GENE PYRAMIDING FOR BACTERIAL BLIGHT RESISTANCE IN RICE VARIETY UMA (Mo 16)

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(2014-11-116)

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DECLARATION

I, hereby declare that this thesis entitled **'Gene pyramiding for bacterial blight resistance in rice variety Uma (Mo 16)' is** a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled '**Gene pyramiding for bacterial blight resistance in rice variety Uma (Mo 16)'** is a record of research work done independently by **Ms. Tintumol Joseph** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Dedicated to my parents

and brother

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LIST OF ABBREVIATIONS

%	Per cent
BB	Bacterial Blight
bp	Base pairs
cm	Centimeter
cM	Centimorgan
CTAB	CetylTrimethyl Ammonium Bromide
COH	College of Horticulture
DBT	Department of Bio Technoilogy
DNA	DeoxyRibo Nucleic acid
EDV	Essentially Derived Variety
g	Gram
GGT	Graphical; Geno Types
IRRI	International Rice Research Institute
ISM	Improved Samba mahsuri
MAB	Marker Assisted Backcrossing
MAS	Marker Assisted Selection
MSL	Mean Sea Level
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
TBE	Tris Boric acid EDTA

Introduction

I. INTRODUCTION

Rice grains form an important source of carbohydrate and staple food for a large section of the world's population. India is the second largest producer and consumer of rice in the world. During 2014-15, rice production in India reached 102.50 million tonnes from an area of 39.35 million hectares (DES, 2015). Sustaining food security is however, a daunting challenge faced by the agricultural community of the country. According to Khush (2005), India needs to produce about 135-140 million tonnes of rice by 2030 to meet its future requirements and remain self-sufficient. The target thus fixed is to be met in the backdrop of limited land, water, labor, fewer chemicals, a continuing battle against new emerging pathogens and pests as well as possible detrimental effects from climate change. Nevertheless, to feed the increasing population it is imperative that the rice productivity in different rice-growing ecosystems in the country be improved.

The rice production and demand scenario in Kerala is no different. The area and production of rice in Kerala has decreased considerably over the years reaching 0.15 million hectares and 0.58 million tonnes respectively during 2014-15 (DES, 2015). In Kerala too, ensuring food security demands an increase in production and productivity of rice from the available limited area overcoming several yield limiting factors.

Apart from abiotic stress imposed by high soil acidity and iron toxicity, productivity of rice in Kerala is under constant threat from insect pests and pathogens. The high humid conditions prevailing in the state for most part of the year favors the prevalence of both insects and pathogens throughout the rice cropping period. As in other rice growing belts of the country, bacterial blight (BB) caused by

Xanthomonas oryzae pv. *oryzae (Xoo)* is one of the most devastating diseases affecting the rice crop in the state.

Measures to check occurrence of BB include disease forecasting, intercultural practices, chemical and biological control, to name a few. But the most economical and environment friendly approach to disease control is host plant resistance, typically conferred by major genes. Currently 39 resistance (R) genes conferring host resistance against various strains of *Xoo* have been identified (Zhang *et al*., 2014). These genes have been designated in series from *Xa1* to *Xa39* and include 27 dominant and 12 recessive R genes respectively.

Pyramiding of resistance genes into rice genotypes is advocated as an efficient strategy to ensure durable resistance against BB pathogen. Through conventional breeding approaches, selection of plants with multiple resistance genes based solely on phenotype is tedious mainly owing to epistasis and /or the masking effect of the genes interacting (Bharani *et al.*, 2010). Marker assisted backcross (MAB) breeding approach has been successfully relied upon for introgression of bacterial blight resistance genes into rice crop. This strategy ensures precise identification of genotypes possessing the genes of interest. It also makes sure that the resultant genotype resembles the recurrent parent in all aspects except for the resistance.

Rice varieties Ptb 39 (Jyothi) and Mo 16 (Uma) are the two major genotypes cultivated widely across the state of Kerala. Uma is a high yielding variety with a yield potential of 6 – 6.5 t/ha. Although Uma exhibits resistance to brown plant hopper, it is highly susceptible to bacterial blight disease leading to drastic reduction in yield. The situation thus warranted improving resistance of this elite cultivar against BB pathogen.

In line with the above, efforts were taken to pyramid three resistance genes (*xa5, xa13* and *Xa21*) into variety Uma from Improved Samba Mahsuri through marker assisted selection. The attempt resulted in production of BC_1F_1 generation pyramided with the resistance genes. Further evaluation of BC_1F_1s and their backcrossing was required to develop a stable pyramided line of Uma exhibiting resistance to bacterial blight. Hence, the present study was formulated with the following objectives:

I. To identify BC_1F_1 lines pyramided with bacterial leaf blight resistant genes (*xa5, xa13* and *Xa21*) using molecular markers.

II. To produce BC_1F_2 and BC_2F_1 generations of the genotypes thus identified.

Review of literature

II. REVIEW OF LITERATURE

The significance of rice in moulding the culture and life in Kerala is nondebatable. As in other rice growing regions worldwide, in Kerala too, various climatic, edaphic, biological, physical, physiological and socio-economic factors influence the area, production and productivity of rice crop. Bacterial blight (BB), an important biotic factor plays a significant role in determining rice productivity in the state. Other than just providing a control measure at the time of incidence, ensuring durable resistance to the disease is both essential and economical to sustain grain yield. The concept of host plant resistance for disease management is being emphasized off late.

Pyramiding of genes into an elite cultivar is an often resorted approach to overcome the phenomenon of counter resistance of a pathogen against a resistant gene. The elite rice varieties in Kerala, Mo 16 (Uma) and Ptb 39 (Jyothy) are highly susceptible to bacterial blight. Drastic reduction in their yield is a common occurrence year after year owing to the susceptibility of these cultivars to the BB pathogen. Effective control of the disease through cultural, mechanical and chemical means is seldom achieved owing to the heavy monsoon showers received during the rice growing seasons in the state. To initialize a resistance breeding programme a thorough understanding of the nature of pathogen, the symptoms generated and control measures adopted is necessary. The brief review of literature available in rice on the above factors is detailed under the following heads:

2.1. Etiology of bacterial blight disease in rice

2.1.1 Ecology of the pathogen

2.1.2 Epidemiology and disease cycle

2.1.3 Symptoms on rice plant

2.1.4 Disease management

2.1.4.1. Host plant resistance

2.1.4.2. Exploiting host plant resistance to combat BB pathogen

2.2. Marker assisted selection to confer resistance to BB pathogen

2.1. Etiology of bacterial blight disease in rice

Bacterial blight of rice, sometimes referred to as bacterial leaf blight, caused by the gram negative bacterium *Xanthomonas oryzae* pv.*oryzae (Xoo)* is a devastating disease with widespread occurrance in the rice growing regions of the world. The pathogen is a member of family Xanthomonadaceae. Almost all the members of the genus, *Xanthomonas* are found to cause diseases in an array of crops. *Xanthomonas oryzae* pv.*oryzae* is found to infect cultivated rice and its wild relatives (Sonti, 1998).

The pathogen was initially discovered by Takaishi in 1908 as a bacterial mass from the dew drops on rice leaves. In 1911, Bokura isolated a bacterium, studied its physiology and morphology and named it *Bacillus oryzae* Hori and Bokura. The pathogen was further studied and redesignated as *Pseudomonas oryzae* Iyeda and Ishiyama by Ishiyama. It was later renamed as *Xanthomonas oryzae.* The pathogen was elevated to the status of species in 1990 and was named *Xanthomonas oryzae* pv. *oryzae* (Swings *et al.,* 1990). The first report of occurrence of the disease in India was from the southern states during 1959 (Parthasarathy *et al*., 2014). Thereafter in 1962, Bihar and other regions of North India were infected with the disease in the form of epidemics. Now the disease is prevalent in all rice growing tracts of the country.

Xanthomonas oryzae pv. *oryzae* is a rod shaped, yellow, slime producing, gram negative bacteria. *X. oryzae* is an obligate aerobic bacterium that does not form spores. The length of individual cells varies from approximately 0.7 - 2.0 µm and width 0.4 -0.7 µm and is covered by a capsule of galactose, glucose, xylose, and

uronic acid. The cells move with the help of single polar flagellum. *Xoo* cells produce copious amount of capsular extra cellular polysaccharides, resulting in the formation of strands or droplets of bacterial exudates from infected leaves. These polysaccharides also provide protection from desiccation and aid in dispersal through wind and rain-water (Swings *et al.*, 1990) of the pathogen. Being catalase positive, it cannot reduce nitrate and weakly produces acids from carbohydrates (Bradbury, 1984).

The *Xoo* pathogen upon infection on rice plant produces certain race specific effectors which triggers the host resistance and also causes infection. The effectors, that target the host cell nucleus, bind to the host resistance or susceptibility genes and causes infection or induce resistance respectively (Horgan and Henderson, 2015).

2.1.1. Ecology of the pathogen

The host range of *Xanthomonas oryzae* pv. *oryzae* includes rice plant and some other graminaceous members. *Leersia oryzoides* var. *japonica*, *L. oryzoides* var. *zizanialatifolia*, and *Pharlaris arundinacea* constitute the species that could be severely infected, whereas, *L. japonica*, *Phragmites communis* and *lsachne globosa* could be slightly infected (Goto *et al.*, 1953). Based on the ecological studies of the pathogen, Mizukami (1961) reported that the habitats of the pathogen include soil of the infected region, seeds and straw. Roots of plants present in the infected region were also found to harbor the pathogen irrespective of whether it is a host plant. Rice straw and stubbles were also found to be the habitats of the pathogen. They survive in these habitats in an inactive form and when the favourable conditions are available, get activated and infest the rice plants on coming in contact with the rice roots.

Rice plants get infected with *Xoo* pathogen from the inoculum harboured in the seeds, tillers or roots that are left behind at harvest, as well as from alternative weed hosts (Tagami *et al.,* 1964). Two hundred and eighty pathogenic bacterial isolates associated with rice seeds collected from the tropical and subtropical region were screened by Xie *et al*., 1999. And the frequency of pathogenic bacteria was about 6 percent in the subtropics and 9 percent in the tropics.

2.1.2. Epidemiology and disease cycle

The entry of pathogen may be through the wounds and other openings like the hydathodes concentrated along the edges of leaf (Ou, 1985). It multiplies within the xylem and travel further to spread the disease. They are present on the leaf in the form of ooze droplets that get collected on the leaf surface. Wind, rain and irrigation water help in dispersal of the pathogen through splashes of the ooze to the uninfected parts or plants. Clipping off the seedling tips during transplanting also favour disease development. In South East Asia and India, the outbreak of bacterial leaf blight is more likely to occur during the monsoon seasons (June to September) than at other times of the year (Liu *et al.,* 2004). The amount (2300 mm) and distribution pattern of rainfall (12-15 days per month) in July-September primarily determine the epidemic build up under Indian conditions. High incidence of rainfall and the high relative humidity (90% and more for 12-14 hours per day) favour rapid disease build up. During wet season a temperature range of 23^0C - 31^0C is quite conductive for disease development. Frequency of kresek (seedling blight) development increases when the temperature ranges between 28^0 C and 35^0 C.

The leaf bacterial population is found to be maximum during the months May to July and is the least during August. According to Tagami *et al.* (1964), the population again increases from maximum tillering to panicle initiation stages. This

fluctuation may be due to the climatic factors like temperature, humidity etc. The bacterial population is found to be abundant on the lower leaf surface initially and later invades the upper surface. Also the population decreases with decrease in metabolic activity of the leaves.

The cultural operations such as nursery preparation, fertilizer application, and selection of rice varieties all contribute to the intensity of disease development (Mizukami and Wakimoto, 1969). Rice seedlings raised in deeply irrigated or flooded nurseries are more likely to be contaminated with the bacterium and therefore, the extend of damage will be higher in rice crop raised from such seedlings. The severity of damage would be lower in paddy fields when seedlings are obtained from semi-irrigated or upland nurseries.

Increased nitrogen fertilizer application favors disease development. It helps in multiplication of the pathogen and lesion enlargement or increases vegetative growth of the plant creating favourable micro-climate for the pathogen. The studies by Reddy *et al.* (1979) concluded that, increased levels of N were associated with increased BB and hence reduced yield. Nitrogen response was in turn negated due to high disease severity. Only when the BB vulnerable and high yielding varieties are protected with bactericide or in the disease free seasons, their genetic potential can be realized through high N application.

The type of soil also is a favourable factor for disease development as it is severe in, clay or clay loam alluvium, sandy loam soils, and negligible in the sandy soil of dune areas. The acidic soils with poor drainage facility were found to be conductive for the disease (Tagami *et al.*, 1966).

The pathogen lives on seeds and dead plants and may move from plant to plant through wind or irrigation. Upon infection of the host plant, the bacterium infiltrates the plant through natural openings or leaf and root wounds.

In temperate regions, *Xoo* pathogen was found to survive through winter in the rhizosphere of weed plants *Leersia* and *Zizania species.* In addition, the pathogen was also reported to survive in the root and base of the tillers of rice stubble (Mizukami and Wakimoto, 1969). *Xoo* is also found to survive in the soil for 1 to 3 months depending on the soil acidity and moisture. However , this is not reported to be an important source of inoculum. *Xoo* is also reported to overwinter in piled straw as well. This source of inoculum may acquire importance in areas where little or no weedy hosts occur (Ou, 1985).

2.1.3. Symptoms of the disease on rice plant.

Symptoms of the disease develop mainly on leaf sheaths, leaf blades, and sometimes on grains. They are characterized as seedling blight (Kresek) and leaf blight according to the stage of infection. Kresek is a seedling blight which occurs after the transplanting of the crop from nurseries to the field (Nino-Liu *et al*., 2006). Tiny water-soaked spots are found to develop along the edges of the older leaves. The spots enlarge and gradually turn yellow. In case of early infection, symptoms appear at third or fourth week after transplanting and eventually spreads upwards as the plant grows. The lesions usually initiate on upper part of the leaf edges where water pores, are more frequently distributed and through which the bacteria can easily invade (Mew, 1987). Within two or three days, the veins show enlarged yellow coloured lesions which turn white or greyish white later on.

The seedlings with kresek symptom show stunted growth and dies within one to six weeks after transplanting (Mew, 1987). Following the infection by the pathogen, the leaves are also found to roll and wither. The disease severity and symptom are highly depended on the variety or physiological condition of the rice plant, climatic conditions and virulence of the pathogen (Tagami *et al*., 1964).Usually the upper half of the leaf or the whole leaf dries rapidly turning pale white before withering.

The symptoms of leaf blight initiate from the leaf blades, develop downwards to the basal part and later, extend through the midribs to sheath. Leaf sheath of severely affected plants becomes discoloured and decays. Discoloured water-soaked leisons usually appear on the glumes of young spikelets which may become conspicuous on young grains, but during the ripening stage, the leisons turn grey to yellowish white in the middle with an indistinct margin (Yoshimura, 1960).

Gnanamanickam *et al.* (1999) observed that though the disease is found to occur at all stages of the crop, it is mostly observed during the maximum tillering to maturity stages. Severe damage is observed when Kresek precedes leaf blight while the grain yield is less affected in case of post flowering infections. Grain development gets severely impaired and increased sterility is also observed when the infection is during panicle initiation or in the pre-flowering stages.

2.1.4. Disease management

The severity of losses due to the disease has necessitated adoption of proper management practices. Several practices like disease forecasting, biological and chemical control and host gene resistance are in practice. Generally, most of these methods are used alone or in combination with each other for combating the pathogen.

However, an economical and effective chemical control approach are yet to be developed for disease management, This may be due to the high variability among the BB pathogen population with respect to their sensitivity to the antibiotics used. The evolution and existence of drug-resistant strains also pose serious problems in delineating appropriate management strategy against the disease. (Gnanamanickam *et al*.,1999).

