MARKER ASSISTED BACKCROSS BREEDING IN TWO-R GENE PYRAMIDED LINES OF RICE VARIETY JYOTHI FOR BACTERIAL BLIGHT RESISTANCE

By

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THESIS

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DECLARATION

I, hereby declare that the thesis entitled 'Marker assisted backcross breeding in two-R gene pyramided lines of rice variety Jyothi for bacterial blight resistance' is a bonafide record of research work done by me during the course of research and the thesis has not previously formed during the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other University or Society.
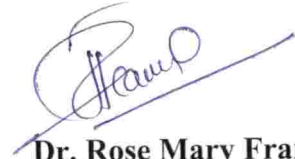
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CERTIFICATE

Certified that the thesis entitled 'Marker assisted backcross breeding in two-R gene pyramided lines of rice variety Jyothi for bacterial blight resistance' is a bonafide record of research work done independently by **Ms. Nayana Nayak** under my guidance and supervision and that it has not been previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.
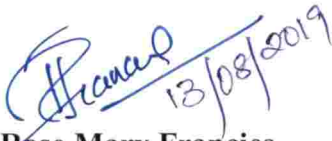
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We, the undersigned members of the advisory committee of **Ms. Nayana Nayak** (2017-11-002), a candidate for the degree of **Master of Science in Agriculture** with major field in **Plant Biotechnology**, agree that the thesis entitled '**Marker assisted backcross breeding in two-R gene pyramided lines of rice variety Jyothi for bacterial blight resistance**' may be submitted by **Ms. Nayana Nayak** in partial fulfilment of the requirement for the degree.

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(Nayana Nayak)

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Dedicated to My Grandparents

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LIST OF ABBREVIATIONS

%	Per cent
BB	Bacterial Blight
bp	Base pairs
cm	Centimeter
cM	Centimorgan
CTAB	Cetyl Trimethyl Ammonium Bromide
COH	College of Horticulture
DBT	Department of Bio Technoilogy
DNA	DeoxyRibo Nucleic acid
EDV	Essentially Derived Variety
g	Gram
GGT	Graphical; Geno Types
IRRI	International Rice Research Institute
ISM	Improved Samba mahsuri
MAB	Marker Assisted Backcrossing
MAS	Marker Assisted Selection
μg	Microgram
μl	Microliter
ml	Milliliter
mm	Millimeter
mM	Millimolar
OD	Optical Density
PCR	Polymerase Chain Reaction
POP	Package of Practices
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism

RLK	Receptor Like Kinase
RM	Rice Microsatellite
SAP	Specific Amplicon Polymorphism
spp	Species
STS	Sequence Tagged Site

Introduction

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the most important food crop of India, Grown in about one-fourth of its total cropped area, it provides food to almost half of the country's burgeoning population which currently stands approximately at 1.37 billion (http://www.worldmeter.info). In 2018-19, India pegged rice production at a record 115.6 million tonnes, 2.4 per cent higher than the previous year (Ghosh, 2019). China and India alone account for about 50 per cent of the rice grown and consumed in the world (Muthayya *et al.*, 2014).

According to Khush (2005), India can remain self-sufficient and meet future demand only when it produces about 130-240 million tonnes of rice by the year 2030. To increase the production and productivity of the crop from limited land, less water, labour and other resources is a daunting challenge. Increasing the production per unit area is the only option to produce more from less land. Hence opting to grow rice varieties with higher yield potential and stability is one approach that can revolutionise rice production in the country.

Rice plays a significant role in moulding the culture and life in Kerala. However, there has been a considerable decrease in area and production of rice in Kerala over the past decade. The rice area and production in the state has declined, respectively from 2.76 lakh ha and 6.30 lakh tons in 2005-06 to 1.71 lakh ha and 4.36 lakh tons, respectively in 2017 (DES, 2017). Despite this decline, rice consumption in Kerala remains high, driven by both population and economic growth.

Kerala faces the challenge of balancing the rice production and demand. Rice farming in the state is under the constant threat of abiotic stress (high soil acidity, iron toxicity and related nutrient deficiencies) as well as biotic stresses. Abundant rainfall and the high humidity favours the incidence of insect pest and disease throughout the farming season.

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is the most economically significant bacterial diseases in rice worldwide. The disease may lead to a yield loss ranging from 20 to 40 per cent and the loss may reach up to 80 to 100 per cent in case of severe incidence (Sonti, 1998). Widespread

incidence of this disease in the state was first reported in 1998 in Palakkad district (Priyadarisini and Gnanamanickam, 1999).

Several options to manage the disease ranging from intercultural practises, application of chemicals, cultivation of resistant varieties and host plant resistance based on multiple genes have been advocated (Chen *et al.*, 2011). The agronomic interventions to manage the disease often fail owing to the extremities of climate prevailing in Kerala. Relying on the use of resistant varieties is the best option as they impart durable resistance against the disease.

Identifying the genes involved in resistance to various strains of *Xoo* races and incorporation of those specific genes into local susceptible cultivar is the best strategy in the development of resistant varieties against specific strain belongs to the particular location. To date, more than 43 host plant resistance (R) genes, listed as *Xa1* to *Xa 42*, have been reported (Zhang *et al.*, 2014; Kim *et al.*, 2015; Hutin *et al.*, 2015; Busungu *et al.*, 2016).

Marker-assisted backcross (MAB) breeding approach is an efficient tool to develop new resistant varieties/lines, especially in rice. However incorporating one gene against the pathogen may not yield desirable protection. Mew *et al.* (1992) reported that the rice varieties with only *Xa4* became susceptible to the *Xoo* pathogen. This makes pyramiding of multiple resistance genes through MAB the best breeding strategy to impart durable resistance to elite rice varieties (Shanti *et al.*, 2010; Salgotra *et al.*, 2012; Baliyan *et al.*, 2018; Das *et al.*, 2018; Sagar *et al.*, 2018). The presence of four *R* genes (*Xa4 + xa5 + xa13 + Xa21*) is reported to protect the rice line NH56, from the Kerala isolate of *Xoo* pathogen (Priyadarisini and Gnanamanickam, 1999).

Among the elite rice cultivars grown in Kerala, Jyothi along with variety Uma covers nearly 80 per cent of the rice-growing belt of the state. Jyothi is highly susceptible to the *Xoo* pathogen. Considering the impact of the BB disease on food security and sustainability of the state, efforts to introgress the three R-genes (*xa5, xa13* and *Xa21*) into the elite cultivar Jyothi (Ptb 39) using Improved Samba Mahsuri (ISM) as the donor parent through Marker Assisted Backcross Breeding (MABB), were initiated at College of Horticulture, Vellanikkara.

Backcrossing of the F_1s to Jyothi (recurrent parent) and further advancement of the population was done to produce BC_2F_4 generation. Resorting to marker assisted selection, Kabade (2017) had identified two 2-R-gene ($xa5+Xa21$) introgressed pyramids (*i.e.*, Plant No. 9 and Plant No. 21) in the BC_2F_4 generation. Backcrossing the two-R gene pyramids to donor parent ISM was done to recover the third R gene ($xa13$), resulting in the production of BC_1F_1s. Simultaneously, selfing of the two 2-R-gene introgressed pyramids was also done to produce the BC_2F_5 generation.

Identification of genotypes pyramided with all the three resistance genes among the BC_1F_1s need to done before resorting to further backcrossing in order to recover the genetic background of recurrent parent Jyothi. Further, the selfed progenies (BC_2F_5) need to be evaluated to identify BB resistant genotypes. The present study was done with the objective to identify BC_1F_1s pyramided with genes ($xa5$, $xa13$ and $Xa21$) conferring resistance to BB using the functional marker and R-gene linked Sequence tagged site (STS) markers. In addition, pathotyping of BC_2F_5 generation was also envisaged.

Review of literature

II. REVIEW OF LITERATURE

Rice is the major source of food for more than half of the world's population. Bacterial blight (BB), which is one of the most important disease in rice, causes significant yield loss (Gnanamanickam *et al.*, 1999; Zhang *et al.*, 2014). In Kerala, severe yield reduction is common in the widely grown elite cultivars Uma (Mo16) and Jyothi (PTB39), owing to the occurrence of BB. The heavy monsoon showers during the rice growing seasons impair the effectiveness of chemical and mechanical control measures. Ensuring durable resistance thus becomes essential and economical to sustain the yield rather than relying on the use of chemical control measures at the time of disease incidence. Presently, exploiting host plant resistance through marker-assisted selection is being emphasised. Enhancing host plant resistance of variety Jyothi by incorporating BB resistance genes through marker assisted backcross breeding (MABB) has been attempted. The literature related to the study is detailed below in brief, under the following headings.

2.1 Bacterial blight (BB) disease in rice

The occurrence of the BB disease in India was reported reported by Srinivas *et al.*, 1959 (Parthasarathy *et al.*, 2014). Thereafter the disease attained the status of epidemics in the year 1962, in Bihar and other regions of North India. Presently, the disease has become prevalent in all rice growing tracts of the country.

2.1.1 The bacterial blight (BB) pathogen

Xanthomonas oryzae pv. *oryzae* (*Xoo*), the causal organism of bacterial blight (BB) disease was initially discovered as a bacterial mass from the dew drops on rice leaves by Takaishi in 1908 and was first isolated by Bokura in 1911. The BB pathogen is a rod-shaped, yellow slime producing non - sporulating flagellate, obligate aerobic bacteria, belonging to the family Xanthomonadaceae.

Other than the single polar flagellum that helps in mobility, the cells are covered with carbohydrates derivatives like galactose, glucose, xylose and uronic acid. The bacterium exudes small droplets rich in these cellular polysaccharide. Wind and rain water act as an active agent for the dissipation of the bacterial exudate containing the bacterium. (Swings *et al.*, 1990).

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Xanthomonas oryzae pv. *oryzae* has a wide host range including other graminaceous species apart from rice. Under unfavourable conditions, the pathogen lives in an inactive form especially on dead plants and seeds and stubbles. They get activated and infect the rice plants during suitable conditions (Mizukami *et al.*, 1961). According to Tagami *et al.* (1964), air and water also show major involvement in the spread of the pathogen inoculum during disease incidence. The presence of *Leersia sayanuka,* which act as an alternative host for the pathogen, is also key to the spread of the disease.

2.1.2 Disease incidence

The pathogen intects plants through the wounds or natural openings or special pores like hydathodes, which are present along the edge of the leaf (Ou, 1985). It enters the water-conducting xylem vessels of plant and starts to proliferate within the vessels and spread to other parts of the host cell. The spread of inoculum can be through soil water or air. Bacteria oozes from the leaf lesions and is spread by wind or rain, especially when strong storms occur and cause wounds to plants. Ooze gets dispersed through irrigation water to uninfected parts of the plants. Rain and stagnant contaminated water are the main ways of dispersal of bacterium. Therefore crop grown in field with poor drainage generally suffer higher infection by BB pathogen. Temperature between from 26°C and 30°C and pH in the range of 6.0 to 6.5 favours the growth of pathogen. However 20°C is best temperature for initial growth.

Disease incidence and severity increases during bad weather (high rainfall and windy conditions), high humidity and temperature fluctuations. Various cultural practises like nursery preparation, transplanting, selection of susceptible rice varieties, *etc.* also favours the disease incidence (Mizukami and Wakimoto, 1969). Clipping of the leaves during transplanting provides a conducive condition for bacterial entry into the host and causes seedling blight in the early stage of the plants the BB incidences are severe in soils with poor drainage among clay or clay loam, and incidence was negligible in the sandy soils, due to good drainage during monsoon season (Tagami *et al.,* 1966). According to Reddy *et al.* (1979), the BB

severity is directly proportional to the amount of nitrogen in the soil and was found to be indirectly proportional to the yield of rice.

The survival of a pathogen between cropping season and its effective dispersal to healthy plants are crucial aspects of the plant disease cycle. Roots, seeds, rice stubbles and the base of the tillers harbour the pathogens and help in their persistance (Mizukami and Wakimoto, 1969). Rhizospheres of weedy plants of *Leersia* and *Zizania* species which acts as an alternative host for the bacterium are considered the most important source of primary inoculum during the winter season of temperate regions (Ou, 1985).

The bacterium induces either wilting of plants or leaf blight according to the stage of the crop. Wilting symptom known as "Kresek" occurs sporadically in the fields causing serious damage in the seedling stage of the plant. Usually, infection occurs within three to four weeks after transplantation of the crop (Nino-Liu *et al.,* 2006). The transplanted seedlings showing Kresek symptoms exhibit wilting of a few leaves or stunted growth and dies within one to six weeks after transplanting (Mew, 1987).

The leaf blight phase predominantly occurs in the late stage of the crop between tillering and heading. Symptoms appear on the leaves of young plants usually towards the tip and margins, as dull greenish to grey-green, water soaked streaks 5 to 10 mm in length. These lesions coalesce and become yellowish-white with wavy edges along either one or both the margins. As the disease progresses, the whole leaf may eventually be affected and then dry. Small droplets of bacterial ooze, pale amber in colour are found on the affected leaves (Yoshimura, 1960).

Although, BB disease is found to affect all stages, starting from the seedling stage to later, the severity increases its infection occurs between maximum during tillering and flowering stage. Infection during grain filling stage may severely affect grain development as well as the fertility of the seed. Post-flowering infection causes less yield loss to compare to pre-flowering infection (Gnanamanickam *et al,*. 1999).

2.1.3   Disease management

Methods of controlling rice BB are limited in their effectiveness. Chemical control has been largely ineffective in minimizing BB infection mainly due to development of resistance to antibiotics due to repeated spraying (Gnanamanickam *et al.*, 1999). Moreover, the applied chemical get washed out due to heavy raining, reducing its retention time and effectivity. Presently the indiscriminate use of chemicals is also a major environment and safety concern. Hence, nowadays the most common method to combat the BB pathogen is the cultivation of rice varieties that can resist *Xoo* infection (Peng *et al.*, 2015).

2.1.3.1 Exploiting host plant resistance

During the last several years many, R- genes conferring resistance against BB disease have been found distributed throughout the rice genome. Receptor-like kinase (RLK) class and nucleotide-binding site-leucine-rich repeat (NBS)-LRR are two important multi R-gene families, related to BB in rice. Approximately a total of 43 resistance genes (Table 1) have been identified so far and named with prefix '*Xa*' or '*xa*' for indicative dominance and recessive nature, respectively. Six R-genes have been cloned (*Xa1, xa5, xa13, Xa21, Xa3/ Xa26* and *Xa27*) and six have been physically mapped (*Xa2, Xa4, Xa7, Xa30, Xa33* and *Xa38*) (Song *et al.*, 1997; Yang *et al.*, 1998; Sun *et al.*, 2003; Gu *et al.*, 2005; Liu *et al.*, 2006; Cheema *et al.*, 2008; Bhasin *et al.*, 2012; Natrajkumar *et al.*, 2012).

The degree of resistance conferred by the gene varies according to the development stage of the crop. The evolution of R-genes in rice for combating new races of the pathogen occurs due to the dynamic interaction between the R-genes and host genome (Horgan and Henderson, 2015).

The resistance in the host against BB pathogen upon infection is activated by production of certain race specific effectors or avirulence factors (transcription activator-like effectors), which targets host cell nucleus and binds to genes that activate resistance to the pathogen (Horgan and Henderson, 2015). The effector-gene binding results in the activation of a series of events finally leads to host resistance. This activated signalling pathways also results in hypersensitivity reactions that check pathogen spread to the rest of the plant by causing localised

cell death or other changes in plants, thereby, leading to a lower degree of disease infection.

Table 1. R-genes conferring resistance against BB pathogen in rice

Gene identified	Resistance source	Origin	Reference
Xa1	Temperate *Japonica*	Japan	Sakaguchi (1967); Yoshimura *et al.* (1998)
Xa2	*Indica*	Vietnam	Kurata and Yamazaki (2006)
Xa3/Xa26	*Japonica*	Japan	Sun *et al.* (2006); Xiang *et al.* (2006)
Xa4	*Indica*	India	Wang *et al.* (2001)
xa5	Aus	Bangladesh	Petpisit *et al.* (1977)
Xa6/Xa3	–	USA	Sidhu *et al.* (1978)
Xa7	Aus	Bangladesh	Sidhu *et al.* (1978); Lee and Khush (2000)
xa8		USA	Sidhu *et al.* (1978); Singh *et al.* (2002)
xa9	–	Laos	Singh *et al.* (1983); Ogawa *et al.* (1988)
Xa10	–	Senegal	Yoshimura *et al.* (1983); Kurata and Yamazaki (2006)
Xa11	*Indica*	Philippines	Kurata and Yamazaki (2006)
Xa12	*Japonica*	Japan	Ogawa (1987)
xa13	–	India	Ogawa *et al.* (1988); Kurata and Yamazaki (2006)

...contd. Table 1. R-genes conferring resistance against BB pathogen in rice

Xa14	Japonica	Taiwan	Sidhu et al. (1978); Kurata and Yamazaki (2006)
xa15	–	–	Nakai et al. (1988); Ogawa (1996)
Xa16	Indica	Vietnam	Kurata and Yamazaki (2006)
Xa17	Japonica	South Korea	Kurata and Yamazaki (2006)
Xa18	Indica, Japonica	Philippines, Japan	Liu et al. (2004); Kurata and Yamazaki (2006)
xa19	–	Japan	Ogawa et al. (1978)
xa20	–	–	Taura et al. (1992); Kurata and Yamazaki (2006)
Xa21	Wild species of Oryza	Mali	Song et al. (1995)
Xa22 (t)	–	China	Sun et al. (2004); Kurata and Yamazaki (2006)
Xa23	Wild species of Oryza	China/Cambodia	Zhang et al. (1998); (2001)
xa24	–	Bangladesh	Khush and Angeles (1999)
xa25(t)	Indica	China	Liu et al. (2011)
xa26(t)	Indica	China	Lee et al. (2003)

Xa27(t)	Wild species of *Oryza*	Philippines	Lee *et al.* (2003); Gu *et al.* (2004)
xa28(t)	*Indica*	Bangladesh	Lee *et al.* (2003)
Xa29(t)	Wild species of *Oryza*	–	Tan *et al.* (2004)
Xa30(t)	Wild spp. of *Oryza*	India	Cheema *et al.* (2008)
Xa31(t)	*Japonica*	China	Wang *et al.* (2009)
Xa32(t)	Wild species of *Oryza*	–	Ruan *et al.* (2008); Zheng *et al.* (2009)
Xa33	Wild species of *Oryza*	–	Natrajkumar *et al.* (2012)
xa33(t)	–	Thailand	Korinsak *et al.* (2009)
xa34 (t)	*Indica*	Sri Lanka	Chen *et al.* (2011)
Xa35 (t)	Wild species of *Oryza*	Philippines	Guo *et al.* (2010)
Xa36(t)	–	China	Miao *et al.* (2010)
Xa38(t)	*Oryza nivara*	–	Bhasin *et al.* (2012)
Xa39	*Oryza rufipogan*	–	Zhang *et al.* (2014)
Xa40(t)	–	Korea	Kim *et al.* (2015)
Xa41(t)	–	–	Hutin *et al.* (2015)
Xa42(t)	–	Japanese	Busungu *et al.* (2016)

Of the 43 R-genes reported so far 29 genes are dominant in their expression. Dominant genes confer more resistance to BB pathogen. The genes introgressed in the present study are:

2.1.3.1.1 *Xa21*

The *Xa21* gene is a class IV resistance gene. Unlike other *Xa* genes, this dominant resistant locus shows resistance to all Indian races of *Xoo* tested. This gene was identified in a wild rice species of *O. longistaminata* and was the first gene to be transferred to the background of *O. sativa* (Khush *et al.*, 1989)Peng *et al.* (2015) revealed that *Xa21* shows a broad spectrum of resistance due to the presence of RLK motif. It has the ability to activate different signalling pathways associated with resistance. RAPD marker RAPD 248 was used for gene tagging (Ronald *et al.*, 1992). A new STS marker pTA 248 was designed at a distance 0.2 cM from *Xa21* on the basis of earlier reported marker RG103. The sequence of the *Xa21* gene revealed that it codes for a receptor kinase domain with serine-threonine specificity. Ronald (1997) used a map based cloning strategy for the first time to clone the disease resistance gene *Xa21* in rice. According to Ponciano *et al.* (2006), *Xa21* shows a high degree of resistance and a moderate degree of resistance during the mature and juvenile growth stage of the rice, respectively. This is mainly because of the complete expression of the gene at the mature stage of plant, whereas, in the juvenile stage, the gene failed to express completely thereby impart moderate degree of resistance.

2.1.3.1.2 *xa5*

xa5 is an important race-specific recessive gene against BB in rice. The gene was mapped onto chromosome 5 of the rice genome (Blair and McCouch, 1997). In Marker assisted backcross breeding (MABB), marker RG556 (RFLP) derived STS markers is used for the confirmation of the gene presence (Huang *et al.*, 1997). Unlike other markers, *xa5* gene specific STS marker does not exhibit polymorphism among the resistant and susceptible plants after PCR amplification. Therefore, for further confirmation, PCR product need to be digested with a restriction enzyme (*Dra 1*) to produce Specific Amplicon Polymorphism (SAP). The gene encodes the gamma subunit of transcription factor IIA (TFIIAγ). Sequencing of (TFIIAγ)

revealed that the production of different amino acids plays a major role in the exhibition of resistance or susceptibility to the pathogen. This indicated that the resistance pathway governed by the *xa5* gene is different from other genes.

2.1.3.1.3 *xa13*

xa13 was mapped on to chromosome 8 of the rice genome. A PCR-based STS marker linked to *xa13* gene was derived from RFLP marker RG136. Zhang *et al.* (1996) tagged the gene with RAPD marker OPAC05900 and RFLP marker RG136. PCR product of RG136 shows monomorphic banding pattern and it is difficult to select the resistant cultivar on this basis. Hence, the PCR product needs to be treated with restriction enzyme *HinfI* to generate SAP.

Conventional backcross breeding approach was used to develop BB resistant rice varieties with *Xa 4* gene (Khush *et al.*, 1989). However, they reported that some of the conventionally bred improved high yielding varieties with BB resistance gene (*Xa 4*) fail to exhibit resistance against bacteria, as new races of the bacterium continue to adapt and overcome the resistance conveyed by the major gene.

Initially, BB resistance varieties like IR36, Karjat, *etc.* were improved through crossing with donor parent TKM6 which act as a source for BB resistance genes in India. Similarly, varieties like Ratnagiri and PR4141 was improved through conventional breeding (Sundaram *et al.*, 2011).

2.1.3.1.4 Pyramiding of multiple genes

The breakdown of genetic resistance by plant pathogen populations is a major setback to the genetic control of crop disease. Increased research efforts revealed that horizontal gene resistance proved more durable than vertical resistance. When grown in Kerala, the rice line IRBB21 (supposed to be resistant) was found to be susceptible to *Xoo* pathogen races isolated from the region (Gnanamanickam *et al.*, 1999). This result suggested that the influx of a single resistant gene is not good enough and might cause a shift in pathogen race regularity leading to the subsequent breakdown of resistance (Khush *et al.*, 1989; Mew *et al.*, 1992; Joseph *et al.*, 2004; Joshi and Nayak, 2010).

Combination of genes provided a wider spectrum of resistance to the pathogen. Gene pyramiding refers to a method aimed at assembling multiple desirable genes from multiple parents into a single genotype for a specific trait (Joshi and Nayak, 2010). This approach works towards the objective of like enhancing the disease resistance durability and broadening the genetic basis of released cultivars (Pink, 2002; McDowell and Woffenden, 2003).

Joseph et al. (2004) observed that the BB resistance genes *Xa4, xa8, xa13* and *Xa21* conferred resistance against four different strains of the pathogen isolated from Basmati growing regions.

The rice line NH56 containing four R-genes (*Xa4* + *xa5* + *xa13* + *Xa21*) was found to resist the isolates of the pathogen from Kerala. (Priyadarisini and Gnanamanickam, 1999).

The presence of genes *xa5, xa13* and *Xa21* was found to give broad spectrum resistance to all predominant races of BB pathogen found in Philippines and Punjab, when introgressed into a susceptible *Indica* rice cultivar PR106. The efficacy of R-gene *Xa21* towards BB was found to be more than *xa5*, while, *xa13* exhibited less effectiveness towards BB compared to the other two genes (*Xa21> xa5>xa13*) (Singh et al., 2001).

2.2 Marker Assisted Selection (MAS) to incorporate bacterial blight resistance in rice

Gene introgression through conventional breeding is a long time experiment. In the conventional approach, the breeder confronts difficulty in delineating the phenotypic reaction to disease, owing to gene epistasis, linkage drag, *etc.* In addition, it would be difficult to identify rice crops with multiple R-genes using the conventional approach alone. Due to the masking effect of genes, identification of plant carrying genes in conventional method does not always ensure the best result (Khan et al., 2014).

Marker-assisted selection (MAS) helps to overcome the disadvantages of conventional breeding However, it cannot serve as a but cannot substitute for conventional breeding (Chukvu, 2019). It is used to enhance the efficiency of

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selection. Basically, it economises the labour cost and the time-consuming phenotypic screening procedure.

The marker and target gene co-segregation and linkage are major factors that determine the effectiveness of MAS (Dekkers, 2004). Salgotra *et al.* (2012) reported that the effectiveness of MAS in BB resistance breeding approach can be enhanced by the use of functional markers which help in the direct selection of R-genes conferring BB resistance. To date, several functional markers have been identified, designed and efficiently used for identifying BB resistance (Song *et al.*, 1995; Yoshimura *et al.*, 1998; Iyer and McCouch, 2004; Chu *et al.*, 2007).

Backcross breeding strategy was employed to transfer the BB resistance (*Xa21*) to rice variety IRBB21 from donor parent *O. longistaminata* using pTA248 marker (Ronald *et al.*, 1992). Similarly resistant gene *xa5* was introgressed from variety DZ192 (Yoshimura *et al.*, 1995) and *xa13* from Nang Som (Zhang *et al.*, 1996) into rice lines IRBB5 and IRBB13, respectively. This transfer was done using markers RG556 and RG136 corresponding to these resistance genes.

Functional markers were found to reduce the risk of false selection in marker assisted selection. Functional markers (STS markers) RG556, RG136 and pTA248 closely linked to the BB resistance genes *xa5, xa13* and *Xa21,* respectively were used successfully to develop pyramided lines in rice (Sundaram *et al.*, 2008; Salgotra *et al.,* 2012; Magar, 2014; Ellur *et al.*, 2016; Kumar *et al.*, 2016.). The success of MAS in developing BB resistance in rice is detailed in Table 2.

