseases into an elite Basmati rice variety, 'Pusa Basmati 1509'. *Euphytica,* **216**: 16. Euphytica (2020) 216:16. https://doi.org/10.1007/s10681-019-2549-4.

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. *Theoretical and Applied Genetics,* **106**(5): 794-803.

Sax, K. 1923. The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. *Genetics,* **8**(6): 552-560.

Scardaci, S. C., Webster, R. K., Greer, C. A., Hill, J. E., William, J. F., Mutters, R. G., Brandon, D. M., Mckenzie, K. S. and Oster, J. J. 1997. Rice blast: a new disease in California. *Agronomy Fact Sheet Series*. (Vol. 86). *Department of Agronomy and Range Science, University of California, Davis*. 86

Scheuermann, K.K., Raimondi, J.V., Marschalek, R., de Andrade, A., Wickert, E. 2012. *Magnaporthe oryzae* genetic diversity and its outcomes on the search for durable resistance. *Molecular Basis Plant Genetic Divers,* 331–356.

Selvaraj, C. I., Nagarajan, P., Thiyagaraj, K., Bharathi, M. and Rabindran, R. 2011. Studies on heterosis and combining ability of well known blast resistant rice genotypes with high yielding varieties of rice (*Oryza sativa* L.). *International Journal of Plant Breeding and Genetics,* **5**(2): 111-129.

Septiningsih, E. M., Pamplona, A. M., Sanchez, D. L., Neeraja, C. N., Vergara, G. V., Heuer, S., Ismail, A. M. and Mackill, D. J. 2009. Development of submergence-tolerant rice cultivars: the Sub1 locus and beyond. *Annals of Botany,* **103**:151–160.

Selisana, S., Yanoria, M., Quime, B., Chaipanya, C., Lu, G., Opulencia, R., Wang, G., Mitchell, T., Correll, J., Talbot, N., Leung, H. and Zhou, B. 2017. Avirulence (AVR) gene-based diagnosis complements existing pathogen surveillance tools for effective deployment of resistance (R) genes against rice blast disease. *Phytopathology,* **107**(6):711–720

Séré, Y., Onasanya, A., Afolabi, A., Mignouna, H. D. and Akator, K. 2007. Genetic diversity of the blast fungus, *Magnaporthe grisea* (Hebert) Barr, in Burkina Faso. *African Journal of Biotechnology,* **6**(22): 2568-2577.

Sesma, A. and Osbourn, A. E. 2004. The rice leaf blast pathogen undergoes developmental processes typical of root-infecting fungi. *Nature,* **431**(7008): 582-586.

Shang, J., Tao, Y., Chen, X., Zou, Y., Lei, C., Wang, J., Li, X., Zhao, X., Zhang, M., Lu, Z., Xu, J., Cheng, Z., Wan, J. and Zhu, L. 2009. Identification of a new rice blast resistance gene, *Pid3*, by genomewide comparison of paired nucleotide-binding site--leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. *Genetics*, **182**(4): 1303-1311.

Sharma, T. R., Gupta, Y. K., Thakur, S., Singh, P. K., Upreti, H. C., Singh, N. K., Singh, U. D., Rathour. R., Kapoor, A. S., Kaushik, R. P., Variar, M., Maiti, D., Mandal, N. P., Prashanti, S. K., and Hanamaratti, N. G. 2009. *Allele mining for important blast resistance genes from Indian land races of rice.* Paper presented at the 6th International Rice Genetics Symposium, Manila, Philipines

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. *Journal of Phytopathology,* **150**(11-12): 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. 2005a. High-resolution mapping, cloning and molecular characterization of the *Pi-kh* gene of rice, which confers resistance to *Magnaporthe grisea*. *Molecular Genetics of Genomics,* **274**:569–578.

Sharma, T.R., Shanker, P., Singh, B.K., Jana, T.K., Madhav, M.S., Gaikwad, G., Singh, N.K., Plaha, P. and Rathour, R. 2005b. Molecular mapping of rice blast resistance gene *Pi-kh* in rice variety Tetep. *Journal of Plant Biochemistry and Biotechnology*, **14**: 127–133.

Sharma, T. R., Rai, A. K., Gupta, S. K. and Singh, N. K. 2010. Broad-spectrum blast resistance gene *Pi-kh* cloned from rice line Tetep designated as *Pi54*. *Journal of Plant Biochemistry and Biotechnology*, **19**(1): 87-89.