Thus although for the management of BB pathogen, disease forecasting, biological, chemical, cultural methods are resorted to, a complete check of the havoc

played by the pathogen on rice crop is not achieved. In addition, as both the major rice growing seasons (*virippu* and *mundakan*) in the state coincides with monsoons, control of the disease through spray application of chemicals or biological agents proves ineffective owing to washing off of the applied materials. Under such circumstances, relying on durable resistance preferably provided by triggering defense against the pathogen within the host is more advantageous.

Peng *et al.* (2015) reported that apart from food safety and environmental issues that the chemical pesticides and bio control agents (plant extracts and antagonistic organisms) can generate, the protection conferred by these agents are far from satisfactory and their effectiveness decreases over passage of time. Till date, the most effective and viable means to combat BB pathogen is to introgress disease resistant genes into rice plants.

2.1.4.1. Host plant resistance

The R gene family which confers resistance to the *Xanthomonas* is reported to be a multigene family with genes distributed throughout the rice genome at multiple loci. The two major R gene classes related to disease development in rice are the receptor like kinase (RLK) class and nucleotide-binding site leucine-rich repeat (NBS)-LRR. The R gene *Xa21* belonging to the RLK class was the first to be cloned to induce broad spectrum resistance against BB pathogens. Hence *Xa21* is the most abundantly used gene in resistance breeding programs in rice. These genes are named with '*Xa'* prefix followed by a specific number assigned upon discovery. 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). About 39 resistant genes (Table 1) have been identified till date (Khan *et al*., 2014; Zhang *et al*., 2014).

The evolution of genes of this multigene family has resulted in development of specific resistance against the multiple races of the newly emerged *Xoo* pathogen. Among the genes, some are dependent on dose, while, a few are dominant, and others recessive. Some are reported to be controlled developmentally (The degree of resistance offered vary according to the developmental stage) while others are expressed constitutively. The genetic functions of these genes are highly diverse and it is an indication of the evolution of R genes in rice for combating the development of new races of *Xoo*. Resistance offered against BB pathogen is not confined to a particular locus or a region or chromosome, but results from the dynamic interaction between the R genes and the host genome (Horgan and Henderson, 2015).

Table 1. List of resistant genes identified for BB resistance

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)

List of resistant genes identified for bacterial blight resistance

Gene identified	Resistance source	Origin	Reference
Xa11	Indica	Philippines	Kurata and Yamazaki (2006)
Xa12	Japonica	Japan	Ogawa (1987)
xa13	–	India	Ogawa *et al*. (1988); Kurata and Yamazaki (2006)
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)
xa20	–	–	Taura *et al*. (1992); Kurata and Yamazaki (2006)
Xa21	Wild spp. of *Oryza*	Mali	Song *et al*. (1995)
Xa22 (*t*)	–	China	Sun *et al*. (2004); Kurata and Yamazaki (2006)
Xa23	Wild spp. 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 spp. of *Oryza*	Philippines	Lee *et al*. (2003); Gu *et al*. (2004), (2005)
xa28(t)	Indica	Bangladesh	Lee *et al*. (2003)
Xa29(t)	Wild spp. 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 spp. of *Oryza*	–	Ruan *et al*. (2008); Zheng *et al*. (2009)

List of resistant genes identified for bacterial blight resistance

Gene identified	Resistance source	Origin	Reference
Xa33	Wild spp. 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 spp. 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)

Source: Khan *et al*. (2014)

Most of the genes conferring resistance to *Xoo* pathogen are dominant while a few are recessive. Some widely studied dominant genes are:

Xa1 gene

The first report of R gene *Xa1* conferring resistance to Japanese race I of *Xoo* was by Sakaguchi (1967). The extensive study of the gene, resulted in tagging the gene locus with RFLP marker XNpb235 and mapping it on to chromosome 4 (Yoshimura *et al*., 1996). Positional cloning of the gene in Japan as part of the rice genome project indicated that, *Xa1* gene was carried by a 340-kb YAC clone (Y5212). Later, it was found that *Xa-1* gene encoded a nucleotide-binding site leucine-rich repeat (NBS-LRR) type of protein Yoshimura *et al*. (1998).

Xa21 gene

Xa-21 is a broad spectrum bacterial blight resistance gene introgressed from a wild species *O. longistaminata* into the background of *O. sativa* (Khush *et al*., 1989). The gene was tagged with RAPD marker RAPD 248 by Ronald *et al*. (1992). The marker RG103 was tightly linked to *Xa21* at a distance of 1.2 cM. , The STS marker pTA248 was designed based on these markers. According to Ronald *et al*. (1992),

pTA248 can be used efficiently in marker-assisted selection. Map based cloning strategy was employed to clone the disease resistance gene *Xa21* in rice for the first time Ronald (1997). The plasmid pC822 was found to contain the gene. *Xa*21. The gene sequencing revealed that Xa21 coded for receptor kinase domain with serine–threonine specificity.

Some of the recessive genes conferring resistance to BB pathogen widely studied include:

***xa5* gene**

The R gene *xa5* was tagged to RFLP markers RG556 and RZ390 and rice microsatellites RM122 and RM390 and mapped on to chromosome 5 (Blair and McCouch, 1997). A PCR-based STS marker was designed using the RFLP marker RG556 for incorporation in marker-assisted breeding programmes (Huang *et al*., 1997). The STS marker on PCR amplification was found to exhibit a monomorphic banding pattern among the resistant and susceptible plants. Therefore, it was suggested that the PCR product are to be digested with a restriction enzyme DraI to generate Specific Amplicon Polymorphism (SAP). The *xa5* region was cloned on chromosome 5 (Sanchez *et al*., 2000). In 2006, Iyer and McCouch developed functional markers for the gene.

***xa13* gene**

The recessive gene *xa13* was reported to confer resistance to the Philippines race 6 of BB pathogen. . The gene was mapped on chromosome 8 (Zhang *et al*., 1996) and tagged with RFLP marker RG136 and RAPD marker OPAC05 900. The RFLP marker RG136 led to the development of a PCR-based STS marker. Similar to RG556 linked to *xa5*, RG136 also produced amonomorphic banding pattern among .

Hence it was suggested that PCR product was to be digested with restriction enzyme HinfI to generate specific amplicon polymorphism.

Rao *et al*. (2002) reported that the pathotype, which is classified into different clusters, show regional variation. Hence, according to them resistance breeding and gene deployment based on the regional variation would be the best strategy. The gene *Xa23*(*t*) was reported from the wild species, *O. rufipogan,* which was collected from the forests of Kerala. The host-pathogen interaction study of the gene *Xa23*(*t*) conducted at the Regional Agricultural Research Station, Pattambi, Kerala by University of Madras showed a high level of resistance to race 10 of *Xoo* pathogen to which gene *Xa21* was found to be highly susceptible. The R gene was also reported to exhibit high levels of resistance to more than 50 southern Indian strains of *Xoo* pathogen (Srinivasan and Gnanamanickam, 2005).

Iyer and McCouch (2004) found that the recessive R-gene *xa5* encoded a novel form of disease resistance. It was found to encode the gamma subunit of transcription factor IIA (TFIIAγ) of the eukaryotes. On sequencing the factor in susceptible and resistant isolines, two nucleotide substitutions were revealed, which resulted in an amino acid change. This relation was conserved across 27 resistant and nine susceptible rice lines in the Aus-Boro group studied by them.

The disease resistance offered by the dominant gene *Xa21* to *Xoo* pathogen is reported to be developmentally controlled in rice and is correlated with pathogenesis related gene expression as studied by Ponciano *et al*. (2006). The pathogenesis related defense genes (OsPR1a, OsPR1b, and OsPR1c) were analysed for their resistance induction at juvenile and adult stage. It was concluded that the leaves in adult stage competently express these genes and that the *Xa21* locus favours higher level of induction of disease resistance. It was also found that the juvenile stage lacks full

resistance due to the lack of activation of defense response. Studies by Peng *et al*. (2015) revealed that *Xa21* is a prime gene conferring resistance to BB pathogen and was responsible for activation of many of the signaling pathways associated with disease resistance.

As detailed earlier, the BB pathogen upon infection produces race specific effectors (transcription activator like effectors) that targets the host cell nucleus and bind to the genes, that activate resistance to the pathogen. Horgan and Henderson (2015) reported that binding of these factors called avirulence factors to the host gene results in the activation of a series of events which may finally lead host resistance. The hypersensitivity reactions may also become evident as a result of the activated signaling pathway, which may cause localized cell death inorder to check pathogen spread to the rest of the plant, or activate other changes leading to lowered pathogen infection.

2.1.4.2. Exploiting host plant resistance to combat BB pathogen

Development of cultivars with resistant genes have been the most effective, environmentally safe and economical strategy for control of BB disease (Huang *et al*., 1997; Jena and Mackill, 2008; Singh *et al*., 2001; Sundaram *et al*., 2008; Rajpurohit *et al*., 2011; Dokku *et al*., 2013; Suh *et al*., 2013).

Conventional breeding was the main method for breeding high yielding BB resistant rice cultivars for a long time. The resistant gene *Xa4* was exploited well for the development of many resistant varieties against *Xoo* by conventional back cross-breeding (Khush *et al*., 1989). Variety TKM6 was initially used as a bacterial blight resistance donor in India. As a result, several varieties like Govind, IR 36, Karjat, Radha, Ramakrishna *etc.* were developed. The varieties Ratnagiri and 68-1 were

developed using the genotype Sigadis. Similarly, BJI was used for developing the varieties PR 4141 and IET 8585 (Ajaya).

R gene *Xa4* was introgressed in many high yielding varieties via conventional breeding. Predominance of *Xoo* races that could overcome resistance conferred by *Xa4* gene has been reported owing to widespread cultivation of varieties carrying *Xa4* (Khush *et al.*, 1989). It is also reported that, cultivation of rice varieties with only a single resistance gene for long term might cause a significant shift in the pathogen-race frequency subsequent to breakdown of resistance (Mew *et al*., 1992).

Plant breeders have options to increase the durability of their resistant cultivars. One of the tangible options to ensure durable resistance is gene pyramiding. Theoretically, pyramiding several 'undefeated' *R* genes into a single cultivar ensures more durable resistance as the probability of simultaneous pathogen mutation to break the resistance is much lower than with a single gene (McDowell and Woffenden, 2003; Pink, 2002). The main strategy in a gene pyramiding scheme is to cumulate the desirable genes identified in multiple parents into a single genotype (Joshi and Nayak, 2010).

Although conventional breeding has had a significant impact on improving resistance of cultivars, the time-consumed in developing a cross and backcross, and the selection of the desired resistant progeny takes between eight and twelve years. However, the resistance of variety developed cannot be guaranteed owing to the evolution of new virulent pathogens (Ragimekula *et al*., 2013).

Novel technologies like DNA markers have enormous potential to improve the speed, and efficiency of conventional plant breeding *via* Marker-Assisted Selection (MAS). Genetic markers are identifiable DNA sequences, found at specific locations of the genome, and transmitted by the standard law of inheritance from one generation to the next. They are abundant, mostly codominant in nature, not stage,

organ or tissue specific; do not have pleiotropic effect and environment influence. They are inherited in mendelian fashion and are highly polymorphic. The success of MAS is influenced by the relationship between the markers and the genes of interest as identified by Dekkers, 2004. The main considerations for the use of DNA markers in MAS are reliability, quantity and quality of DNA required, technical procedure for marker assay, level of polymorphism and cost (Mohler and Singrun, 2004).

Microsatellite markers linked to a resistance gene *xa5* have been reported by Blair and McCouch (1997). Microsatellite markers were developed for most of the resistance genes. These markers would be extremely helpful in efficient and effective marker-assisted selection of resistance genes as reported by Davierwala *et al.* (2001). A functional marker is expected to enhance the reliability of MAS, as it helps in direct selection of genes involved in BB resistance (Salgotra *et al.*, 2012). They were successfully designed within coding sequences of different resistance genes, conferring rust resistance in flax (Hausner *et al*., 1999). Several functional markers were developed as a result of cloning some of the identified BB resistance genes (*Xa1, xa5, xa13, Xa21, Xa26* and *Xa27*) (Song *et al*., 1995; Yoshimura *et al*., 1998; Iyer and McCouch, 2004; Chu *et al*., 2007).

2.2. Marker assisted selection (MAS) to confer resistance to BB pathogen

MAS enhance the precision of plant breeding by reducing the reliance on laborious and fallible screening procedures. DNA markers can aid in detecting the presence of allelic variation in the genes underlying the economic traits. In absence of markers, identifying backcross plants carrying these genes would be cumbersome due to masking effects (Khan *et al.*, 2014). The introgression of several *R*-genes into a single cultivar from various sources during a crossing program can be effectively tracked by molecular markers and probe. Also the desired genes can be fixed in a homozygous state early through Marker Assisted Backcross Breeding (MABB).

During transfer of target allele from a donor variety to a popular cultivar by backcrossing, there are also chances that some undesirable segments from the donor are also present in the cultivar, consequently the new genotype developed would fail to perform like the popular cultivar, thus limiting its appeal to farmers. Integrating the use of molecular markers in a breeding programme can greatly reduce such linkage drags (Frisch *et al.*, 1999; Joshi and Nayak, 2010). In addition, MAS can also be effectively used for selection of plants with multiple resistance genes. Selection of a resistant genotype solely based on phenotype can be misleading because of epistasis and/or the masking effect of genes, wherein the action of a gene conferring resistance to many races of the pathogen may mask the action of another resistance gene (Tanksley *et al.*, 1989; Davierwala *et al.*, 2001; Rao *et al.*, 2002; Akhtar *et al*, 2010).

Marker assisted backcross breeding involves three levels of selection. Selection for target gene (foreground selection), background selection to accelerate the recurrent parent genotype reconstruction, and minimize linkage drag (recombinant selection). Recombinant selection helps in selecting the backcross progeny possessing the target gene with tightly-linked flanking markers. Foreground selection may be useful for traits with laborious or time consuming phenotypic screening procedures and also to select for reproductive stage traits in the seedling stage, allowing the best plants to be identified for backcrossing. The recessive alleles can also be selected, which is difficult to do using conventional methods. Background selection accelerates the recovery of recurrent parent while recombinant selection reduces the size of segment of donor chromosome containing the target locus (Akhtar *et al.*, 2010).

Gene pyramiding for BB resistance by MAS was for the first time employed by Abenes *et al*. (1993). Later, success of the strategy led to its wide spread adoption and more attention was focused on the identification of resistant genes. IRBB21 variety was developed by introgression of *Xa-21* gene using pTA248 marker from the

germplasm of *O. longistaminata* (Ronald *et al*., 1992). The RG556 marker aided the transfer of *xa5* to IRBB5 from DZ192 variety (Yoshimura *et al*. 1995). R gene *xa13* gene was incorporated from long grain variety, Nang Som into IRBB13 variety using RG136 marker (Zhang *et al*., 1996).

Gnanamanickam *et al*. (1999) observed a sub-population of *Xoo* virulent to rice line IRBB21 from a pathogen isolate from Kerala. According to Priyadarisini and Gnanamanickam (1999), rice line NH56 carrying four *R* genes, (*Xa4 + xa5 + xa13 + Xa21*) was found to be resistant to Kerala isolate of the *Xoo* pathogen.

The usefulness of MAS in gene pyramiding was demonstrated by the studies of Sanchez *et al.* (2000). Three bacterial blight resistance genes, *xa5*, *xa13*, and *Xa21*, were successfully transferred to three new plant type (NPT) rice lines - IR65598-112, IR65600-42 and IR65600-96 *via* a marker-aided backcrossing procedure. The markers RG556 and RG207 were used for *xa5*; RM136 for *xa13* and pTA 248 for *Xa21*. Marker polymorphism for *xa5* was detected after digestion of RG556 and RG207 with *MaeII* restriction enzyme while in case of *xa13,* restriction digestion of RG136 with *HinfI* enzyme was resorted to. This attempt highlighted the usefulness of MAS of desirable genotype, particularly when recessive genes such as *xa5* and *xa13* were involved. Identification of lines with recessive genes is difficult through conventional breeding in the presence of a dominant gene such as *Xa21*.