Table 2. The success of MAS in developing BB resistance in rice

Sl. No.	Variety improved	Donor parent	Genes incorporated	MAS approach employed	Reference
1	IR65600-96, IR65598-112 and IR65600-42	IRBB59	*xa5, xa13* and *Xa21*	MABB using markers RG207 and RG556 for *xa5* gene, RM136 for *xa13* and	Sanchez *et al.* (2000)

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				pTA248 for *Xa21*	
2	PR106	IRBB62	*xa5, xa13* and *Xa21*	MABB using markers RG556 for *xa5* gene, RM136 for *xa13* and pTA248 for *Xa21*	Singh *et al.* (2001)
3	IR 50	C101A51	*Xa21* and *Xa4*	MAS	Narayanan *et al.* (2002)
4	IR 24	Nang Som	*xa5, xa13* and *Xa21*	MABB using STS markers RG556 for *xa5*, RG136 for *xa13* and pTA248 for *Xa21*	Nguyen and Nguyen (2004)
5	Improved Samba Mashuri (ISM)	SS1113	*xa5, xa13* and *Xa21*	MABB using STS markers RG556 for *xa5*, RG136 for *xa13* and pTA248 for *Xa21*	Sundaram *et al.* (2008)
6	Jyothi and IR50	NH56	*Xa4, xa5, xa13,* and *Xa21,*	MABB using markers RG207 and	Bharathkumar *et al.* (2008)

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				RG556 for *xa5* gene, RFLP marker G181 for *Xa4* RM136 for *xa13* and pTA248 for *Xa21*	
7	ADT43 and ADT47	IRDB60	*xa5*, *xa13* and *Xa21*	MABB using markers RM122 (*xa5*); RG136 (*xa13*) and pTA248 (*Xa21*)	Bharani *et al.* (2010)
8	Parental hybrid lines KMR3, IR58025B, PRR78, Mahsuri and Pusa 6B	IRBB60	*Xa4*, *xa13*, *Xa21* and *xa5*	MAS using STS markers Npb 181 for *Xa4*, RG136 for *xa13*, pTA248 for *Xa21* and RM122 (SSR) for *xa5*	Shanti *et al.* (2010)
9	Basmati breeding line IRS 5441	IRBB59	*xa13* and *Xa21*	MABB using marker RG136 for *xa13* and pTA248 for *Xa21*	Salgotra *et al.* (2012)
10	Basmati 370	IRBB55	*xa13* and *Xa21*	MABB using marker RG136	Bhat *et al.* (2015)

				for *xa13* and pTA248 for *Xa21*	
11	RD6	IR62266	*xa5*	MABB using SSR marker RM122/ RM159	Pinta *et al.* (2013)
12	Mangeumb yeo,	IRBB57	*Xa4, xa5* and *Xa21*	MAS using PCR specific MP1+MP2 for *Xa4*, 10603.T10Dw for *xa5* and U1/I1 for *Xa21*	Suh *et al.* (2013)
13	MTU1010.	B95-1	*xa13* and *Xa21*	MABB using marker *xa13 promoter* for *xa13* and *pTA 248* for *Xa21*	Magar *et al.* (2014)
14	local varieties in the Iranian provinces	5/DV85, IR24	*Xa7, Xa14* and *Xa21*	MABB	Khoshkdam an *et al.* (2014)
15	Swarna	IR64	*xa5, xa13* and *Xa21*	MABB using markers RG556 for *xa5* gene, RM136 for *xa13* and pTA248 for *Xa21*	Pradhan *et al.* (2015)

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16	Native variety of Pakistan (IR 24)	IRBB4 (carrying Xa4), IRBB5 (carrying xa5) and IRBB21 (carrying Xa21)	Xa21, xa5 and Xa4	MAS using marker pTa 248 for Xa21 , RM122 for xa5 and MP1 for Xa4	Sabar et al. (2016)
17	Jalamagna	Swarna	Xa21, xa13 and xa5	MABB using markers RG556 for xa5 gene, RM136 for xa13 and pTA248 for Xa21	Pradhan et al. (2015b)
18	CRMAS26 21-7-1	IRBB60	xa5, xa13 and Xa21	MABB using markers RG556 for xa5 gene, RM136 for xa13 and pTA248 for Xa21	Das et al. (2015)
19	MTU1010	Improved Samba Mashuri	Xa21 and xa13	using the co-dominant markers, pTA248 for Xa21 and xa13-prom for xa13	Arunakumari et al. (2016)
20	Wanhui 6725 (WH6725)	IRBB27	Xa27	MABB using marker M124 used for Xa27	Luo et al. (2016)

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21	Improved Tapaswini	IR64	xa5, xa13, and Xa21	MABB using markers RG556 for xa5 gene, pTA248 for Xa21 and xa13 promoter for xa13	Dash et al. (2018)
22	Tainung 82	IR BB66	Xa4, xa5, Xa7, xa13 and Xa21	MABB using Primer Xa4, RM604, Primer Xa7, Xa13 and Xa21	Yap et al. (2016)
23	Karma Mashuri	Indica	Xa4, xa5 and Xa21	MABB using markers RM224 for Xa4, xa5 SR for xa5 and pTA248 for Xa21	Verma (2016)
24	RPHR-1005	DRRH-3	Xa21 and Xa33	MABB	Kumar et al. (2016)
25	Maintainer lines CO 2B, CO 23B, and CO 24B	IRBB60	xa5, xa13, and Xa21	MABB using Gene specific markers	Ramalingam et al. (2017)
26	BRRIdhan 52	IRBB60	Xa21	MABB using Gene specific markers	Kabir et al. (2017)
27	Karma Mahsur	IRBB59	xa5, xa13 and Xa21	MABB using markers xa5R,	Deshmukh et al. (2017)

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				xa5S, xa13 promoter and *Xa21*F/R	
28	Basmati variety CSR-30	IRBB- 60	*Xa21, xa13 and xa5*	MABB using markers pTA248, RG136 and RG556	Baliyan *et al.* (2018)
29	Pusa Basmati 1509	Pusa 1790	*xa13* and *Xa21*	MABB using markers used xa13 prom for *xa13* and pTA248 for *Xa21*	Sagar *et al.* (2018)
30	*Japonica* rice	IR65482-7-126-1-2	*Xa40+Xa3*	MABB using markers SSR and CAPS markers are used	Reinke *et al.* (2018)
31	APMS 6B	PR 114	*Xa38*	MABB using Gene specific marker	Yugander *et al.* (2019)
32	Jagatial Sannalu (JGL 1798)	Improved Samba Mahsuri	*xa13* and *Xa21*	MABB using markers xa13 promoter for *xa13* and PTA248 *for Xa21* used	Swathi *et al.* (2019)
33	Yuehui9113	YH (Y58S/Hua15)	*Xa21*	Marker pTA248 for *Xa21* used	He *et al.* (2019)

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| 34 | DRR17B | ISM and 'Samba Mahsuri' (FBR1-15EM) | *Xa21* and *Xa33* | MABB using markers pTA248 for *Xa21* and RMWR7.6 for *Xa33* | Balachiranj eevi *et al.* (2018) |

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Materials and Methods

III. MATERIALS AND METHODS

Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* is the most important disease of rice. Rice cultivar Jyothi, the most popular rice variety grown in the State of Kerala, suffers severe yield loss due to BB infection. Incidence of this disease causes great economic loss to the farmers as well. In order to confer resistance to rice variety Jyothi against bacterial blight, efforts to pyramid three R genes (*xa5, xa13* and *Xa21*) into the variety from donor parent Improved Samba Mashuri (ISM) through Marker Assisted Backcross Breeding (MABB) was carried out at the College of Horticulture, Vellanikara, Thrissur. The backcross progenies (BC_1F_1 and BC_2F_5) thus generated, formed the basis of the present investigations. The study comprised of four experiments listed below:

I. Genotyping of BC_1F_1 population

II. Morphological characterisation of pyramided lines (BC_1F_1 population)

III. Production of BC_2F_1's and BC_1F_2's

IV. Morphological characterisation and pathotyping of BC_2F_5 population, and production of BC_2F_6 population

3.1 Experimental location

The study was conducted in the Department of Plant Biotechnology, College of Horticulture, Kerala Agricultural University, Thrissur, between 2017 and 2019. The experimental site is located 40 m above MSL between $10°31'$N latitude and $76°13'$E longitude and experiences humid tropic climate. The laboratory and field facilities of the Department of Seed Science and Technology, College of Horticulture, Vellanikara, Thrissur were also utilised for conducting the experiment.

3.2 Experimental material

Improved Samba Mashuri (ISM), is an essentially derived variety developed through MABB at the Indian Institute of Rice Research (formerly the Directorate of Rice Research), Hyderabad. Improved Samba Mashuri has been introgressed with three major bacterial blight resistance genes *Xa21*, *xa13* and *xa5*. Pyramiding three bacterial blight resistance genes from Improved Samba Mahsuri into popular

cultivar Jyothi was first initiated under the DBT project: 'Rice-Gene pyramiding to develop cultivars with durable resistance to bacterial blight through Marker assisted selection' (MAS) at the College of Horticulture, Vellanikkara,Thrissur. Backcrossing of the F_1s to Jyothi (recurrent parent) and further advancement of the population was done to produce BC_2F_4 generation. Resorting to the marker-assisted selection, Kabade (2017) identified two 2-R-gene ($xa5+Xa21$) introgressed pyramids (*i.e.*, Plant No. 9 and Plant No. 21) in the BC_2F_4 generation. Backcrossing the two-R gene pyramids to donor parent ISM was done to recover the third R gene ($xa13$). This resulted in the production of BC_1F_1s. Simultaneously, selfing of these individuals was also done to produce the BC_2F_5 generation. The details of the parental material (Table 3) and the genesis of the experimental population is traced in Fig.1

Table 3. Genotypes used to generate the experimental material

Genotype	Parentage	Year of production/ release	Salient features
Recurrent parent: Jyothi (Ptb 39)	Ptb 10 × IR8(HS)	1974	Medium duration (110 - 125) Red kernled rice variety
Donor parent: Improved Samba Mahsuri (ISM)	Samba Mahsuri × 4/SS1113	2008	Long duration (135 - 140) White kernelled rice variety
BC_2F_4 Plant No. 9	Jyothi (Ptb 39) × Improved Sama Mahsuri (ISM)	2017	Long duration (140 days) Red kernled rice variety
BC_2F_4 Plant No. 21	Jyothi (Ptb 39) × Improved Sama Mahsuri (ISM)	2017	Long duration (135 days) Red kernled rice variety

3.3 Experiment 1: Genotyping of BC_1F_1 population

Experiment block of BC_1F_1's (Table 4) was raised in January 2019. Seeds of BC_1F_1 Plant No. 9 and Plant No. 21 as well as the seeds of the donor parent ISM

Jyothi (Ptb 39)

Improved Samba Mahsuri (ISM)
($xa5 + xa13 + Xa21$)

F_1s X   Jyothi (Ptb 39)
(Recurrent parent)

BC_1F_1s

Backcrossing and selfing

BC_2F_4s

Genotyping

Two -R gene *(xa5* and *Xa21*) introgressed (**BC₂F₄'s Plant No. 9 and 21**) were identified   X   ISM Donor parent

Selfing

Backcrossing

BC_2F_5s seeds obtained
(212 Nos.)

Plant No. 9: 165 seeds

Plant No. 21: 47 seeds

BC_1F_1 seeds obtained
(27 Nos.)

Plant No. 9: 22 seeds

Plant No. 21:05 seeds

Figure 1. Preamble to the material used in the study

and recurrent parent Jyothi, were sown in separate trays containing sterilised soil. Twenty-one-day-old seedlings of backcross progenies as well as parents were then transferred in to pots of 30 cm height and 25 cm diameter. To obtain a good crop stand, standard agronomic practices as per package of practices (KAU, 2016) were followed during the entire crop period.

Table 4. Details of BC$_1$F$_1$s used

Source of BC$_1$F$_1$s	BC$_1$F$_1$s seeds sown (No.)	Number of surviving plants studied
BC$_2$F$_4$ Plant No. 9 × ISM	22	21
BC$_2$F$_4$ Plant No. 21× ISM	5	3
Total	27	24

3.3.1 DNA isolation, analysis of quality and quantity

Quality and quantity estimation of the cellular DNA extracted from BC$_1$F$_1$s and the recurrent and donor parents were done as enumerated below.

3.3.1.1 Extraction of genomic DNA

The young emerging leaf samples were collected from plants grown in net house in the morning. Extraction of DNA from the collected samples was done by following the Modified CTAB method of Dellaporta *et al.* (1983), as per the protocol detailed below.

Required reagents

1. **CTAB buffer**

Table 5. CTAB Extraction buffer (2%)

Contents	Concentration
CTAB (W/V)	2 %
NaCl	1.4 M
EDTA (pH 8)	20 mM
Tris HCL (pH 8)	100 mM
Polyvinylpyrrolidin (PVP)	1 %
B-mercaptoethanol	10 mM

2. Chloroform - Isoamyl alcohol (24:1)

3. 70 per cent ethanol

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4. Chilled isopropanol (100 %)

5. RNAses (10 mg/mL)

Procedure

1. Before extracting the DNA, the leaf samples were thoroughly washed with distilled water as well as wiped with 70 per cent ethanol. Leaf samples (0.2 g) were then weighed out from the sterilised samples.

2. The leaf sample was cut into small pieces and ground into a fine paste using 1 mL CTAB (Cetyl Trimethyl Ammonium Bromide) buffer along with 50 μL of β-mercaptoethanol and a pinch of Polyvinylpyrrolidine (PVP), in a pre-chilled mortar and pestle.

3. After the samples homoginised with gentle inversion, they were transferred to an autoclaved 2 mL centrifuge tube and incubated at 65°C in a water bath for 15 to 20 minutes.

4. On incubation, equal volume (1 mL) of chilled chloroform: isoamyl alcohol (24:1,) was added to the sample and the contents inverted mixing. The samples were centrifuged using high-speed refrigerated centrifuge (Eppendorf 5804 R) at 10,000 rpm for 10 minutes at 4°C. After centrifugation, the content was separated into three distinct layers.

   Aqueous top layer – DNA with a small quantity of RNA

   Middle layer – Protein and other cell debris

   Bottom layer – containing chloroform and some pigments, *etc.*

5. Without disturbing the three layers, only the upper aqueous layer was carefully transferred into a new centrifuge tube. To this 1/10th volume of 10 per cent CTAB solution and an equal volume of chloroform: isoamyl alcohol (24:1) were added. Centrifuged at 12,000 rpm for 15 minutes at 4°C.

6. After centrifugation transferred the top layer was transfered to the fresh tube and 2 μL of RNase (10 mg/mL) was added and incubated in water bath for 20 minutes at 37°C.

7. On centrifugation, once again, an equal volume of chloroform: isoamyl alcohol (24:1) was added to the samples and centrifuged at 10,000 rpm for 10 minutes.

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8. The top aqueous phase was transferred to a 1.5 mL tube and an equal volume of ice-cold isopropanol was added. The samples were then incubated at -20 °C overnight for precipitation of DNA.

9. After 24 hours, the samples were centrifuged at 10,000 rpm for 10 minutes at 4°C and the supernatant was decanted carefully, retaining only the pellet.

10. To the pellet, 200 μL of 70 per cent ethanol was added and the contents centrifuged again at 10,000 rpm at 4°C for 10 minutes. Washing with 90 per cent ethanol was repeated. After centrifugation the ethanol was drained without disturbing the pellet. The pellets were air-dried, dissolved in the 50-100 μL distilled water and stored in vials at -20°C.

3.3.1.2 Determination of quality and quantity of isolated DNA

The quality and quantity of the DNA isolated were determined based on the absorbance at 260 nm using the Nanodrop Spectrophotometer Nano Drop® ND1000, which works on the principle of Beer-Lambert's law. Nucleic acids show absorption maxima around 260 nm whereas proteins shows peak absorbance at 280 nm. The DNA purity is indicated by the ratio OD 260/280.

3.3.2 Foreground selection

The functional marker xa13 pro and xa5 SR was used to confirm the presence of genes *xa13* and *xa5,* respectively (Table 6). Three STS markers, RG556, RG136 (restriction digestion based) and pTA248, closely linked to the BB resistance genes *xa5, xa13* and *Xa21*, respectively, was used to confirm the presence of the resistance allele of each gene in the backcross generation as per the procedure given by Sundaram *et al.* (2008).

Table 6. Markers used for foreground selection

Gene	Primer name	Primer sequence (5' -3')	Marker distance (cM)	Product size (bp)	AT (˚C)	Reference
xa5	xa5SR	F: AGCTCGCCATT CAAGTTCTTGAG R: TGACTTGGTTC TCCAAGGCTT	0.0	410, 310, 180	61.0	Petpisit *et al.* (1977)

	RG 556	F: ATACTGTCACA CACTTCACGG R: GAATATTTCAGT GTGTGCATC	0.1	440, 410	53.5	
xal3	RG 136	F: TCCCAGAAAGCT ACTACAGC R: GCAGACTCCAGT TTGACT TC	3.8	530, 490	54.0	Sundaram *et al.* (2008)
	xal3 pro	F: GGCCATGGCTCA GTG TTTAT R: GAGCTCCAGCTC TCCAAATG	0.7	500	61.0	
Xa21	pTA 248	F: AGACGCGGAAGG GTGGTTCCCGGA R: AGACGCGGTAAT CGAAAGATG AAA	0.2	1000	65.0	

3.3.2.1 Thermal cycling

It is an *in vitro* method for the enzymatic synthesis of specific DNA sequences widely employed to generate multiple copies of good quality isolated DNA. Temperature for annealing (AT) was standardised based on the primers used (Table 6). PCR amplification was done using thermal cyclers (model- Veriti 96 Well Thermal Cycler, made: Applied Biosystems and model- Master Ggradient, made: Eppendorf).

The amplification of diluted genomic DNA was carried out in 0.2 mL PCR tubes using a 20 μL reaction mixture as detailed in (Table 7) as per the profile detailed.

Table 7. Composition of the thermal cycling reaction mixture (20μL)

Components	Quantity(μL)
Genomic DNA	1.5
10X Taq assay buffer B	2
$MgCl_2$	0.7
dNTP mix	1.5
Taq DNA polymerase (3 U)	0.3
Primers (Forward and Reverse)	1 μL each
Chilled autoclaved distilled water	12.0
Total	**20**

The PCR programme followed

a.	94 °C for 4 min.	:	Initial denaturation
b.	94 °C for 45 sec.	:	Denaturation
c.	50 °C to 55 °C for 1 min.	:	Primer annealing
d.	72 °C for 2 min.	:	Primer extension
e.	72 °C for 8 min.	:	Final extension
f.	4 °C hold for infinity	:	Storage

Items b, c, d bracketed as: 35 Cycles

3.3.2.2 Restriction digestion of PCR product of STS markers

The PCR amplified product (10 µL) of STS markers RG556 and RG 136 before restriction digestion was used to detect the amplification of DNA. The remaining 10 µL of the amplified DNA was subjected to restriction digestion with enzymes *Hinf*1 and *Dra*1, respectively. The reaction mixture (30 µL) detailed in Table 8 was then centrifuged for one minute and incubated at 37 °C for two hours.

Table 8. Reaction mixture for restriction digestion

Aliquot	Quantity(µL)
Distilled water	17
10X- Fast digest green buffer	2
DNA samples after PCR	10
Fast digest enzyme (Dra1/Hinf1)	1
Total	**30**

3.3.2.3 Gel electrophoresis of PCR products

The amplified PCR products (STS and SSR) of markers used for foreground and background selection were separated in a horizontal gel electrophoresis unit (GeNie) using 1.5 per cent and 2 per cent agarose gel, respectively.

Reagents used:

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1) 50X TAE buffer (Table 9)

Table 9. Composition for TAE buffer

Contents	50X TAE (1 Litre stock solution)
Tris base	242 g
Glacial acetic acid	57.1 mL
EDTA	100 mL of 500 mM (pH 8)
Distilled water	842.9 mL

Working stock (1X TAE) 1 litre was prepared by dissolving 20 mL of 50X TAE in 980 mL distilled water.

2) Agarose 1.5 per cent and 3 per cent

3) Ethidium Bromide (0.5 µg/mL)

Procedure:

Agarose gel was prepared by melting respective amount of agarose in 1X TAE buffer. The mixture was heated in a microwave or hot plate until agarose was dissolved and the solution was clear. The solution was allowed to cool to about 42°C to 45°C and ethidium bromide was added (1 µL for each 10 mL of gel) at this point before pouring it carefully into the gel casting tray. The comb was then carefully inserted into the gel before it solidified. The set up was left undisturbed to cool for about half an hour at room temperature after which the comb was gently removed. The solidified gel was then placed along with the tray in an electrophoresis chamber, taking care to immerse it completely in the electrophoresis buffer (1X TAE). The PCR digested samples were then loaded into the wells. A 100 bp DNA ladder was also loaded in one of the wells. Electrophoresis was done for approximately 45 minutes at 80 volts and stopped when the dye has migrated two third the length of the gel. The electrophoresed gel was then transferred to gel documentation unit to observe the banding pattern under UV exposure.

3.3.2.4 Visualisation of PCR product and screening

Proper visualisation of bands in the electrophoresed gel and scoring of the well resolved and distinct amplicons for monomorphism /polymorphism, was done

using gel documentation software (GeNei[TM] – UVITEC Fire Reader, Merck, UK + Dell computer system).

3.3.3 Background selection

One hundered and eighty six rice microsatellite (RM) (Appendix I) primers were used to study the parental polymorphism. Of these 58 primers were observed to produce polymorphism between the studied parents. The polymorphic 58 RM primers (Table 10) were selected for background selection for background selection of BC_1F_1s. The list of background markers used is described in Table 10.

Table 10. Markers used for background selection

Sl. no.	Marker name	Chromosom No.	Forward primer Reverse primer (5' -3')	AT °C	Expected size product (bp)
1	RM11069	1	F:GGTACAATGAAGCTTGGCAACG R:CGGTGGAGTAGAACCACGAAGC	63.0	279
2	RM24	1	F:GAAGTGTGATCACTGTAACC R:TACAGTGGACGGCGAAGTCG	53.8	192
3	RM583	1	F:AGATCCATCCCTGTGGAGAG R: GCGAACTCGCGTTGTAATC	56.0	192
4	RM493	1	F:TAGCTCCAACAGGATCGACC R:GTACGTAAACGCGGAAGGTG	53.0	211
5	RM9	1	F:GGTGCCATTGTCGTCCTC R: ACGGCCCTCATCACCTTC	55.5	136
6	RM243	1	F:GATCTGCAGACTGCAGTTGC R: AGCTGCAACGATGTTGTCC	58.5	116
7	RM1	1	F:GCGAAAACACAATGCAAAAA R: GCGTTGGTTGGACCTGAC	55.0	113
8	RM11313	1	F:TGAGGCTGATAGAAAGCAGAATGC R:CCCGTTTCTTCCATATCATGTCG	64.0	388
9	RM13910	2	F:GAGCGAGCTATACCACCGTGACC R:ATCGCGTCCAAGAAAGGTGTCG	61.0	188
10	RM3340	2	F:TCTTGGCAAGCTCTCCTCTC R: CCATCATCTCGATCTTGACG	58.3	117
11	RM263	2	F:CCCAGGCTAGCTCATGAACC R:GCTACGTTTGAGCTACCACG	55.5	199

12	RM324	2	F:CTGATTCCACACACTTGTGC	52.3	175
			R: GATTCCACGTCAGGATCTTC		
13	RM207	2	F:CCATTCGTGAGAAGATCTGA	52.8	118
			R: CACCTCATCCTCGTAACGCC		
14	RM214	3	F:CTGATGATAGAAACCTCTTCTC	54.0	112
			R:AAGAACAGCTGACTTCACAA		
15	RM16	3	F:CGCTAGGGCAGCATCTAAA	54.0	181
			R: AACACAGCAGGTACGCGC		
16	RM85	3	F:CCAAAGATGAAACCTGGATTG	55.5	107
			R: GCACAAGGTGAGCAGTCC		
17	RM14723	3	F:GCAAAGTCCTTTGGACAGGTAGC	55.5	195
			R:CGTCCCAGATCAAAGTACACTCTTCC		
18	RM14487	3	F:TGCACACTCTGCCTAAATTTGC	55.5	391
			R:CGAGAGTGTCTGTCTAGATTTCAGG		
19	RM411	3	F:ACACCAACTCTTGCCTGCAT	52.6	110
			R:TGAAGCAAAAACATGGCTAGG		
20	RM7	3	F:TTCGCCATGAAGTCTCTCG	50.4	180
			R: CCTCCCATCATTTCGTTGTT		
21	RM307	4	F:GTACTACCGACCTACCGTTCAC	59.0	174
			R: CTGCTATGCATGAACTGCTC		
22	RM6679	4	F:TTTAGGCCGTAAGAGCGAAC	58.5	141
			R:GAATTTGAGTAGCTGGCTCC		
23	RM5586	4	F:CTCCATAATCAAGGAAGCTA	57.3	134
			R: ATGAGTTCTTTCGTCAGTGT		
24	RM261	4	F:CTACTTCTCCCCTTGTGTCG	52.8	125
			R: CTACTTCTCCCCTTGTGTCG		
25	RM19218	5	F:CGGAGGGAGTAGGTACGTAGGG	57.5	169
			R:CCCATTCCATTCTACACTGACG		
26	RM20158	6	F:ACTCACCGTACGAACTCGATGC	51.9	238
			R:ATCTGTCCTGAACCCGATACTGC		
27	RM3628	6	F:AATCATGCCTAGAGCATCGG	55.0	126
			R:GTTCAACATGGGTGCAGATG		
28	RM7488	6	F:ACCTCCATAAGGGACAAATG	56.0	184
			R:GATTTAGGAGGGTTTTGAGG		

29	RM204	6	F:GTGACTGACTTGGTCATAGGG	56.0	169
			R: GCTAGCCATGCTCTCGTACC		
30	RM541	6	F:TATAACCGACCTCAGTGCCC	53.8	158
			R: CCTTACTCCCATGCCATGAG		
31	RM402	6	F:GAGCCATGGAAAGATGCATG	53.8	133
			R:TCAGCTGGCCTATGACAATG		
32	RM3859	7	F:TTGCAGATCGGTTTCCACTG	53.8	191
			R: GGTCCTGGATTCATGGTGTC		
33	RM248	7	F:TCCTTGTGAAATCTGGTCCC	57.3	102
			R: TCCTTGTGAAATCTGGTCCC		
34	RM72	8	F:CCGGCGATAAAACAATGAG	54.0	166
			R:GCATCGGTCCTAACTAAGGG		
35	RM433	8	F:TGCGCTGAACTAAACACAGC	57.3	224
			R:AGACAAACCTGGCCATTCAC		
36	RM25	8	F:GGAAAGAATGATCTTTTCATGG	50.5	146
			R:CTACCATCAAAACCAATGTTC		
37	RM331	8	F:GAACCAGAGGACAAAAATGC	50.0	176
			R:CATCATACATTTGCAGCCAG		
38	RM6070	8	F:TTGCTAGTGCTTACCACCCC	50.0	114
			R: TCCCAGTCACCCTGCTACTC		
39	RM337	8	F:GTAGGAAAGGAAGGGCAGAG	52.5	192
			R:CGATAGATAGCTAGATGTGGCC		
40	RM524	9	F:TGAAGAGCAGGAACCGTAGG	57.5	198
			R: TCTGATATCGGTTCCTTCGG		
41	RM242	9	F:GGCCAACGTGTGTATGTCTC	52.5	225
			R:TATATGCCAAGACGGATGGG		
42	RM410	9	F:GCTCAACGTTTCGTTCCTG	57.5	183
			R:GAAGATGCGTAAAGTGAACGG		
43	RM205	9	F:CTGGTTCTGTATGGGAGCAG	59.0	122
			R:TCGGTGAGACCTAGAGAGCC		
44	RM7545	10	F:GTATCCGCTCCGTTTTCATC	64.5	225
			R:GAGGGGGGGGTGTAGAATAG		
45	RM228	10	F:CTGGCCATTAGTCCTTGG	55.5	154
			R: GCTTGCGGCTCTGCTTAC		

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46	RM24866	10	F:CCCTTTCATTTGCGCTTTATGG	59.0	342
			R:GGGTTATTTCAGTCCGTGATTGC		
47	RM222	10	F:CTTAAATGGGCCACATGCG	53.5	213
			R: CAAAGCTTCCGGCCAAAAG		
48	RM244	10	F:CCGACTGTTCGTCCTTATCA	52.0	163
			R: CCGACTGTTCGTCCTTATCA		
49	RM271	10	F:TCAGATCTACAATTCCATCC	57.5	101
			R:TCGGTGAGACCTAGAGAGCC		
50	RM224	11	F:ATCGATCGATCTTCACGAGG	54.0	157
			R:TGCTATAAAAGGCATTCGGG		
51	RM26213	11	F:GCCACAGGAGACAGCAAGAACC	66.5	345
			R:CGATCCAATTCCAGCCTAGATAGC		
52	RM332	11	F:GCGAAGGCGAAGGTGAAG	57.5	183
			R:CATGAGTGATCTCACTCACCC		
53	RM254	11	F:AGCCCCGAATAAATCCACCT	56.4	165
			R:CTGGAGGAGCATTTGGTAGC		
54	RM5961	11	F:GTATGCTCCTCCTCACCTGC	55.0	129
			R:ACATGCGACGTGATGTGAAC		
55	RM202	11	F:CAGATTGGAGATGAAGTCCTCC	57.0	189
			R:CCAGCAAGCATGTCAATGTA		
56	RM206	11	F:CCCATGCGTTTAACTATTCT	52.7	147
			R: CGTTCCATCGATCCGTATGG		
57	RM229	11	F:CACTCACACGAACGACTGAC	56.5	116
			R:CGCAGGTTCTTGTGAAATGT		
58	RM19	12	F:CAAAAACAGAGCAGATGAC	55.0	226
			R:CTCAAGATGGACGCCAAGA		

The amplification of DNA samples with microsatellite markers (Table 11) and the product visualisation during background selection was done as per the procedure detailed under 3.3.2.4.

3.3.4 Experiment II: Morphological characterisation of pyramided lines (BC₁F₁ population)

Morphological characterisation of BC₁F₁s was done along with the recurrent parent Jyothi and donor parent ISM, as per the descriptor for rice (IRRI, 1996).

3.3.5 Experiment III: Production of BC₂F₁s and BC₁F₂s

5/

3.3.5.1 Production of BC₁F₂s

A few panicles of the BC_1F_1 plants that were found to be introgressed with all the three R-genes (identified under Experiment I), were selfed to obtain BC_1F_2s. At maturity, the seeds were harvested, dried and stored.

3.3.5.2 Development of BC₂F₁s hybrids

The genotypes identified to be introgressed with all the three R- genes in BC_1F_1s under Experiment I were backcrossed to the recurrent parent Jyothi following the procedure detailed below, to generate BC_2F_1s.

3.3.5.1.1 Hybridisation

Early in the morning (5.30 am - 6.00 am), the spikelets that would dehisce on the respective day were selected for emasculation. The top one-third of the spikelets selected for emasculation was carefully clipped off with the help of scissors to expose anthers. The anthers were removed without rupturing the anther wall and damaging the bilobed stigma. Care was taken not to leave any anther in the floret. The emasculated panicles were covered with paper bags. Pollen from selected male plants were collected on the day of anthesis in a petri dish containing distilled water. With the help of a brush, the pollen grain solutions was transferred to the stigma of each emasculated spikelets of the female lines. The pollinated panicle was then re-bagged in butter paper bags to avoid contamination by foreign pollen and proper tagging was done. At maturity, the crossed seeds were harvested, dried and stored.

3.3.6 Experiment IV: Morphological characterisation and pathotyping of BC₂F₅ population, and production of BC₂F₆ population

3.3.6.1 Pathotyping of BC₂F₅ population

Morphological characterisation of the 2-R gene pyramided BC_2F_5s (Table 11.) and pathotyping the population to evaluate their reaction to bacterial blight pathogen was done following the standard protocol *i.e.,* leaf clipping method advocated by IRRI (1996) enumerated below.

Table 11. Details of BC₂F₅s used in the study

Source of BC_2F_5 inoculation	No. of BC_2F_5 seeds sown	No. of BC_2F_5 seeds survived	No. of BC_2F_5 seeds characterised
BC_2F_4 Plant No. 9	165	123	86
BC_2F_4 Plant No. 21	47	31	25
Total	**212**	**154**	**111**

3.3.6.1.1 Preparation of bacterial culture (*Xanthomonas oryzae* pv. *oryzae* isolates)

The isolate of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) culture collected from Regional Agricultural Research Station (RARS), KAU, Pattambi, Kerala, was used to screen the BC_2F_5 population along with the donor and recurrent parents for bacterial blight resistance.