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. *Agricultural Research,* **1**(1): 37-52.

Sharma, T. R., Shanker, P., Singh, B. K., Jana, T. K., Madhav, M. S., Gaikwad, K., Singh, N. K., Plaha, P. and Rathour, R. 2012. Molecular mapping of rice blast resistance gene *Pi-kh* in the rice variety Tetep. *Journal of Plant Biochemistry and Biotechnology,* **14**(2): 127-133.

Sherlock, J., Cirigliano, V., Petrou, M., Tutschek, B., and Adinolfi, M. 1998. Assessment of diagnostic quantitative fluorescent multiplex polymerase chain reaction assays performed on single cells. *Annals of Human Genetics,* **62**(Pt 1): 9-23.

Shuber, A. P., Skoletsky, J., Stern, R. and Handelin, B. L. 1993. Efficient 12-mutation testing in the CFTR gene: a general model for complex mutation analysis. *Human Molecular Genetics,* **2**(2): 153-158.

Singh, S., Sidhu, J.S., Huang. N., Vikal, Y., Li, Z., Brar, D. S., Dhaliwal, H. S. and Khush, G.S. 2001. Pyramiding three bacterial blight resistance genes (*xa5, xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR106. *Theoretical and Applied Genetics,* **102**:1011–1015

Singh, A., Gopalakrishnan, S., Singh, V., Prabhu, K., Mohapatra, T., Singh, N. K., Sharma, T., Nagarajan, M., Vinod, K., Singh, D., Singh, U. D., Chander, S., Atwal, S., Seth, R., Ellur, R. K., Anand, D., Khanna, A., Yadav, S., Goel, N., Shikari, A. B., Singh, A. and Marathi, B. 2011. Marker assisted selection: a paradigm shift in Basmati breeding. *Indian Journal of Genetics and Plant Breeding,* **71**: 120-128.

Singh, A., Singh, V.K., Singh, S.P., Pandian, R.T.P., Ellur R.K., Singh, D., Bhowmick, P.K., Gopala Krishnan, S., Nagarajan, M., Vinod, K.K., Singh, U.D., Prabhu, K.V., Sharma, T.R., Mohapatra, T and Singh, A.K. 2012. Molecular breeding for the development of multiple disease resistance in Basmati rice. *Annals of Botany Plants*, 1-13. doi: 10.1093/aobpla/pls029.

Singh, P. K., Thakur, S., Rathour, R., Variar, M., Prashanthi, S. K., Singh, A. K., Singh, U. D., Sharma, V., Singh, N. K. and Sharma, T. R. 2014. Transposon-based high sequence diversity in *Avr-Pita* alleles increases the potential for

pathogenicity of *Magnaporthe oryzae* populations. *Functional and Integrative Genomics*, **14**:419–429

Singh, S. K., Bhati, P. K., Sharma, A. and Sahu, V. 2015. Super hybrid rice in China and India: current status and future prospects. *International Journal of Agriculture and Biology*, **17**: 221-232.

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

Sprague, S. J., Balesdent, M. H., Brun, H., Hayden, H. L., Marcroft, S. J., Pinochet, X., Rouxel, T. and Howlett, B. J. 2006. Major gene resistance in *Brassica napus* (oilseed rape) is overcome by changes in virulence of populations of *Leptosphaeria maculans* in France and Australia. *European Journal of Plant Pathology*, **114**:33–40.

Srinivasarao, K., Hari, Y., Lahagas., Virakthamath, B. C., Mishra, B., Hariprasad, A. S., Nataraj, K. P., Mohan, K. M., Prasad, M. S., and Sundaram, R. M. 2009. *Marker assisted introgression of bacterial blight and rice blast resistance genes into hybrid rice parental lines.* Paper presented at the Proceedings of 6th International Rice Genetics Symposium, IRRI, Phillippines. PP 3-56.

Stam, J. M., Kroes, A., Li, Y., Gols, R., van Loon, J. J., Poelman, E. H. and Dicke, M. 2014. Plant interactions with multiple insect herbivores: from community to genes. *Annual Review of Plant Biology*, **65**: 689-713.

Steele, K. A., Price, A. H., Shashidhar, H. E. and Witcombe, J. R. 2006. Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. *Theoretical and Applied Genetics*, **112**(2): 208-221.