The resistant genes *xa5*, *xa13*, and *Xa21* were reported to provide sufficient wide spectrum of resistance to all predominant races of *Xoo* from Punjab and a few from Philippines when pyramided into a susceptible *indica* rice cultivar, PR106 (Singh *et al.*, 2001). Among the genes, *Xa21* was the most effective followed by *xa5* while *xa13* was the least effective. The advanced backcross lines were with high yield advantage.

Narayanan *et al*. (2002) succeeded in improving IR50, an elite *indica* rice line, by molecular breeding approach through marker-aided selection (MAS) and genetic transformation for resistance against blast (BL) and bacterial blight (BB). In their study a blast resistant near isogenic line C101A51 was used as the donor parent. On confirming blast resistance further resistance to bacterial blight was provided by transforming the blast resistant isolines with *Xa21* gene. Bioassay data showed that transgenic IR50 is resistant to blast and blight pathogens. The *Xa4* gene, which may show an increased level of resistance to the BB pathogen along with the transformed *Xa21* gene, was found endogenously present in IR50.

Backcross generations were developed using IR24 as recurrent parent to transfer the R- genes against BB pathogen from Nang Som (donor). The target genes selected were, two recessive genes, *xa-5* and *xa-13*, and a dominant one, *Xa-21*, conferring resistance to different BB races. The STS markers pTA248, RG556 and RG136 were used for *Xa21*, *xa5* and *xa13* genes respectively. Of the 160 plants resistant banding pattern as in the donor was observed in 11 plants on using the pTA248 marker. However, only the *Xa21* gene was found to be transferred from the donor (Nguyen and Nguyen, 2004).

Sundaram *et al.* (2008) attempted to confer durable BB resistance to Samba Mahsuri (BPT5204), a medium slender grained *indica* rice variety with high yield and excellent cooking quality. PCR based molecular markers were used in the backcross-breeding program to introgress three major BB resistance genes (*xa5*, *xa13* and *Xa21*) into Samba Mahsuri from a donor line (SS1113) in which all the three genes are present in a homozygous condition. The three STS markers used *viz*., pTA248, RG136 and RG556, were closely linked to the BB resistance genes, *Xa21*, *xa13* and *xa5*, respectively. Background selection was done using microsatellite markers which showed polymorphism between the donor and recurrent parents.

About 97 per cent recovery of the Samba Mahsuri genome in three gene pyramided lines were reported. The study concluded that the two gene and three gene pyramided lines exhibited higher level of disease resistance. Also, on BB infection significant yield advantage over the parent was exhibited by the three gene pyramided lines. .

Shanti *et al.* (2010) introgressed four BB resistant genes *Xa4, xa5, xa13* and *Xa21* into the hybrid rice parental lines KMR3, IR58025B, PRR78, Mahsuri and Pusa 6B. IRBB60 acted as donor of the four resistance genes. Foreground selection was done using the markers and background selection by conventional breeding. According to the study, the pyramids with four resistance genes showed very high level of resistance to 10 highly virulent isolates of the *Xoo* pathogen. Also, they resembled the recurrent parent phenotype in characters like the grain quality. This four gene combination was found to be the most effective gene combination to combat the ever-changing pathogen population. These pyramided lines can either be used directly or as donors of bacterial blight (BB) resistance breeding.

Bharani *et al*. (2010) introgressed three BB resistant genes (*Xa21, xa13* and *xa5*) from IRBB60 into high yielding, short duration but susceptible cultivar ADT43 and ADT47. IRBB60 provides resistance to six isolates of races of the pathogen. The parents and the improved resistant lines were tested against two *Xoo* isolates (*Xoo12 and Xoo17*) prevalent in Tamil Nadu. The resistance offered by the pyramided lines and their hybrid derivatives was much higher than those of the hosts with single resistance gene. In F_3 generation of ADT43/ IRBB60 a genotype introgressed with the three R genes (*Xa21, xa13* and *xa5*) in homozygous state were identified. It exhibited a high level of resistance against two prevalent isolates of Tamil Nadu. It was also reported that *Xa21* is strongly preferred compared to other resistance genes for the development of resistant genotypes against widely prevalent isolates in the state.

Use of functional markers for MAS reduces the risk of false selection (Salgotra *et al.,* 2012). The study was aimed at introgression of BB resistant genes and the important basmati quality traits combining phenotypic selection and MAS. The genes *xa5*, *xa13* and *Xa21* were successfully introduced into basmati breeding line IRS 5441-2 from the non-basmati donor of BB resistance, IRBB59. The BC_1F_3 recombinants derived in this study were found effective against the most virulent BB isolates. The higher level of resistance offered by introducing more than two resistance genes may be the result of gene interaction or quantitative complementation between resistant genes (Yoshimura *et al*., 1995; Huang *et al*., 1997; Sanchez *et al*., 2000, Sundaram *et al.*, 2008). The study successfully identified superior recombinants for three BB resistance genes (*Xa21, xa13* and *xa5*) along with aromatic gene in the homozygous condition. Development of advanced basmati breeding lines through MAS and phenotypic selection from the improved genotypes is expected to confer durable resistance to bacterial blight in Basmati genotypes..

RD6 is a high quality and popular fragrant glutinous rice cultivar among rice growers in North and Northeast Thailand. But it is found to be highly susceptible to BB pathogen. Several attempts made to provide varietal resistance failed due to high variability among the pathotypes in the region. Introgression of resistance genes into RD6 using IR62266 as the donor was attempted. About twelve lines were successfully enhanced with resistance to BB and benefitted the farming community (Pinta *et al.,* 2013).

The strategy of introduction of multiple R genes followed by simultaneous foreground and phenotypic selection helps to reduce the cost and the time required for the isolation of desirable recombinants with target resistance genes in rice (Suh *et al.*, 2013). Three resistance genes *Xa4* + *xa5* + *Xa21* were transferred from an *indica* donor (IRBB57), using marker-assisted backcross breeding strategy, into a BB susceptible elite *japonica* rice cultivar, Mangeumbyeo. Three elite advanced backcross breeding lines with three resistance genes were developed and confirmed

by foreground and phenotypic selection in a *japonica* genetic background without linkage drag. The background genome recovery was about 92.1 per cent. The pyramided lines showed extremely high resistance to the *Xoo* races compared to that provided by individual genes. Also the combination of two dominant (*Xa4* and *Xa21*) and one recessive gene did not create any negative effects on the yield and other agronomic traits.

Guvvala *et al.* (2013), evaluated nine stabilized, four-gene pyramided families of Mahsuri for agronomic characters, yield under natural and disease pressure conditions. Three different spacing were also evaluated to find out the optimum spacing under disease free and disease pressure conditions. The results revealed that the parent exhibited high susceptibility under heavy disease pressure whereas the pyramid families were highly resistant. Yield loss was found less under wider spacing when compared to dense planting under BB infestation in case of parent. No such yield loss was reported in the pyramid families. The pyramids insulated the yield loss against bacterial leaf blight and help farmers to overcome the heavy yield losses due to this disease. They have the potential to replace the parents and can be used directly or as donors for resistant genes.

Magar *et al*. (2014), conducted a study to develop a high yielding, fine grain, short duration rice variety resistant to BB by introgression of two BB resistance genes *xa13* and *Xa21* from B95-1 into the genetic background of MTU1010 (Cottondora Sannalu) – a rice variety released from Andhra Pradesh Rice Research Institute (APRRI), in 1999. The F_1 plants confirmed as true hybrids for both the genes were advanced to F_2 generation and foreground selection was done using gene linked markers *xa13* promotor and *pTA 248*. Genetic analysis in F_2 populations confirmed that the genes (*xa13* and *Xa21*) governing BB resistance followed mendelian pattern of inheritance.

The local varieties in the Iranian provinces were highly susceptible to the disease compared to the improved lines which were moderately resistant. The lines pyramided with the genes *Xa7*, *Xa14* and *Xa21* were resistant to most of the cultivars. Pyramided rice lines with two to five resistant genes were reported to provide higher level of disease resistance compared to the two or three gene pyramided lines (Khoshkdaman *et al*., 2014).

The studies on six, three-gene (*xa5*, *xa13*, *Xa21*) pyramided lines in the background of Swarna and IR64 under different hotspots across the country to identify lines with broad spectrum resistance was conducted by Pradhan *et al*. (2015a). The results revealed superiority of the pyramided line CRMAS2232-85 in agronomic performance along with higher level of resistance to BB as compared to parental lines. The pyramided line also showed similar agro-morphologic and quality traits like the recipient parent. Hence, it was concluded that development and release of pyramided lines with broad-spectrum resistance can provide better resistance against the disease.

Materials & Methods

III. MATERIALS AND METHODS

Aiming to confer resistance to rice variety Uma (Mo 16) against bacterial blight, the variety was hybridized with donor 'Improved Samba Mahsuri' (ISM) under the DBT project: 'Rice-Gene pyramiding to develop cultivars with durable resistance to Bacterial Leaf Blight through Marker Assisted Selection'. The F_1s were then backcrossed to the recipient parent (Uma) to obtain BC_1F_1 generation. The present investigation 'Gene pyramiding for bacterial blight resistance in rice variety Uma (Mo 16)' was conducted in the department of Plant Breeding and Genetics, College of Horticulture, during 2014-2016 using the backcross generation (BC_1F_1) thus generated. The study comprised of four major experiments *viz*., I) Genotyping of BC_1F_1 population, II) Morphological characterization of BC_1F_1s, III) Production of BC_2F_1s and IV) Production of BC_1F_2s. The details of the material used and methods employed in the present investigation are presented below.

3.1 Experimental location

The experimental site was located at the College of Horticulture (COH), Kerala Agriculture University, Vellanikkara P.O., Thrissur 680 656, 40m above MSL between 10º 31'N latitude and 76º 13'E longitude and experiencing humid tropical climate.

The laboratory and field facilities under Department of Seed Science and Technology, College of Horticulture, Vellanikkara, Thrissur 680 656 were used for the study.

3.2 Experimental material

One hundred and thirty BC_1F_1 individuals [Mo16 (Uma)/ Improved Samba Mahsuri (ISM)/ Mo 16], along with the recipient parent [Mo16 (Uma)] and donor parent [Improved Samba Mahsuri (ISM)] formed the basis of the study (Table 2). Improved Samba Mahsuri, an essentially derived variety (EDV) developed from variety Samba Mahsuri was used as the source of bacterial blight resistance genes *xa5, xa13* and *Xa21*. ISM was developed at the Indian Institute of Rice Research (former Directorate of Rice Research), Hyderabad through marker assisted backcross breeding programme.

Table 2. Details of genotypes used to develop the BC_1F_1 generation

Variety	**Parentage**	**Year of release**	**Salient features**
Recurrent parent: Uma (Mo16)	Mo 6 x Pokkali	1998	Medium duration (115 – 120 days) red kernelled rice variety
Donor parent: Improved Samba Mahsuri (ISM)	Samba Mahsuri x 4/SS1113	2008	Long duration (135 – 140 days) white kernelled rice variety

3.3 Methods

3.3.1. Experiment I: Genotyping of BC_1F_1 population

A non-replicated BC_1F_1 block was laid out during October – December, 2015. Seeds of BC_1F_1s (130 nos.) and parents (Uma and ISM) were sown in trays containing sterile sand and transferred the 95 germinated seedlings to pots (30 cm diameter) on 14th day after sowing. Staggered sowing of the recurrent parent (Uma) was also done at weekly intervals from 8th October 2015 to 24th December 2015 to

ensure pollen load for the production of BC_2F_1s. Standard agronomic practices as per package of practices (KAU, 2011) were followed during crop growth period to raise a good crop.

Genotyping of BC_1F_1 generation was done using STS and Rice Microsatellite (RM) markers available at database www.gramene.org. A set of 22 RM markers that were reported to be polymorphic between the parents in the earlier mentioned DBT project were selected for background selection. Care was taken to ensure that the markers selected for background selection covered most of the 12 linkage groups in rice. The list of markers used for foreground and background selection is detailed in Table 3 and Table 4 respectively.

Table 3. List of markers used for foreground selection

Gene	Primer name	Primer sequence	Marker distance (cM)	Product size (bp)	Reference
xa5	xa5SR F	AGC TCG CCA TTC AAG TTC TTG AG	0.0	410, 310, 180	Petpisit *et al.* (1977)
	xa5SR R	TGA CTT GGT TCT CCA AGG CTT			
	RG 556 F	ATA CTG TCA CAC ACT TCA CGG	0.1	440, 410	
	RG 556 R	GAA TAT TTC AGT GTG TGC ATC			
xa13	RG 136 F	TCC CAG AAA GCT ACT ACA GC	3.8	530, 490	Sundaram *et al.* (2008)
	RG 136 R	GCA GAC TCC AGT TTG ACT TC			
	xa13 pro F	GGC CAT GGC TCA GTG TTT AT	0.7	500	
	xa13 pro R	GAG CTC CAG CTC TCC AAA TG			
Xa21	pTA 248 F	AGA CGC GGA AGG GTG GTT CCC GGA	0.2	1000	Sundaram *et al.* (2008)
	pTA 248 R	AGA CGC GGT AAT CGA AAG ATG AAA			

Table 4. List of polymorphic markers used for background selection

Primer	Sequence		Annealing temperature (°C)	Product size (bp)
	Forward	Reverse		
RM 1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	55	113
RM 16	CGCTAGGGCAGCATCTAAA	AACACAGCAGGTACGCGC	55	181
RM 205	CTGGTTCTGTATGGGAGCAG	CTGGCCCTTCACGTTTCAGTG	55	122
RM 214	CTGATGATAGAAACCTCTTCTC	AAGAACAGCTGACTTCACAA	55	112
RM 252	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	55	216
RM 254	AGCCCCGAATAAATCCACCT	CTGGAGGAGCATTTGGTAGC	55	165
RM 307	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC	55	174
RM 5586	CTCCATAATCAAGGAAGCTA	ATGAGTTCTTTCGTCAGTGT	55	134
RM 11554	AGG ACT TAG GGT ACG TTT GAA TCT CC	GAC GAT GAT TGT CTC CTA AGT CTG C	55	318
RM 10871	TGA GGC TGT AAC GTA GAC GAT GAA CC	AAG CCT GCT AGA GAG GCC CAA CC	55	234
RM 13910	GAG CGA GCT ATA CCA CCG TGA CC	ATC GCG TCC AAG AAA GGT GTC G	55	188
RM 14725	CCA CAT AAG TAT TGG AGT GCA TCG	AGA TGT TAA CCC ACG AGG AAT GG	55	469
RM 15026	GCA TGC TCT TCC ATG ACT GC	CAT ATC AGA GGG TAC GAA ATG ACC	55	378
RM 15303	GAA TCG GGT CTA CGG TTT AGG	AAA GGA AGA GAA GAG GCA ACG	55	199
RM 15561	ATT AGC TTG GGC GTC TTC CTC TGG	TGC AAA CAA TGG CTT CAC ATC G	55	266
RM 15583	CCC AAA TAG TCA CCA GCA TTA TCG	TTG CCT GTG CAA CCT TAT GAA CC	55	174
RM 17182	TGCAGCGTCTCATCATAAAGTCG	GCTTAGTGCTGTGAACTGTGAAGACC	55	199
RM 19199	GCTCTACCAGGTATTATAGCCGATCC	AACTCCTCCAAGGTTCCATAGCC	55	158
RM 24616	CACCTTGGCCAACTAACTAATCG	GGGCAAGAGGAATTCACAACC	55	287
RM 26213	GCCACAGGAGACAGCAAGAACC	CGATCCAATTCCAGCCTAGATAGC	55	345
RM 26868	CAACTGTACTGTGCTGACCATCG	AGTAGGGACGAGGATTTCATGG	55	168
RM 28267	GCATAGCCCTGTTTGTTGCATGG	CGGTCCTTCCTCTTCTGTCATAACG	55	382

3.3.1.1. Isolation of DNA and assessment of quality and quantity of extracted DNA

Total cellular DNA (deoxy ribonucleic acid) of parents and backcross population (BC_1F_1s) was extracted and determined their quality and quantity.