3.3.6.1.2 Bacterial inoculation

Bacterial suspension used for inoculating the plants was prepared by mixing two loops of the pure strain of *Xoo* bacteria in 500 mL distilled water (Kauffman *et al.*, 1973). The turbid suspension (10^8 cfu/ mL) was immediately used for inoculation.

As advocated by Kauffman *et al.* (1973), the artificial inoculation of the pathogen through leaf cliffing method was done at the maximum tillering stage (50 days after transplanting). A minimum of three leaves per plant were inoculated early in the morning by cutting off 1-2 cm of the leaf tip with scissors which was dipped in the bacterial suspension. After each cut, the scissors was placed back in the beaker containing the bacterial inoculum.

3.3.6.1.3 Phenotypic evaluation of BC_2F_5 population

Disease reaction was scored after 15 days of pathogen inoculation. Measurement of bacterial blight lesion length was done and the per cent infected leaf area was calculated. The disease score was worked out as per IRRI Standard Evaluation System (IRRI-SES) scale (IRRI, 1996).

3.3.7 Production of BC_2F_6 s

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BC_2F_5s exhibiting resistance or moderate resistance to bacterial blight pathogen as per 3.3.4.1, were selfed to produce BC_2F_6 population. The selfed seeds harvested at maturity, dried and stored.

3.4 Observations recorded

3.4.1. Genotyping of BC_1F_1 population

3.4.1.1. Quality and quantity of DNA isolated

Quality and quantity of DNA isolated was assessed as per the procedure enumerated under 3.3.1.2. The purity of the DNA samples was assessed based on the A_{260}/A_{280} ratio. The ratio of 1.8 to 2 indicated pure DNA. Protein contamination was inferred if the ratio was less than 1.8 and for values greater than 2, the presence of RNA contamination was confirmed. The quantity of DNA obtained was calculated based on the relation that optical density (OD) for a DNA sample with a concentration of 50 µg/mL (double-stranded) at 260 nm equals one.

3.4.1.2. Nature of amplification

UVITEC Fire Reader software, gel documentation system was used to capture the image for analysing the banding pattern resolved by gel electrophoresis. Based on the nature of the banding pattern, the marker was categorised as monomorphic or polymorphic.

3.4.1.3. Number of amplicons

UVITEC Fire Reader software used for gel documentation was used to count the number of amplicons resolved on the electrophoresed gel.

3.4.1.4. Size of amplicons

Using UVITEC Fire Reader software, the size of amplicons for each marker resolved on the gel was estimated in base pairs (bp) by comparison with a known molecular weight marker that was run along with the PCR product.

3.4.2. Pathotyping of BC_2F_5 population

The length of the leaf and the lesion length was measured in centimetre and per cent diseased area was worked out based on the total leaf area. The resistant and susceptible plants were categorised based on scores 1-9 corresponding to lesion length (IRRI, 2002) as in Table 12.

Table 12. Scale for rating BB resistant lines and varieties

Scale	Diseased leaf area (%)	Description
1	1-5	Resistant (R)
3	6 – 12	Moderately resistant (MR)
5	13 – 25	Moderately susceptible (MS)
7	26 – 50	Susceptible(S)
9	>50 %	Highly susceptible (HS)

3.4.3. Morphological characterisation of BC_1F_1 population

1. Plant height (cm)

Distance between the plant base to the tip of flag leaf at maturity stage was measured and expressed in centimetre.

2. Days to flowering

The days taken from sowing to the start of anthesis in the individual plants was recorded.

3. Leaf blade width (cm)

Measurement across the broadest point of the leaf lamina of three randomLy selected leaves were taken and the average expressed in centimetre.

4. Leaf blade length (cm)

The length of the leaf lamina (in centimetre) of the fully expanded leaves (5 nos.) was measured from leaf base to tip.

5. Productive tillers

At the maturity stage, the total number of grain-bearing tillers in each plant was counted.

6. Panicle length (cm)

The length of four randomLy selected panicles was measured from the panicle base to the tip and expressed in centimetre.

7. Spikelets/ panicle

The number of spikelets in three randomLy selected panicles was counted and the average computed.

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3.5 Statistical analysis

3.5.1. Genotyping of BC₁F₁ population

Analysis of the genomic contribution of the parent in the BC_1F_1s based on (background) SSR molecular data was carried out using Graphical GenoTypes (GGT) Version 2.0 (Van Berloo, 1999) software. The similarity matrix was generated as per Sneath and Sokal (1973) and clusters based on default similarity coefficient and dendrogram were generated.

3.6.2. Parameters of variability

The variability in morphology of backcross progenies was estimated.

1. Mean

The mean is the average of the numbers (observation), recorded. It is the sum of observation divided by the count of observation (N)

$$X = \sum X_i / N$$

Where,

X_i - any observation in the ith treatment

N - Total number of

observations

2. Range

In statistics, the range can tell basic details as to the lowest and the highest values present in the group of sample observation.

3. Diseased leaf area (%)

The ratio of lesion length (cm) of the clipped leaf to total length (cm) of the same leaf expressed in percentage

Results and discussion

IV. RESULTS AND DISCUSSION

Rice serves as staple food and source of energy for more than half of the world's population. Of late, yield loss in rice associated with the occurrence of biotic and abiotic stress has become quite frequent world over.

Bacterial Blight caused by the bacteria *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious diseases affecting rice crop in Kerala. The climatic conditions prevailing in the state enhance the occurrence and spread of bacterial blight disease. Spraying of antibiotics (streptomycin) or cow dung extract (KAU, 2016) or chemical fertilizers is not much effective, as it can be washed-off with monsoon showers during both the rice growing season (*Kharif* and *rabi*). Apart from this, both researchers and consumers are concerned about the decrease of chemical pesticides efficiency and effectiveness food safety as well as its negative impact on the environment (Peng *et al.*, 2015). Moreover, according to Gnanamanickam, (1999), the evolution and occurrence of drug-resistant strains also impair the effectiveness of chemical management.

Exploiting host-plant resistance through pyramiding multiple R-genes would impart durable and broad-spectrum stable resistance to varities against various pathotypes (Chen *et al.*, 2011; Reink *et al.*, 2018). To date, about 43 resistant genes imparting resistance to BB have been reported (Zhang *et al.*, 2014; Khan *et al.*, 2014; Busungu *et al.*, 2016). Gene introgression through conventional breeding is a laborious and time-consuming approach. In a backcross breeding programme, chances of introgression of some undesirable segments from the donor parent is high. Such linkage drags can be greatly reduced by integrating the use of molecular markers in the breeding programmes (Joshi and Nayak., 2010; Frisch *et al.*, 1999). MAS therefore has been reported to be an effective alternative to conventional breeding approach for pyramiding R-genes (Tanksley *et al.*, 1989; Frisch *et al.*, 1999; Davierwala *et al.*, 2001; Rao *et al.*, 2002; Akhtar *et al*, 2010; Joshi and Nayak 2010; Salgotra *et al.*, 2012; Pinta *et al.*, 2013; Khoshkdaman *et al.*, 2014; Pradhan *et al.*, 2015b; Sabar *et al.*, 2016; Arunakumari *et al.*, 2016; Luo *et al.*,

2016;Ramalingam *et al.*, 2017; Das *et al.*, 2018; Sagar *et al.*, 2018; Baliyan *et al.*, 2018; Chukwu *et al.*, 2019).

Considering the impact of the BB disease on food security and sustainability, efforts to introgress three R-genes (*xa5, xa13 and Xa21*) into the elite cultivar Jyothi (Ptb 39) using Improved Samba Mahsuri (ISM) as the donor parent through (MABB) were initiated at College of Horticulture, Vellanikkara.

The present study aimed to identify R-gene pyramided plants in BC_1F_1 population (27 Nos.) and to develop stable pyramided BC_2F_1 lines of variety Jyothi with low linkage drag from the donor parent (ISM). It also aimed to evaluate BC_2F_5 population (212 Nos.) for resistance to bacterial blight pathogen (pathotyping) and production of next-generation breeding populations (BC_2F_1s and BC_1F_2s) of R gene introgressed lines. The results obtained are enumerated and discussed below.

4.1 Genotyping of BC_1F_1s

4.1.1. Quality and quantity of DNA isolated

Quality and quantity for the genomic DNA of 24 BC_1F_1s, recurrent parent Jyothi and donor parent ISM are detailed in Table 13, Appendix II and III.

The ratio of UV absorbance of the 21 BC_1F_1 plants derived from BC_2F_4 Plant No. 9 ranged from 1.91 to 2.02, while, it varied between 2.03 to 2.05 in case of the three BC_1F_1 plants derived from BC_2F_4 Plant No. 21. The average values of parents Jyothi and ISM were 2.04 and 2.02, respectively. The results indicated that the genomic DNA extracted from the parents as well as BC_1F_1 plants were of good quality as the A260/A280 ratio of DNA extracts ranged between 1.8 and 2.0. A value less than 1.8 or greater than 2.0 would have indicated a high degree of protein contamination and RNAse, respectively (Manchester, 1996).

The quantity of the isolated DNA in the 21 BC_1F_1 plants derived from BC_2F_4 Plant No. 9 varied from 345.81 μg/ml to 987.37.6 μg/ml, while it varied between 580.09 μg/ml to 957.03 μg/ml in case of the three BC_1F_1 plants derived from BC_2F_4 Plant No. 21. In case of parents, Jyothi and ISM, the concentration of DNA extracted was 574.57 μg/ml and 290.56μg/ml, respectively. The

(a)

(b)

(c)

(d)

(e)

Plate 1. Staggered sowing of BC_1F_1s (a, b, c, d, e)

concentration of DNA (50 µg/ml DNA) required for genotyping was obtained by dilution of the samples based on the optical density (OD) values and DNA quantity (µg/ml).

Table 13. Quality and quantity of genomic DNA of BC_1F_1s and parents

Genotype	Quantity of DNA (µg/ml)			Quality of DNA		
	Max	Min	Mean	Max	Min	Mean
ISM (Donor parent)	349.25	231.87	290.56	2.17	1.91	2.04
BC_1F_1s of BC_2F_4 Plant No. 9	987.37	345.81	666.59	2.02	1.91	1.96
BC_1F_1s of BC_2F_4 Plant No. 21	574.57	290.56	432.56	2.02	2.04	2.03
Jyothi (Recurrent parent)	998.34	150.8	574.57	2.08	1.97	2.02

4.1.2. Foreground selection of BC_1F_1s

The good quality isolated DNA from BC_1F_1s (24 Nos.) and parents (ISM and Jyothi) were subjected to foreground selection.

4.1.2.1. Foreground selection for R-gene *xa5*

Sundaram *et al.* (2008) reported that, STS marker RG556 is located at a distance of 0.1 cM from the BB resistance gene *xa5*. The functional marker xa5SR also act as a tool for confirmation of the presence of the R-gene *xa5* (Pradhan *et al.*, 2015b; Verma *et al.*, 2016).

Analysis of the restriction digested (digested with *DraI*) PCR product of marker RG556 linked to *xa5* revealed the presence of specific amplicon polymorphism (SAP) in all the 24 BC_1F_1s, recurrent parent Jyothi and donor parent ISM. The banding pattern indicated the presence of amplicons of size 673bp, 484bp and 468bp (Plate No. 2, Table 14 and Table 15) in all the individuals studied. The results, thus indicated the endogenous presence of R-gene *xa5* in both the parents as well as all the 24 individuals BC_1F_1s.

For the further confirmation of the above result, PCR analysis with functional marker xa5SR was done. It revealed monomorphic banding pattern in all the 24 BC_1F_1s as well as recurrent parent Jyothi and donor parent ISM (Plate 3). The presence of a single amplicon of size 167bp (resistance allele of *xa5* gene) was

Plate 2. Foreground selection of BC₁F₁s using STS marker RG556 on restriction digestion with *Dra* I

Plate 3. Foreground selection of BC₁F₁s using functional marker xa5 SR

ISM: Donor parent	1- 21: BC₁F₁s of BC₂F₄ Plant No.9
Jyothi:Recurrent parent	22-24: BC₁F₁s of BC₂F₄ Plant No.21

observed in all the individuals. Results thus confirmed the presence of resistance allele (167bp) of *xa5* gene it not only confirmed the endogenous presence of resistance allele in both the recurrent parent Jyothi and donor parent ISM, but also its presence in all the 24 BC₁F₁s studied.

The endogenous presence of R- gene (*xa5*) in Jyothi, ISM and the backcross progenies derived from these have been observed by the earlier workers (Kabade, 2017; Megha, 2018). The endogenous presence of *xa5* gene in genotypes have also been reported by earlier workers (Narayanan *et al.*, 2002; Joseph *et al.*, 2016; Megha *et al.*, 2018). Monomorphic banding pattern was also observed for the *xa5* gene by Tuyen and Lang (2004) and Ullah *et al.* (2012).

It is to be noted that in spite of the presence of resistant allele of R-gene *xa5*, the recurrent parent is susceptible to BB pathogen. This revealed that the *xa 5* gene failed to confer resistance against BB pathogen prevalent in Kerala. Li *et al.* (2001) and Sundaram *et al.* (2008) had mentioned that *xa5* provides only moderate resistance to BB pathogen. The gene *xa5* displays partial dominance (*Li et al.* 2001). According to Lyer *et al.* (2004), *xa5* shows haplo-insufficient and dose-dependent resistance. Dose-dependent activation of a particular signalling pathway by *xa5* gene inside the host cell will decide the resistance or susceptible nature of the plant. This may be reason for the susceptible reaction of cultivar Jyothi to BB pathogen inspite of the presence of resistance allele of R-gene *xa5* gene. Pyramiding of multiple BB resistance gene into lines have been confirmed to confer higher level of resistance to the *Xoo* pathogen compared to those with single resistance gene (Yoshimura *et al.*, 1996; Huang *et al.*, 1997; Sundaram *et al.*, 2008; Pradhan *et al.*, 2015a, Baliyan *et al.*, 2018).

4.1.2.2. Foreground selection for R-gene *xa13*

Introgression of R-gene *xa13* was confirmed by using functional marker xa13 promoter and STS marker RG136.

Monomorphic banding pattern was observed in the BC₁F₁s and parents when the samples were amplified with of STS marker RG136 and resolved in agarose gel 1.5 per cent. However, further digestion of the PCR products using restriction enzyme *Hinf1* generated polymorphic banding pattern (Plate 4). The
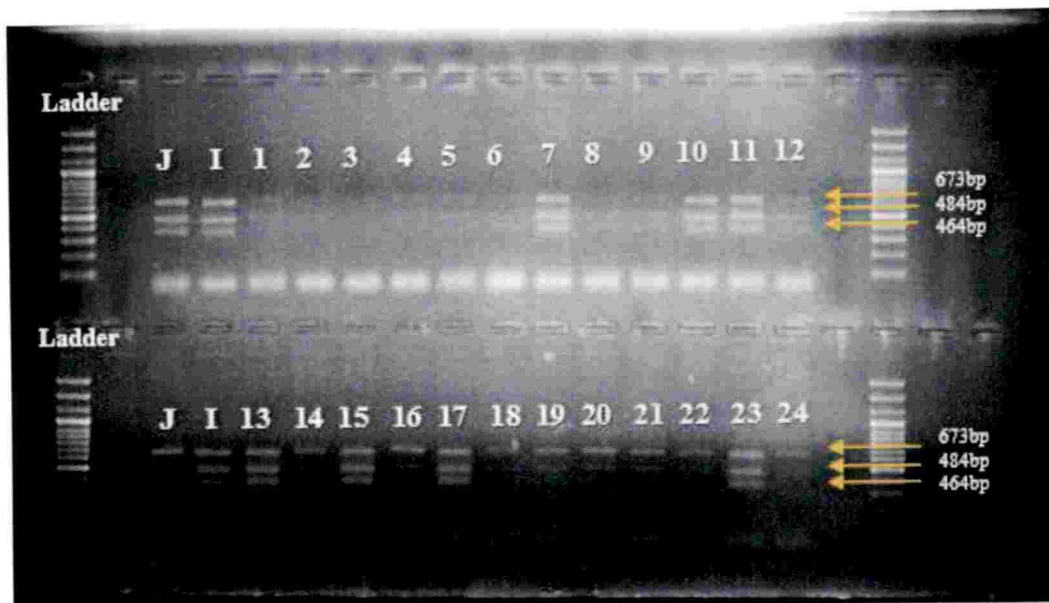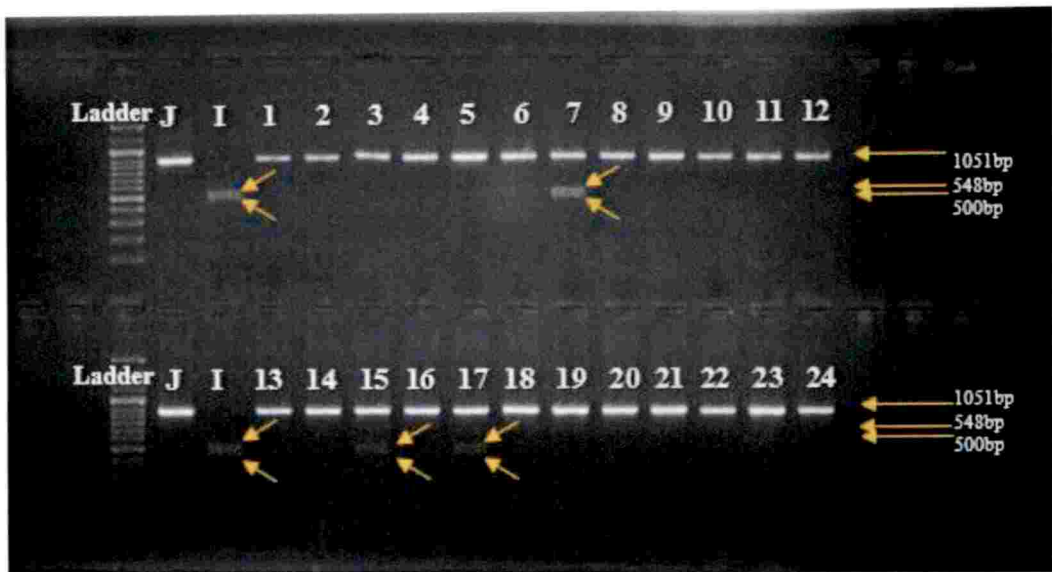
Plate 4. Foreground selection of BC₁F₁s using marker RG136 on restriction digestion with *Hinf*I
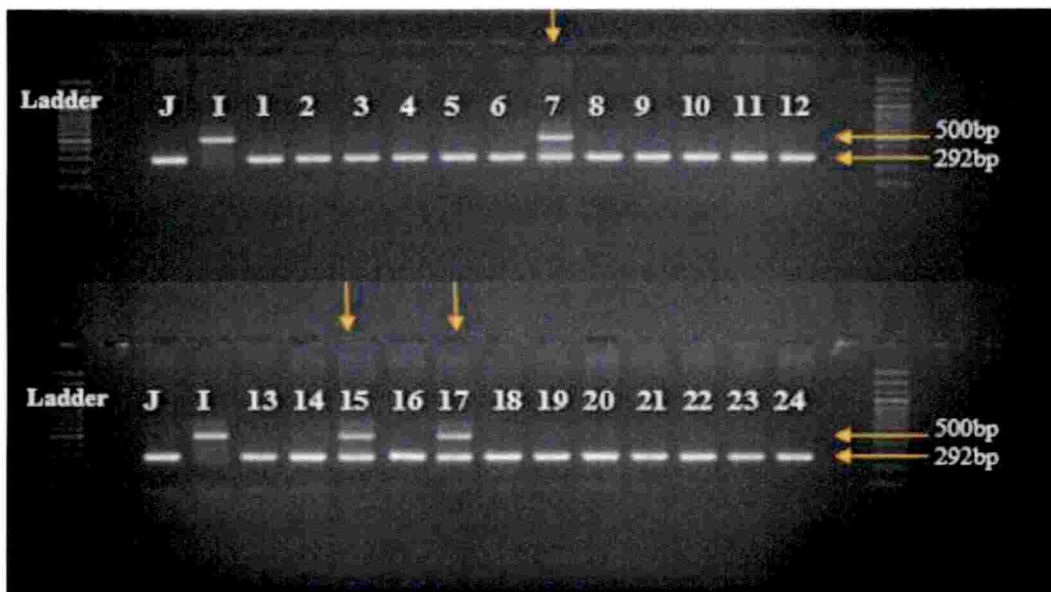
Plate 5. Foreground selection of BC₁F₁s using functional marker xa 13 promoter

ISM: Donor parent	1- 21: BC₁F₁s of BC₂F₄ Plant No.9
Jyothi:Recurrent parent	22-24: BC₁F₁s of BC₂F₄ Plant No.21

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restriction digestion generated a fragment of size 1051bp and 548bp, 500bp in recurrent parent Jyothi and donor parent ISM respectively (Table 14 and 15).

Three BC₁F₁ plants *i.e.,* Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17 exhibited banding pattern similar to both donor parent ISM as well as the susceptible parent Jyothi. All the other BC₁F₁ plants exhibited banding pattern similar to susceptible recurrent parent Jyothi. Being a co-dominant marker, STS marker RG136 helps differentiate a heterozygote from a homozygote. Thus, the results pointed out that the BC₁F₁ Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17 were heterozygous for the R-gene *xa13*.

The presence of *xa13* gene was further analysed using functional marker xa13promoter (Plate 5). Amplicons of sizes 500bp and 292bp were respectively produced in the donor parent ISM and the recurrent parent Jyothi. All the BC₁F₁s except BC₁F₁ Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17, exhibited banding pattern similar to the recurrent parent Jyothi. Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17, possessed bands of sizes corresponding to both Jyothi and ISM. Thus, the heterozygous nature of the R-gene *xa13* present in the above-mentioned plants was confirmed.

The result indicated that, out of the 24 BC₁F₁s screened, three plants (Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17) were 2-R-gene pyramids (*xa5 + xa13*), while, all the others possessed only a single R gene (*xa5*) owing to the endogenous presence of R-gene *xa 5* in both the parents.

The gene *xa13* being a recessive gene, the resistance conferred is evidently expressed only in homozygous condition. As the identified BC₁F₁ Plant No. 9.7, 9.15 and 9.17 was heterozygous at *xa13* loci, it would exhibit susceptibility to BB pathogen if subjected to pathotyping, as the resistance expression conferred by *xa13* would be suppressed by its dominant allele. Hence, MAS has helped in identifying the presence of heterozygous loci for the recessive gene *xa 13,* which may have gone unnoticed during conventional screening programmes.

Recessively inherited R-genes like *xa5* and *xa13* cannot be efficiently identified by conventional breeding methods (Singh *et al.,* 2015, Sundaram *et al.,*

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2008). Pradhan *et al.* (2015a) and Arunakumari *et al.* (2016), had also observed backcross individuals heterozygous for *xa13* locus in their studies.

According to Singh *et al.* (2000) resistance conferred by recessive R-gene *xa 13* is less effective against the BB pathogen compared to that bestowed by the other two R-genes (*Xa21* and *xa 5*). The R-gene *xa 13* mediated resistance has been reported to overcome by some pathotypes of the *X. oryzae pv oryzae, i.e., xa13* shows race-specific resistance (Lee *et al.*, 2003; Chu *et al.*, 2006). Homozygous condition of *xa 13* enhances the host defence mechanism when compared with the heterozygous condition of the R-gene.

The action of R- gene *xa13* gene, however is boosted when dominant genes are present along with it. This is because some defence-responsive genes activated in *xa13*-mediated resistance are not directly involved in resistance, but, is mediated by dominant R genes (*Xa4, Xa10* and *Xa26*) (Chu *et al.*, 2007). The *xa13* gene was found to confer host-plant resistance to several strains of *Xoo* BB pathogen effectively in combination with other genes (*Xa21, xa5, Xa4*) (Lore *et al.*, 2011 and Li *et al.* 2001). The presence of multiple genes, thus make sure the presence of broad spectrum resistance to a wide range of races (Dokku *et al.*, 2013a, 2013b; Das *et al.*, 2015; Das *et al.*, 2018; Mallikarjuna and Kotasthane, 2018; Swathi *et al.*, 2019).

4.1.2.3. Foreground selection for R-gene *Xa21*

Dokku *et al.* (2013a) reported that STS marker pTA248 is tightly linked to a dominant resistance gene *Xa 21* at a distance of 0.2cM. Among the 24 BC_1F_1s progenies studied, amplicons of size 966bp and 660bp corresponding to parents Jyothi and ISM respectively were observed in three plants (*i.e.,* Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17). All other BC_1F_1 individuals registered only one amplicon (660 bp) corresponding to the susceptible recurrent parent Jyothi (Plate 6 and Table 15). This revealed the presence of R-gene *Xa21* in the heterozygous condition in BC_1F_1 Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17 and the absence of resistance allele of gene *Xa21* in all other BC_1F_1 plants studied.

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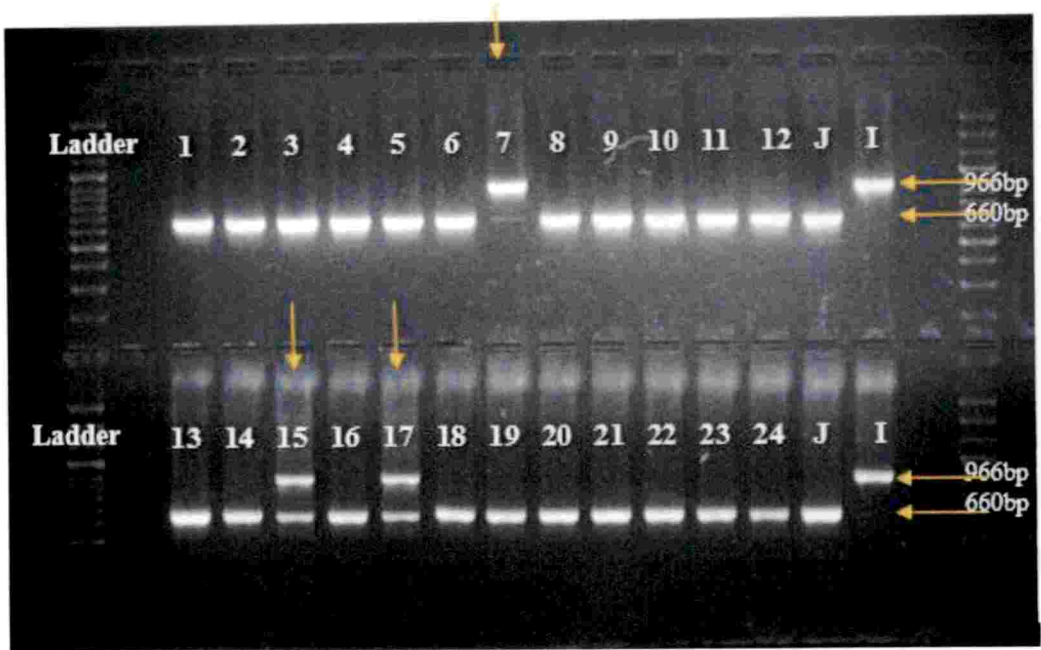
Plate 6. Foreground selection of BC₁F₁s using STS marker pTA248

ISM: Donor parent	1- 21: BC₁F₁s of BC₂F₄ Plant No.9
Jyothi:Recurrent parent	22-24: BC₁F₁s of BC₂F₄ Plant No.21

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Several studies revealed that *Xa 21* was the most effective dominant R- gene because of its widespread resistance against BB pathogen races (Huang *et al.*, 1997; Sanchez *et al.*, 2000; Singh *et al.*, 2001; Lee *et al.*, 2009; Antony *et al.*, 2010; Pradhan *et al.*, 2016; Sagar *et al.*, 2018). Zhang *et al.* (2006), reported that the plants with homozygous genotypes are more resistant to the pathogen compared to individuals with heterozygous alleles at the marker locus. The presence of an interactive additive effect of *Xa 21* gene with other genes makes sure that the resistance conferred after pyramiding it with other R-genes (recessive *xa5* and *xa13* or other dominant R-genes) is more effective than when they were interacting with pathogen individually (Baliyan *et al.*, 2018; Singh *et al.*, 2015). Li *et al.* (2001) opined that pyramiding of *Xa 21* with *xa13* enhances the defence response of *xa13* gene against BB pathogen.

4.1.2.4. Summary of foreground selection

Twenty four BC_1F_1s were subjected to foreground selection to identify the plants introgressed with all the three R-genes (*Xa21+xa13+xa5*). Results indicated that three BC_1F_1 plants *i.e.*, Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17, were the only 3-R-gene among the 24 BC_1F_1 individuals investigated. The other BC_1F_1s possessed a single recessive R-gene *xa5*. Endogenous presence of resistance allele of *xa5* in the susceptible recurrent parent Jyothi was also confirmed.

In all the three identified 3-R-gene pyramids, the R-gene *xa5* was present in homozygous state while, the other two R-genes *viz.*, *xa13* and *Xa21*, were introgressed in the heterozygous condition (*xa5xa5 +Xa13 xa13 + Xa21xa21*).

The heterozygous condition, however, will not confer complete resistance expression of the 3-R-gene pyramided plant. Hence, further selfing of the R-gene pyramided plants was suggested for attaining homozygosity for the alleles concerned.