Su, J., Wang, W., Han, J., Chen, S., Wang, C., Zeng, L., Feng, A., Yang, J., Zhou, B., and Zhu, X. 2015. Functional divergence of duplicated genes results in a novel blast resistance gene *Pi50* at the *Pi2/9* locus. *Theoretical and Applied Genetics*, **128**(11): 2213-2225.

Suh, J. P., Roh, J. H., Cho, Y. C., Han, S. S., Kim, Y. G. and Jena, K. K. 2009. The pi40 gene for durable resistance to rice blast and molecular analysis of pi40-advanced backcross breeding lines. *Phytopathology*, **99**(3): 243-250.

Sundaram, R.M., Vishnupriya, R.M., Biradar, S.K., Laha, G.S., Ashok Reddy, G.,

Shobha Rani, N., Sarma, N.P. Sonti, R.V. 2008. Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica,* **160:**411-422.

Swamy, B. P. M., and Sarla, N. 2011. Meta-analysis of Yield QTLs Derived from inter-specific crosses of rice reveals consensus regions and candidate genes. *Plant Molecular Biology Reporter,* **29**(3): 663-680.

Swathi, G., Durga Rani, C. V., Md, J., Madhav, M. S., Vanisree, S., Anuradha, C., Kumar, N. R., Kumar, N. A. P., Kumari, K. A., Bhogadhi, S. C., Ramprasad, E., Sravanthi, P., Raju, S. K., Bhuvaneswari, V., Rajan, C. P. D. and Jagadeeswar, R. 2019. Marker-assisted introgression of the major bacterial blight resistance genes, *Xa21* and *xa13*, and blast resistance gene, *Pi54*, into the popular rice variety, JGL1798. *Molecular Breeding,* **39**(4).

Tacconi, G., Baldassarre, V., Lanzanova, C., Faivre-Rampant, O., Cavigiolo, S., Urso, S., Lupotto, E. and Valè, G. 2010. Polymorphism analysis of genomic regions associated with broad-spectrum effective blast resistance genes for marker development in rice. *Molecular Breeding,* **26**(4): 595-617.

Takahashi, A., Hayashi, N., Miyao, A. and Hirochika, H. 2010. Unique features of the rice blast resistance *Pish* locus revealed by large scale retrotransposon-tagging. *BMC Plant Biology,* **10**: 175.

Tanksley, S. D. and McCouch, S. R. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. *Science,* **277**(5329): 1063-1066.

Tanweer, F. A., Rafii, M. Y., Sijam, K., Rahim, H. A., Ahmed, F., Ashkani, S. and Latif, M. A. 2015. Introgression of blast resistance genes (putative *Pi-b* and *Pi-kh*) into elite rice cultivar MR219 through marker-assisted selection. *Frontiers in Plant Science,* **6**: 1002.

Thakur, S., Singh, P. K., Das, A., Rathour, R., Variar, M., Prashanthi, S. K., Singh, A. K., Singh, U. D., Chand, D., Singh, N. K. and Sharma, T. R. 2015. Extensive sequence variation in rice blast resistance gene *Pi54* makes it broad spectrum in nature. *Frontiers in Plant Science,* **6**: 345.

Usatov, A. V., Kostylev, P. I., Azarin, K. V., Markin, N. V, Makarenko, M. S, Khachumov, V. A. and Bibov, M. Y. 2016. Introgression of the rice blast

resistance genes *Pi1*, *Pi2* and *Pi33* into Russian rice varieties by marker-assisted selection. *Indian Journal of Genetics and Plant Breeding*, **76**:18–23.

Valent, B., and Chumley, F.G. 1991. Molecular genetic analysis of the rice blast fungus, *Magnaporthe grisea*. *Annual Review of Phytopathology*, **29**: 443–467.

Valent, B. and Khang, C. H. 2010. Recent advances in rice blast effector research. *Current Opinion in Plant Biology*, **13**(4): 434-441.

Van Berloo, R. 1999. Computer note. GGT: software for the display of graphical genotypes. *Journal of Heredity*, **90**(2): 328-329.

Vasudevan, K., Vera Cruz, C. M., Gruissem, W. and Bhullar, N. K. 2014. Large scale germplasm screening for identification of novel rice blast resistance sources. *Frontiers in Plant Science*, **5**: 505. doi: 10.3389/fpls.2014.00505.

Veneault-Fourrey, C., Barooah, M., Egan, M., Wakley, G. and Talbot, N. J. 2006. Autophagic fungal cell death is necessary for infection by the rice blast fungus. *Science*, **312**(5773): 580-583.