3.3.1.1.1. Extraction of genomic DNA

Young leaves from the actively growing tip of plants were collected early in the morning. The collected leaves were labeled, covered with aluminium foil and immediately transported to the laboratory after placing in an ice box to prevent deterioration. Leaf surface was cleaned by washing with sterile distilled water followed by wiping with 70 per cent ethanol. The samples were weighed and stored at -20^{o} C until used for DNA extraction. Modified CTAB method advocated by Dellaporta *et al*, 1983 was used for the extraction of good quality DNA.

Reagents used

1. **CTAB buffer**

Table 5. Composition of CTAB buffer

Contents	**Concentration**	**Quantity for 500 ml**
CTAB (W/V)	2%	10g
Nacl	1.4M	40.6g
EDTA (pH 8)	20mM	3.7g
Tris base (pH 8)	100mM	6.07g
Polyvinyl pyrrolidin (PVP)	1%	5g
β mercaptoethanol	10mM	0.5 ml
Distilled water	-	500ml

2. **Chloroform – isoamyl alcohol (100ml)**

   Chloroform – 96 ml

   Isoamyl alcohol – 4ml

3. **3M sodium acetate (100ml)**

   Dissolved 24.6g sodium acetate in 50ml distilled water. Kept on magnetic stirrer for proper mixing. A pH of 5.2 was ensured and the volume made up to 100ml.

4. **70% ethanol**

   70ml – Ethanol

   30ml – Distilled water

5. **Chilled isopropanol (100%)**

Procedure

1. One gram leaf sample was weighed and surface sterilized using 70 per cent ethanol.
2. The leaf was cut into small pieces and ground into a fine paste in a pre-chilled mortar and pestle using CTAB (Cetyl Trimethyl Ammonium Bromide) buffer (500µl/g) and transferred to a centrifuge tube.
3. The sample was incubated at 65º C in water bath for 15 minutes ensuring shaking of the tubes at 5 minute intervals.
4. Equal volume of chloroform: Isoamyl alcohol (24:1) was added to the sample and contents were gently mixed. The sample was then centrifuged at 10,000 rpm for 10 minutes The content in the tubes will be separated into three phases

   Aqueous top layer - DNA with small quantity of RNA

   Middle layer - Protein and other cell debris

   Bottom layer - Chloroform, pigments etc.

   The aqueous phase was transferred to a fresh tube.

5. Equal volume of ice cold isopropanol and 3M sodium acetate (1:10) was added to the aqueous solution and incubated for 24 hrs at -20ºC for DNA precipitation.
6. The sample was centrifuged at 10,000 rpm at 4ºC for 10 minutes and the supernatant was carefully discarded after spin to retain only the pellet.
7. To the pellet obtained, 100-200 µl of 70 per cent ethanol was added and again centrifuged at 10,000 rpm at 4 ºC for 10 minutes.
8. If any sediment was found remained in the centrifuge tube, the steps 6 and 7 were repeated.
9. The supernatant was discarded and the tubes were air dried. The pelleted DNA was resuspended in 30-50µl buffer or 100-200µl sterilized water and stored in vials.

3.3.1.1.2 Determination of quality and quantity of isolated DNA

3.3.1.1.2.1 Assessing quality by agarose gel electrophoresis

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

Reagents used

1. Agarose - 1.5 per cent
2. 10X TBE buffer

Table 6. Composition of TBE buffer

Contents	10X stock concentration	Quantity for 1 L
Tris base	890mM	108g
Boric acid	890mM	55g
EDTA	20mM	3.72g

3. Tracking dye

4. Ethidium bromide (0.5µg /ml stock)

Procedure

Agarose gel (1.5 per cent) was prepared by melting 2.25g agarose in 150ml of 0.5X TBE buffer. Ethidium bromide was added (0.5µg/ml) and mixed well when the agarose cooled. The gel casting tray was wiped with 100 per cent alcohol and the comb was placed. The melted agarose was poured into the casting tray and allowed to set for 30 minutes. The comb was then removed and the tray was kept in the electrophoresis unit. 0.5X TBE buffer was added to the well. The DNA sample was diluted with millipore water in 1:9 ratio. The DNA sample (5µl) along with 3µl of tracking dye was added into the wells using a micropipette. A DNA ladder of molecular weight 100bp was loaded in one of the wells as a standard marker for easy detection and interpretation of results. Electrophoresis of gel was done for 45 minutes at 80 Volts until the tracking dye reaches 2/3rd of the gel. Electrophoresed gel was carefully transferred to gel documentation unit (Gel Doc Fire Reader Documentation System, UVITEC, Merck, UK) and observed.under UV exposure. Presence of highly resolved high molecular weight thick bands near the wells indicates the presence of DNA. RNA contamination can be observed as presence of thick bands around 100 bp region while a thick white patch observed inside the well indicated the presence of protein. UVITEC Fire reader software provided by Merck, UK was used to analyze the electrophoresed agarose gel.

3.3.1.1.2.2 Assessing quality and quantity by spectrophotometry

Further confirmation of the quality and quantity of the DNA isolated was analysed using spectrophotometer (Merck, UK Model: Spectroquant Pharo 300). The maximum absorbance of nucleic acids and proteins occurs at 260 nm and 280nm respectively. Absorbance of the samples (1µl) was measured at wavelengths 260 nm and 280 nm. The purity of DNA was assessed based on the OD_{260}/OD_{280} ratio. A ratio of 1.8 to 2.0 indicated pure DNA. A ratio greater than 1.8 indicated protein

contamination and greater than 2.0 indicated RNA contamination. The quantity of DNA was calculated based on the relation that Optical density (OD) for a DNA sample with a concentration of 50 µg/ml (double stranded) at 260 nm equals one.

i.e., 1 OD_{260} = 50 µg/ml (ds)

Therefore, Quantity of DNA (µg/ml) = Absorbance at OD_{260}* 50

3.3.1.1.2.3. Dilution of DNA for PCR

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

3.3.1.2. Polymerase Chain Reaction (PCR)

The good quality DNA isolated from the leaf samples were diluted to a concentration of 50 ng/µl and were used for polymorphism study. The DNA was amplified for both foreground and background selection. PCR amplification was performed using thermal cycler Eppendorf Master cycler (Eppendorf, Germany Model: Hamburg 22331).

3.3.1.2.1. Foreground selection

Three STS markers RG556, RG136 and pTA248, closely linked to the BLB resistance genes *xa5, xa13 and Xa21*, respectively were used to confirm the presence of the resistance allele of each gene in the backcross generation. The marker RG556 is located ~ 0.1 cM from *xa5*. RG136 marker is located ~3.8 cM from *xa13* while pTA248 marker is ~ 0.2 cM from *Xa21*. Restriction digestion of the marker RG556 with restriction enzyme Dra1 and marker RG136 with enzyme Hinf1was done after PCR amplification as advocated by Sundaram *et al.* (2008). In addition, the

functional marker xa5 and xa13 prom was also used to confirm the presence of xa5 and xa13 respectively.

3.3.1.2.1.1 Primer dilution and PCR

The primers were first diluted with distilled water. Same quantity of water as the concentration of the primer was added to make it 1M. Later it was diluted to a ratio 1:9 using 10 µl of the primer and 90 µl distilled water. Amplification of the diluted genomic DNA was performed using 15 µl reaction mixture in 0.2 ml PCR tubes. Required number of PCR tubes was arranged and the PCR reaction mixture for each tube was prepared as detailed in Table 7. The tubes were then centrifuged for 1 minute at 4ºC and placed in the thermal cycler as per the profile provided by Sundaram *et al.,* 2008 (Table 8).

Table 7. Composition of PCR reaction mix

Aliquot	**Quantity**
10x Taq buffer	2µl
dNTP mix	1 µl
$Mgcl_2$(25mM)	1.5 µl
Taq DNA polymerase (1U)	0.3 µl
Primers (Forward and Reverse)	2µl each
DNA sample	3µl
Distilled water	3.2 µl
Total	**15 µl**

Table 8. The PCR reaction profile followed

Stage	**Temperature (ºC)**	**Time**	**Number of cycles**
Initial denaturation (hot start)	94	5 min	
Denaturation	94	30 sec	35 cycles
Primer annealing	55	30 sec	
Primer elongation	72	1 min	
Final extension	72	7 min	
Cold storage	4	Infinity	

3.3.1.2.1.2 Restriction digestion of PCR product

Restriction digestion was done for the PCR amplified DNA samples. Five µl of the PCR amplified product was used initially to detect the amplification of DNA. The remaining DNA was used for restriction digestion with enzymes Dra1 and Hinf1 respectively for the STS primers RG 556 and RG 136. The reaction mixture (30 µl) as enumerated in Table 10 was centrifuged for 1 minute and incubated at 37ºC for 4 – 5 hours.

Table 9. Reaction mixture for restriction digestion

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

3.3.1.2.1.3. Screening and analysis of PCR products

The restriction digested PCR products were separated by gel electrophoresis along with 1kb ladder (Gene ruler ladder, Fermentas) on 1.5 per cent agarose gel stained with ethidium bromide. Banding pattern obtained was visualized using gel documentation unit (GeNei^{TM}- UVITEC, Merck, UK + Dell computer system) under UV exposure. The gel pictures were saved in image format for further scoring and detection of polymorphism among amplicons.

3.3.1.2.2. Background selection

Twenty-two rice microsatellite (RM) markers (Simple Sequence Repeats) reported to exhibit polymorphism between Uma and Improved Samba Mahsuri were selected to genotype BC_1F_1 generation to ascertain the genotypic background of the

pyramided lines. The DNA samples were amplified by PCR and the PCR products were separated by agarose gel electrophoresis and images captured as enumerated under 3.3.1.2.1.1 and 3.3.1.2.1.3. However, no restriction digestion of PCR product was done for markers used for background selection.

3.3.1.2.3 Analysis of bands and amplification data analysis

The banding pattern observed in the electrophoresed gels was scored for polymorphism/ monomorphism. The UVITEC Fire Reader software (Merck, UK) was used to give optimum exposure and proper visualization of the bands as well as to save the gel images. Well resolved and distinct amplicons were scored. Location of amplicon position and molecular weight of bands was visually assessed initially in comparison with the known molecular weight markers that were run along with the amplified samples. Amplicons of same size were scored as monomorphic bands while bands of different size were scored as polymorphic for interpretation of results. The banding pattern results obtained were further processed using Graphical Geno Types (GGT) *version* 2.0 (Van Berloo, 1999) software.

3.3.2 Experiment II: Morphological characterization of BC_1F_1s and parents.

Observations on the morphological characters of each BC_1F_1 plants and parents (10 plants each) raised were recorded at appropriate growth stages as per IRRI (1996).

3.3.3 Experiment III: Production of BC_2F_1s

3.3.3.1. Hybridisation

The BC_1F_1s identified to contain all the three BB resistance genes *xa5*, *xa13* and *Xa21* under 3.3.1 were backcrossed to the recurrent parent Mo16 (Uma) to obtain

BC_2F_1 seeds. Hand pollination of the female (BC_1F_1) spikelets, emasculated through clipping method, was done to obtain BC_1F_2 seeds.

3.3.3.2. Emasculation

Panicles of female parent (BC_1F_1s) that exhibited fifty to sixty per cent emergence out of the flag leaf were selected for emasculation. The leaf sheath was slightly detached from the panicle to expose the spikelets and for ease of emasculation. Emasculation was done late in the afternoon (after 3 p.m.). Very young florets from the bottom of the panicle, in which the height of the anthers was less than half the floret, were cut away. Florets that were likely to open the next day (with the height of anthers being equal or more than half the florets) were selected for emasculation. The top one-third of each floret selected for emasculation was clipped with scissors to expose the anthers. The anthers were then removed using the tip of the forceps prong by pressing them against the side of the floret and lifting out. The emasculated panicles were then bagged in butter paper bags, tagged and labeled. The butter paper bags were held securely in place by folding its bottom edge against the peduncle before tagging.

3.3.3.3. Pollination

At about 8 a.m. on the subsequent of emasculation, panicles about to dehisce were selected from the male parent variety Uma (recurrent parent) and enclosed in a petridish. Pollen grains were collected by gently tapping the top of the petridish. The collected pollen grains were then transferred to the stigma with the help of a thin camel brush. The pollinated panicles were re-bagged to avoid contamination by foreign pollen. Seed set was checked on the fifth day after hybridization. Maximum seed set was observed when on pollination was done on the day subsequent to emasculation, although the stigma remained receptive for three to seven days. The seeds were harvested at maturity, dried to 13 per cent and stored.

3.3.4. Experiment IV: Production of BC_1F_2s

Atleast one panicle of each BC_1F_1s plant identified to be introgressed with all the three BB resistance genes *xa5, xa13* and *Xa21* (described under 3.3.1) was selfed to obtain BC_1F_2 seeds. The seeds were harvested at maturity and dried to 13 per cent before storage.

3.4. Observations recorded

3.4.1. Genotyping of BC_1F_1 population

3.4.1.1 Quality and quantity of DNA isolated

Purity of DNA was assessed using the OD_{260}/OD_{280}. A ratio of 1.8 – 2.0 indicated pure DNA.

The concentration of nucleic acid in the sample was calculated based on Beer-Lambert law using the formula:

OD 260 = 1 is equivalent to 50 µg of double stranded DNA

1 OD at 260nm = 50µg/ml DNA

Therefore OD 260 x 50 gives the quantity of DNA in µg/ml

3.4.1.2 Nature of amplification

The banding pattern resolved on the gel for each marker was observed using the image captured by the gel documentation system (UVITEC Fire Reader software). The nature of band amplification was recorded either as monomorphic or polymorphic.

3.4.1.3 Number of amplicons

The number of amplicons resolved on the gel for each marker was counted using the image captured by the gel documentation system (UVITEC Fire Reader software).

3.4.1.4 Size of amplicons

Uvitec Fire Reader software (GeNei^{TM} – UVITEC, Merck, UK) estimates the size of amplicons resolved on the gel for each marker in base pairs (bp).

3.4.2 Morphological characterization of BC_1F_1s / parents

1. **Plant height (cm)**

   Measured from the ground level to the tip of flag leaf at maturity and expressed in centimeter.

2. **Days to 50 per cent flowering**

   The number of days taken from the date of sowing to the date of first panicle emergence in 50 per cent of the population was recorded.

3. **Leaf width (cm)**

   Measured across the leaf lamina at the broadest point of ten random leaves and average computed and expressed in centimeter.

4. **Leaf blade length (cm)**

   Measured from the base to tip of ten representative leaves and average computed and expressed in centimeter.

5. **Productive tillers**

   The total number of grain bearing tillers per plant was counted at maturity and average computed.

6. **Panicle length (cm)**

Length of main axis of panicle was measured from the panicle base to the tip and expressed in centimeter.

7. **Spikelets/panicle**

Number of spikelets/panicle was counted on three randomly selected panicles From each of the ten representative plants at maturity and the average computed.

8. **Grains /panicle**

Number of filled grains/panicle was counted at maturity on three randomly selected panicles from each of the ten representative plants at maturity and the average computed.

9. **1000 grain weight (g)**

Random sample of 1000 well-developed, whole grains, was weighed after harvest and the average computed and expressed in grams.

10. **Grain length (mm)**

Length of grains was measured from ten random seeds to obtain the grain length in millimeter.

11. **Grain width (mm)**

Width of grains was measured from ten random seeds to obtain the grain length in millimeter.

12. Decorticated grain length (mm)

The seeds were decorticated and the length measured from ten random seeds and expressed in millimeter.

13. Decorticated grain width (mm)

The seeds were decorticated and the width measured from ten random seeds and expressed in millimeter.

14. Grain yield/ plant (g)

Total grain yield from each plant was weighed and the average value expressed in grams.