Many researchers have attempted introgression of R-genes conferring resistance to BB pathogen in the susceptible varieties through MABB approach. Recovery of varying R-gene combinations have been reported (Guvalla *et al.*, 2013; Nayak *et al.*, 2015; Pradhan *et al.*, 2016; Yap *et al.*, 2016; Arunakumari *et al.*, 2016; Das *et al.*, 2018; Sagar *et al.*, 2018) .

Table 14. Distribution of alleles of PCR marker loci linked to BB resistance (R-genes) in the BC₁F₁s and parents

Target genes	xa5		xa13		Xa21
Marker	RG 556	Xa5 SR	RG 136	Xa13 pro	pTA 248
Donor parent (ISM)	A	A	A	A	A
Recurrent parent (Jyothi)	A	A	B	B	B
BC₂F₄ Plant No.9					
9.1	A	A	B	B	B
9.2	A	A	B	B	B
9.3	A	A	B	B	B
9.4	A	A	B	B	B
9.4	A	A	B	B	B
9.5	A	A	B	B	B
9.6	A	A	B	B	B
9.7	A	A	H	H	H
9.8	A	A	B	B	B
9.9	A	A	B	B	B
9.1	A	A	B	B	B
9.11	A	A	B	B	B

A –Allele similar to donor parent   B – Allele similar to recurrent parent

H – Heterozygous loci

...contd. Table 14. Distribution of alleles of PCR marker loci linked to BB resistance (R-genes) in the BC₁F₁s and parents

Target genes	xa5		xa13		Xa21
Marker	RG 556	Xa5 SR	RG 136	Xa13 pro	pTA 248
Donor parent (ISM)	A	A	A	A	A
Recurrent parent (Jyothi)	A	A	B	B	B
BC₂F₄ Plant No.9					
9.12	A	A	B	B	B
9.13	A	A	B	B	B
9.14	A	A	B	B	B
9.15	A	A	H	H	H
9.16	A	A	B	B	B
9.17	A	A	H	H	H
9.18	A	A	B	B	B
9.19	A	A	B	B	B
9.2	A	A	B	B	B
9.21	A	A	B	B	B
BC₂F₄ Plant No. 21					
21.22	A	A	B	B	B
21.23	A	A	B	B	B
21.24	A	A	B	B	B

A –Allele similar to donor parent   B – Allele similar to recurrent parent

H – Heterozygous loci

Table 15. Segregation of molecular markers during foreground selection in 3-R-gene introgressed BC$_1$F$_1$s and parents

Gene	Markers employed in foreground selection	Nature of amplification	Number of amplicons	Size of the amplicon				
				Donor parent	BC$_1$F$_1$			Recurrent parent
				ISM	Plant No. 9.7	Plant No. 9.15	Plant No. 9.17	Jyothi
xa5	xa5 SR	Monomorphic	1	167	167	167	167	167
	RG 556	Monomorphic	3	673, 484 and 468	673, 484 and 468	673, 484 and 468	673, 484 and 468	673, 484 and 468
xa13	xa13 Pro	Polymorphic	2	500	500 and 292	500 and 292	500 and 292	292
	RG 136	Polymorphic	3	548 and 500	1051and 548,500	1051and 548,500	1051and 548,500	1051
Xa21	pTA 248	Polymorphic	2	966	966 and 660	966 and 66O	966 and 660	660

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4.1.3. Background selection (Parental polymorphism survey)

The three BC_1F_1s (Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17) identified to be introgressed with all the three R-genes through foreground selection were selected for background selection. Background selection helps to assess the recovery per cent of recurrent parent genome and indirectly helps to know the extent of linkage drag from the donor parent and accelerate the efficiency of selection in a plant breeding programme (Joseph *et al.*, 2004).

4.1.3.1. Allele distribution in R-gene introgressed BC_1F_1s

The study aimed to improve the host resistance against bacterial blight disease and retain the agro-morphological character of elite cultivar Jyothi.

A set of 186 Rice Microsatellites (RM) (SSR markers) distributed across the 12 chromosomes of rice were initially used to study the allelic distribution among the donor parent ISM and recurrent patent Jyothi. Among these, 58 markers (Table 10) that exhibited polymorphism between the parents were used for background selection in the 3-Rgenes pyramids (BC_1F_1s Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17) along with both the parents (Plate 7).

Of the 58 polymorphic SSR markers used, 21 markers (RM 224, RM 524, RM 242, RM 72, RM 25, RM 331, RM 6070, RM 3859, RM 204, RM 19218, RM 307, RM 6679, RM 261, RM 16, RM 14723, RM 7, RM 13910, RM 324, RM 2017, RM 11069, RM 24), exhibited the same allelic pattern in the recurrent parent (Jyothi) as well as the three R-gene pyramided BC_1F_1s (Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17), indicating the similarity in alleles at these marker loci in recurrent parent Jyothi and the 3-R gene pyramided BC_1F_1s .

Monomorphism was observed in both the donor parent (ISM) and R-gene introgressed BC_1F_1s (Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17) in 11 (RM 19, RM 5961, RM 7545, RM 222, RM 271, RM 541, RM 402, RM 5586, RM 214, RM1 1313, RM 583) out of 58 markers used for background selection indicating that the BC_1F_1s and the donor had identical alleles at these marker loci.

Presence of alleles of both the parents were observed in the BC_1F_1s Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17 with respect to eight SSR markers (RM 26213, RM 202, RM 24866, RM 14487 and RM 411, RM 3340, RM 263, RM 9).
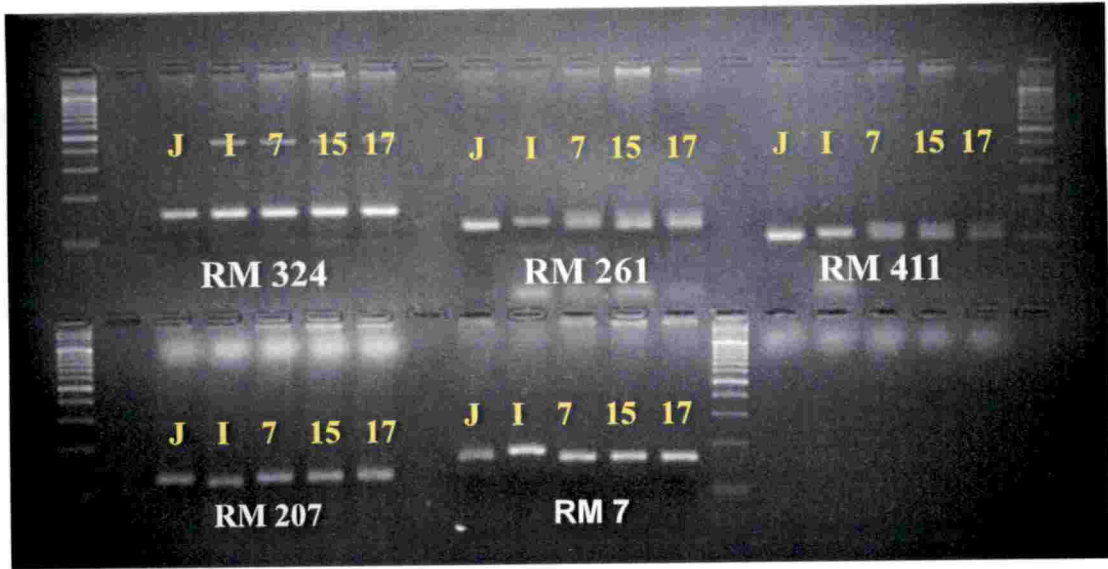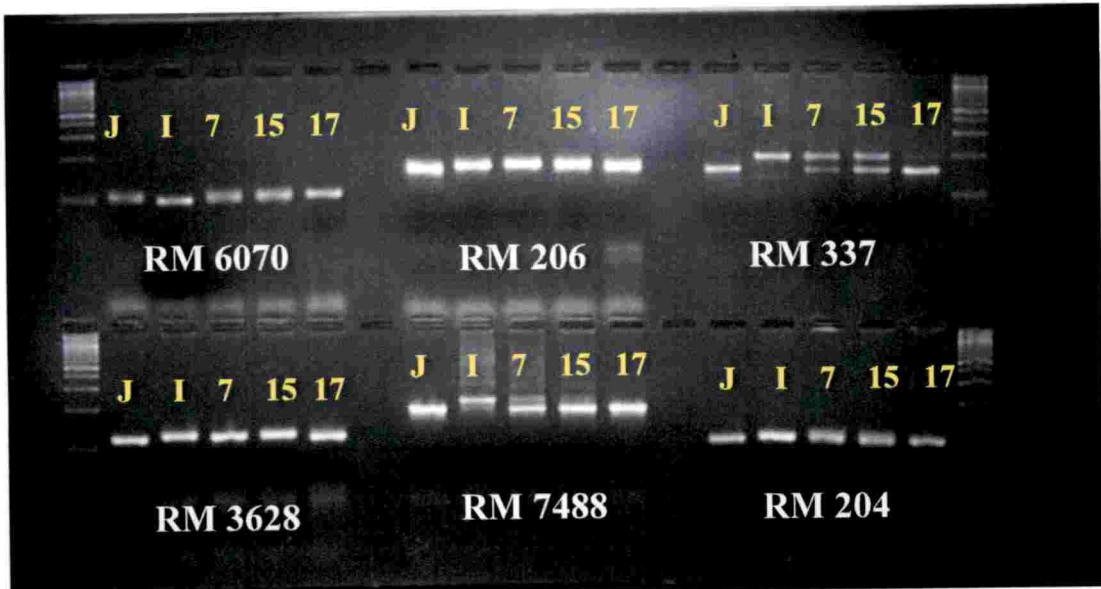
7: BC₁F₁ Plant No. 9.7   15: BC₁F₁ Plant No. 9.15   17: BC₁F₁ Plant No. 9.17

I: Improved Samba Mahsuri   J: Jyothi

Plate 7(a). Back ground selection in the 3-R-gene pyramids
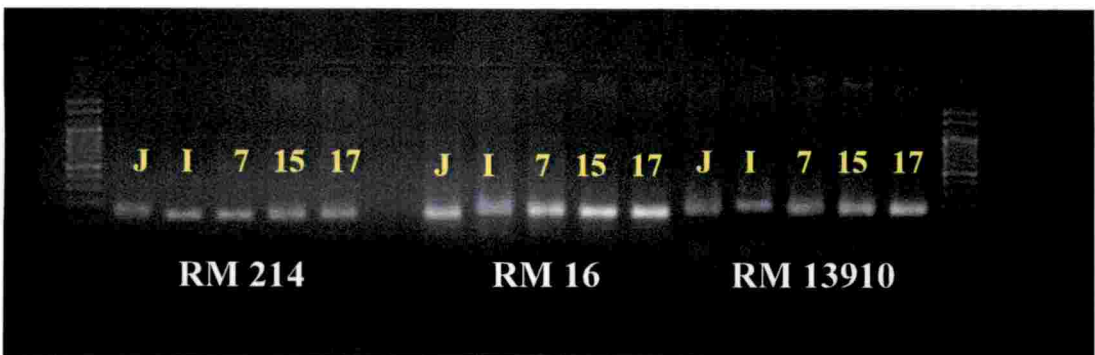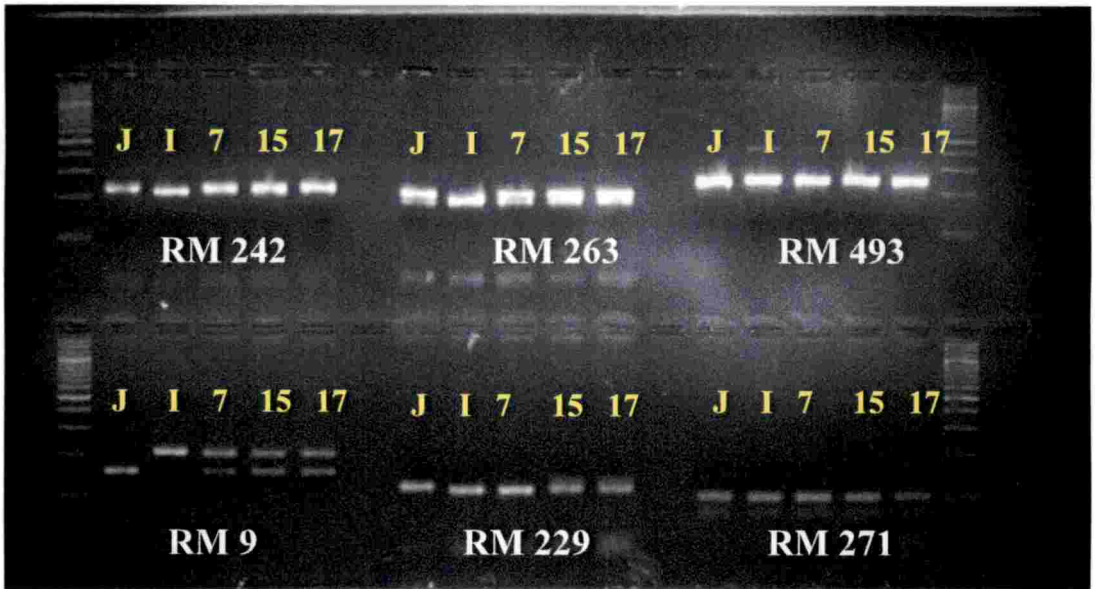
J I 7 15 17   **J I 7 15 17**   **J I 7 15 17**

RM 19218   RM 248   RM228

J I 7 15 17   **J I 7 15 17**   **J I 7 15 17**

RM 242   RM 263   RM 493

J I 7 15 17   **J I 7 15 17**   **J I 7 15 17**

RM 9   RM 229   RM 271

J I 7 15 17   **J I 7 15 17**   **J I 7 15 17**

RM 214   RM 16   RM 13910

7: BC$_1$F$_1$ Plant No. 9.7   15: BC$_1$F$_1$ Plant No. 9.15   17: BC$_1$F$_1$ Plant No. 9.17

I: Improved Samba Mahsuri   J: Jyothi

Plate 7(b). Back ground selection in the 3-R-gene pyramids
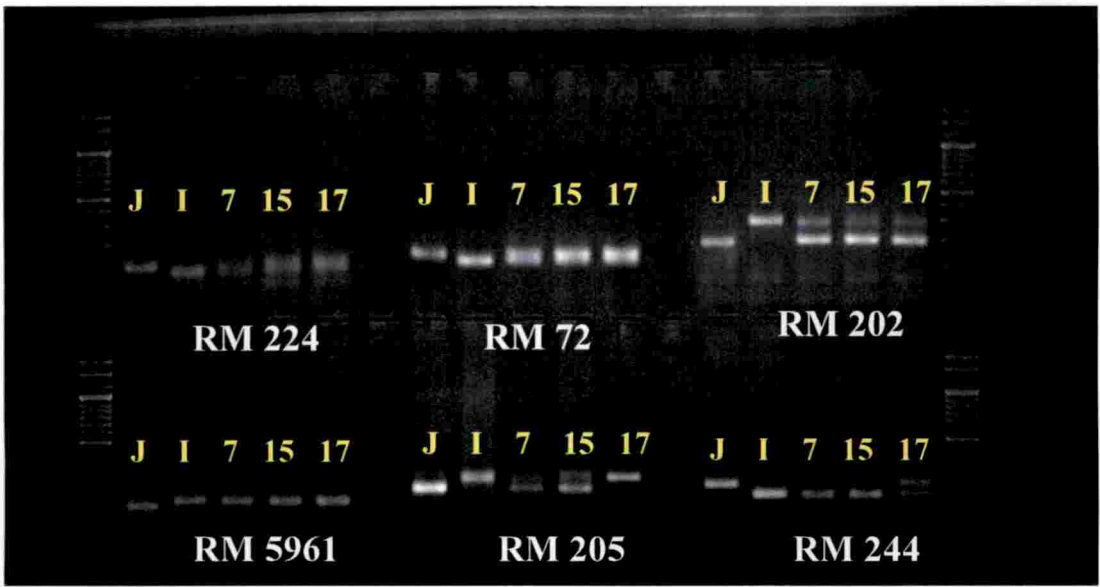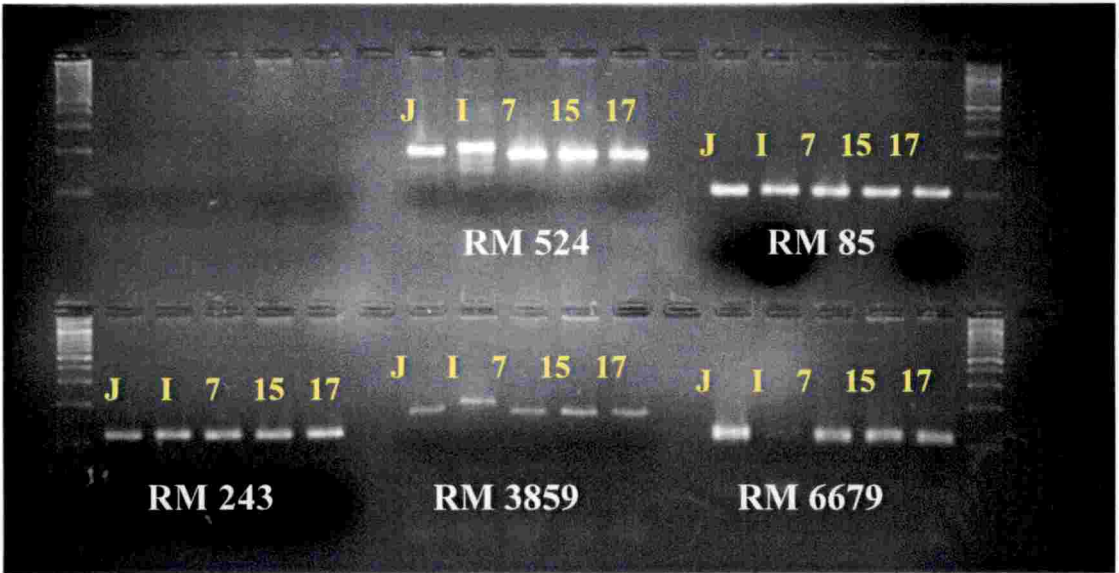
| 7: BC₁F₁ Plant No. 9.7 | 15: BC₁F₁ Plant No. 9.15 | 17: BC₁F₁ Plant No. 9.17 |
| I: Improved Samba Mahsuri | | J: Jyothi |

Plate 7(c). Back ground selection in the 3-R-gene pyramids
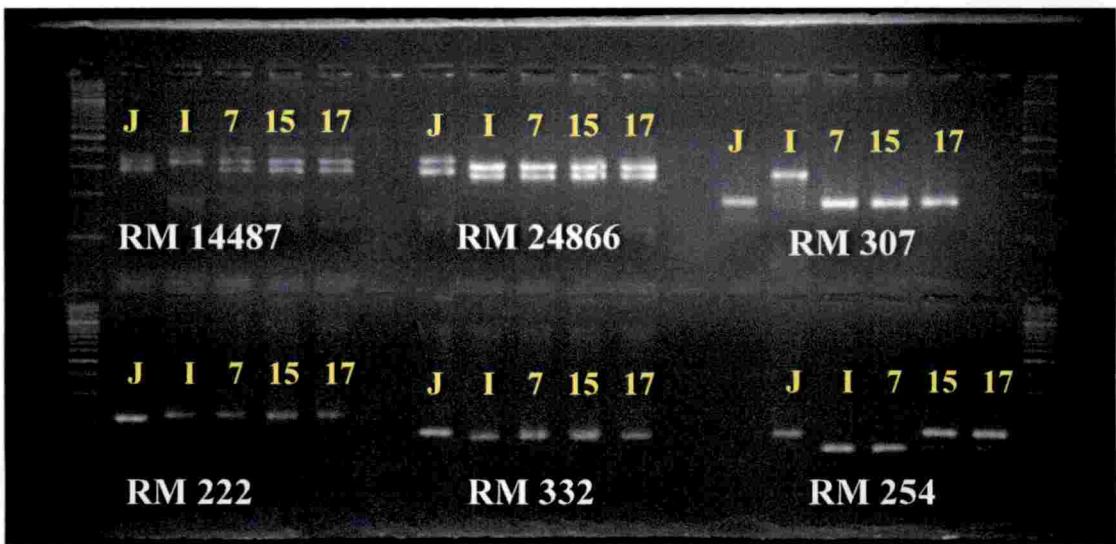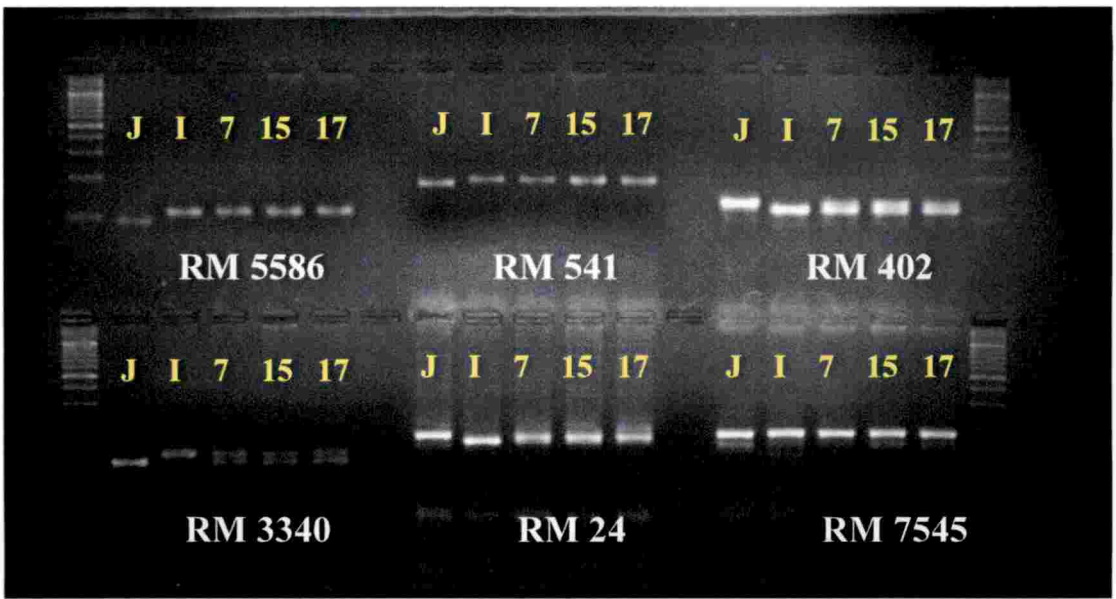
7: BC$_1$F$_1$ Plant No. 9.7   15: BC$_1$F$_1$ Plant No. 9.15   17: BC$_1$F$_1$ Plant No. 9.17

I: Improved Samba Mahsuri   J: Jyothi

Plate 7(d). Back ground selection in the 3-R-gene pyramids
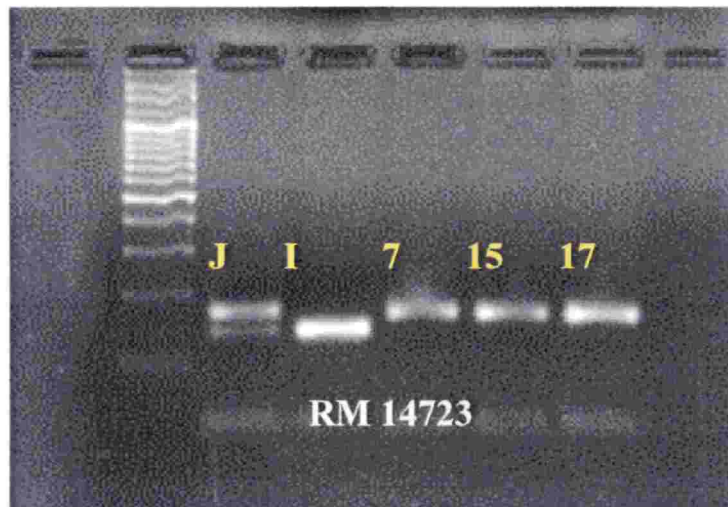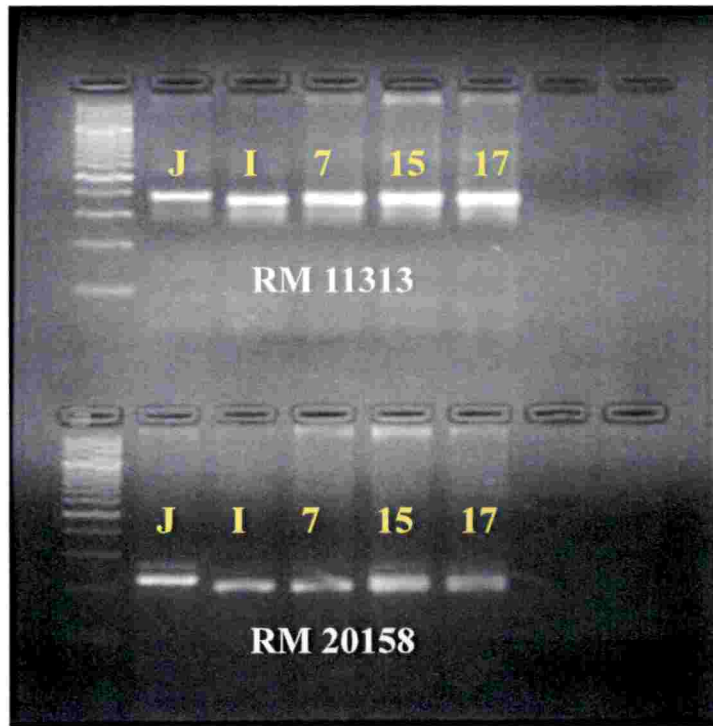
RM 11313

RM 20158

RM 14723

7: BC$_1$F$_1$ Plant No. 9.7   15: BC$_1$F$_1$ Plant No. 9.15   17: BC$_1$F$_1$ Plant No. 9.17

I: Improved Samba Mahsuri   J: Jyothi

Plate 7(e). Back ground selection in the 3-R-gene pyramids
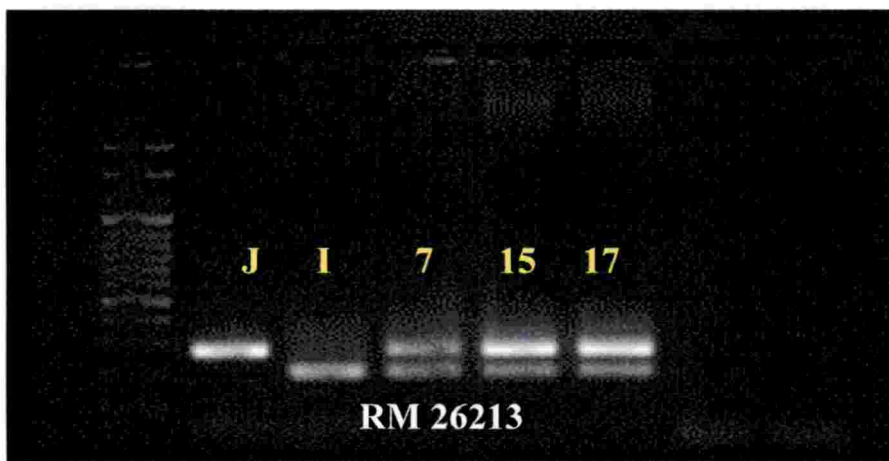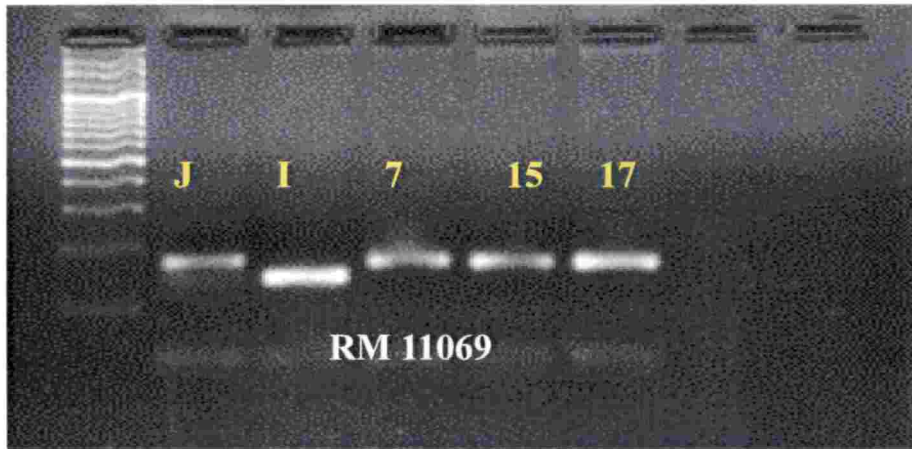
RM 11069

RM 26213

7: BC$_1$F$_1$ Plant No. 9.7   15: BC$_1$F$_1$ Plant No. 9.15   17: BC$_1$F$_1$ Plant No. 9.17

I: Improved Samba Mahsuri   J: Jyothi

Plate 7(f). Back ground selection in the 3-R-gene pyramids

This revealed the heterozygous nature of above eight marker loci in the three R-gene pyramided of BC_1F_1s (Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17).

Background selection thus revealed that, the R-gene introgressed BC_1F_1 Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17 were similar to recurrent parent Jyothi respectively at 24, 22 and 34 marker loci. Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17 were found to be heterozygous at 13, 15 and 12 marker loci respectively. The BC_1F_1 Plant No. 9.7 and No. 9.15 were similar to donor parent ISM at 21 marker loci each, while, Plant No. 9.17 was identical to the donor parent at 12 marker loci only. The result thus indicated that, of the three 3-R-gene introgressed BC_1F_1s, Plant No. 9.17 was more similar to recurrent parent Jyothi.