Viraktamath, B. C. 2009. Hybrid Rice in India –Current status and future prospects. Directorate of Rice Research, Hyderabad.

Viraktamath, B .C., Madhavi, R. K., Prasad, M. S., Laha, G. S. and Mohan, K. M. 2011. Combining blast and bacterial blight resistance in rice cultivar improved Sambamashuri. *Indian Journal of Plant Protection* **39**:124-29.

Wang, B. H., Ebbole, D. J., and Wang, Z. H. 2017. The arms race between *Magnaporthe oryzae* and rice: Diversity and interaction of *Avr* and *R* genes. *Journal of Integrative Agriculture*, **16**(12): 2746-2760.

Wang, G. L., Mackill, D. J., Bonman, J. M., McCouch, S. R., Champoux, M. C. and Nelson, R. J. 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. *Genetics*, **136**(4): 1421-1434.

Wang R., Fang N., Guan C., He W., Bao Y. and Zhang H. 2016 Characterization and fine mapping of a blast resistant gene *Pi–jnw1* from the japonica rice landrace Jiangnanwan. *PLoS One* 11, e0169417.

Werner, K., Friedt, W. and Ordon, F. 2005. Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2). *Molecular Breeding,* **16**(1): 45-55.

Wiesner-Hanks, T. and Nelson, R. 2016. Multiple disease resistance in plants. *Annual Review of Phytopathology*, **54**: 229-252.

Wilson, R. A. and Talbot, N. J. 2009. Under pressure: investigating the biology of plant infection by *Magnaporthe oryzae*. *Nature Reviews Microbiology,* **7**(3): 185-195.

Wu, J., Kou, Y., Bao, J., Li, Y., Tang, M., Zhu, X., Ponaya, A., Xiao, G., Li, J., Li, C., Song, M. Y., Cumagun, C. J., Deng, Q., Lu, G., Jeon, J. S., Naqvi, N. I., and Zhou, B. 2015. Comparative genomics identifies the *Magnaporthe oryzae* avirulence effector *AvrPi9* that triggers *Pi9*-mediated blast resistance in rice. *New Phytologist,* **206**(4): 1463-1475.

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. *Theoretical and Applied Genetics,* **111**(1): 50-56.

Wu, Y., Xiao, N., Chen, Y., Yu, L., Pan, C., Li, Y., Zhang, X., Huang, N., Ji, H., Dai, Z., Chen, X. and Li, A. 2019. Comprehensive evaluation of resistance effects of pyramiding lines with different broad-spectrum resistance genes against *Magnaporthe oryzae* in rice (*Oryza sativa* L.). *Rice (N Y),* **12**: 11. doi: 10.1186/s12284-019-0264-3.

Xiao, W.M., Luo, L. X., Wang, H., Guo, T., Liu, Y. Z., Zhou, J.Y., Zhu, X. Y., Yang, Q.Y. and Chen, Z. Q. 2016. Pyramiding of *Pi46* and *Pita* to improve blast resistance and to evaluate the resistance effect of the two R genes. *Journal of Integrative Agriculture,* **15**(10): 2290-2298.

Xiao, W., Yang, Q., Sun, D., Wang, H., Guo, T., Liu, Y., Zhu, X. and Chen, Z. 2015. Identification of three major R genes responsible for broad-spectrum blast resistance in an indica rice accession. *Molecular Breeding,* **35**: 49. https://doi.org/10.1007/s11032-015-0226-4.

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

Xu, X., Hayashi, N., Wang, C. T., Kato, H., Fujimura, T. and Kawasaki, S. 2008. Efficient authentic fine mapping of the rice blast resistance gene *Pik-h* in the *Pik* cluster, using new *Pik-h*-differentiating isolates. *Molecular Breeding,* **22**(2): 289-299.

Yadav, M. K., Aravindan, S., Ngangkham, U., Raghu, S., Prabhukarthikeyan, S. R., Keerthana, U., Marndi, B. C., Adak, T., Munda, S., Deshmukh, R., Pramesh, D., Samantaray, S. and Rath, P. C. 2019. Blast resistance in Indian rice landraces: Genetic dissection by gene specific markers. *PLoS One,* **14**(1): e0211061.

Yadav, M. K., S, A., Ngangkham, U., Shubudhi, H. N., Bag, M. K., Adak, T., Munda, S., Samantaray, S. and Jena, M. 2017. Use of molecular markers in identification and characterization of resistance to rice blast in India. *PLoS One*, **12**(4): e0176236.