15. Straw yield/ plant (g)

Total straw yield from each plant was weighed and the average value expressed in grams.

3.5. Statistical analysis

3.5.1. Genotyping of BC_1F_1 population

Graphical Geno Types (GGT) *version* 2.0 (Van Berloo, 1999) software was used for the assessment of the genomic contribution of the parent in the selected recombinants based on SSR data. The software generates similarity matrix as per Sneath and Sokal (1973) and clusters based on default similarity coefficient and dendrogram were generated.

3.5.3. Morphological characterization of BC_1F_1 population

3.5.3.1. Parameters of variability

1. Mean

The mean value of each observation was worked out by dividing the total of individual values of each observation by corresponding number of observation made:

$$\mathbf{X} = \frac{\sum X_i}{N}$$

Where,

X_i - any observation in i^{th} treatment

N - Total number of observations

2. Range

The range of each observation was noted as the lowest and highest values present in the observations of a sample.

Results

IV. RESULTS

A devastating disease of rice, bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae (Xoo)* is a common occurrence in the rice belts of Kerala. The elite variety Mo16 (Uma) being susceptible to the BB pathogen, huge economic loss is incurred by the rice farming community of the state each year. Considering the impact of the disease on rice production and productivity, three BB resistance genes (*xa5, xa13* and *Xa21*) were introgressed into variety Uma from the donor, Improved Samba Mahsuri (ISM), followed by backcrossing the resultant F_1s to variety Uma to recover the recurrent parent genome. The present investigation aimed to evaluate the morphological and molecular characteristics of the BC_1F_1 population thus generated, identify the R-gene introgressed individuals and produce selfed generation BC_1F_2s as well as the second backcross generation (BC_2F_1s).

Subsequently, a non-replicated BC_1F_1 block was laid out with the seeds of 130 BC_1F_1s along with the parents (Uma and ISM). The ninety-five BC_1F_1 plants that germinated were subjected to morphological characterization and genotyping. The results obtained are detailed below.

1.1.Genotyping of BC_1F_1 population

4.1.1. Quantity and quality of extracted genomic DNA of BC_1F_1s and parents

The concentration of extracted DNA of the BC_1F_1 individuals (Table 10, Appendix I) ranged from 53µg/ml in plant no. 5.3.2 to 98µg/ml in plant no. 4.3.2. The DNA concentration in the recurrent parent Uma and donor parent ISM was 75µg/ml and 69 µg/ml respectively.

The quality of DNA in the BC_1F_1 plants (Table 10, Appendix I) ranged from 1.81 to 1.98.The quality of DNA in the recurrent parent (Uma) was 1.83 and 1.95 in the donor parent (ISM).

Table 10. Quantity and quality of genomic DNA of BC_1F_1s and parents

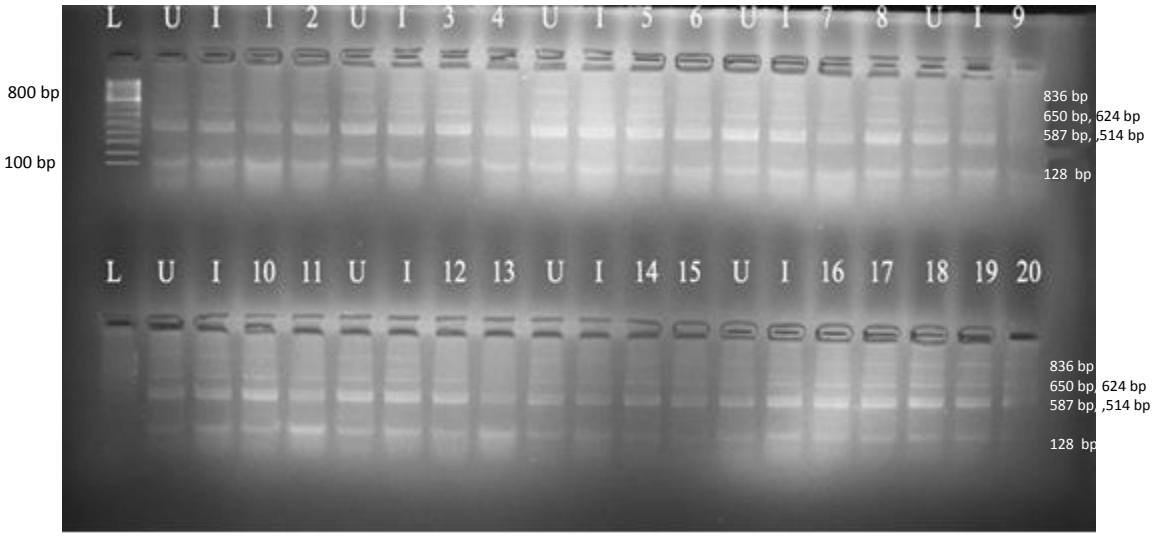
Individuals	Quantity of DNA (µg/ ml)			Quality of DNA		
	Mean	Range		Mean	Range	
		Max.	Min.		Max.	Min.
Uma (Recurrent parent)	75	94	58	1.83	1.98	1.8
ISM (Donor parent)	69	90	55	1.95	1.91	1.87
BC_1F_1s	77	98	53	1.88	1.98	1.81

4.1.2 Foreground selection

4.1.2.1. Foreground selection for *xa5*

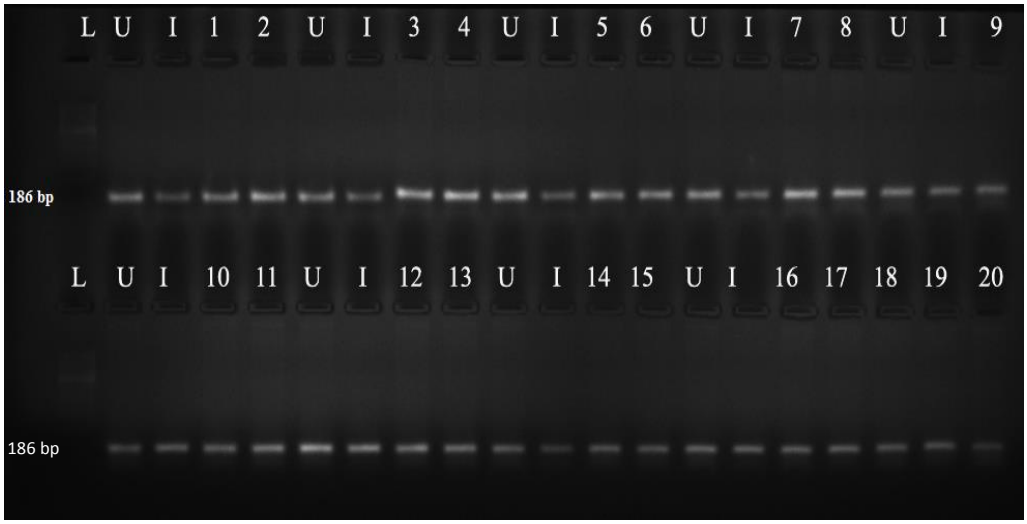
STS marker RG 556 and functional marker xa5 SR were used to confirm the presence of the resistant allele of *xa5* gene in each of the backcross individuals. The amplified product of RG 556 when resolved on 1.5 per cent agarose gel did not produce any polymorphism between the parents and the backcross populations screened. The PCR products were therefore digested with restriction enzyme Dra1 and examined for specific amplicon polymorphism (SAP). Dra1 has been reported to be effective in generating SAP which helps in differentiating the resistant and susceptible genotypes (Huang *et al*., 1997). Restriction digestion (Plate1, Table 11) produced six fragments of size 128 bp, 514 bp, 587 bp, 624 bp, 650 bp and 836 bp associated with the resistant allele in the homozygous state in both the parents as well as all the BC_1F_1 plants analysed.

Similarly foreground selection for the resistance gene *xa5* using the functional marker xa5SR, produced a 186 bp amplicon in both the parents as well as the BC_1F_1 plants analysed (Plate 2, Table 11).



L: Ladder U: Uma I: ISM Plant 1-20: BC_1F_1 plants

Plate 1. Specific amplicon polymorphism in BC_1F_1s on restriction digestion of PCR product of *xa5* linked STS marker RG 556



L: Ladder U: Uma I: ISM 1-20: BC_1F_1 plants

Plate 2. Foreground selection of BC_1F_1s using *xa5* linked functional marker xa5 SR

Table 11. Distribution of alleles of PCR marker loci linked to bacterial blight resistance (R) genes in the BC_1F_1 plants and parents

Target genes	***xa5***		***xa13***		***Xa21***
Marker	**RG 556**	**Xa5 SR**	**RG 136**	**Xa13 pro**	**pTA 248**
Donor parent (ISM)	+	+	+	+	+
Recurrent parent (Uma)	+	+	-	-	-
BC_1F_1 plants					
1.1.1	+	+	-	-	-
1.1.2	+	+	-	-	-
1.1.3	+	+	-	-	-
1.1.4	+	+	-	-	-
1.1.5	+	+	-	-	-
1.1.6	+	+	-	-	-
1.1.7	+	+	-	-	-
1.1.8	+	+	-	-	-
1.1.9	+	+	-	-	-
1.1.10	+	+	-	-	-
1.1.11	+	+	-	-	-
1.1.12	+	+	-	-	-
1.1.13	+	+	-	-	-
1.1.14	+	+	-	-	-
1.1.15	+	+	-	-	-
1.1.16	+	+	-	-	-
1.1.17	+	+	-	-	-
2.1.1	+	+	-	-	-
2.1.2	+	+	-	-	-
2.1.3	+	+	-	-	-
2.2.1	+	+	-	-	-
2.2.2	+	+	-	-	-
2.2.3	+	+	-	-	-
3.1.1	+	+	-	-	-
3.1.2	+	+	-	-	-
3.1.3	+	+	-	-	-
3.1.4	+	+	-	-	-
3.1.5	+	+	-	-	-
3.1.6	+	+	-	-	-
3.2.1	+	+	-	-	-
3.2.2	+	+	-	-	-
3.2.3	+	+	-	-	-
3.2.4	+	+	-	-	-
3.2.5	+	+	-	-	-
4.1.1	+	+	-	-	-
4.1.2	+	+	-	-	-
4.1.3	+	+	-	-	-
4.2.1	+	+	-	-	-
4.2.2	+	+	-	-	-

Distribution of alleles of PCR marker loci linked to bacterial blight resistance (R) genes in the BC_1F_1 plants and parents

Marker	RG 556	Xa5 SR	RG 136	Xa13 pro	pTA 248
4.3.1	+	+	-	-	-
4.3.2	+	+	-	-	-
5.1.1	+	+	-	-	-
5.1.2	+	+	-	-	-
5.1.3	+	+	-	-	-
5.3.1	+	+	-	-	-
5.3.2	+	+	-	-	-
5.3.3	+	+	-	-	-
5.3.4	+	+	-	-	-
5.3.5	+	+	-	-	-
6.1.1	+	+	-	-	-
6.1.2	+	+	-	-	-
6.1.3	+	+	-	-	-
6.1.4	+	+	-	-	-
6.1.5	+	+	-	-	-
6.2.1	+	+	-	-	-
6.2.2	+	+	-	-	-
7.1.1	+	+	-	-	-
7.1.2	+	+	-	-	-
7.1.3	+	+	-	-	-
7.1.4	+	+	-	-	-
7.4.1	+	+	-	-	-
7.4.2	+	+	-	-	-
7.4.3	+	+	-	-	-
8.1.1	+	+	-	-	-
8.1.2	+	+	-	-	-
8.1.3	+	+	-	-	-
8.3.1	+	+	-	-	-
8.3.2	+	+	+	+	+
8.3.3	+	+	+	+	+
8.3.4	+	+	-	-	-
8.3.5	+	+	-	-	-
8.3.6	+	+	-	-	-
8.3.7	+	+	-	-	-
8.3.8	+	+	-	-	-
8.3.9	+	+	+	+	+
12.1.1	+	+	-	-	-
12.1.2	+	+	-	-	-
14.2.1	+	+	-	-	-
14.2.2	+	+	-	-	-
14.2.3	+	+	-	-	-
14.3.1	+	+	-	-	-
14.3.2	+	+	-	-	-
21.1.1	+	+	-	-	-
21.1.2	+	+	-	-	-
21.1.3	+	+	-	-	-
21.1.4	+	+	-	-	-
21.1.5	+	+	-	-	-

Distribution of alleles of PCR marker loci linked to bacterial blight resistance (R) genes in the BC_1F_1 plants and parents

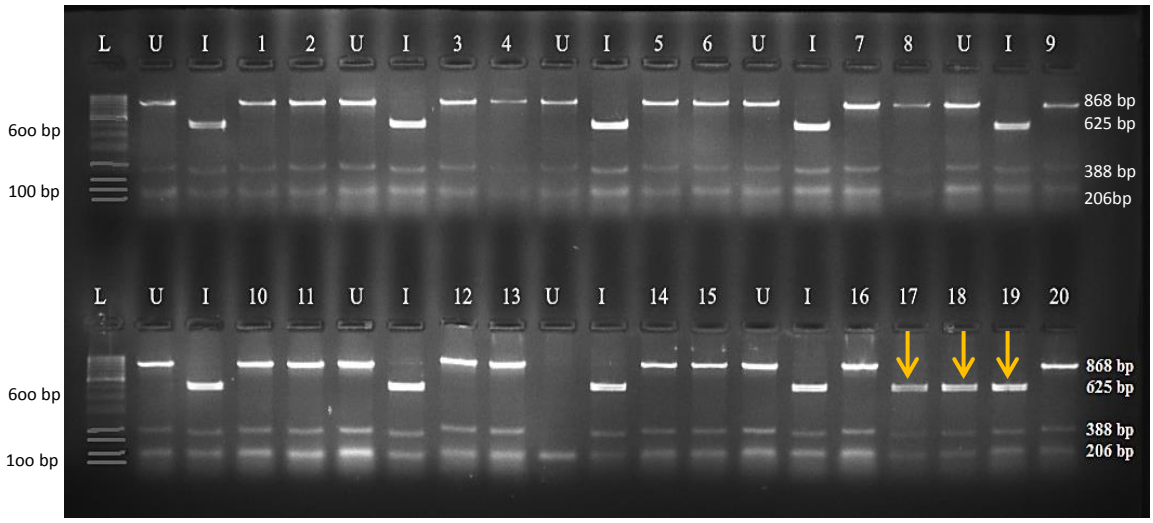
Marker	RG 556	Xa5 SR	RG 136	Xa13 pro	pTA 248
21.2.1	+	+	-	-	-
21.2.2	+	+	-	-	-
21.2.3	+	+	-	-	-
21.2.4	+	+	-	-	-
21.2.5	+	+	-	-	-
21.3.1	+	+	-	-	-
21.3.2	+	+	-	-	-
21.3.3	+	+	-	-	-

+ indicates presence of the gene; - indicates absence of the gene

4.1.2.2. Foreground selection for *xa13*

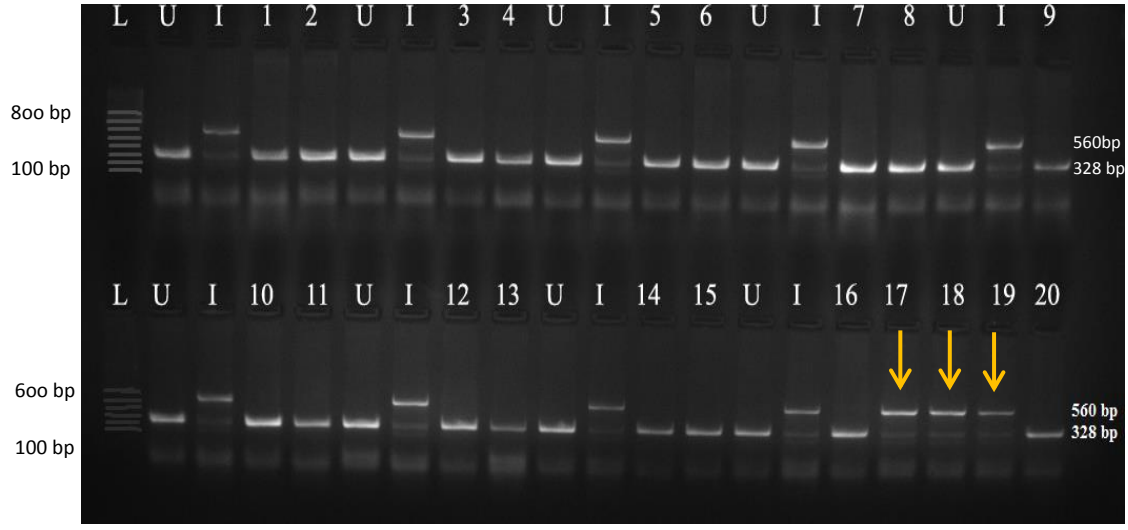
Foreground selection for the resistance gene *xa13* was analysed using the STS marker RG 136 and functional marker xa13 pro. Similar to STS marker RG 556 linked to *xa5* gene, RG 136 linked to *xa13* did not produce any polymorphism among the population studied. Hence, restriction digestion of the PCR amplified product using enzyme Hinf1 was resorted to. Hinf1 has been reported to distinguish the resistant and susceptible genotypes by production of SAP in the resistant genotypes. Restriction digestion of the PCR product generated polymorphism (Plate 3, Table 11) between the parents, and the BC_1F_1s studied. Three amplicons of size 625 bp, 388 bp and 206 bp were detected in the resistant donor parent ISM, while, in the recurrent parent, three bands of size 868 bp, 388 bp and 206 bp were observed. Of the 95 BC_1F_1s studied, 92 BC_1F_1s carried the three alleles as observed in the recurrent parent Uma. Only three BC_1F_1 individuals (Lane 17, 18 and 19, *i.e.*, BC_1F_1 plant no. 8.3.2, plant no.8.3.3 and plant no. 8.3.9 respectively) carried the same allele as that of the donor parent.