Similarity between donor and R-gene introgressed backcross individuals at various marker loci and heterozygosity at a few other marker loci have been reported earlier (Sundaram *et al.,* 2008; Guvvala *et al.,* 2013, Nayak *et al.,* 2015; Pradhan *et al.,* 2015a and 2015b; Pradhan *et al.,* 2016; Baliyan *et al.,* 2018; Das *et al.,* 2018; Sagar *et al.,* 2018).

Table 16 Distribution of alleles of marker loci used for background selection in the 3-R-gene introgressed BC_1F_1s.

Sl. No.	RM Marker	Jyothi	Pl. No. 7	Pl. No. 15	Pl. No. 17	ISM
1	RM19	A	B	B	B	B
2	RM224	A	A	A	A	B
3	RM26213	A	H	H	A	B
4	RM332	A	A	B	A	B
5	RM254	A	B	A	A	B
6	RM5961	A	B	B	B	B
7	RM202	A	H	H	H	B
8	RM206	A	B	B	A	B
9	RM229	A	B	A	B	B
10	RM7545	A	B	B	B	B
11	RM228	A	H	H	A	B
12	RM24866	A	A	A	A	B
13	RM222	A	A	A	A	B

...contd. Table 16. Distribution of alleles of marker loci used for background selection in the 3-R-gene introgressed BC$_1$F$_1$s.

Sl. No.	RM Marker	Jyothi	Pl. No. 7	Pl. No. 15	Pl. No. 17	ISM
14	RM244	A	A	B	B	B
15	RM271	A	B	B	B	B
16	RM205	A	H	H	B	B
17	RM524	A	A	A	A	B
18	RM242	A	A	B	B	B
19	RM410	A	B	B	A	B
20	RM72	A	B	A	A	B
21	RM433	A	B	B	A	B
22	RM25	A	A	A	A	B
23	RM331	A	A	A	A	B
24	RM6070	A	B	B	B	B
25	RM337	A	H	H	B	B
26	RM3859	A	A	A	A	B
27	RM248	A	H	H	A	B
28	RM541	A	B	B	B	B
29	RM402	A	B	B	B	B
30	RM20158	A	B	B	B	B
31	RM3628	A	B	B	A	B
32	RM7488	A	A	A	A	B
33	RM204	A	A	A	A	B
34	RM19218	A	A	A	A	B
35	RM307	A	A	A	A	B
36	RM6679	A	A	A	A	B
37	RM5586	A	B	B	B	ISM
38	RM261	A	B	A	A	B
39	RM214	A	B	B	B	B
40	RM16	A	A	A	A	B
41	RM85	A	B	B	A	B
42	RM14723	A	A	A	A	B
43	RM14487	A	H	H	H	B
44	RM411	A	H	H	H	B
45	RM7	A	A	A	A	B

…contd. Table 16. Distribution of alleles of marker loci used for background selection in the 3-R-gene introgressed BC_1F_1s.

Sl. No.	RM Marker	Jyothi	Pl. No. 7	Pl. No. 15	Pl. No. 17	ISM
46	RM13910	A	A	A	A	B
47	RM3340	A	H	H	H	B
48	RM263	A	H	H	H	B
49	RM324	A	A	A	A	B
50	RM207	A	A	A	A	B
51	RM1	A	B	H	H	B
52	RM11313	A	B	B	B	B
53	RM11069	A	A	A	A	B
54	RM24	A	B	B	B	B
55	RM583	A	B	B	B	B
56	RM493	A	A	H	H	B
57	RM9	A	H	H	H	B
58	RM243	A	B	B	A	B

A –Allele similar to donor parent   B – Allele similar to recurrent parent

H – Heterozygous loci

Table 17. Segregation of polymorphic markers during background selection in 3-R-gene introgressed BC₁F₁s

| Sl. No. | Markers | Number of amplicon | Recurrent parent | BC₁F₁ | | | Donor parent |
| | | | | | | | |
			Jyothi	Pl. No. 9.7	Pl. No. 9.15	Pl. No. 9.17	ISM
1	RM19	2	240	223	223	223	223
2	RM224	2	107	107	107	107	103
3	RM26213	2	174	174 and 85	174 and 85	174 and 85	85
4	RM332	2	152	152	136	152	136
5	RM254	2	136	101	136 and 101	136 and 101	101
6	RM5961	2	186	182	182	182	182
7	RM202	2	216	216 and 322	216 and 322	216 and 322	322
8	RM206	2	146	152	152	146	152
9	RM229	2	112	100	112	112	100
10	RM17545	2	152	149	149	149	149
11	RM228	2	127	127and 100	127and 100	127	100
12	RM24866	2	247	247 and 268	247 and 268	247 and 268	268
13	RM222	2	213	217	217	217	217
14	RM244	2	141	141	130	141 and 130	130
15	RM271	2	75	66	66	66	66
16	RM205	2	224	261 and 224	261 and 224	261	261
17	RM524	2	160	160	160	160	181
18	RM242	2	228	228	228	228	207
19	RM410	2	120	146	146	120	146

...cond. Table 17. Segregation of polymorphic markers during background selection in 3-R-gene introgressed BC₁F₁s

Sl. No.	Markers	Number of amplicon	Recurrent parent	BC₁F₁			Donor parent
			Jyothi	Pl. No. 9.7	Pl. No. 9.15	Pl. No. 9.17	ISM
20	RM72	2	161	161	161	161	135
21	RM433	2	141	123	123	141	123
22	RM25	2	143	143	143	143	164
23	RM331	2	155	155	155	155	115
24	RM6070	2	110	110	110	110	97
25	RM337	2	128	128 and 158	128 and 158	128	158
26	RM3859	2	172	172	172	172	195
27	RM248	2	103	103 and 81	103 and 81	103	81
28	RM541	2	151	170	170	170	170
29	RM402	2	104	88	88	88	88
30	RM20158	2	224	203	203	224 and 203	203
31	RM3628	2	173	186	186	173	186
32	RM7488	2	173	200 and173	200 and173	173	200
33	RM204	2	90	90	90	90	93
34	RM19218	2	189	189	189	189	215
35	RM307	2	127	127	127	127	195
36	RM6679	2	80	80	80	80	110
37	RM5586	2	94	110	110	110	110
38	RM261	2	122	122	122	122	133

Sl. No.	Markers	Number of amplicon	Recurrent parent	Size of amplicon (bp)			Donor parent
				BC$_1$F$_1$			
			Jyothi	Pl. No. 9.7	Pl. No. 9.15	Pl. No. 9.17	ISM
39	RM214	2	180	123	123	123	123
40	RM16	2	172	172	172	172	129
41	RM85	2	81	81	87	81	87
42	RM14723	2	175	175	175	175	140
43	RM14487	2	289	289 and 324	289 and 324	289 and 324	324
44	RM411	2	74	91 and 74	91 and 74	91 and 74	91
45	RM7	2	163	163	163	163	182
46	RM13910	2	142	142	142	142	200
47	RM3340	2	116	139 and 116	139 and 116	139 and 116	139
48	RM263	2	185	185 and 153	185 and 153	185 and 153	153
49	RM324	2	161	161	161	161	150
50	RM207	2	135	135	135	135	123
51	RM1	2	70	100	100 and 70	100 and 70	100
52	RM11313	2	364	337	337	337	337
53	RM11069	2	120	120	120	120	147
54	RM24	2	169	169	169	169	146
55	RM583	2	112	96	96	96	96
56	RM493	2	211	215	215	211	215
57	RM9	2	151	151 and 200	151 and 200	151 and 200	200
58	RM243	2	96	103	103	96	103

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Repeated backcrossing and simultaneous background selection using MABB leads to the speedy recovery of recurrent parent genome (Sundaram *et al.*, 2008; Salgotra *et al.*, 2012; Suh *et al.* 2013).

4.1.2.2. Recovery of recurrent parent (RP) genome in 3-R-gene introgressed BC₁F₁ Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17

The highest recovery of the recurrent patent genome was found in BC_1F_1 Plant No. 9.17 (93.00%), followed by Plant No. 9.7 and 9.15 (89.00%) each. Thus, the result of the Graphical genotyping software GGT version 2.0 also indicated a greater similarity between Plant No. 9.17 and recurrent parent genome.

According to Singh *et al.* (2001) linkage drag from the donor parent and recovery per cent of the recurrent parent genome after each backcrossing shows an inverse relationship with each other. The recovery of the recurrent parent genome (RPG) in the backcross progenies increases by one-half with each backcross and therefore, the RPG of in BC_1F_1 is estimated to be 75 per cent.

The proportion of the RP genome is recovered at a rate of $1- (1/2) t+1$ for each of the generations of backcrossing. However, any specific backcross progeny may deviate from the expected during the crossing over resulting in greater RPG, which is not possible to be detected phenotypically. In BC_1F_1 population, theoretically the average per cent of the RP genome is 75 per cent for the entire population. But some individuals possess more or less of the RP genome than others (Harlan, 1922; Hassan *et al.*, 2015).

The per cent recovery of recurrent parent genome in the 3-R-gene introgressed BC_1F_1s were more than expected recovery of 75.00 per cent. Unlike the results of the present study, Meksem *et al.* (2009) had noticed a lower recovery of recurrent parent genome in BC_1F_1 generation, than the expected (75%). Similarly, several workers found that the recurrent parent genome contribution in the first backcross progeny was less than the expected in backcross population (Sundaram *et al.*, 2008; Gopalkrishna *et* al., 2008; Rajpurohit *et al.*, 2011).

However, several studies revealed that the recovery was greatly enhanced in advanced backcross generations. Basavaraj *et al.* (2010) revealed that the extent of RPG recovery of in two BC_2F_1 plants was 87.75 and 90.02 per cent compared to

71 per cent to 79 per cent, respectively in BC_1F_1 generation. The RPG in the study conducted by Joseph (2016) and Megha (2018) was to the extent of 21.80 per cent and 81.82 per cent, respectively in BC_1F_1 and BC_2F_1. It was felt that use of very less number (22) of SSR marker during background selection may be the reason for the low RPG in their study. Background selection had helped to identify progenies with 65.40 per cent and 95.78 per cent recurrent parent genome recovery respectively in BC_1F_1 and BC_3F_3 generation (Rahman et al., 2018).

Swathi et al. (2019) had reported, that 136 polymorphic SSR markers were deployed between parents for accelerating background genome recovery. Results revealed that 90 per cent of the recurrent parent genome was recovered after the second backcross.

Table.18 Contribution of recurrent parent genome (Jyothi) in 3-R-gene introgressed plants

Sl. No.	Details of genome	Per cent recovery of parent genome
1	BC_1F_1 Plant No. 9.7	89.00
2	BC_1F_1 Plant No. 9.15	89.00
3	BC_1F_1 Plant No. 9.17	93.00

The GGT software output indicated that (Fig.2 to 5) maximum donor parent genomic regions among the three carrier chromosomes were present on chromosome 8 and chromosome 11 in regions flanking the R-genes *xa13* and *Xa21* introgression, owing to greater linkage drag.

Balachiranjeevi et al. (2015) found minimum unwanted gene transfer from the donor parent with highest RPG recovery (95%) on R-gene carrier chromosomes of improved lines. Sundaram et al. (2008) opined that during the transfer of a target trait, background selection using a limited number of polymorphic SSR markers (approximately 50) in conjunction with four backcrosses is sufficient to recover the agro- morphological characteristics of the recurrent parent.

Several reports of variable extent of recovery of RP genome background in the R-gene introgressed lines obtained through MAS has been reported (Rajpurohit et al., 2010; Dokku et al., 2013a and 2013b; Nayak et al., 2015; Arunakumari et al., 2016; Kumar et al., 2016; Sagar et al., 2018).
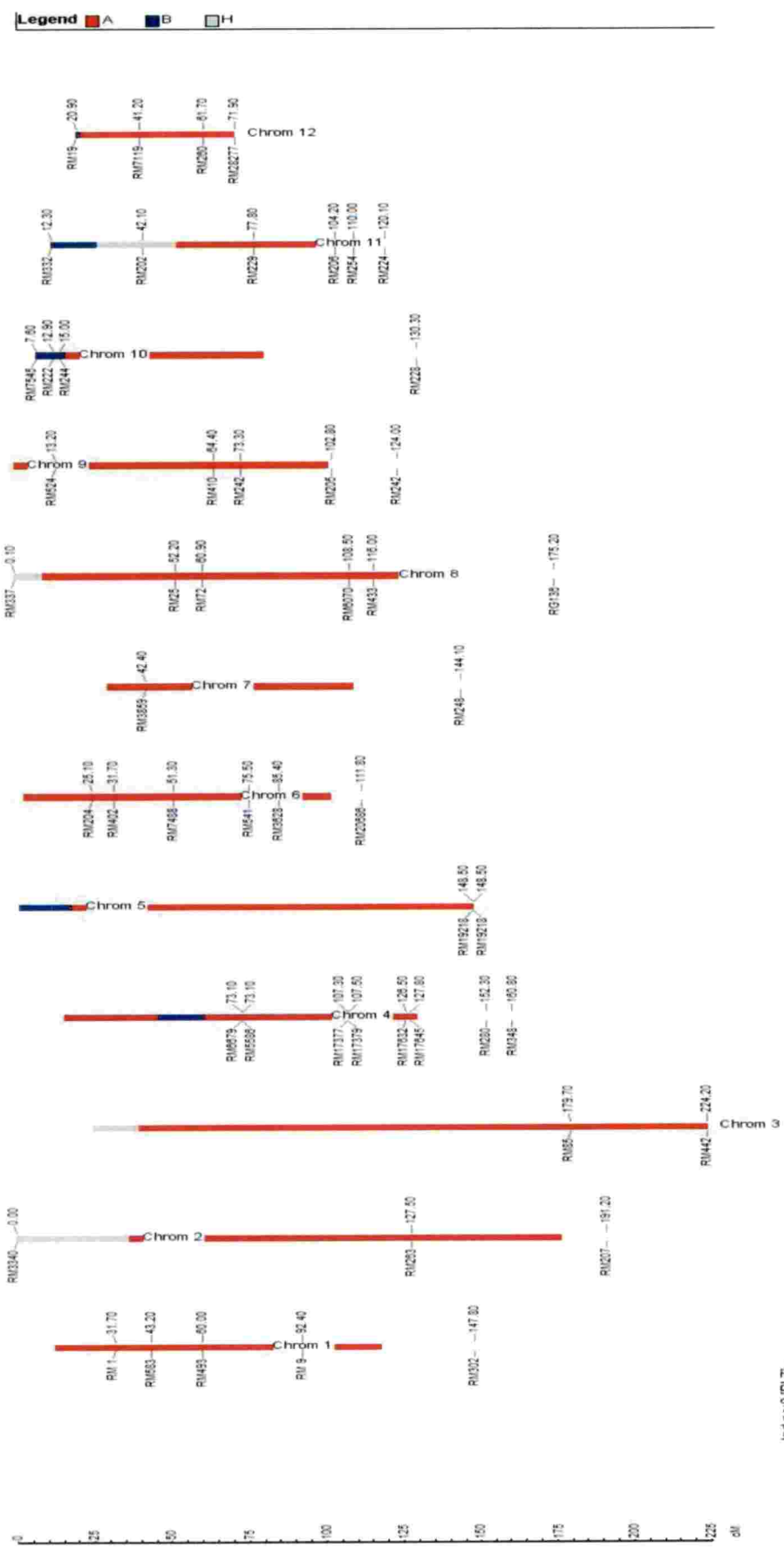
Figure 2. Graphical genotyping of 3-R-gene introgressed BC$_1$F$_1$ Plant No. 9.7

Donor parent genome

Recurrent parent genome

Heterozygous locus
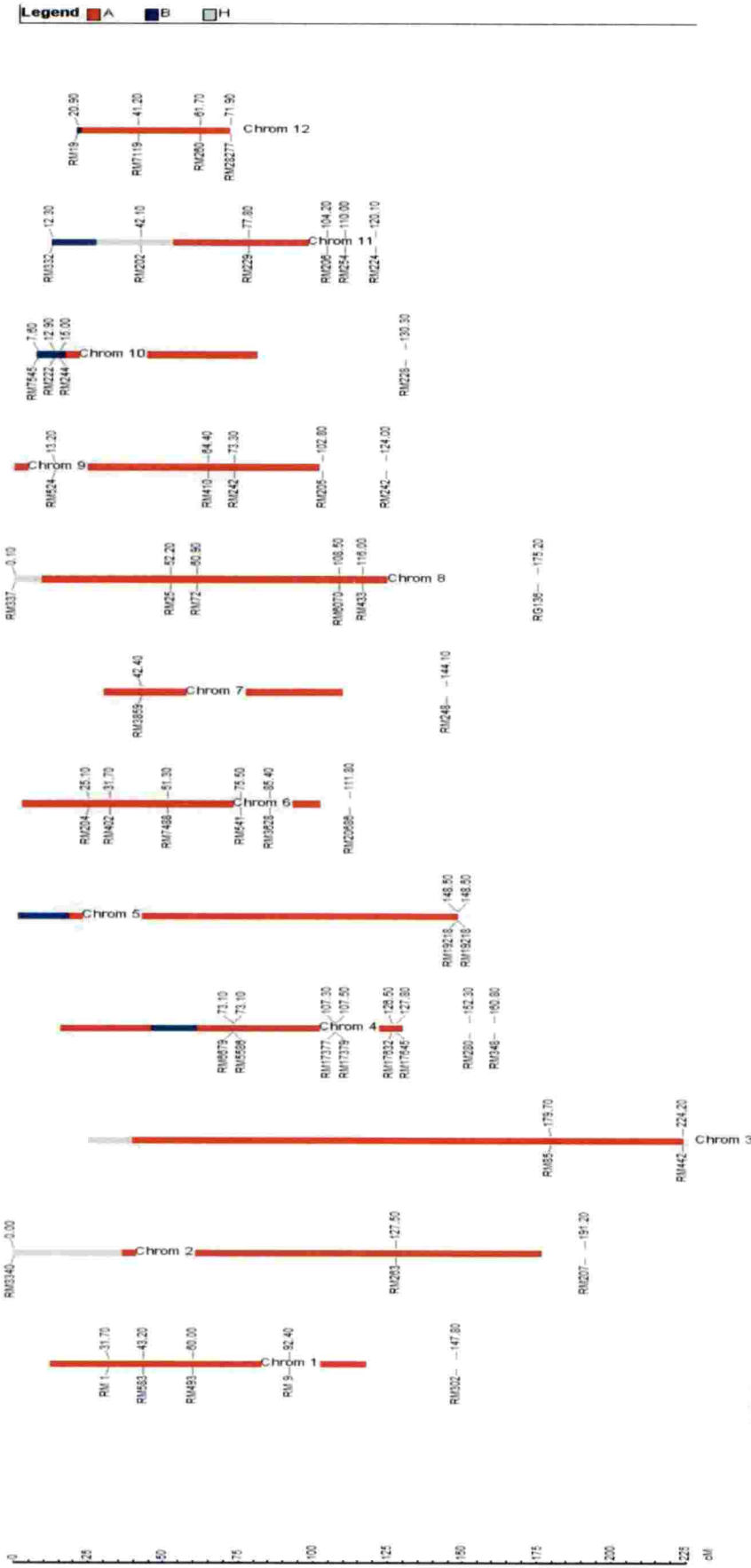
Figure 3. Graphical genotyping of 3-R-gene introgressed BC₁F₁ Plant No. 9.15

Donor parent genome   **Recurrent parent genome**   **Heterozygous locus**
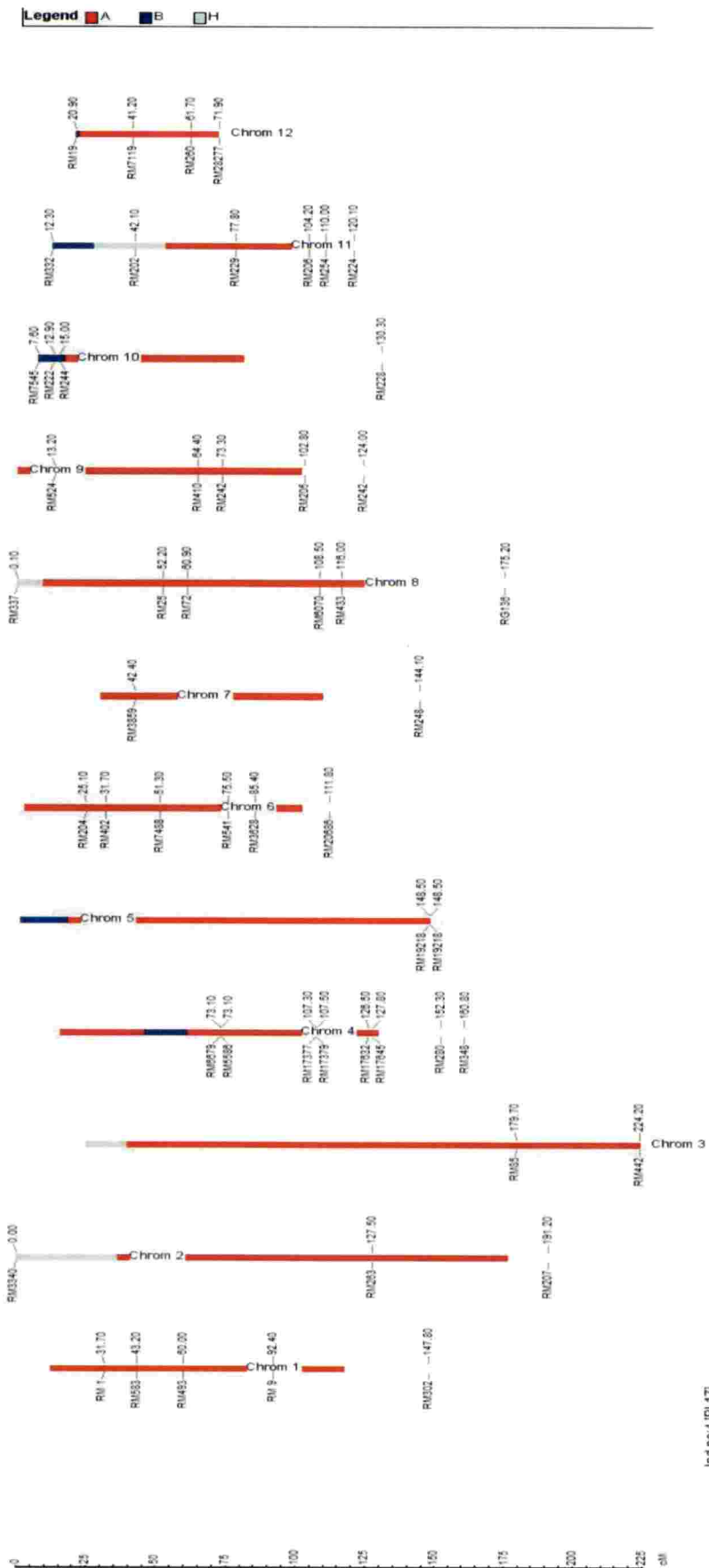
Figure 4. Graphical genotyping of 3-R-gene introgressed BC₁F₁ Plant No. 9.17
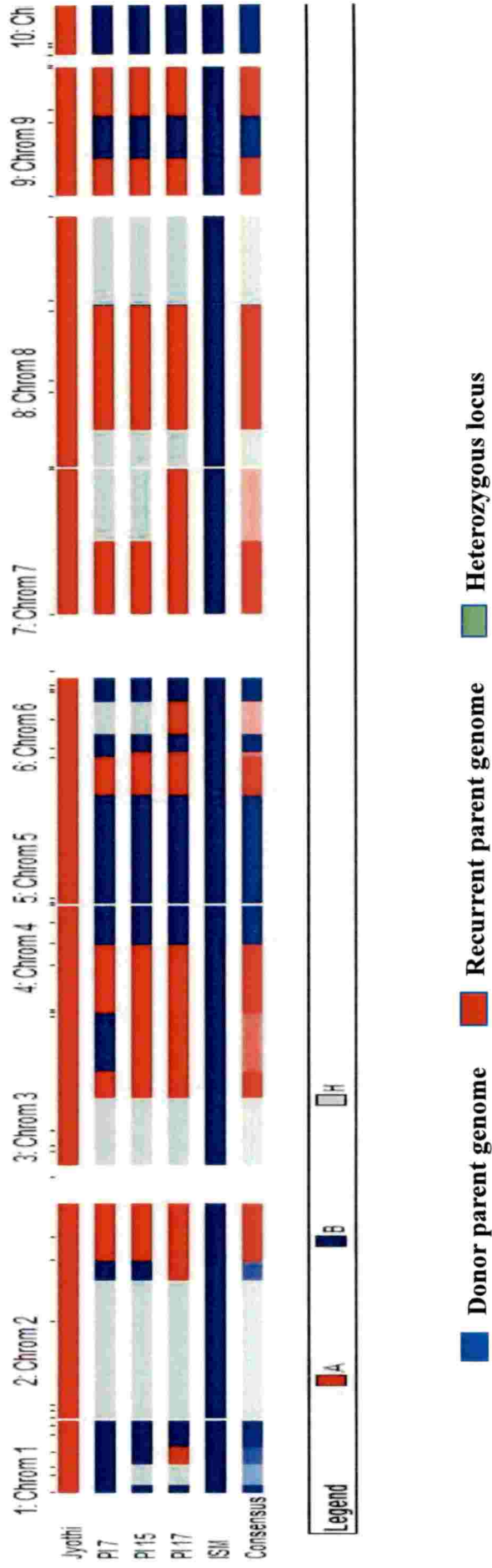
Figure 5. Graphical representation of recovery of recurrent parent genome 3-R-gene pyramided (BC₁F₁ Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17)

The dendrogram based on molecular data grouped the three R-gene pyramided individuals and parents into two clusters; cluster I being monogenic with only the donor parent ISM and cluster II comprised of the three R-gene pyramided plants (Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17) as well as the recipient parent Jyothi. Sub-clusters of Cluster II consisted of a monogenic group with only the Plant No. 9.17 and a subgroup 2 with Plant No. 9.7 and Plant No. 9.15 together exhibiting the same similarity coefficient. This pointed out that Plant No. 9.7 and Plant No. 9.15 were more similar to each other. These had exhibited a similarity coefficient 80 per cent and 82 per cent respectively with Plant No. 9.17 (Fig. 6).

As in the present study, Baliyan *et al.* (2018) grouped 15 pyramided lines and two parents into two clusters while the donor parent IRBB grouped into a monogenic cluster I. Similarly the cluster tree generated in the study of Dokku *et al.* (2013a) also showed less genetic similarity between both the parents. The 10 pyramided lines and two parents were grouped into two clusters.

Many factors like the number of backcrosses, the size of the population, and the position as well as the number of background markers helps to determines the effectiveness of marker-assisted breeding and recurrent parent genome recovery (Frisch and Melchinger; 2005; Hasan *et al.,* 2015; Deshmukh *et al.,* 2017). According to Rajpurohit *et al.* (2011), Ye (2010) and Suh *et al.* (2013), the best strategy to pyramid or introduce multiple genes and recover a maximum recurrent parent background effect in the shortest time will be to take up the transfer of genes simultaneously, generate a large backcross population and select the target genes through foreground selection and flanking marker analysis, to reduce the linkage drag. Hence, it is advisable to grow a large back cross population to recover multi-gene pyramids.

4.2 Production of BC_2F_1s

Minimum linkage drag for the resistance genes (*xa5, xa13* and *Xa21*) results in higher recovery of the recurrent parent genome in the later backcross generations (Pradhan *et al.*, 2015a; Suh *et al.*, 2013; Dokku *et al.*, 2013a; Basavaraj *et al.,*2010). Hence, successful backcrossing of the 3-R-gene introgressed BC_1F_1 Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17, with recurrent parent Jyothi will results in a

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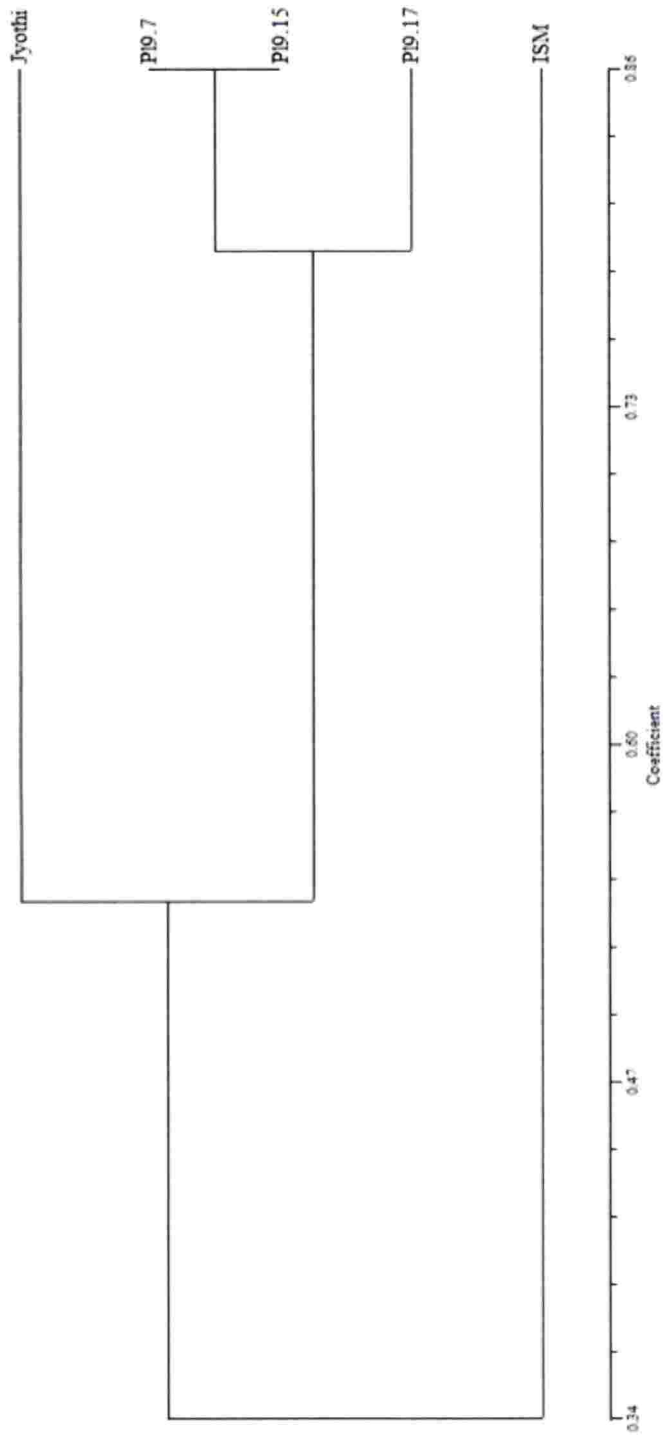
Figure 6. Clustering of R gene introgressed BC₁F₁s and parents based on molecular profils

(BC₁F₁ Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17)

cultivar with durable resistance to BB pathogen with a high recovery of the recurrent parent in following generation.