Yang, Y., Zhang, H., Xuan, N., Chen, G., Liu, X., Yao, et al. 2017. Identification of blast resistance genes in 358 rice germplasms (*Oryza sativa* L.) using functional molecular markers. *European Journal of Plant Pathology*, **148**: 567–576.

Yap, R., Hsu, Y. C., Wu, Y. P., Lin, Y. R., Kuo, C. W. and Li, Z. K. 2016. Multiplex PCR genotyping for five bacterial blight resistance genes applied to marker-assisted selection in rice (Oryza sativa). *Plant Breeding,* **135**(3): 309-317.

Yasuda, N., Mitsunaga, T., Hayashi, K., Koizumi, S., and Fujita, Y. 2015. Effects of pyramiding quantitative resistance genes *pi21*, *Pi34*, and *Pi35* on rice leaf blast disease. *Plant Disease,* **99**(7): 904-909.

Yoshida, K., Saitoh, H., Fujisawa, S., Kanzaki, H., Matsumura, H., Yoshida, K., Tosa, Y., Chuma, I., Takano, Y., Win, J., Kamoun, S. and Terauchi, R. 2009. Association genetics reveals three novel avirulence genes from the rice blast fungal pathogen *Magnaporthe oryzae*. *Plant Cell,* **21**(5): 1573-1591.

Yu, J., Hu, S., Wang, J., Wong, G. K., Li, S., Liu, B., Deng, Y., Dai, L., Zhou, Y., Zhang, X., Cao, M., Liu, J., Sun, J., Tang, J., Chen, Y., Huang, X., Lin, W., Ye, C., Tong, W., Cong, L., Geng, J., Han, Y., Li, L., Li, W., Hu, G., Huang, X., Li, W., Li, J., Liu, Z., Li, L., Liu, J., Qi, Q., Liu, J., Li, L., Li, T., Wang, X., Lu, H., Wu, T., Zhu, M., Ni, P., Han, H., Dong, W., Ren, X., Feng, X.,

Cui, P., Li, X., Wang, H., Xu, X., Zhai, W., Xu, Z., Zhang, J., He, S., Zhang, J., Xu, J., Zhang, K., Zheng, X., Dong, J., Zeng, W., Tao, L., Ye, J., Tan, J., Ren, X., Chen, X., He, J., Liu, D., Tian, W., Tian, C., Xia, H., Bao, Q., Li, G., Gao, H., Cao, T., Wang, J., Zhao, W., Li, P., Chen, W., Wang, X., Zhang, Y., Hu, J., Wang, J., Liu, S., Yang, J., Zhang, G., Xiong, Y., Li, Z., Mao, L., Zhou, C., Zhu, Z., Chen, R., Hao, B., Zheng, W., Chen, S., Guo, W., Li, G., Liu, S., Tao, M., Wang, J., Zhu, L., Yuan, L. and Yang, H. 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp. indica). *Science*, **296**(5565): 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. *Theoretical and Applied Genetics*, **81**(4): 471-476.

Yuan, B., Zhai, C., Wang, W., Zeng, X., Xu, X., Hu, H., Lin, F., Wang, L. and Pan, Q. 2011. The *Pik-p* resistance to Magnaporthe oryzae in rice is mediated by a pair of closely linked CC-NBS-LRR genes. *Theoretical and Applied Genetics,* **122**(5): 1017-1028.

Zeigler, R. S. T., Nelson, J., Levy, R. and Victoria, M. F. J. C. 1994. Lineage exclusion: A proposal for linking blast population analysis to breeding. In: Zeigler, R. S., Leong, S. A., Teeng, P. S (Eds.). Rice Blast Disease. Wallingford: CAB International: 267–292.

Zhai, C., Lin, F., Dong, Z., He, X., Yuan, B., Zeng, X., Wang, L. and Pan, Q. 2011. The isolation and characterization of *Pik*, a rice blast resistance gene which emerged after rice domestication. *New Phytologist*, **189**(1): 321-334.

Zhang, S., Wang, L., Wu, W., He, L., Yang, X. and Pan, Q. 2015. Function and evolution of *Magnaporthe oryzae* avirulence gene *AvrPib* responding to the rice blast resistance gene *Pib*. *Scientific Reports*, **5**: 11642. doi: 10.1038/srep11642.