Screening the BC_1F_1s with xa13 pro revealed the presence of 560bp alleles in donor parent ISM while in the recurrent parent Uma an amplicon of size 328 bp was detected (Plate 4, Table 11). Among the BC_1F_1s, in 92 plants xa13 pro amplified a 328 bp fragment similar to that found in the recurrent parent while in the plants in lane 17, 18 and 19, *i.e.*, BC_1F_1 plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9 an amplified fragments of size 560 bp as in the donor parent was observed.



L: Ladder; U: Uma; I: ISM ; 1-20: BC_1F_1 plants; Lanes 17,18,19: *xa13* introgressed BC_1F_1s

Plate 3. Specific amplicon polymorphism in BC_1F_1s on restriction digestion of PCR product of *xa13* linked STS marker RG 136



L: Ladder; U: Uma; I: ISM; 1-20: BC_1F_1 plants; Lanes 17, 18 ,19: *xa13* introgressed BC_1F_1s

Plate 4. Foreground selection of BC_1F_1s using *xa13* linked functional marker xa13 pro

4.1.2.3. Foreground selection for *Xa21*

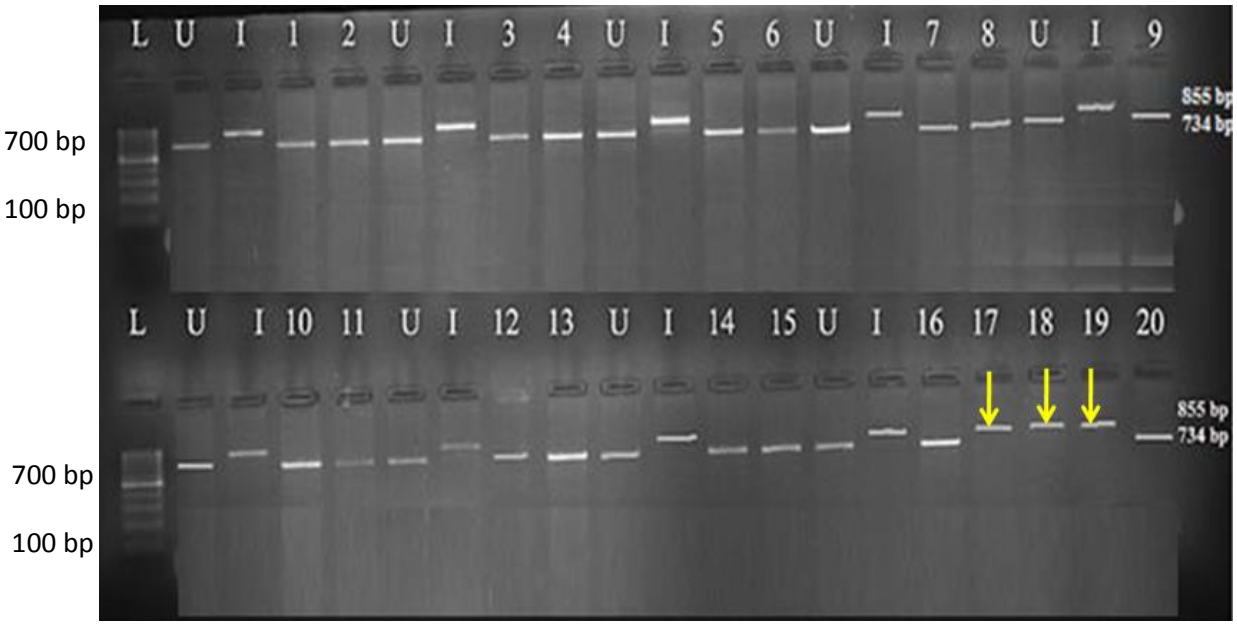
Ninety five BC_1F_1plants along with the parents were analysed for the presence of *Xa21* gene using the STS marker pTA 248 (Plate 5, Table 11). Three BC_1F_1individuals (lane 17, 18 and 19, *i.e*., BC_1F_1plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9respectively) exhibited amplicons of size 855 bp as in the donor parent ISM. The remaining 92 BC_1F_1 individuals exhibited a banding pattern similar to that in the susceptible recurrent parent Uma producing an amplicon of size 734 bp.

4.1.3. Background selection

The list of RM (Rice microsatellite) markers used for parental polymorphism survey (recurrent parent Uma and donor ISM) in the project: 'Rice-Gene pyramiding to develop cultivars with durable resistance to Bacterial Leaf Blight through Marker Assisted Selection,' is detailed in appendix II. Twenty two rice microsatellite markers reported to exhibit polymorphism between the recurrent parent Uma and the donor parent ISM in the above study were used for background selection (Plates 6a, 6b, 6c; Table12). Out of the 95 BC_1F_1s subjected to foreground selection, three, R-genes pyramided plants were identified (plants in lane 17, 18 and 19 *i.e*., plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9 respectively) under section 4.1.1. For ease and economy only the 3 R-genes pyramided plants (plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9) found to be introgressed with all the three R-genes (*xa*5, *xa13* and *Xa21*) were subjected to background selection. The result obtained is detailed below.

Marker RM 1 amplified a 412 bp fragment in the donor parent ISM and a 374 bp fragment in the recurrent parent Uma. The three R-genes introgressed BC_1F_{1s} also exhibited the allele of size 412 bp as in the donor.

The three R-genes introgressed BC_1F_1s as well as the donor parent were found to possess an allele of size 497 bp when analysed with RM 16 while a 410 bp amplicon was observed in recurrent parent Uma.



L : Ladder; U: Uma; I: ISM; 1 -20: BC_1F_1 s; Lanes 17, 18 and 19 - *Xa21* introgressed BC_1F_1s

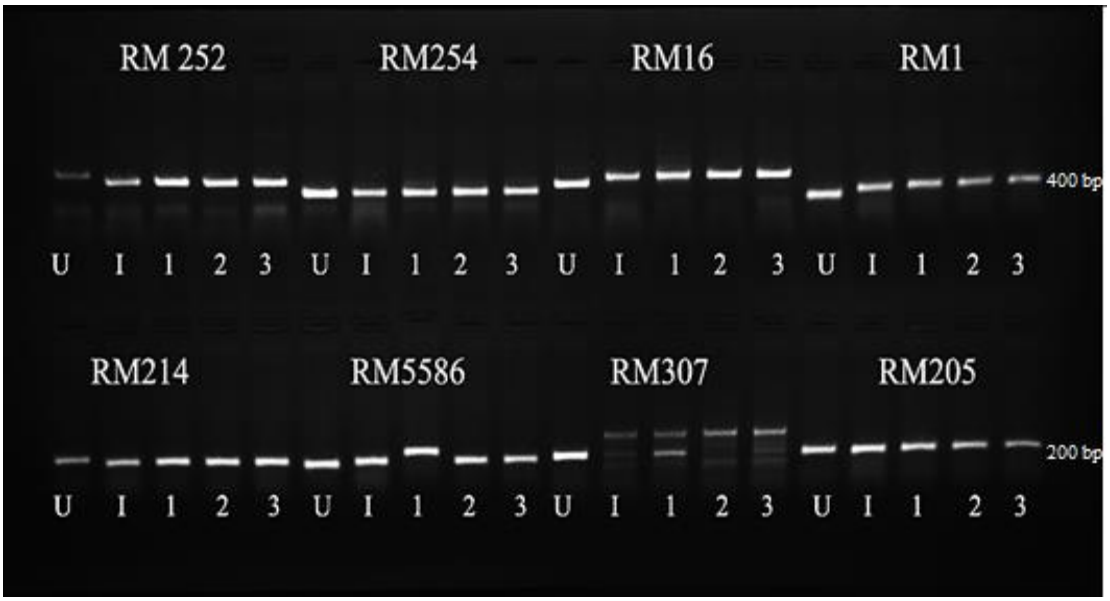
Plate 5. Foreground selection of BC_1F_1s using *Xa21* using linked STS marker pTA248

Table 12. Distribution of alleles of PCR marker loci used for background selection in the R-genes introgressed BC_1F_1s and parental genotypes

Sl. No	RM marker	Recurrent parent	BC_1F_1 8.3.2	BC_1F_1 8.3.3	BC_1F_1 8.3.9	Donor parent
1	RM 1	A	B	B	B	B
2	RM16	A	B	B	B	B
3	RM 205	A	A	A	A	A
4	RM 214	A	B	B	B	B
5	RM 252	A	B	B	B	B
6	RM 254	A	B	B	B	B
7	RM 307	A	H	B	B	B
8	RM 5586	A	B	B	B	B
9	RM 10871	A	B	B	B	B
10	RM 11554	A	B	B	B	B
11	RM 13910	A	B	B	B	B
12	RM 14725	A	B	B	B	B
13	RM 15026	A	B	B	B	B
14	RM 15303	A	B	B	B	B
15	RM 15561	A	B	B	B	B
16	RM 15583	A	A	A	A	A
17	RM 17182	A	B	B	B	B
18	RM 19199	A	B	B	B	B
19	RM 24616	A	B	B	B	B
20	RM 26213	A	B	B	B	B
21	RM 26868	A	B	B	B	B
22	RM 28267	A	B	B	B	B

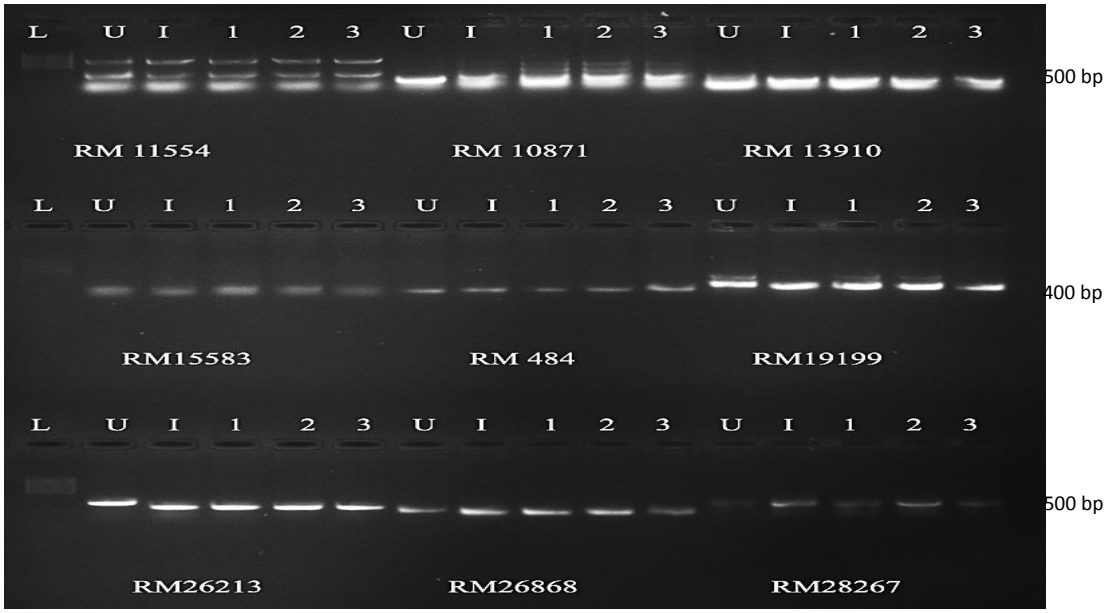
A: Allele of recurrent parent B: Allele of donor parent H: Heterozygous locus

The marker RM 205, on the other hand, amplified an allele of size 327 bp fragment in both the parents as well as the three BC_1F_1s. Similarly, monomorphic bands of size 255 bp were observed in the parents as well as in the three BC_1F_1 when amplified with marker RM 15583.



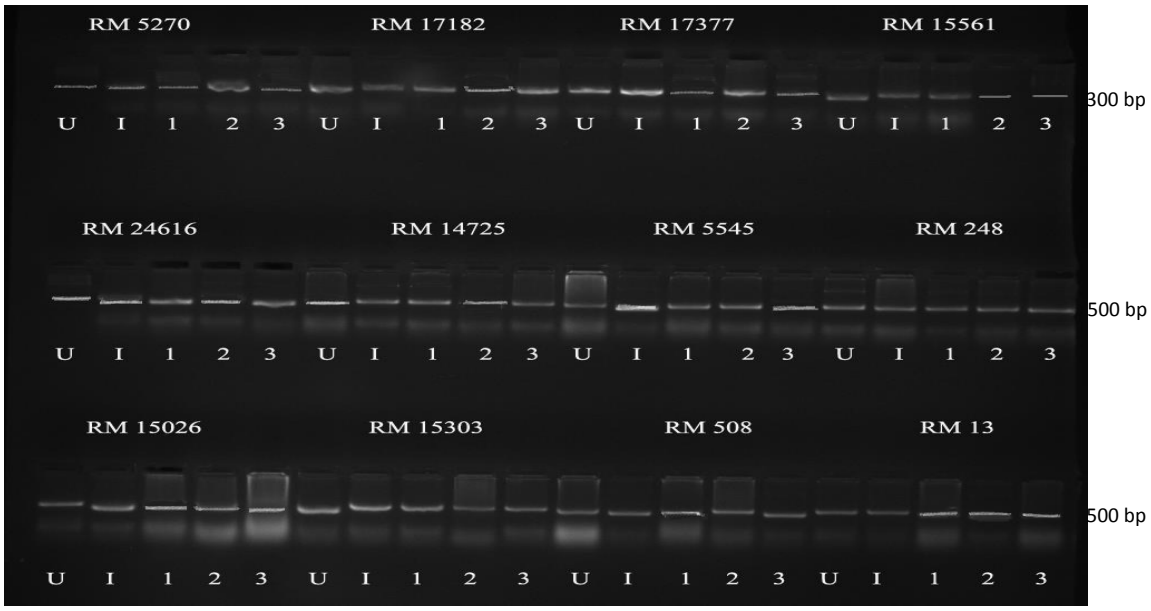
U: Uma; I: ISM; 1: BC_1F_1 plant no. 8.3.2; 2: plant no. 8.3.3; 3: plant no. 8.3.9

Plate 6 (a). Background selection of the R-genes introgressed BC_1F_1s using microsatellite markers -I



U: Uma; I: ISM; 1: BC_1F_1 plant no. 8.3.2; 2: plant no. 8.3.3; 3: plant no. 8.3.9

Plate 6 (b). Background selection of the R-genes introgressed BC_1F_1s using microsatellite markers- II



U: Uma; I: ISM; 1: BC_1F_1 plant no. 8.3.2; 2: plant no. 8.3.3; 3: plant no. 8.3.9

Plate 6 (c). Background selection of the R-genes introgressed BC_1F_1s using microsatellite markers -III

The marker RM 214 amplified fragments of size 299 bp in the susceptible recurrent parent Uma. A 206 bp fragment was amplified by RM 214 in the resistant donor parent ISM as well as in the three R-genes pyramided BC_1F_1s.