Considering the above, backcrossing of the R-gene introgressed BC_1F_1 Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17, to recipient parent (Jyothi) was done. It resulted in the production of BC_2F_1s (Table 19). Only five BC_2F_1 seeds were obtained. The reduced seed set observed on backcrossing of BC_1F_1 Plant No. 9.17 to recurrent parent Jyothi and the absence of seed setting in other two 3- R-gene pyramided lines (Plant No. 9.7 and 9.15), may be due to high temperature during grain filling stage. Floral abnormalities induced by heat stress leading to spikelet sterility causes reduction in grain production. The BC_2F_1s (Plate 8)obtained may be further subjected to the foreground and background profiling to identify and recover the 3-R-gene pyramided lines with maximum recovery of the recurrent parent.

4.3 Production of BC_1F_2s

In the present study, three BC_1F_1s (Plant No. 9.7; 9.15 and 9.17) were found to be 3-R gene pyramids. The R-genes *xa13* and *Xa21* were confirmed to be present in them in heterozygous condition. Resistance against pathogen is more effective in homozygous rather than heterozygous condition of the gene. Selfing act as a power full tool for achieving homozygosity at the gene loci in a heterozygous individual. Joseph *et al.* (2004) resorted to combined phenotypic selection along with MAS after selfing, resulting in the recovery of 80.40 to 86.70 per cent recurrent parent genome of Pusa Basmati 1 in BC_1F_3 generation with two BB resistant genes, *xa13* and *Xa21* along with the grain and cooking quality characteristics and desirable agronomic features of recurrent parent.

To attain homozygosity for the three R-gene heterozygous loci, selfing of BC_1F_1 Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17 was attempted. This resulted in the production of 67, 100 and 53 BC_1F_2 seeds respectively from Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17 (Table 20).

Plate 8. BC2F1s produced from R- gene pyramided

BC1F1 Plant No. 9.17

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Table 19. BC₁F₂ and BC₂F₁ progenies of the 3 R-gene introgressed

BC₁F₁s	BC₁F₂ seeds obtained (No.)	BC₂F₁ seeds obtained (No.)
Plant No. 9.7	67	0
Plant No. 9.15	100	0
Plant No. 9.17	53	5
Total	220	5

4.4 Morphological characterization of BC₁ F₁s

The major intention of backcross breeding programme is the transfer of one or few desirable genes into an otherwise popular elite cultivar. The expression of traits of economic importance is not only influenced by the genetic background but also to some extent by the external environment. Hence, resorting to both the genotypic and phenotypic selection, gives a better output in breeding programmes.

Morphological characterisation of the recurrent parent Jyothi, donor parent ISM and the three 3-R gene pyramided plants (Plate 9 and 10) indicated vide variation for various morphological traits studied (Fig. 7 to 11).

Plant height of recurrent parent (RP) Jyothi was 69.00 cm while it was 85.00 cm in the donor. Flowering occurred early in the recurrent parent (80 days) compared to the donor parent (DP) (120 days). The leaves were longer (RP: 33.14 cm; DP: 36.60 cm) and thinner in the donor parent (RP: 1.15 cm; DP: 0.95 cm). However, panicle length (RP: 20.20cm; DP: 22.23 cm) was higher in donor than RP, even though the number of spikelets per panicle (RP: 102; DP: 87) were higher in RP than the donor.

The length of the grain (RP: 9.36 mm; DP: 6.81mm) and kernels (RP: 6.99 mm; DP: 4.79 mm) varied in both the parents. But, the width of grain (RP: 3.02 mm; DP: 1.87 mm) as well as kernel (RP: 2.58 mm; DP: 1.55mm) of donor was slender in comparison to the recurrent parent (Jyothi). Recurrent parent had dark red colour kernels whereas the donor parent ISM possessed creamish white kernel.

BC₁F₁ Plant No. 9.7

BC₁F₁ Plant No. 9.15

BC₁F₁ Plant No. 9.17

Plate 9. Three R- gene pyramided BC₁F₁s

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Improved samba Mashuri
(Donor parent)

Jyothi
(Recurrent parent)

Plate 10. Parental genotypes

Plant height (cm)

(a) Plant height (cm)
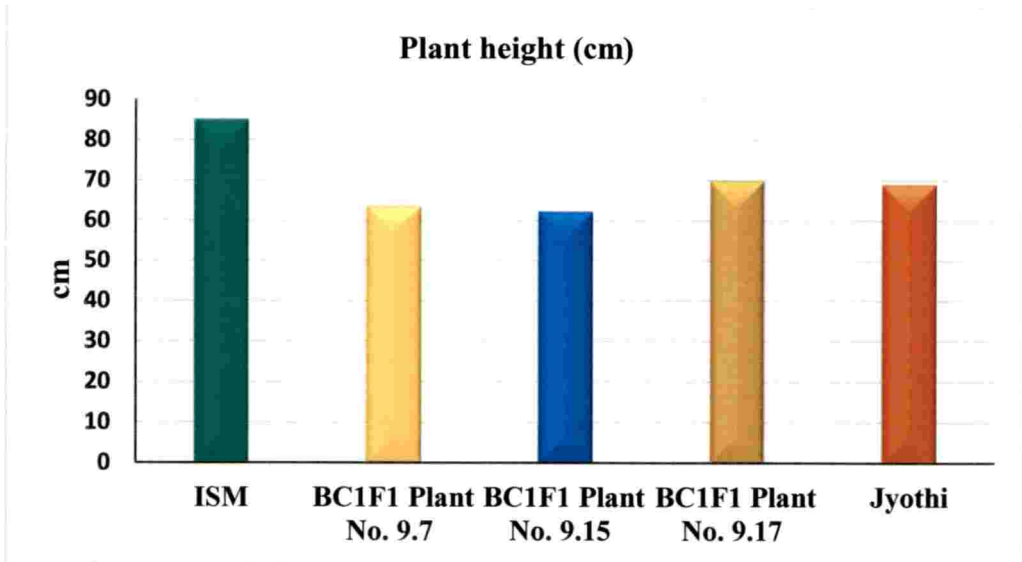
Days to 50% flowering
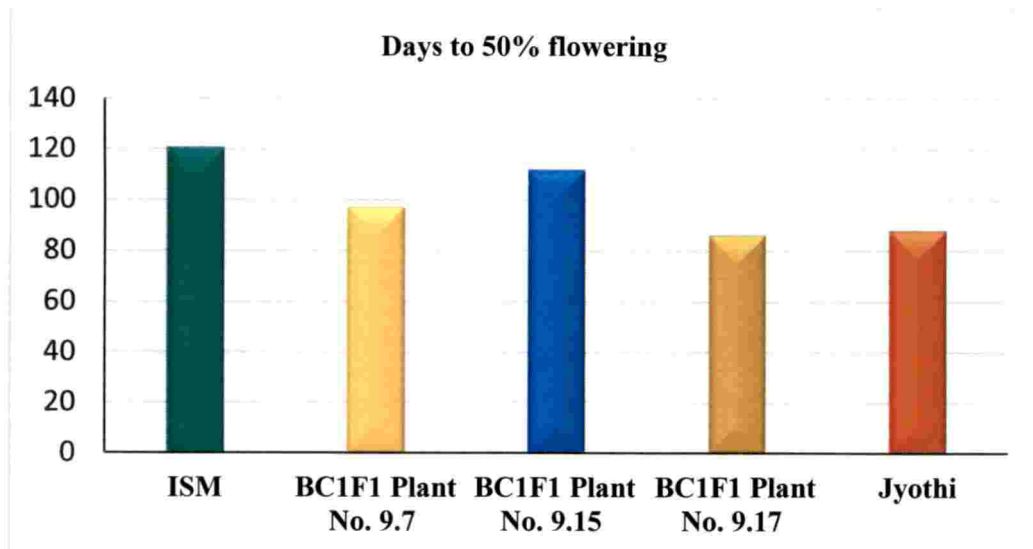
(b) Days to flowering

Figure 7 (a) and (b). Plant height and days to flowering of 3-R gene pyramided BC₁F₁s and parents

Leaf blade width (cm)

(c) Leaf blade width
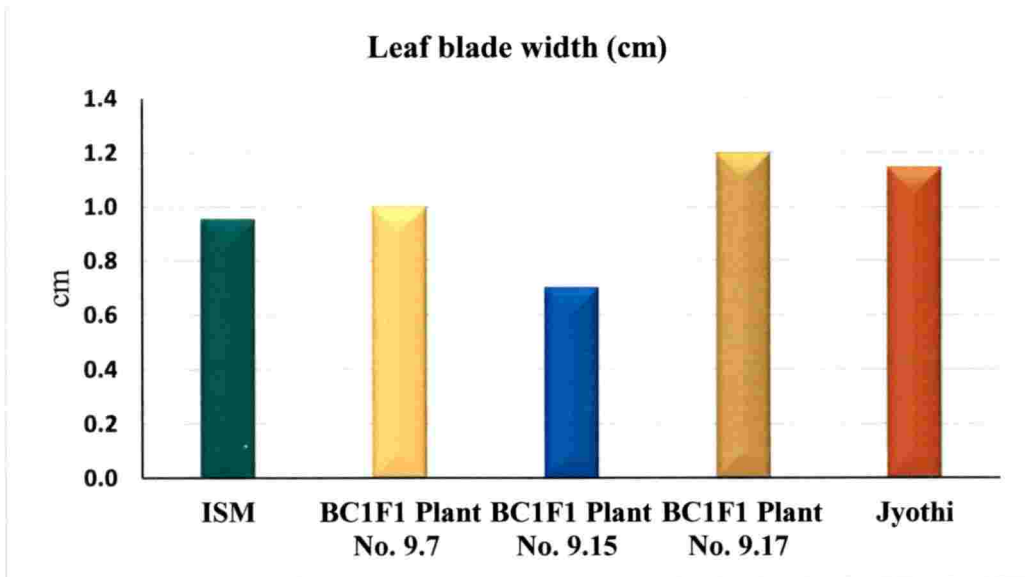
Leaf blade length (cm)
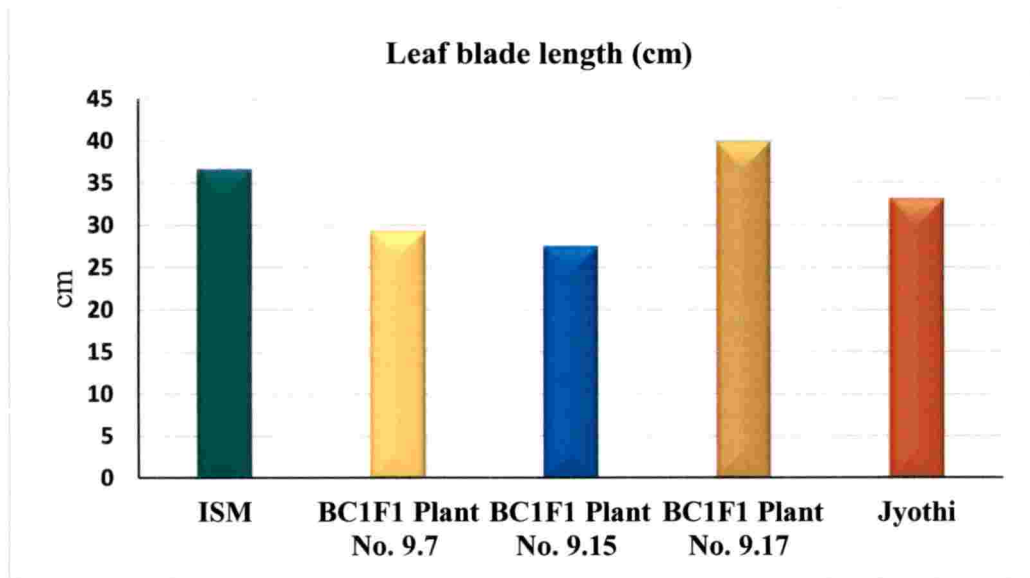
(d) Leaf blade length

Figure 8 (c) and (d). Leaf dimensions of 3-R-gene pyramided BC₁F₁s and parents

Panicle length (cm)
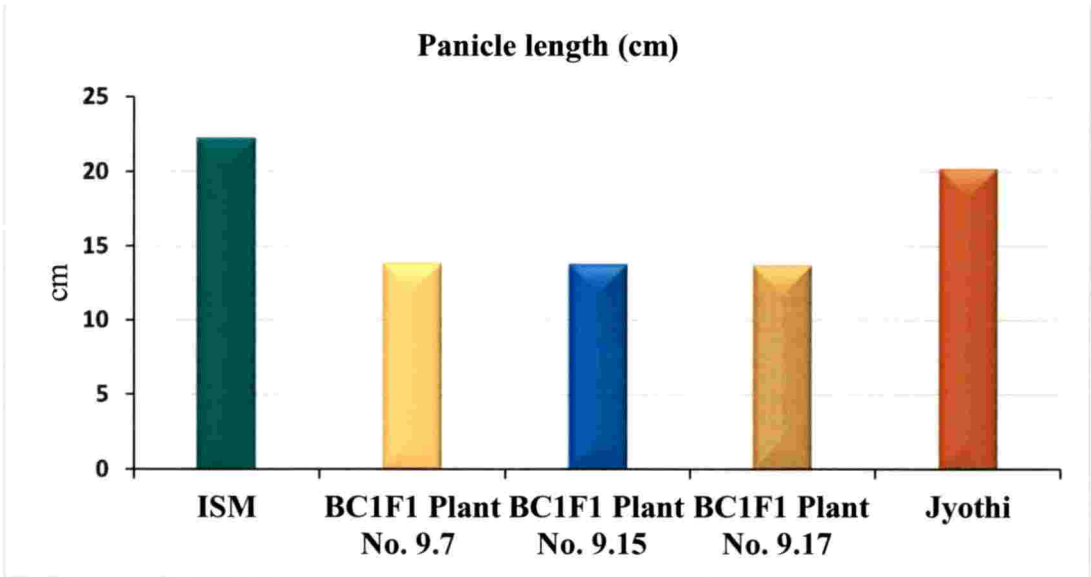
(e) Panicle length
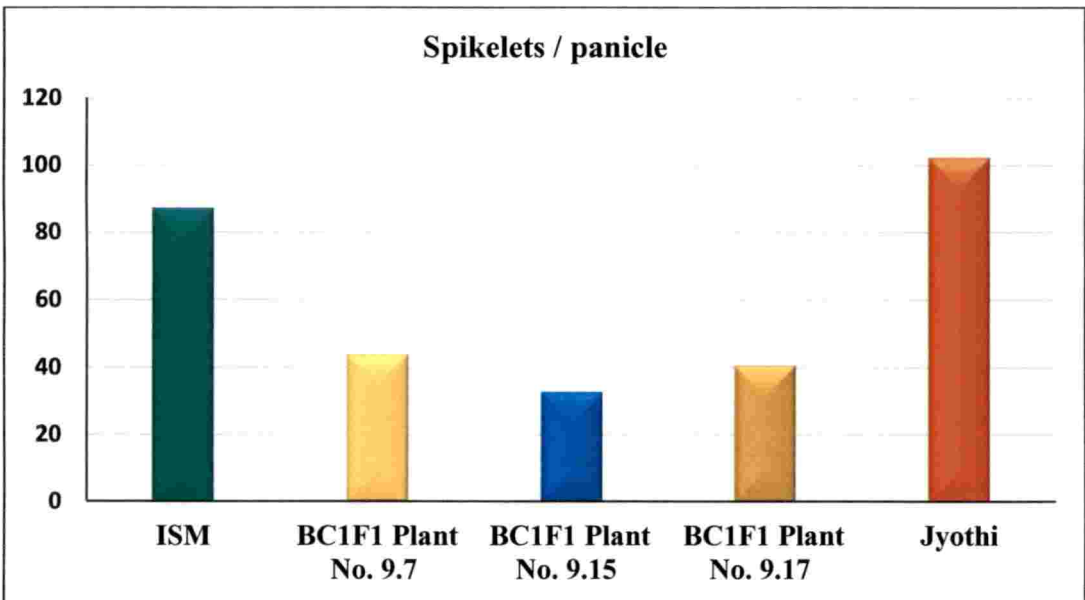
Spikelets / panicle

(f) Spikelet /panicle

Figure 9 (e, f). Panicle characteristics of 3-R-gene pyramided BC$_1$F$_1$s and parents
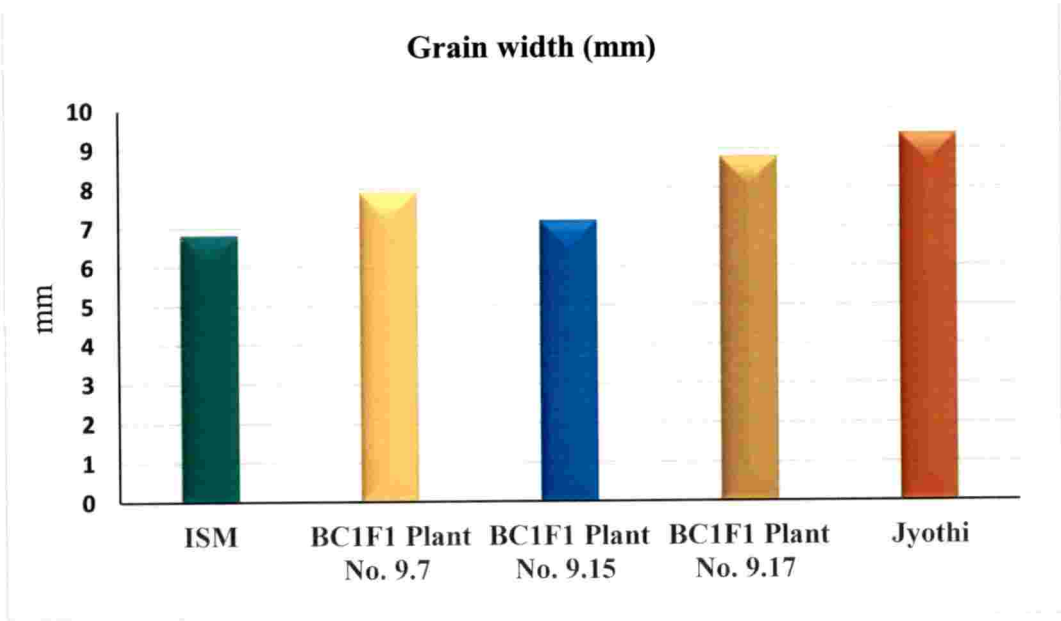
(g) Grain width (mm)
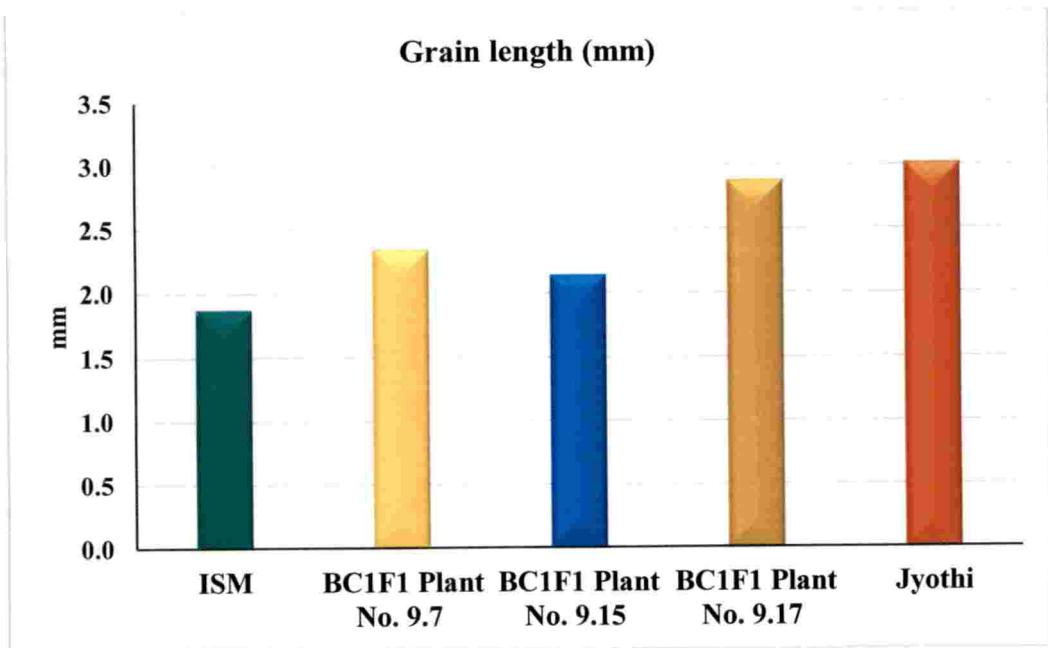
(h) Grain length (mm)

Figure 10 (g, h). Dimensions of 3-R gene pyramided BC₁F₁s and parents

Decorticated grain length (mm)

(i) Decorticated grain length (mm)
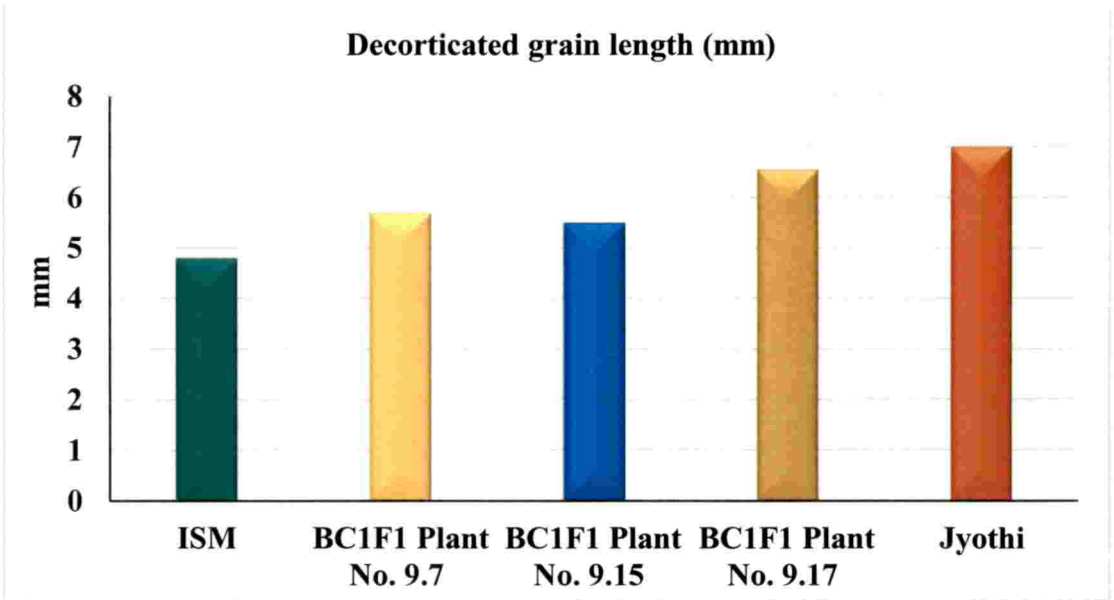
Decorticated grain width (mm)
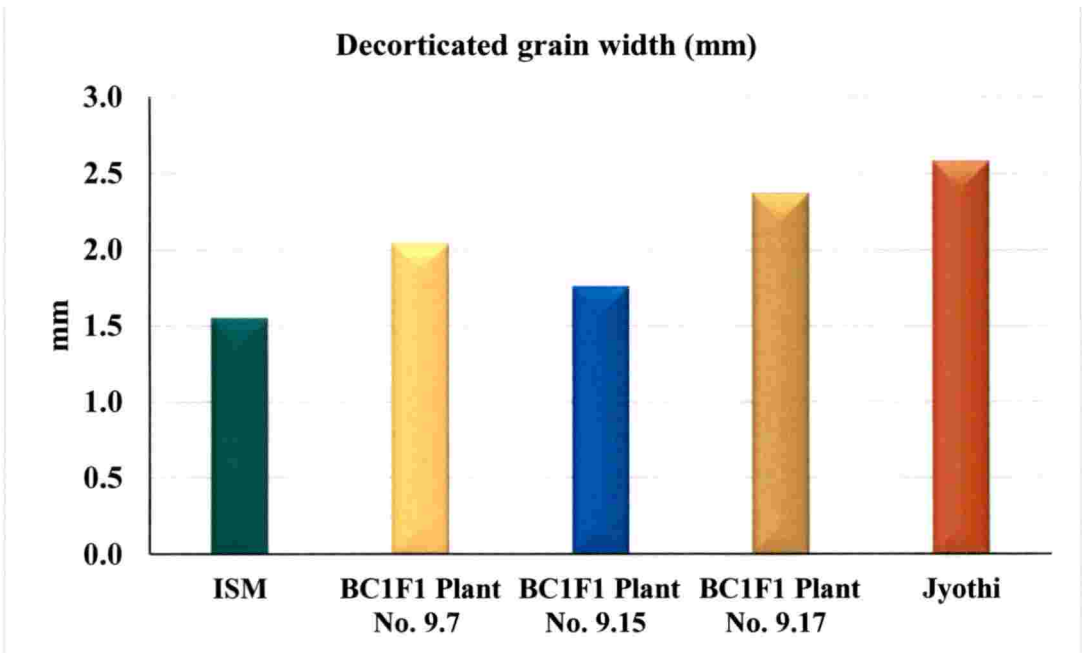
(j) Decorticated grain width (mm)

Figure 11 (i) and (j). Kernel dimensions of 3-R-gene pyramided BC₁F₁s and parents

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Comparison of the three 3 R-gene pyramided BC_1F_1s revealed that, the selected BC_1F_1s Plant No. 9.7 and Plant No. 9.15 were intermediate between the two parents for characters like panicle length, spikelets per panicle, days to flowering, plant height, length and width of the leaf blade and panicle length.

Results thus indicated that the Plant No.9.17 was near similar to the recurrent parent Jyothi with respect to the days to flowering, plant height, seed length and width, and kernel colour. The panicle of selected BC_1F_1s was found to be lower than that observed in both the parents panicle length and spikelets per panicle. Among 3 R-gene pyramided BC_1F_1s, Plant No. 9.17, was found to be morphologically similar to the recurrent parent Jyothi, except for the number of panicle and spikelets per panicle. All the three pyramids possessed reddish brown kernel colour similar to the recurrent parent. The intensity of red colour was higher in Plant No. 9.17 followed by that observed in Plant No. 9.15 and Plant No. 9.7 (Plate 11).

According to Swathi *et al.* (2019), phenotype-based selection coupled with marker-assisted selection, helps to recover the desirable plant type and grain type in a backcross programme. Baliyan *et al.* (2018) mentioned that most three gene pyramids in their study were similar or superior to the recurrent parent CSR-30 for the agronomic traits. Resorting to phenotype-based selection coupled with marker-assisted selection, Sagar *et al.* (2018) identified novel segregants with basmati grain qualities in backcross population through MAS aiming to introgress resistance to both BB and blast disease in early maturing basmati rice variety PB 1509. Swathi *et al.*, (2019) had reported the improved lines developed through MABB exhibited significant superiority for agro-morphological trait when compared with the recurrent parent. Earlier, Pradhan *et al.* (2015b) had reported complete recovery of yield and grain quality traits in Jalamagna along with transfer of three BB resistance genes through MAS. Arunakumari *et al.* (2016) could recover the desirable plant and grain type in an improved version of MTU1010.