Zhang, Y., Luo, L., Xu, C., Zhang, Q. and Xing, Y. 2006. Quantitative trait loci for panicle size, heading date and plant height co-segregating in trait-performance derived near-isogenic lines of rice (Oryza sativa). *Theoretical and Applied Genetics*, **113**(2): 361-368.

Zhao, H., Wang, X., Jia, Y., Minkenberg, B., Wheatley, M., Fan, J., Jia, M. H., Famoso, A., Edwards, J. D., Wamishe, Y., Valent, B., Wang, G. L. and Yang, Y. 2018. The rice blast resistance gene *Ptr* encodes an atypical protein required for broad-spectrum disease resistance. *Nature Communications,* **9**: 2039. https://doi.org/10.1038/s41467-018-04369-4.

Zheng, W., Wang, Y., Wang, L., Ma, Z., Zhao, J., Wang, P., Zhang, L., Liu, Z. and Lu, X. 2016. Genetic mapping and molecular marker development for *Pi65(t),* a novel broad-spectrum resistance gene to rice blast using next-generation sequencing. *Theoretical and Applied Genetics,* **129**(5): 1035-1044.

Zhou, B., Qu, S., Liu, G., Dolan, M., Sakai, H., Lu, G., Bellizzi, M. and Wang, G. L. 2006. The eight amino-acid differences within three leucine-rich repeats between *Pi2* and *Piz-t* resistance proteins determine the resistance specificity to *Magnaporthe grisea*. *Molecular Plant-Microbe Interactions,* **19**(11): 1216-1228.

Zhu, D., Kang, H., Li, Z., Liu, M., Zhu, X., Wang, Y., Wang, D., Wang, Z., Liu, W. and Wang, G. L. 2016. A genome-wide association study of field resistance to *Magnaporthe oryzae* in rice. *Rice (N Y),* **9**: 44. https://doi.org/10.1186/s12284-016-0116-3.

Zhu, Y., Chen, H., Fan, J., Wang, Y., Li, Y., Chen, J., Fan, J., Yang, S., Hu, L., Leung, H., Mew, T. W., Teng, P. S., Wang, Z. and Mundt, C. C. 2000. Genetic diversity and disease control in rice. *Nature,* **406**(6797): 718-722.

Zimmermann, K., Schogl, D., Plaimauer, B. and Mannhalter, J. W. 1996. Quantitative multiple competitive PCR of HIV-1 DNA in a single reaction tube. *Biotechniques,* **21**(3): 480-484.

Zou, S., Stansfield, C. and Bridge, J. 1998. Identification of new influenza B virus variants by multiplex reverse transcription-PCR and the heteroduplex mobility assay. *Journal of Clinical Microbiology,* **36**(6): 1544-1548.

Vita

CURRRICULUM VITA

Name of the student	**:**	Usha Kiran
Father's name	**:**	Sh. Subhash Chander Motan
Mother's name	**:**	Smt. Garo Motan
Nationality	**:**	Indian
Date of Birth	**:**	05-03-1988
Permanent home address	**:**	C/O Sh. S.C Motan, Village Mehlu, R.S Pura, Jammu
E- mail	:	Kiranjmu@gmail.com

EDUCATIONAL QUALIFICATIONS

Bachelor's degree	**:**	B.Sc. Biotechnology
University and year of award	**:**	Guru Nanak Dev University, 2008
Percentage	**:**	70.89%
Masters degree	**:**	M.Sc. Biotechnology
University and year of award	**:**	Guru Nanak Dev University, 2010
Percentage	**:**	66.21%
Doctor of Philosophy	**:**	Ph.D. Biotechnology
University and year of award	**:**	Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu
OGPA	**:**	7.58
Award	**:**	CSIR (NET-LS) 2013 GATE 2011, 2012, 2013
Scholarship	**:**	Rajiv Gandhi National Fellowship (UGC)

CERTIFICATE-IV

Certified that all the necessary corrections as suggested by the external examiner and the Advisory Committee have been duly incorporated in the thesis entitled **"Molecular Marker Assisted Pyramiding of *Pi9* and *Pi54* Blast Resistance Genes in Rice Cultivar K 343"** submitted by **Ms. Usha Kiran,** Registration No. **J-14-D-21-Biot.**

24/12/2020

Dr. Manmohan Sharma
Major Advisor

Place : Chatha, Jammu
Date : 24/12/2020

Dr. R.K. Salgotra
Coordinator
Division of School of Biotechnology