Marker RM 252 was found to exhibit polymorphism between the donor and the recurrent parent producing alleles of size 497 bp and 410 bp respectively. The three R- gene introgressed BC_1F_1s also exhibited a banding pattern similar to the donor parent.

The marker RM 254 produced amplicons of size 353 bp in the recurrent parent and 374 bp in the donor parent. Amplicon of size 374 bp as in the donor parent was found in the three, 3 R-genes introgressed individuals.

Analysing the parents and BC_1F_1 individuals using the marker RM 307 revealed that two (plant no. 8.3.3 and plant no. 8.3.9) out of three R-genes introgressed BC_1F_1were homozygous for the allele 386 bp similar to the donor parent ISM. However the BC_1F_1 plant no. 8.3.2 was found to be heterozygous with alleles of size 386 bp and 206 bp. RM 307 had amplified a 206 bp fragment in the recurrent parent.

Marker RM 5586 amplified a 327 bp fragment in the donor parent ISM and 206 bp in the recurrent parent Uma. The three R-genes introgressed BC_1F_1s also exhibited an allele of size of 327 bp as in the donor parent.

An amplicon of size 409 bp in the donor parent ISM and 393 bp in the recurrent parent Uma was produced by the marker RM 10871. Amplicon size similar to that of the donor parent was also observed in the three R-genes introgressed BC_1F_1s.

On analysis using the RM marker 11554, the resistant donor parent ISM and the three gene introgressed BC_1F_1s were found to possess alleles of size 467 bp and 220 bp while the recurrent parent showed a 453 bp allele.

The marker RM 13910 produced amplicons of size 393 bp in the donor parent, ISM and in the three R-genes introgressed BC_1F_1s while a 254 bp amplicon was observed in the recurrent parent Uma.

The marker RM 14725 was found to exhibit polymorphism between the donor and the recurrent parent producing alleles of size 508 bp and 476 bp respectively. The three gene introgressed BC_1F_1s also showed a banding pattern similar to the donor parent.

The rice microsatellite marker RM 15026 amplified a 528 bp amplicon in the recurrent Uma. A 443 bp amplicon was observed in the donor parent ISM as well as in the three gene introgressed BC_1F_1s.

Amplicon size of 528 bp was produced by the marker RM 15303 in the resistant parent ISM and the three gene introgressed BC_1F_1s while a 443 bp fragment was found amplified in Uma.

Both the markers RM 15561 and RM 17182 produced alleles of size 357 bp in the donor parent as well as the three gene introgressed BC_1F_1s while a 284 bp amplicon was produced in the recurrent parent Uma.

The marker RM 19199 amplified a fragment of size 532bp in the susceptible parent Uma. The donor parent ISM and the three R-genes introgressed BC_1F_1s produced amplicons of size 476.

Using the marker 24616 an amplicon of size 476 bp was produced in the three R-genes introgressed BC_1F_1s and the donor parent while in the recipient parent the amplicon size was 524 bp.

The marker RM 26213 was found to show polymorphism between the recurrent parent Uma and the donor parent ISM producing amplicons of 504 bp and 458 bp respectively. The three gene pyramided BC_1F_1s also showed a banding pattern similar to the donor parent.

The amplicon generated by the marker RM 26868 was of size 443bp in susceptible parent and 410 bp in the resistant parent. The three R-genes pyramided BC_1F_1s also produced amplicons of the same size as that of the donor parent.

The marker RM 28267 amplified a 524 bp fragment in ISM and the three gene introgressed BC_1F_1s. In the recurrent parent an amplicon of size 504 wasproduced.

4.1.4. Recovery of recurrent parent genome

The recovery of the recurrent parent genome in each of the three, R-genes introgressed BC_1F_1s (plant no 8.3.2, plant no. 8.3.3 and plant no. 8.3.9) was estimated from the results of the background profiling of these plants using 22 markers used. The per cent recovery of recurrent parent was assessed through graphical genotyping software GGT version 2.0.

The results (Table 13, Figure 1) indicated that the per cent recovery of recurrent parent genome varied among the three R-genes pyramided lines. The magnitude of recovery of recurrent parent genome was found to be higher in plant no 8.3.2 (23.90 %) while it was 21.80 per cent each, in plant no. 8.3.3 and plant no. 8.3.9.

Table 13. Contribution of recurrent parent genome in the R-genes introgressed BC_1F_1s

Plant number	Recurrent parent genome in BC_1F_1s (%)	
	Estimated recovery	Expected recovery
8.3.2	23.90	75
8.3.3	21.80	
8.3.9	21.80	

Based on the marker data, similarity co-efficient was calculated and a dendrogram showing the genetic similarity between the parents and the pyramids was

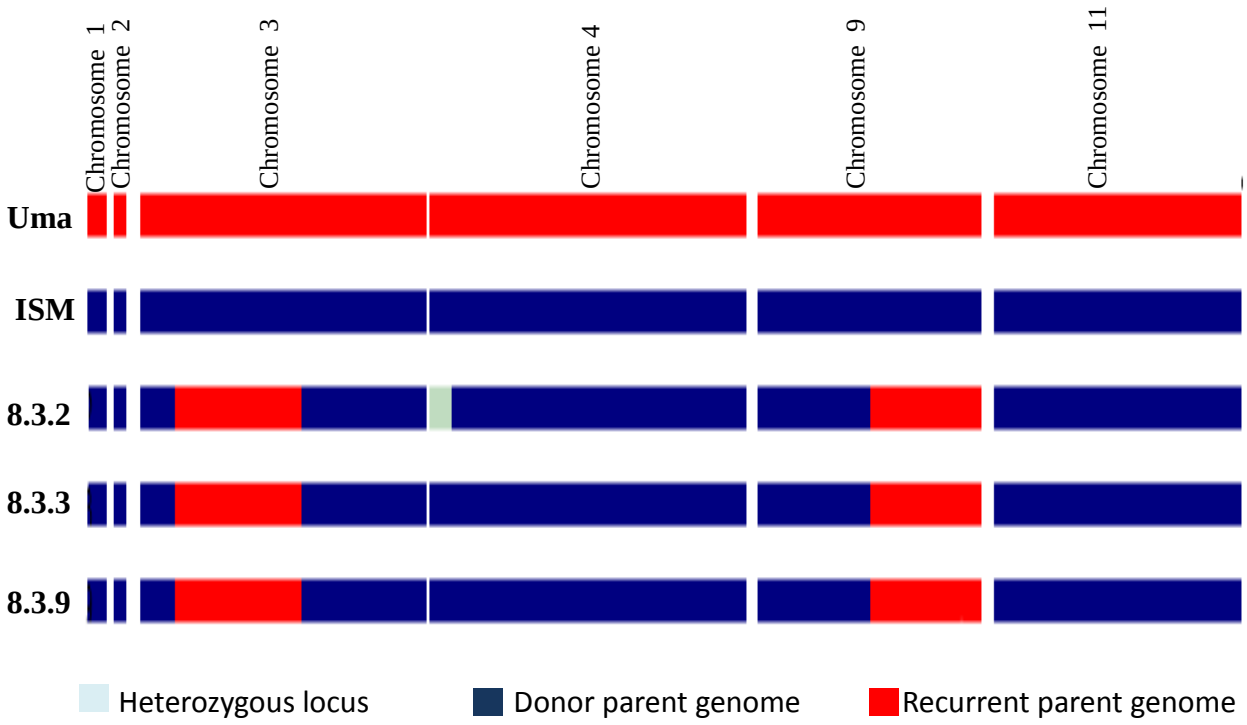


Figure 1. Recovery of recurrent parent genome in R-genes introgressed BC_1F_1s

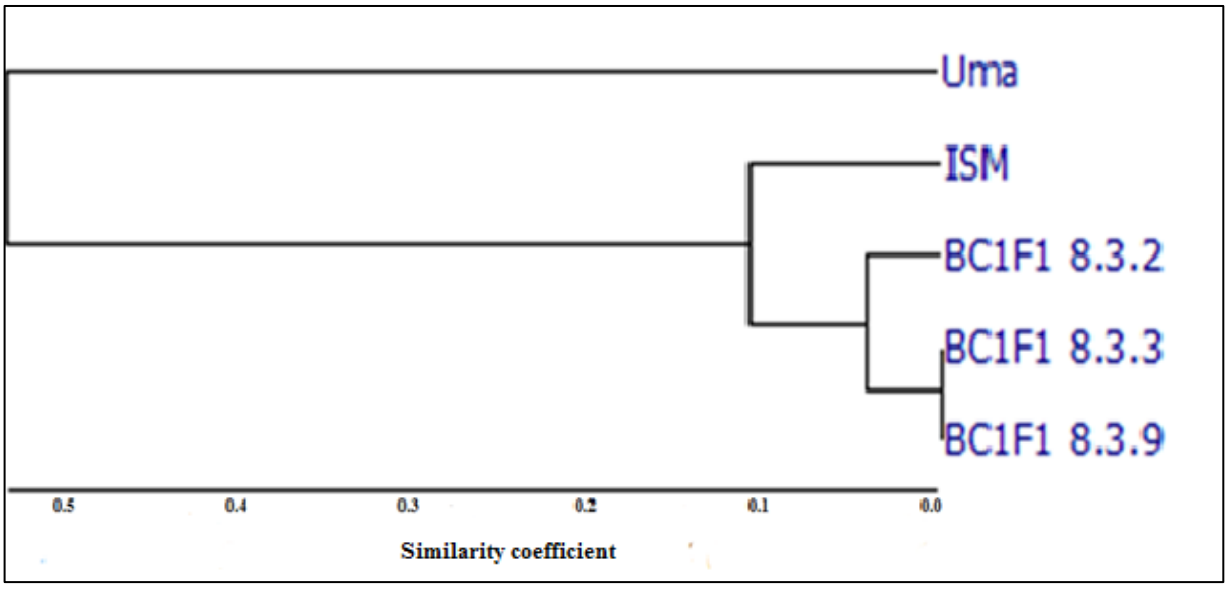


Figure 2. Clustering of R-genes introgressed BC_1F_1s and parents based on molecular data