Plate 11. Grains of parents and the 3-R-gene pyramided
BC₁F₁s
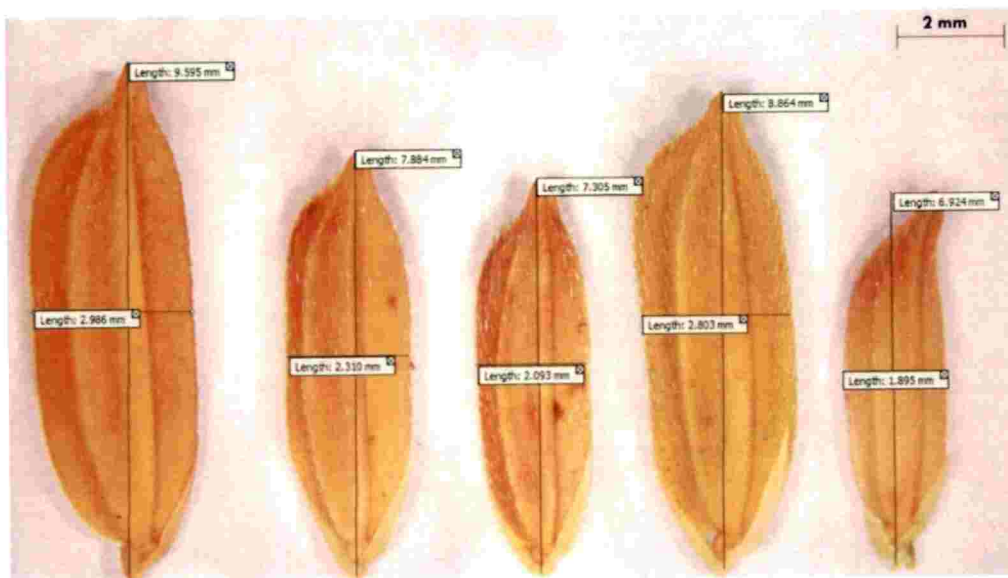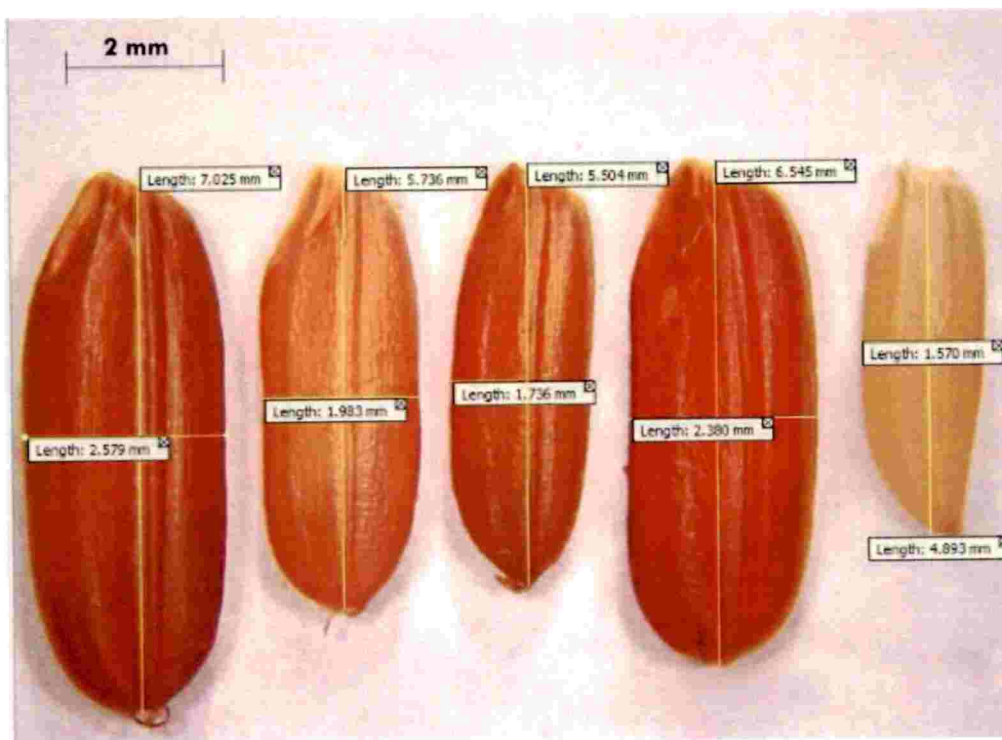
I: Improved Samba Mashuri   J: Jyothi
9.7: Plant No. BC₁F₁ 9.7   9.15: Plant No. BC₁F₁ 9.15
9.17: Plant No. BC₁F₁9.17
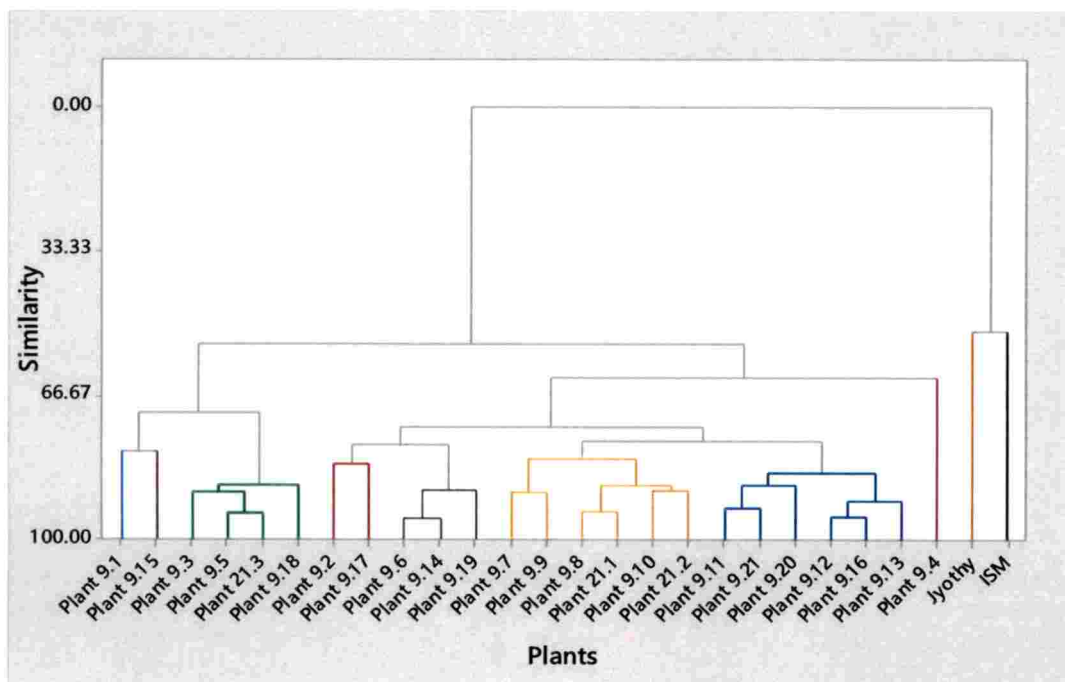
Figure 12. Clustering of parents and BC₁F₁ based on morphological characters
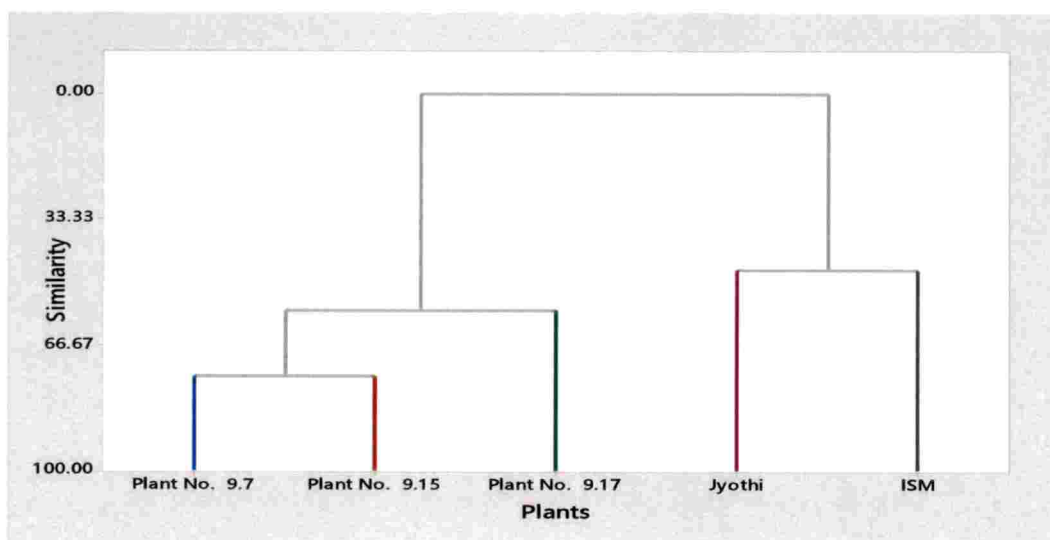
Figure 13. Clustering of BC₁F₁ 3-R gene pyramids and parents based on morphological characters

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4.4.2. Clustering of parents and three 3-R gene pyramided BC_1F_1s based on morphological characters

Based on morphological characters, clustering of parents and the 24 BC_1F_1s was done (Appendix IV and Fig.12). At 80 per cent similarity coefficient, the BC_1F_1s and parents grouped into 10 major clusters.

The 3-R-gene introgressed BC_1F_{1s} Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17 exhibited less than 60 per cent similarity to the recurrent parent Jyothi and the donor parent ISM (Fig.13).

Suh *et al.* (2013) had reported that the insertion of even a small chromosome segment from the donor may greatly affect the phenotype of the plant, thus, even though the R-gene introgressed individual exhibited high RP genome recovery, the donor parent segments may influence in the genome. Hence, the pyramids may not resemble the phenotype of the recurrent parent under the influence of the external environment.

4.5. Phenotypic screening of BC_2F_5s against Bacterial blight

Pathotyping of BC_2F_5s produced from the 2-R-gene pyramided BC_2F_4s (Plant No. 9 and Plant No. 21) in the earlier experiment (Kabade, 2017) was done.

The 212 BC_2F_4s (165 BC_2F_5s from BC_2F_4 Plant No. 9 and 47 BC_2F_5s from Plant No.21) were sown (Plate 12) to evaluate their resistance to BB pathogen (Table 20). Only 111 seedlings survived. These were screened for resistance to bacterial blight pathogen. Inoculation of BC_2F_5s with the bacterial suspension was done through Leaf clip method advocated by IRRI (2002) at maximum tillering stage (45 to 50 days) using Kerala isolates of *Xoo* (Plate 13) during November 2018 (plate 14). The lesions length was scored 15 days after inoculation based on the per cent of diseased leaf area (DLA) as per the Standard Evaluation System (SES) for rice (IRRI, 2002) (Plate 15).

Plate 12. Pathotyping population (BC$_2$F$_5$s)

Plate 13. Bacterial culture

(*Xanthomonas oryzae* pv. *oryzae* (*Xoo*)

Plate 14. Pathotyping through leaf clipping method
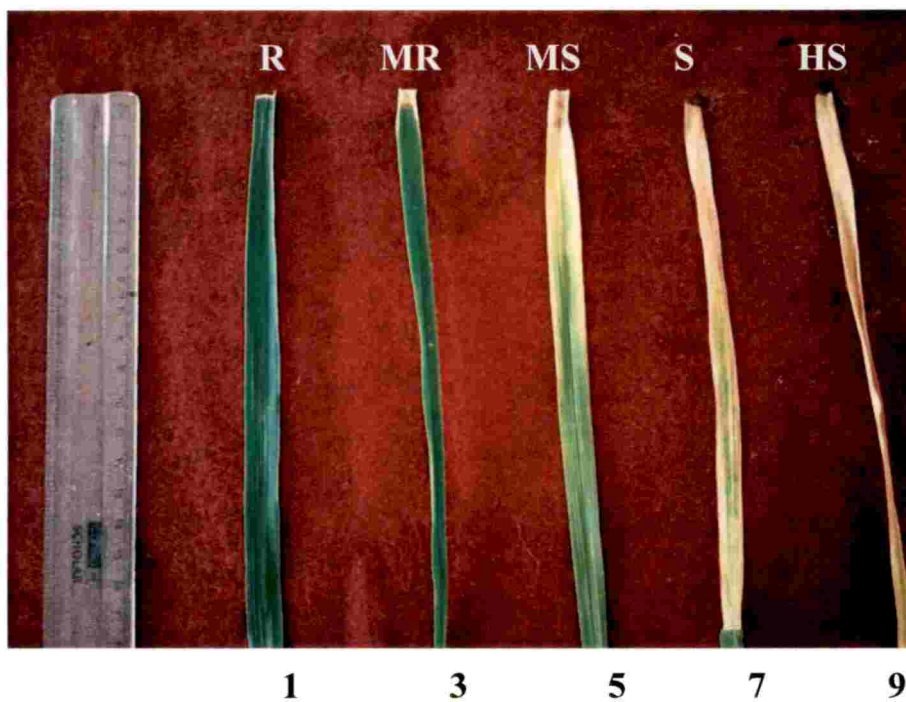
Plate 15.   Scale for categorising BB in rice based on per cent of lesion length

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Resistance: 1

Moderately Resistance: 3

Moderately susceptible: 5

Susceptible: 7

Highly susceptible: 9

Plate 16. Response of BC$_2$F$_5$s to infection by bacterial blight pathogen (Disease reaction based on scale chart)

Jyothi

Improved samba Mashuri

Plate 17. Response of parental genotypes to infection by bacterial blight pathogen

Table 20. Pathotyping of BC_2F_5s

Sl. No.	Plant No.	Number of seeds sown	Number of seeds germinated	Number of plants screened for BB resistance
1	BC_2F_5 from Plant No. 9	165	123	86
2	BC_2F_5 from Plant No. 21	47	31	25
Total		**212**	**154**	**111**

It was observed that out of the 111 BC_2F_5 progenies studied, 11.21 per cent (13 Nos.) of the progenies from BC_2F_4 Plant No. 9 and BC_2F_4 Plant No. 21, exhibited resistance reaction to BB infection, while, 10 plants were moderately resistant, 24 moderately susceptible, 21 susceptible and 43 were highly susceptible (Table 21).

Table 21. Grouping of BC_1F_2 progenies of BC_2F_4 Plant. No. 9 and 21

Diseased Leaf area (%)	Scale	Description	BC_2F_5 progenies	Diseased leaf area (%)		
				BC_2F_5 progeny	ISM	Jyothi
0-5	1	Resistant (R)	13	11.71	100	0
6-12	3	Moderately resistant (MR)	10	9.00	0	0
13-25	5	Moderately susceptible (MS)	24	21.62	0	0
26-50	7	Susceptible (S)	21	18.91	0	0
>50 %	9	Highly susceptible (HS)	43	38.73	0	100
Total			**111**			

Unlike in the present study, an earlier study on pathotyping of BC_1F_2s derived from the 3-R-gene pyramided BC_1F_1s of Uma variety revealed that more than half of the population (52%) exhibiting resistance to the BB in the field condition (Megha, 2018).

This results obtained indirectly indicated that the presence of appropriate R-gene combinations in the individuals exhibiting resistant to a moderately resistant

reaction to BB pathogen. It may be predicted that the resistant individuals may possess the gene combination $xa5xa5 + xa13xa13 + Xa21Xa21$.

Chukwu *et al.*, (2019) opined that marker-assisted selection with phenotypic screening can be combined in order to maximize the genetic gain. Similar to the present study, phenotypic screening was resorted to during attempts to improve resistance to BB pathogen in cultivars through MAS (Sanchez *et al.*, 2000; Singh *et al.*, 2001; Zhang *et al.*, 2001; Guvvala *et al.*, 2013; Das *et al.*, 2015; Pradhan *et al.*, 2015a and 2015b; Luo *et al.*, 2016; Mubassir *et al.*, 2016; Xiao Y *et al.*, 2016; Baliyan *et al.*, 2018; Das *et al.*, 2018; Reinke *et al.*, 2018; He C *et al.*, 2019).

4.6. Production of BC$_2$F$_6$s

Selfing of the BC$_2$F$_5$ individuals that exhibited resistance or moderate resistance reaction to BB infection (Plate 18) was done resulting in production of 1425 BC$_2$F$_6$ seeds (Table 22). These are to be evaluated further through MAS as well as characterised agro-morphological to isolate novel genotypes with BB resistance.

Table 22. Number of seeds obtained from resistance and moderately resistance plants of backcross generation

BC$_2$F$_5$ population	No. of Seeds from resistance plants (BC$_2$F$_6$)	No. of Seeds from Moderately resistance plants (BC$_2$F$_6$)	Total
BC$_2$F$_4$ Plant No.9	900	103	1003
BC$_2$F$_4$ Plant No.21	221	200	421

4.7 Morphological characterisation of BC$_2$F$_5$s

Agronomic traits evaluation of BC$_2$F$_5$s was done after the artificial inoculation of Bacteria (Table 23 and Appendix V). The plant height among the population ranged between 28 cm and 86 cm in BC$_2$F$_5$s compared to 55 cm in the recurrent parent Jyothi. The BC$_2$F$_5$s plants possessed a high number of effective tillers per plant in comparison to recurrent parent Jyothi. The number of spikelets per panicle ranged from 0 to 45. Most plants showed higher days to flowering than the recurrent parent. The better growth observed in BC$_2$F$_5$ plants compared to Jyothi
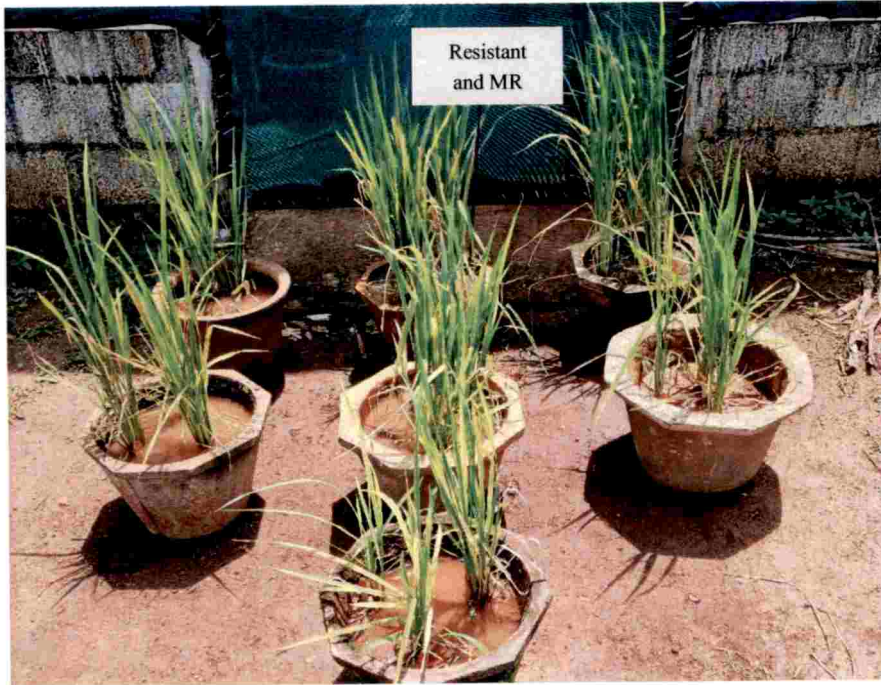
Plate 18. Resistant and moderately Resistant plants(BC$_2$F$_5$) for BC$_2$F$_6$ production

on inoculation with BB pathogen may be indicative of their tolerance to BB infection in comparison to the recurrent parent Jyothi. However, a few BC$_2$F$_5$ plants were inferior to recurrent parent Jyothi.

Table 23. Morphological characterisation of BC$_2$F$_5$ and parents

Sl. No.	Traits	BC$_2$F$_5$			Jyothi	ISM
		Min	Max	Mean		
1	Plant height (cm)	28.50	83.60	56.07	43.75	50.30
2	Days to flowering	88.00	117.00	102.00	90.00	111.00
3	Leaf width (cm)	0.60	1.30	0.95	0.93	0.90
4	Leaf blade length (cm)	11.80	49.50	30.65	22.74	26.10
5	Productive tillers /plant	0.00	15.00	7.50	5.00	8.00
6	Panicle length	0.00	15.50	7.75	8.15	17.92
7	Spikelet's/panicle	0.00	45.50	22.75	23.00	70.00

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Summary

V. Summary

The research programme 'Marker assisted backcross breeding in two-R gene pyramided lines of rice variety Jyothi for bacterial blight resistance' was carried out between 2017 and 2019 in the Department of Plant Biotechnology, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur.

Jyothi is one among the most widely cultivated high yielding rice variety in Kerala. Since, yield loss due to occurrence of bacterial blight (BB), is a recurring phenomenon, efforts to introgress resistance genes ($xa5 + xa13 + Xa21$) imparting resistance to bacterial blight from donor Improved Samba Mahsuri into variety Jyothi (PTB 39), through marker assisted backcross breeding programme were taken. It resulted in the production of BC_1F_1s and BC_2F_5s. In the present study, an attempt was made to identify BC_1F_1s pyramided with the 3-R genes. Pathotyping of the BC_2F_5s to assess their response to BB pathogen was also aimed at. The study comprised of four experiments listed below:

I: Genotyping of BC_1F_1 population

II: Morphological characterization of pyramided lines (BC_1F_1 population)

III: Production of BC_2F_1's and BC_1F_2's

IV: Morphological characterisation and pathotyping of BC_2F_5 population and production of BC_2F_6 population

The results obtained are summarized below:

I: Genotyping of BC_1F_1 population
➢ Adequate quantity of good quality total genomic DNA was extracted from 24 BC_1F_1s and the parents (Recurrent parent: Jyothi; Donor parent: Improved Samba Mahsuri (ISM)).

A). Foreground selection of BC_1F_1s
➢ Result of the restriction digested (digested with *DraI*) PCR product of marker RG 556 linked *xa5* revealed the presence of amplicons of size 673 bp, 484 bp and 468 bp in all the 24 BC_1F_1s as well as the recurrent parent Jyothi and donor parent ISM.

- Foreground analysis with functional marker xa5SR revealed monomorphic banding pattern (amplicon size 167 bp) in all the individuals studied.

- The results thus indicated the endogenous presence of R-gene *xa5* in both the parents as well as all the 24 BC₁F₁ individuals.

- The PCR product of STS marker RG 136 linked to *xa13* when digested with restriction enzyme *HinfI* produced amplicons of size 1051 bp and 536 bp in recurrent parent Jyothi and donor parent ISM respectively, while, in three BC₁F₁s *i.e.,* Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17, alleles of both donor parent ISM as well as the susceptible parent Jyothi was observed.

- The functional marker xa13 promoter produced amplicons of size 500bp and 292 bp respectively in the donor parent ISM and the recurrent parent Jyothi.

- As in restricted digested product of STS marker RG 136 linked to *xa13,* the three BC₁F₁ plants *i.e.,* Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17, exhibited banding pattern similar to both donor parent ISM as well as the susceptible parent Jyothi.

- The results, thus revealed that the above mentioned three BC₁F₁s (Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17) were heterozygous at the R-gene *xa13* locus.

- The result also indicated that among the 24 BC₁F₁ plants studied, three plants *i.e.,* Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17, were introgressed with the two R-genes *xa5* and *xa13*.

- Among the 24 BC₁F₁s progenies studied, amplicons of size 966bp and 660bp corresponding to parents Jyothi and ISM respectively were observed in three BC₁F₁ plants (*i.e.,* Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17) when analysed with marker pTA248 linked to R-gene *Xa21*.

- This not only indicated the presence of R-gene *Xa21* in the heterozygous state in BC₁F₁ Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17, but also pointed out that these three BC₁F₁s were 3-R gene pyramids.

- Results of foreground selection, thus indicated that the three BC₁F₁ plants *i.e.,* Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17, were the only 3-R-

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gene pyramids among the 24 BC_1F_1 individuals investigated. The other BC_1F_1s possessed only a single recessive R-gene *xa5*.

➢ The 3-R-genes introgressed BC_1F_1s (Plant No. 9.7; Plant No. 9.15 and Plant No. 9.17) were homozygous at the R-gene *xa5* locus but were heterozygous at the other two R-gene (*xa5xa5 + Xa13xa13 + Xa21xa21*).

B). Background selection of the 3-R-gene introgressed BC_1F_1s

➢ Background selection of the three BC_1F_1 3-R gene pyramids (Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17) using 58 markers that were found to be polymorphic between the parents ISM and Jyothi, revealed that the R-gene introgressed BC_1F_1s were similar to recurrent parent Jyothi and donor parent ISM respectively at 24 and 21, 22 and 21, 34 and 12 marker loci.

➢ The BC_1F_1 Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17 were heterozygous at 13, 15 and 12 marker loci respectively.

➢ The result thus indicated that, among the three 3-R-gene introgressed BC_1F_1s, Plant No. 9.17 was more similar to recurrent parent Jyothi.

➢ The result of the Graphical genotyping also indicated a greater similarity between Plant No. 9.17 and the recurrent parent Jyothi.

➢ The highest recovery of the recurrent patent genome was found in BC_1F_1 Plant No. 9.17 (93.00%), followed by Plant No. 9.7 and 9.15 with a recovery of 89.00% each.

➢ Dendrogram based on molecular data grouped the three R-gene pyramided individuals and parents into two clusters. Cluster I was monogenic with only the donor parent ISM, while, cluster II comprised of the three R-gene pyramided plants (Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17) as well as the recurrent parent Jyothi. Sub-clusters of cluster II was monogenic with only Plant No. 9.17 and the second sub-group comprised of with Plant No. 9.7 and Plant No. 9.15. This pointed out that Plant No. 9.7 and Plant No. 9.15 were more similar to each other. These had exhibited a similarity coefficient 80 per cent and 82 per cent respectively with Plant No. 9.17.

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II. Morphological characterization of pyramided lines (BC₁F₁ population)

> The 3-R-gene pyramided BC₁F₁s (Plants No. 9.7, Plant No. 9.15 and Plant No. 9.17) exhibited about 60 per cent similarity to the recurrent parent Jyothi and the donor parent ISM.

> Comparison of the three 3 R-gene pyramided BC₁F₁s with recurrent parent and donor parent revealed that the 3-R-gene introgressed BC₁F₁s Plant No. 9.7 and Plant No. 9.15 were intermediate between the two parents for characters like plant height, length and width of the leaf blade, days to flowering, panicle length and spikelets per panicle.

> Plant No.9.17 was near similar to the recurrent parent Jyothi with respect to the days to flowering, plant height, seed length and width and kernel colour.

> The length of panicles and the number of spikelets per panicle in the 3-R-gene introgressed BC₁F₁s were found to be less than that of the parents.

> The intensity of red colour in grain was higher in Plant No. 9.17 compared to Plant No. 9.15 and Plant No. 9.7.

> The above observation indicated that among the three 3-R-gene BC₁F₁s, Plant No. 9.17 was more similar to the recurrent parent Jyothi in morphology.

III. Production of BC₂F₁'s

> BC₂F₁ seeds (5 Nos.) were produced by backcrossing the 3-R-gene introgressed BC₁F₁s to recurrent parent Jyothi.

IV. Production of BC₁F₂s

> Selfing of the three 3-R-gene pyramids (BC₁F₁ Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17) resulted in the production of 220 BC₁F₂ seeds

IV. Morphological characterisation and pathotyping of BC₂F₅ population, and production of BC₂F₆ population

A). Screening of BC₂F₅s against BB pathogen

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- Pathotyping of 111 BC_2F_5s through Leaf clip method advocated by IRRI (2002) revealed that 11.71 per cent (13 Nos.) of the progenies exhibited resistance reaction to BB infection, while, 10 (9.00%) were moderately resistance, 24 (21.62%) moderately susceptible, 21 (18.91%) susceptible and 43 (38.73%) highly susceptible.
- It may be predicted that the resistant individuals may possess the R-gene combination $xa5xa5 + xa13xa13 + Xa21Xa21$.

Morphological characterisation of BC_2F_5s

- Better growth parameters observed in a few BC_2F_5 plants compared to Jyothi on inoculation with BB pathogen, may be indicative of their tolerance to BB infection.

Production of BC_2F_6s

- Selfing of the BC_2F_5 individuals that exhibited resistance or moderate resistance reaction to BB infection was done resulting in production of 1425 BC_2F_6 seeds.

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Appendices

Appendix I. Markers list

Sl.No.	Marker name	Chromosome number	Forward sequence (5' -3')	Reverse sequence(5' –3')	AT (°C)
1	RM493	1	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG	57.6
2	RM428	1	AACAGATGGCATCGTCTTCC	CGCTGCATCCACTACTGTTG	56.9
3	RM6340	1	AACGGAGATCGAGATCGATG	TGCTTCCTCATCTCCCTCAC	55.0
4	RM340	1	GGTAAATGGACAATCCTATGGC	GACAAATATAAGGGCAGTGTGC	54.8
5	RM294	1	TTGGCCTAGTGCCTCCAATC	GAGGGTACAACTTAGGACGCA	56.0
6	RM5919	1	AAACAGTCAGCGGCTTTGTC	ATAGCGTTTGACGGGACAAC	58.0
7	RM246	1	GAGCTCCATCAGCCATTCAG	CTGAGTGCTGCTGCGACT	56.0
8	RM302	1	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC	58.0
9	RM10871	1	TGAGGCTGTAACGTAGACGATGAACC	AAGCCTGCTAGAGAGGCCCAACC	56.0
10	RM11342	1	CCATCCATGCACATTTAGGAGTAGG	TGTACAATCACGTCGCTCTACACG	57.0
11	RM12038	1	AGACGCACGCAACATCACTCG	GGGTATACCATACTCCCTCCGTTGC	56.0
12	RM12941	2	TTATGCCATGTGGTCCAATCAGC	ATTTGAACCATTTGGGCCTTGG	55.0
13	RM497	2	TCCTCTTCACCTATGGGTGG	GCCAGTGCTAGGAGAGTTGG	57.0
14	RM250	2	GGTTCAAACCAAGCTGATCA	GATGAAGGCCTTCCACGCAG	55.0
15	RM482	2	TCTGAAAGCCTGACTCATCG	GTCAATTGCAGTGCCCTTTC	58.0
16	RM279	2	GCGGGAGAGGGATCTCCT	GCGGGAGAGGGATCTCCT	56.0
17	RM166	2	CGGTCCTGGGTCAATAATTGGGTTAC	TTGCTGCATGATCCTAAACCGG	56.0
18	RM250	2	GGTTCAAACCAAGCTGATCA	GATGAAGGCCTTCCACGCAG	55.0
19	RM233	2	CCAAATGAACCTACACATGTTG	GCATTGCAGACAGCTATTGA	57.0
20	RM208	2	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGACC	56.0
21	RM263	2	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	57.7
22	RM442	3	CTTAAGCCGATGCATGAAGG	ATCCTATCGACGAATGCACC	58.0
23	RM231	3	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG	57.0
24	RM186	3	TCCTCCATCTCCTCCGCTCCCG	GGGCGTGGTGGCCTTCTTCGTC	58.0
25	RM7076	3	TGGTTCGATTCGGATTTC	AAGCTATTCACAAGCAGCTC	59.0
26	RM60	3	AGTCCCATGTTCCACTTCCG	ATGGCTACTGCCTGTACTAC	59.0
27	RM338	3	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC	55.0

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...cond. Appendix I. Markers list

28	RM60	3	AGTCCCATGTTCCACTTCCG	ATGGCTACTGCCTGTACTAC	59.0
29	RM251	3	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCGATC	57.0
30	RM17377	4	ATATTACTTCGACGCTGGATCAGG	GTCAGTTCGTCAGGCACAACG	57.0
31	RM17379	4	TCGGACTCAGCACTACGTTACCC	GAGCACGTAATGGAATCTGATGG	55.0'
32	RM17182	4	TGCAGCGTCTCATCATAAAGTCG	GCTTAGTGCTGTGAACTGTGAAGACC	55.0
33	RM17632	4	ACAGCATGCGCACCACATAAAGG	CGTGGTTCACACACTTACATTGTTGG	56.0
34	RM16854	4	TCGTAGATCGACTCGTAGTCGTAGG	AGAGAGGGAGTATAACGGAATGACG	58.0
35	RM17645	4	GCTTTGTTGGGTGATCGTCTAGG	GGCGATCTACTGTTCTTGTCACC	59.0
36	RM17620	4	ACCATCTCGTATTTGGCTCATCC	AACATGCACTGGATGATCTCTCG	56.0
37	RM16883	4	TGCCATGATATGATTCCTGTGG	GGTCCTATTACAAGCATGCAGTCC	56.0
38	RM16866	4	AACTCGCGAGGAGTCGGAGTCG	ATGCGCGGGATTTCACCTACTTCC	57.0
39	RM17162	4	GATGTACCAGTCCAGTTACAAAGACC	CCTTCAGAGTCTGCACACAGG	57.0
40	RM16556	4	TTGGACCAGGAGATCAATGAAGG	GTGCGCACACTCTTCTATGTGC	56.0
41	RM280	4	ACACGATCCACTTTGCGC	TGTGTCTTGAGCAGCCAGG	56.0
42	RM413	4	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC	56.0
43	RM470	4	TCCTCATCGGCTTCTTCTTC	AGAACCCGTTCTACGTCACG	56.0
44	RM119	4	CATCCCCCTGCTGCTGCTG	CGCCGGATGTGTGGGACTAGCG	56.0
45	RM6089	4	CCACCGAATCGAATAAACCAC	ATGGCCAGCGTGATCTCC	56.0
46	RM252	4	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	56.0
47	RM348	4	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC	56.0
48	RM19218	5	CGGAGGGAGTAGGTACGTAGGG	CCCATTCCATTCTACACTGACG	55.0
49	RM19221	5	CCGATAATCACCTCCATTCCTAGC	AATGGAGTAGACGGAGCACTAATCG	56.0
50	RM18384	5	GCAGCAGAAAGGGAGAGAGTATGG	CAGCAACGTACGTACCAACAGG	56.0
51	RM18353	5	AGATCTCACTATTGAGTAGCCCATGC	CACCTTGCCCTTAAATACCAACC	55.0
52	RM18004	5	CTCGAAGCTATTAGCCGGGATCG	ATCTTCTTCCGCGCGTCTTCC	55.5
53	RM18353	5	AGATCTCACTATTGAGTAGCCCATGC	CACCTTGCCCTTAAATACCAACC	56.0

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...cond. Appendix I. Markers list

54	RM17998	5	GAACACTAGGGCGCATCCATTCC	ATTAGGGAGCGTTGGATTGTTCC	59.0
55	RM18647	5	ATTTCTAGCCCTCACGGTAAATGTGG	GGGTGAAACGGTGTCTGACTGG	61.0
56	RM18222	5	TGATTCCTCTATATGCAGCCTTGG	TATCGTGGTTTCATCGTGTGTGC	56.0
57	RM18382	5	GGAATTAAATGTGCGGGAATGC	TGTAAGTACAAATCCGGCACCTATGG	57.0
58	RM18204	5	GAAACTAGAGATGCACACATCC	ATGGTAAGTACTCCCTCCATCC	58.0
59	RM18618	5	TGCTACCGATAGTAGAAGTGATCG	GCATGTGTACAGGAGGAAGC	58.0
60	RM18639	5	CATCATGTGGTAAGTGTGCAACG	GGTTGCGATGAGATTACGAGACC	57.0
61	RM169	5	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTCATCCCTCC	57.0
62	RM163	5	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT	56.0
63	RM178	5	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTTCCCTCCGCCTGC	56.0
64	RM18941	5	GTGAAGTGCAGCCGAAGAGC	ATCGATCTCTCATCACGATCAACC	54.0
65	RM18225	5	CGACAGGAGGGAGAGAGGAAGG	GGTTTGACCGTGGTTTGACTAACG	57.2
66	RM18919	5	AGGAGTTCAGTTTCTGCAAGTCAGG	CAGCATGCCGTAGTTCACACC	55.0
67	RM20182	6	CCTTATTGGGCCAGAGATAGTTGG	CAGTGTTGTGACGGTACAATGC	55.0
68	RM20023	6	CTGACCTGACGGCTGACATGACC	CAAGCAACCTTTCGGGATTTGC	57.0
69	RM19483	6	CCAACTAAACAAGCCCTGACTATGG	GGTTGTCCCGTCAATAAAGTACCC	58.0
70	RM20168	6	GAATATCCTTGGCTCTCTAGACTTGG	TGGGACTTGACTTGGACTATTTGC	57.0
71	RM20158	6	ACTCACCGTACGAACTCGATGC	ATCTGTCCTGAACCGATACTGC	57.0
72	RM20037	6	TGTGCCAAACAGGCTCTTAGTATAGG	CAGACTGTCGTTCCTCCGTTGG	56.0
73	RM20686	6	ATGCACACATAGTCAACAGCTTCC	GTGATCACCACACAGACTGAAACC	55.0
74	RM20409	6	GGCCAACCTAATGATATACTCC	GAGTGACTCGAGTGTGTGACC	55.0
75	RM20683	6	ATGATGATCCCTTCAGCCTTTCG	TGTCAGTGCCTCCTCTTCATTCC	59.0
76	RM20190	6	ATAACGTACTCAGGGTGCGGTTAGC	GGCGGAAGGTTGTGATTAGATAAAGG	56.0
77	RM508	6	GGATAGATCATGTGTGGGGG	ACCCGTGAACCACAAAGAAC	55.8
78	RM589	6	ATCATGGTCGGGTGGCTTAAC	CAGGTTCCAACCAGACACTG	55.8
79	RM217	6	ATCCGCAGCAATGCCTCGT	GGGTGTGAACAAAGACAC	56.0

III

80	RM314	6	CTAGCAGGAACTCCTTCAGG	AACATTCCACACACACACGC	58.0
81	RM253	6	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC	56.0
82	RM238	6	GATGGAAAGCACGTGCACTA	ACAGGCAATCCGTAGACTCG	57.0
83	RM21452	7	GGTTATCCAACCGGGACTACC	CATACACCTGAGTGTACGAAAGAGC	57.0
84	RM21470	7	TCTTGCCATCACATAGCAACAGG	ACTCGGTGAGCATCCAATGTCC	57.0
85	RM21320	7	CGTGCAACCCTATATGTAGATTGTGG	GGAGCCCGGAGTAATTTCTAAAGC	58.0
86	RM21661	7	CTCCGCAGGGTCTGTTTAGTTTCC	GACGATATTGTTGCAAGCGTGAGG	57.0
87	RM180	7	CTACATCGGCTTAGGTGTGTAGCAACACG	ACTTGCTCTACTTGTGGTGAGGGACTG	55.0
88	RM478	7	CAGCTGGGGAAGAGAGAGAG	TCAGAAACTAAACGCACCCC	57.0
89	RM180	7	CTACATCGGCTTAGGTGTGTAGCAACACG	ACTTGCTCTACTTGTGGTGAGGGACTG	55.0
90	RM234	7	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	59.0
91	RM18	7	TTCCCTCTCATGAGCTCCAT	GAGTGCCTGGCGTGTAC	53.0
92	RM295	7	CGAGACGAGCATCGGATAAG	GATCTGGTGGAGGGGAGG	57.0
93	RM23099	8	GACACGCCTGGAGACAATAGTAGG	TTTATTCGGGATGCGTGATGC	57.0
94	RM23645	8	CATACAGCATGCTCACAGTTGATCG	CATCAGCATCTGGGACCTCTCC	56.0
95	RM23080	8	CAACCTCCCGCCCTAACTACC	ATCAACAGAAGAAACCGGCTACC	57.0
96	RM23096	8	AAATAGACTACTGGGGTGCGTTCG	GTGCAATCATGTTCACATCAGC	59.0
97	RM22905	8	CACTGCTCACTGCTGCCTTGC	CACGGGAGCTTCTGTCAGTGG	55.0
98	RM22903	8	GGATCTTCTGGATTGTCTAACG	AGGAGCTCATATCTCTTCAACC	54.0
99	RM256	8	GACAGGGAGTGATTGAAGGC	GACAGGGAGTGATTGAAGGC	57.0
100	RM339	8	GTAATCGATGCTGTGGGAAG	GAGTCATGTGATAGCCGATATG	57.0
101	RM6070	8	TTGCTAGTGCTTACCACCCC	TCCCAGTCACCCTGCTACTC	57.7
102	RM407	8	GATTGAGGAGACGAGCCATC	CTTTTCAGATCTGCGCTCC	57.0
103	RM3309	8	ACCATTCACTGCTCCTCCTC	GGGTATAAACGGCAACCACC	56.0
104	RM230	8	GCCAGACCGTGGATGTTC	CACCGCAGTCACTTTCAAG	57.0
105	RM223	8	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG	56.0

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...cond. Appendix I. Markers list

106	RM308	8	GGCTGCACACGCACACTATA	TTACGCATATGGTGAGTAGGC	57.0
107	RM23087	8	GATATTAGCTAGACATGGCACTCTGC	GTACATCCGCATGAATAGAGTGG	53.4
108	RM5545	8	CAGCACTCCTCCCTACCAG	GGCTAAGTCAGCGTGAGACC	57.0
109	RM23096	8	AAATAGACTACTGGGTGCGTTCG	GTGCAATCATGTTCACATCAGC	59.0
110	RM22905	8	CACTGCTCACTGCTGCCTTGC	CACGGGAGCTTCTGTCAGTGG	55.0
111	RM22903	8	GGATCTTCTGGATTGTGTCTAACG	AGGAGCTCATATCTCTTCAACC	54.0
112	RM23996	9	TACTTCTAACGCCGGCCAGTGC	ATCGTTCCTAGCGCGCAACACC	55.0
113	RM23659	9	ATTCGTCTCGCGGTGTACTGACG	TCACGCGGATCTAAACACAACC	58.0
114	RM242	9	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG	56.0
115	RM107	9	AGATCGAAGCATCGCGCCCGAG	ACTGCGTCCTCTGGGTTCCGG	56.0
116	RM23998	9	CTGCACGTACGGTCAAGTCTACC	GCATTGCAAGGGTTGAAGTGG	56.5
117	RM216	10	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA	57.0
118	RM25066	10	GTTGTTAGGTGTGAGCCGTGTAGG	GTACACCAATAACTGTGGAAGAGC	58.0
119	RM484	10	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTC	56.0
120	RM6440	11	CTGAGAGAAATGCCGATAGTG	TCTCCATCTCCATTCATCC	59.0
121	RM332	11	GCGAAGGGCGAAGGTGAAG	CATGAGTGATCTCACTCACCC	58.0
122	RM27184	11	ATGTGACCTCGTCGATCTTGTTCC	CCGAGTACAGCAGCACACAGC	59.0
123	RM260	12	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG	57.0
124	RM28277	12	TGCACCACCTATTTCAATCCACTCC	CCTTCCTCAAGGGAAATCACAGAAGC	56.0
125	RM277	12	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG	55.0
126	RM247	12	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG	57.0
127	RM7119	12	AGGCTGAGGCTTATAGGCAG	GGATGATACAACTTGACCCC	57.0
128	RM20	12	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG	55.0

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Appendix II. Quantity and quality of DNA of BC_1F_1

Progeny No.	Quantity($\mu g/ml$)	Quality(A_{260}/A_{280})
BC_1F_1s		
1	345.81	2.20
2	224.30	2.16
3	729.68	2.13
4	663.59	2.05
5	346.80	2.04
6	442.43	2.11
7	350.26	2.01
8	789.00	2.13
9	448.40	2.11
10	648.58	2.08
11	960.34	2.11
12	758.00	2.17
13	823.57	2.07
14	636.83	2.09
15	865.00	2.05
16	813.81	2.03
17	841.86	2.05
18	987.37	2.08
19	681.35	2.07
20	480.36	1.99
21	957.03	2.02
22	574.57	2.04
23	340.09	2.05
24	290.56	2.03

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Appendix III. Quality and quality of DNA of parents

Genotype	Quantity(μg/ml)	Quality (A_{260}/A_{280})
Jyothi		
1	998.34	2.01
2	654.83	2.08
3	207.70	1.97
4	150.80	2.02
Improved Samba Mahsuri		
1	349.25	2.17
2	232.00	1.91
3	231.87	2.01

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Appendix IV. Morphological characterisation of BC₁F₁

Plant number	Plant height (cm)	Days to Flowering	Leaf width (cm)	Leaf blade length (cm)	Productive tillers / plant	Panicle length (cm)	Spikelets / panicle
BC₁F₁ generation (Progeny of BC₂F₄ Plant No. 9)							
1	56.06	100.00	0.80	18.90	9.00	9.96	30.20
2	66.20	96.00	1.20	45.70	11.00	12.00	36.6
3	58.80	97.00	0.70	28.05	9.00	12.60	27.66
4	77.50	94.00	0.90	47.90	7.00	12.70	23.66
5	64.00	96.00	1.20	35.30	9.00	9.80	26.33
6	75.25	97.00	0.70	35.20	9.00	14.25	34.00
7	63.55	97.00	1.00	29.30	9.00	13.85	43.75
8	70.30	94.00	0.80	25.40	8.00	14.00	40.40
9	61.25	95.00	0.70	33.50	8.00	15.45	36.50
10	74.60	90.00	1.20	30.80	13.00	14.60	39.00
11	70.50	89.00	0.90	36.40	13.00	15.20	33.75
12	63.00	88.00	0.80	27.30	9.00	12.70	31.60
13	62.70	85.00	0.70	33.50	9.00	14.70	30.00
14	75.10	94.00	0.90	33.40	11.00	13.90	34.25
15	62.20	112.00	0.70	27.50	11.00	13.80	32.66
16	65.87	87.00	0.80	28.90	10.00	13.27	33.75
17	69.89	86.00	1.20	39.90	18.00	13.69	40.50
18	58.40	93.00	0.70	35.40	8.00	10.5	21.33
19	72.99	98.00	1.00	38.40	13.00	10.89	28.50
20	71.00	89.00	1.40	29.00	7.00	12.40	32.60
21	67.90	93.00	0.80	35.90	13.00	12.10	32.10
BC₁F₁ generation (Progeny of BC₂F₄ Plant No. 21)							
22	72.43	97.00	0.40	29.10	9.00	13.33	39.60
23	66.00	93.00	0.70	31.20	15.00	12.80	38.30
24	61.75	98.00	0.90	34.20	12.00	11.55	28.50
Jyothi	69.00	88.00	1.15	33.14	8.38	20.20	102.38
ISM	85.06	120.00	0.95	36.60	8.63	22.23	87.00

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Appendix V. Morphological characterisation of BC₂F₅

Plant number	Plant height (cm)	Days to Flowering	Leaf width (cm)	Leaf blade length (cm)	Productive tillers / plant	Panicle length (cm)	Spike lets / panicle
			Progenies of BC₂F₄ Plant No. 9				
1	69.62	97.00	1.20	42.20	6.00	12.37	23.50
2	65.03	97.00	1.10	40.10	4.00	14.03	36.25
3	83.65	98.00	1.10	48.00	6.00	15.55	39.25
4	68.87	110.00	0.90	28.00	10.00	15.07	24.25
5	81.47	95.00	1.20	49.50	10.00	13.17	33.75
6	46.82	95.00	0.90	25.40	10.00	13.92	33.00
7	65.54	95.00	0.90	48.60	0.00	0	0
8	79.58	96.00	0.80	36.90	10.00	12.58	35.25
9	81.90	88.00	0.80	37.40	7.00	15.10	34.50
10	46.10	89.00	0.60	23.50	7.00	11.50	26.30
11	67.20	93.00	0.70	34.50	6.00	13.80	33.50
12	69.60	93.00	0.90	36.80	6.00	11.70	30.00
13	42.42	93.00	0.70	18.20	2.00	11.82	30.00
14	75.47	93.00	1.10	31.30	6.00	15.07	37.75
15	83.30	106.00	1.30	48.30	9.00	13.90	35.25
16	70.70	98.00	1.10	46.10	8.00	12.30	31.50
17	80.00	98.00	0.90	48.30	7.00	14.10	35.00
18	73.63	103.00	0.80	33.20	5.00	13.03	38.50
19	75.11	102.00	0.80	34.90	6.00	14.41	30.50
20	57.80	102.00	0.70	28.90	8.00	11.20	30.25
21	28.50	102.00	0.70	11.80	5.00	7.80	20.00
22	61.10	100.00	0.80	28.40	7.00	12.80	31.00
23	70.40	99.00	0.90	29.40	8.00	11.30	31.25
24	75.50	98.00	1.10	34.90	10.00	14.10	42.50
25	49.48	98.00	0.70	18.50	6.00	12.08	31.50
26	56.36	94.00	0.80	26.80	6.00	14.16	37.60
27	51.99	94.00	0.70	28.40	7.00	11.86	35.30
28	56.45	94.00	0.70	27.70	7.00	12.25	25.25
29	60.31	97.00	0.60	33.10	6.00	14.10	40.00
30	61.25	95.00	0.70	33.50	8.00	15.45	36.50
31	59.50	114.00	0.80	21.40	9.00	13.20	33.50
32	76.90	112.00	0.90	33.20	10.00	15.90	35.25
33	50.40	96.00	0.70	30.10	7.00	12.40	25.50
34	80.25	92.00	1.00	30.90	13.00	14.05	37.00
35	74.60	90.00	1.20	30.80	13.00	14.60	39.00
36	70.50	89.00	0.90	36.40	13.00	15.20	33.75
37	66.75	89.00	0.90	32.80	8.00	12.45	32.75

38	63.00	88.00	0.80	27.30	9.00	12.70	31.60
39	78.23	93.00	0.80	40.80	7.00	15.23	45.00
40	62.70	85.00	0.70	33.50	9.00	14.70	30.00
41	75.10	94.00	0.90	33.40	11.00	13.90	34.25
42	63.27	85.00	0.80	30.10	15.00	13.07	38.20
43	62.20	112.00	0.70	27.50	11.00	13.80	32.66
44	64.20	95.00	0.80	26.40	9.00	11.70	30.33
45	68.00	98.00	0.80	28.20	9.00	13.00	35.00
46	67.45	98.00	0.70	23.80	8.00	13.75	46.00
47	70.80	112.00	0.90	33.10	9.00	13.00	65.50
48	65.87	87.00	0.80	28.90	10.00	13.27	33.75
49	61.75	98.00	0.90	34.20	12.00	11.55	28.50
50	69.89	86.00	1.20	39.90	18.00	13.69	40.50
51	72.99	98.00	1.00	38.40	13.00	10.89	28.50
52	64.00	96.00	1.20	35.30	9.00	9.80	26.33
53	75.25	97.00	0.70	35.20	9.00	14.25	34.00
54	49.10	94.00	0.80	33.80	5.00	13.40	31.25
55	71.57	97.00	0.90	25.40	8.00	13.57	38.25
56	72.43	97.00	0.60	29.10	9.00	13.33	39.60
57	63.55	97.00	1.00	29.30	9.00	13.85	43.75
58	79.92	88.00	0.90	36.40	10.00	13.72	45.50
59	57.35	84.00	0.70	35.80	9.00	11.95	37.50
60	66.00	100.00	0.80	29.00	7.00	12.90	37.60
61	70.30	94.00	0.80	25.40	8.00	14.00	40.40
62	90.70	100.00	0.90	28.00	16.00	32.40	34.30
63	74.87	97.00	0.80	37.50	11.00	13.45	31.75
64	81.20	97.00	0.80	40.20	18.00	13.90	32.75
65	74.82	98.00	0.70	39.70	14.00	14.42	35.50
66	65.97	96.00	0.80	35.40	9.00	13.77	34.25
67	70.20	96.00	0.80	44.00	10.00	13.80	36.00
68	71.00	89.00	1.40	29.00	7.00	12.40	32.60
69	84.57	102.00	0.80	47.40	7.00	14.17	38.25
70	67.90	93.00	0.80	35.90	13.00	12.10	32.10
71	66.00	93.00	0.70	31.20	15.00	12.80	38.30
72	69.53	96.00	0.60	29.90	19.00	13.23	38.30
73	68.06	94.00	0.60	28.00	9.00	12.86	37.00
74	57.50	95.00	0.70	26.50	4.00	13.40	34.66
74	43.20	98.00	0.70	18.20	5.00	13.00	29.30
76	45.10	88.00	0.70	18.00	7.00	12.10	27.70
77	58.83	82.00	0.70	16.50	9.00	13.63	30.66
78	46.50	110.00	0.70	17.80	6.00	12.30	26.33
79	54.80	106.00	0.80	22.40	6.00	13.40	33.60
80	80.32	115.00	1.10	46.50	9.00	11.82	35.00

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81	69.10	94.00	1.10	47.20	9.00	11.60	28.33
82	58.40	93.00	0.70	35.40	8.00	10.50	21.33
83	79.00	86.00	1.30	48.20	10.00	13.70	41.00
84	49.25	84.00	0.70	20.10	9.00	11.05	27.09
85	50.70	98.00	0.70	36.40	7.00	10.50	27.00
86	54.50	100.00	0.70	24.00	6.00	10.40	23.66
Progenies of BC$_2$F$_4$ Plant No. 21							
1	56.06	100.00	0.80	18.90	9.00	9.96	30.20
2	58.80	97.00	0.70	28.50	9.00	12.60	27.66
3	70.50	92.00	0.70	40.10	8.00	12.30	35.25
4	64.85	95.00	0.90	35.60	10.00	11.45	30.75
5	57.95	95.00	0.90	33.20	8.00	11.85	25.30
6	62.00	94.00	0.80	28.10	15.00	10.70	37.00
7	71.90	94.00	1.00	27.90	8.00	12.80	31.30
8	64.26	93.00	1.10	40.00	8.00	12.06	32.67
9	74.20	93.00	1.10	39.60	9.00	11.30	44.00
10	66.20	96.00	1.20	45.70	11.00	12.00	36.6
11	69.59	95.00	1.10	45.00	12.00	10.99	29.00
12	56.10	98.00	0.90	23.50	12.00	15.50	39.00
13	50.46	110.00	0.80	18.90	4.00	11.56	29.66
14	59.20	113.00	0.70	25.00	6.00	11.70	28.50
15	60.80	109.00	0.70	26.40	4.00	13.50	35.33
16	60.17	106.00	0.90	25.80	7.00	13.37	18.33
17	77.50	94.00	0.90	47.90	7.00	12.70	23.66
18	36.00	117.00	0.60	15.40	10.00	12.90	34.00
19	69.60	95.00	0.90	28.10	5.00	13.30	36.60
20	66.76	97.00	0.80	28.00	10.00	14.26	43.60
21	63.10	93.00	0.70	35.20	7.00	15.10	29.00
22	43.85	93.00	0.70	18.90	8.00	13.65	39.50
23	66.95	110.00	0.80	31.20	5.00	13.65	39.50
24	69.60	96.00	0.90	35.70	7.00	13.65	39.50
25	47.23	96.00	0.80	19.50	5.00	10.53	22.60
Parental genotypes							
Jyothi	55.34	90.00	0.93	23.20	4.33	9.27	21.83
ISM	66.04	114.67	0.92	25.30	8.17	17.89	68.83

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MARKER ASSISTED BACKCROSS BREEDING IN TWO-R GENE PYRAMIDED LINES OF RICE VARIETY JYOTHI FOR BACTERIAL BLIGHT RESISTANCE

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Rice variety Ptb 39, also known as Jyothi, is extremely popular amongst farmers and consumers of Kerala. Despite its popularity, the variety is highly susceptible to bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The climatic conditions prevailing in the state enhances the occurrence and spread of the bacterial blight disease. In both rice growing seasons (*Kharif* and *rabi*), spraying of antibiotics is not much effective, as it is often washed-off during the monsoon showers. Moreover, both the researchers and the consumers are concerned about the food safety aspects as well as the negative impact of plant protection chemicals on the environment. Hence, the best alternative is to exploit host-plant resistance by pyramiding multiple R-genes to impart durable and broad-spectrum stable resistance to varieties against the pathogen.

Considering the impact of the BB disease on food security and sustainability, efforts to introgress the three R-genes (*xa5, xa13* and *Xa21*) into the elite cultivar Jyothi (Ptb 39) through Marker Assisted Selection (MAS) were initiated at College of Horticulture, Vellanikkara. Improved Samba Mahsuri (ISM) was used as the donor parent. The present study aimed to identify R-gene pyramided plants in BC_1F_1 population and to develop stable pyramided BC_2F_1 lines of variety Jyothi with low linkage drag from the donor parent (ISM). It also aimed to evaluate the BC_2F_5 population for resistance to BB pathogen (pathotyping) and production of next-generation backcross populations (BC_2F_1s, BC_1F_2s and BC_2F_6).

The good quality DNA, isolated from BC_1F_1s (24 Nos.) and parents (ISM and Jyothi) was subjected to foreground selection to identify the plants introgressed with the three R-genes, using markers RG556 and xa5 SR, RG136 and xa13 promoter, and pTA248 linked to *xa5, xa13* and *Xa21,* respectively. Monomorphic banding pattern was observed in all the BC_1F_1s as well as the parents on analysis with markers RG556 and xa5 SR, pointing to the endogenous presence of R-gene *xa5* in all the individuals tested. Screening of BC_1F_1 individuals with STS marker RG 136 linked to R-gene *xa13* and functional marker xa13 promoter revealed that

the three BC₁F₁s *i.e.,* Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17 were heterozygous at *xa13* locus while, all other BC₁F₁ individuals possessed alleles similar to that of the recurrent parent (RP) Jyothi. The result thus pointed out that BC₁F₁ Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17 were 2-R-gene pyramids (*xa5xa5 + Xa13xa13*). Foreground selection with STS marker pTA 248 to detect the presence of *Xa21* gene revealed that the above mentioned three BC₁F₁s were heterozygous at *Xa21* locus. Results of foreground selection, thus indicated that three BC₁F₁ plants, *i.e.,* Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17 were the only 3-R-gene pyramids (*xa5xa5 + Xa13xa13 + Xa21xa21*) among the 24 BC₁F₁ individuals investigated. The other BC₁F₁s possessed only a single recessive R-gene *xa5*.

Background selection of the three BC₁F₁ 3-R gene pyramids was done using 58 microsatellite markers. The profiles revealed that BC₁F₁ Plant No. 9.7, Plant No. 9.15 and Plant No. 9.17 were similar to recurrent parent Jyothi and donor parent ISM at 24 and 21 marker loci, 22 and 21 marker loci, and 34 and 12 marker loci, respectively and heterozygous at 13, 15 and 12 marker loci respectively. Dendrogram based on molecular data, further suggested that the 3-R-gene introgressed BC₁F₁s were more similar to the recurrent parent Jyothi than to the donor parent ISM. The recovery of the recurrent patent genome was found to be the highest in Plant No. 9.17 (93.00 %) while, it was 89.00 per cent each in Plant No. 9.7 and Plant No. 9.15.

Morphological characterisation revealed that the 3-R-gene introgressed BC₁F₁s Plant No. 9.7 and Plant No. 9.15 and Plant No. 9.17 exhibited about 60 per cent similarity to the parents Jyothi and ISM. BC₁F₁ Plant No.9.17 was near similar to the recurrent parent Jyothi with respect to the days to flowering, plant height, seed length and width as well as kernel colour. However, the length of panicles and the number of spikelets per panicle in the selected BC₁F₁s were found to be less than that of the parents.

The identified 3-R-gene pyramids (BC₁F₁ Plant No. 9.7; 9.15 and 9.17) were backcrossed to recurrent parent Jyothi as well as selfed resulting in production of BC₂F₁s (5 Nos.) and BC₁F₂s (220 Nos.).

Wide variability was observed among the BC_2F_5 individuals (111 Nos.) for various morphological traits. Pathotyping of BC_2F_5 population through leaf clipping method, as suggested by IRRI (2002), revealed that 11.71 per cent (13 Nos.) of the progenies exhibited resistance reaction to BB infection, while, 10 (9.00 %) were moderately resistant, 24 (21.62 %) moderately susceptible, 21 (18.91%) susceptible and 43 (38.73 %) highly susceptible. Selfing of the BC_2F_5 individuals exhibiting resistance and moderate resistance to BB pathogen resulted in production of 1425 BC_2F_6 seeds.