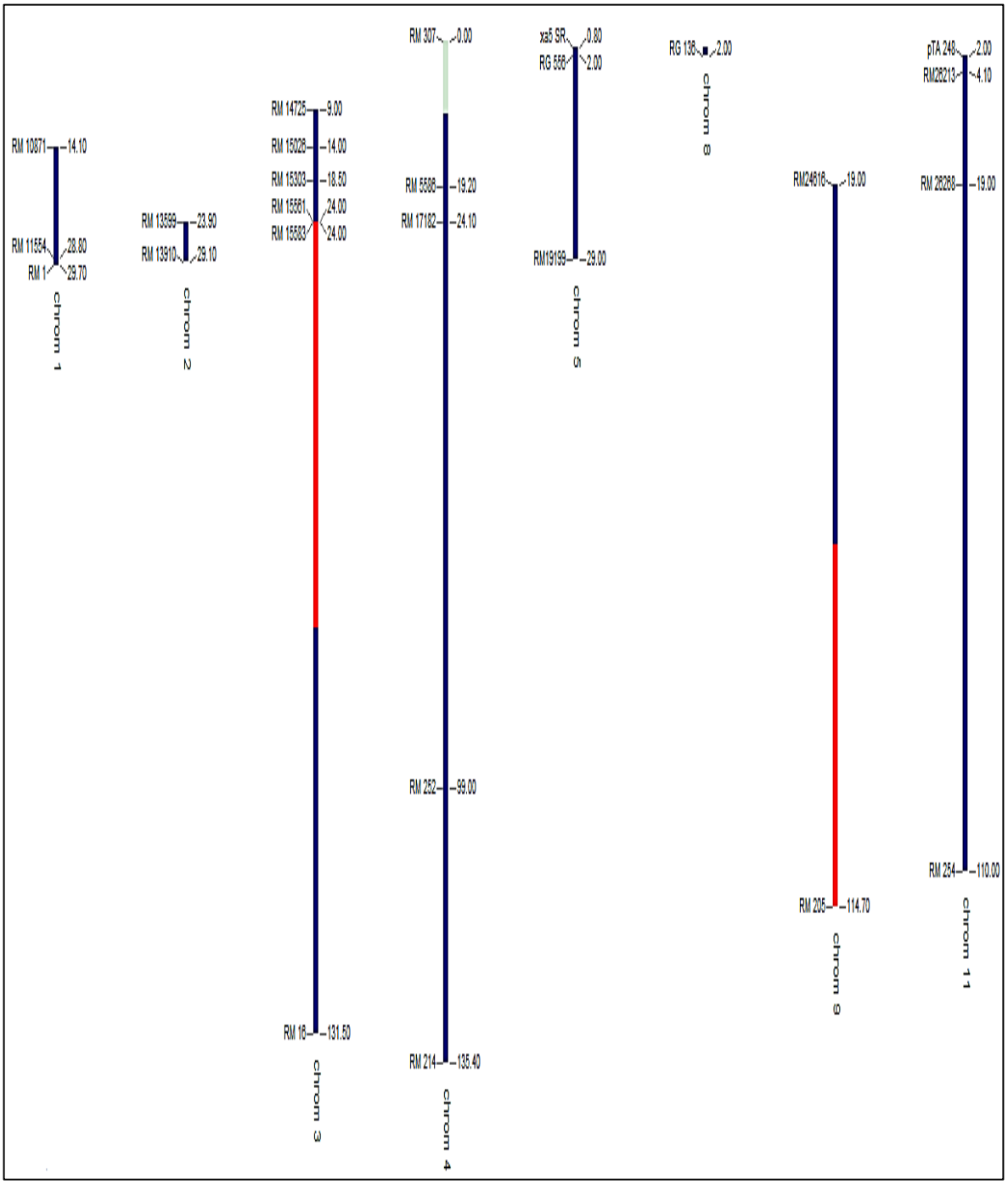


Figure 3. Graphical genotyping of R-genes introgressed BC_1F_1 plant no. 8.3.2

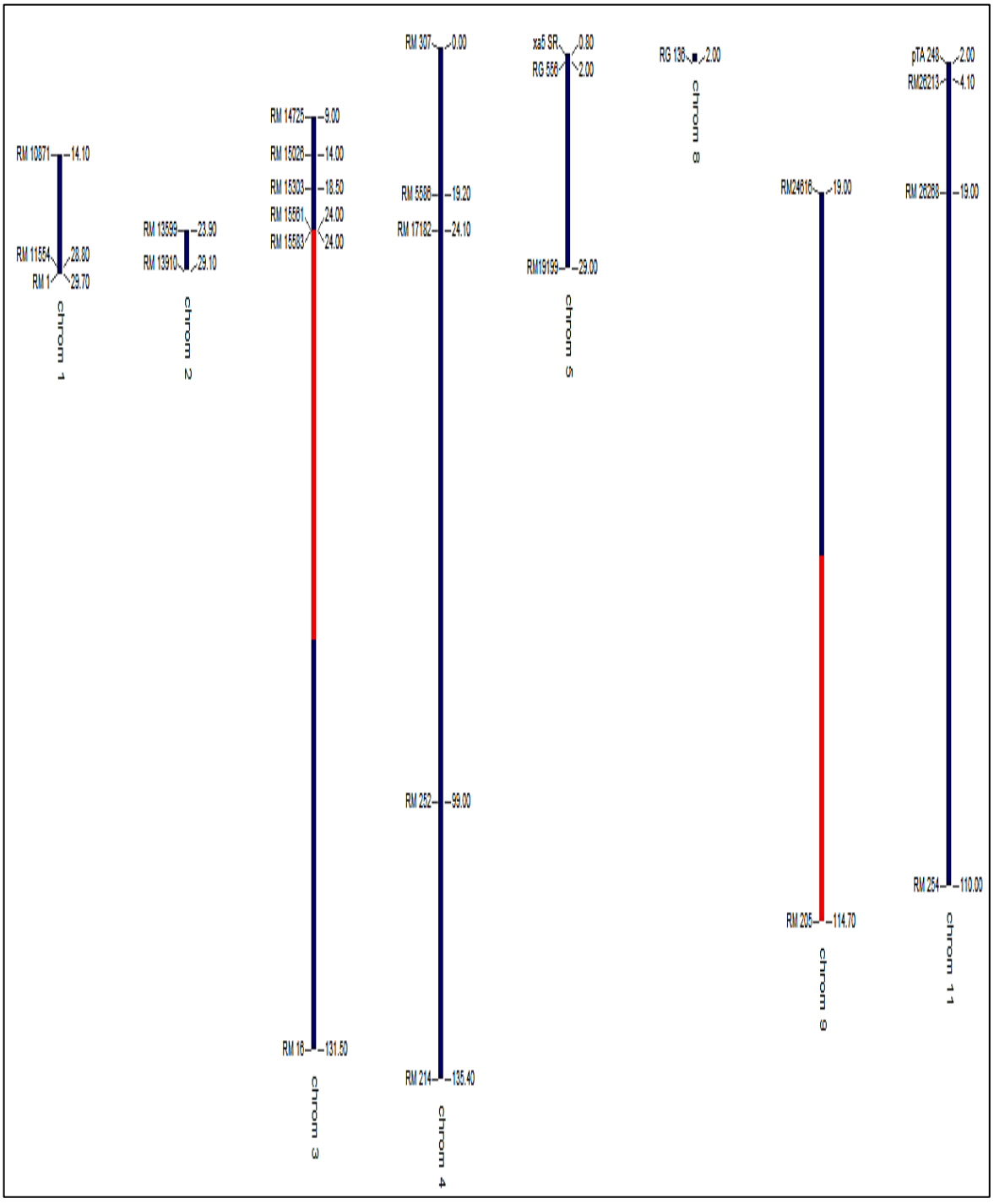


Figure 4. Graphical genotyping of R-genes introgressed BC_1F_1 plant no. 8.3.3

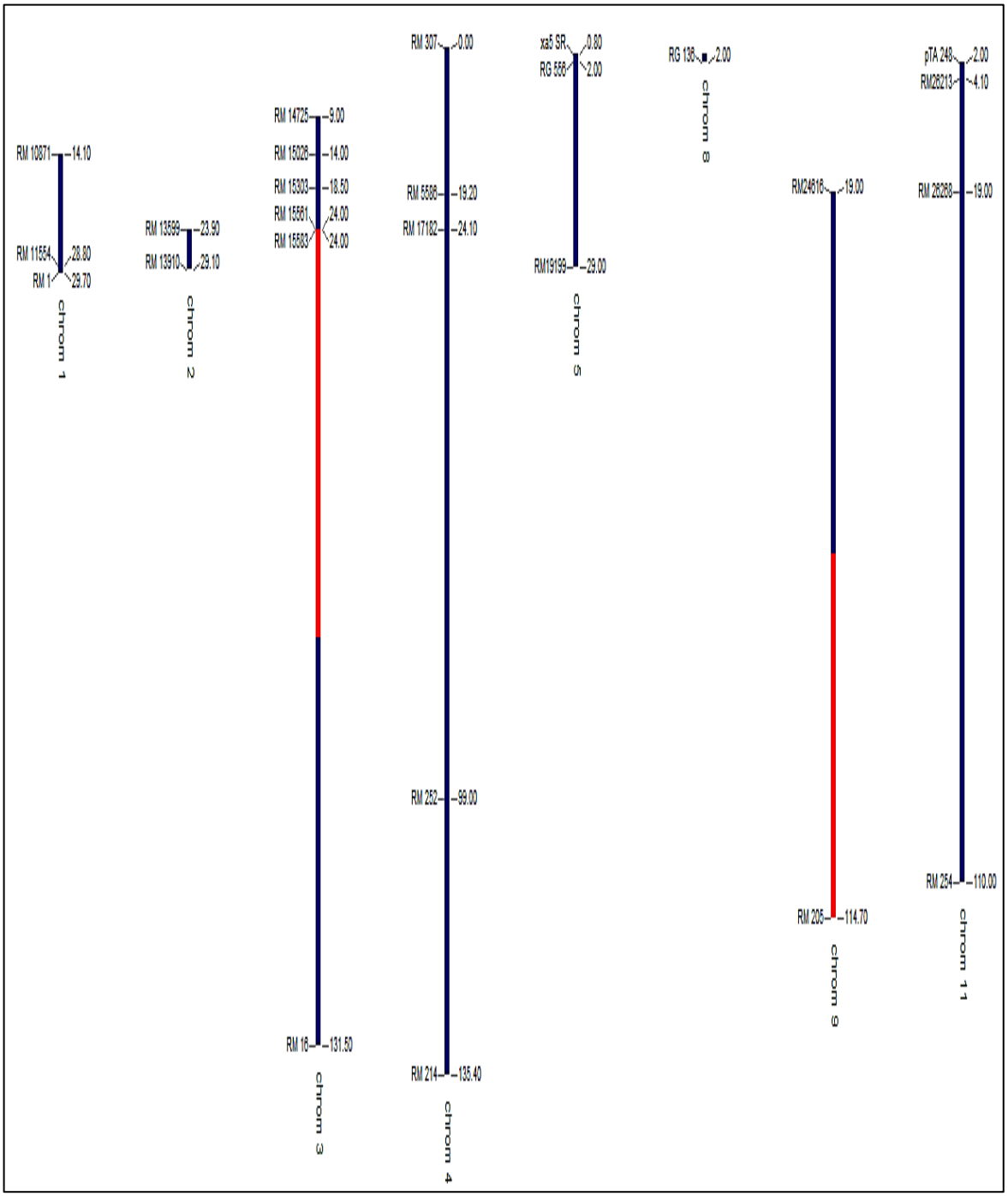


Figure 5. Graphical genotyping of R genes introgressed BC_1F_1 plant no. 8.3.9

generated (Figure 2). The dendrogram grouped the individuals into two major clusters; cluster 1 was monogenic with only the recurrent parent Uma at 40 per cent similarity with the second cluster. Cluster 2 comprised of the donor parent ISM and the three R- gene pyramided BC_IF_1s. Highest similarity was observed between the R gene pyramids 8.3.3 and 8.3.9.

The graphical representation of the results of genotyping of the BC_1F_1s done using the GGT software (Figures 3, 4 and 5) indicated similar pattern for the plants BC_1F_1 plant no. 8.3.3 and plant no. 8.3.9 unlike in plant no. 8.3.2.

4.2. Morphological characterization of BC_1F_1s and parents

The BC_1F_1s were evaluated for their agro-morphological characteristics. The results obtained are (Table 14, Appendices III, IV and V) detailed below:

4.2.1. Plant height

Plant height of the BC_1F_1 individuals varied between 39.6 cm and 71.2 cm with an average value of 53.08 cm. The height of the three R genes introgressed BC_1F_1s *i.e.*, plant no.8.3.2, plant no.8.3.3 and plant no.8.3.9 were found to be 48.97 cm, 51.12 cm and 43.21 cm respectively. The plant height of parents ISM and Uma was 47.98 cm and 66.29 cm respectively.

4.2.2. Days to 50 per cent flowering

The average days to 50 per cent flowering, in the donor parent ISM was 147.78 days. The recurrent parent Uma flowered in 127.54 days. Among the BC_1F_1s, days to 50 per cent flowering varied between 126.00 days in plant no.1.1.8 and 234 days in plant no.8.3.2. Days to flowering was found to be high in the three R genes introgressed BC_1F_1s namely plant no.8.3.2 (234 days), plant no.8.3.3 (228 days) and plant no. 8.3.9 (158 days).

4.2.3. Leaf width

Average leaf width of 1.00 cm, 1.03 cm and 0.94 cm was recorded in the BC_1F_1s, Uma and ISM was respectively. Leaf width of the BC_1F_1s ranged from 0.67 cm (plant no. 1.1.15) to 1.60 cm (plant no. 8.3.3). In the three R-genes introgressed BC_1F_1s leaf width was 1.40 cm (plant no.8.3.2), 1.60 cm (plant no.8.3.3) and 1.10 cm (plant no. 8.3.9).

4.2.4 Leaf blade length

Leaf blade length of the BC_1F_1s ranged from 20.66 cm to 50.60 cm with an average value of 32.40 cm. Leaf blade length in the three R-genes introgressed BC_1F_1s varied between 44.88 cm (plant no. 8.3.2) and 37.51 cm (plant no. 8.3.9) while in plant no. 8.3.3 the leaf blade length was found to be 43.96 cm. The average length of leaf blade of the recurrent parent was 48.39 cm and that of the donor parent was 36.69 cm.

4.2.5. Number of productive tillers

The number of productive tillers per plant in the donor parent ISM (8.65) was, higher than in the recurrent parent Uma (8.27). Among the 95 BC_1F_1s, it ranged between 5.00 and 12.The average number of productive tillers per plant in BC_1F_1s was 7.31. The three R-genes introgressed BC_1F_1s recorded 7 (plant no. 8.3.2), 12(plant no. 8.3.3) and 9 (plant no. 8.3.9) productive tillers per plant.

4.2.6. Panicle length

The average length of panicle in the BC_1F_1s was 17.88 cm and it varied from 13.57 cm in plant no. 7.4.1 to 21.63cm in plant no. 8.3.3. The recurrent parent Uma recorded a panicle length of 17.88 whereas the average panicle length in donor parent ISM was 22.16 cm long. Panicle length of the three R-genes pyramided lines *i.e.*, plant no.8.3.2, plant no.8.3.3 and plant no.8.3.9 was 20.38 cm, 21.63 cm and 19.98 cm respectively.

4.2.7. Spikelets / panicle

The average number of spikelets per panicle of BC_1F_1s was 98.03 and it was found to range from 86.40 (plant no. 5.3.3 and plant no. 7.4.3) to 112.50. The average number of spikelets/ panicle in the recurrent parent Uma was 97.16 while it was 98.86 in the donor parent ISM. The three R-genes introgressed BC_1F_1s recorded 87.57 (plant no. 8.3.2), 87.66 (plant no. 8.3.3) and 90.11 (plant no. 8.3.9) spikelets/ panicle.

4.2.8. Grains / panicle

The average number of grains per panicle recorded in parents Uma and ISM was 85.35 and 85.49 respectively. The number of grains per panicle in the BC_1F_1s ranged from 66.57 in plant no. 8.3.2 to 99.80 in plant no. 5.1.1 with an average value of 85. Grains/ panicle in the R-genes pyramided lines *i.e*., plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9 was 66.57, 79.25 and 87.44 respectively.

4.2.9. 1000 grain weight

The BC_1F_1s registered an average 1000 grain weight of 18.73 g. Thousand grain weight ranged between 12.32 g (plant no. 8.3.9) 24.20 g (plant no. 1.1.14) in BC_1F_1s. The recurrent parent Uma registered an average 1000 grain weight of 18.69 g while it was found to be 10.32 g in the donor parent ISM. Thousand grain weight in the three R-genes introgressed BC_1F_1s was 18.90 g (plant no. 8.3.2), 17.65g (plant no. 8.3.3) and 18.32 g (plant no. 8.3.9).

4.2.10. Grain length

Average length of grain in the recurrent parent Uma was 6.15 mm and that of the donor parent ISM 6.58 mm. The BC_1F_1s recorded an average grain length of 6.73 mm ranging between 6.12 mm and 7.00 mm. Grain length in the three R-genes introgressed BC_1F_1s was 6.12 mm (plant no. 8.3.2), 6.18 mm (plant no. 8.3.3) and 6.20 mm (plant no. 8.3.9).

4.2.11. Grain width

An average grain width of 2.79 mm was recorded in the recurrent parent Uma while it was 1.78 mm in donor parent ISM. Average grain width observed in the BC_1F_1s was 2.75 mm and it ranged from 2.40 (Plant no. 1.1.10) mm to 2.90 mm. Grain width in the three R-genes introgressed BC_1F_1s was 2.76 mm (plant no. 8.3.2), 2.80 mm (plant no. 8.3.3) and 2.75 mm (plant no. 8.3.9).

4.2.12. Decorticated grain length

The average grain length of the susceptible parent Uma after decortication was 5.85 mm and that of the resistant parent ISM was 4.96 mm. Decorticated grain length of the BC_1F_1s ranged between 5.10 mm (Plant no. 1.1.2) and 6.50 mm (Plant no. 3.2.5) with an average value of 5.77 mm. Decorticated grain length in the three R-genes introgressed BC_1F_1s was 5.81mm (plant no. 8.3.2), 5.78 mm (plant no. 8.3.3) and 5.79 mm (plant no. 8.3.9).

4.2.13. Decorticated grain width

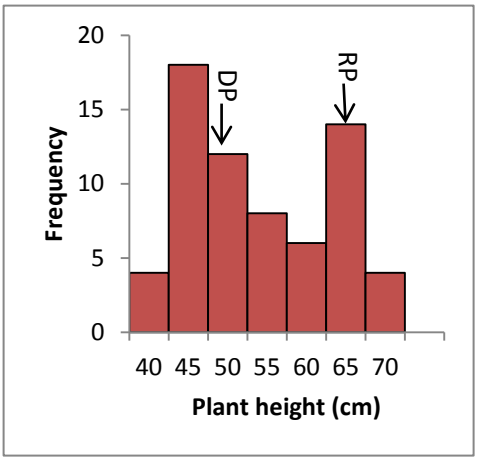
The average decorticated grain width of the BC_1F_1s was 2.36 mm and ranged between 2.10 mm and 2.60 mm. Average grain width after decortication in the recurrent parent was 2.34 mm while it was 1.47 mm in the donor parent. Decorticated grain width in the three R-gene introgressed BC_1F_1s was 2.21 mm (plant no. 8.3.2), 2.22 mm (plant no. 8.3.3) and 2.31 mm (plant no. 8.3.9).

4.2.14. Grain yield/ plant

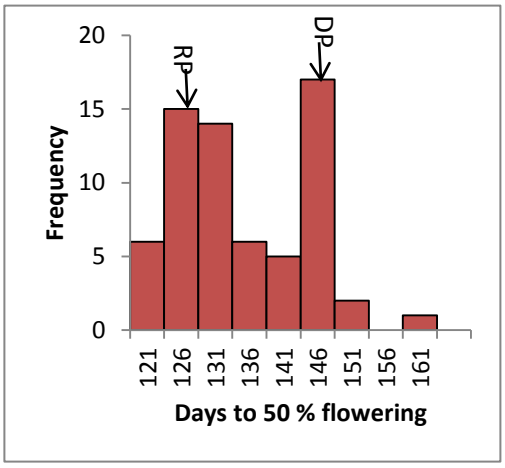
Average grain yield/ plant in Uma was15.79 g and 8.69 g in ISM. The average grain yield of the BC_1F_1s was 16.01 g and ranged between11.91g in plant no. 7.4.3 and 19.88 g in plant no. 4.3.1.

Table 14. Variability in morphological characteristics among BC_1F_1s and the parents

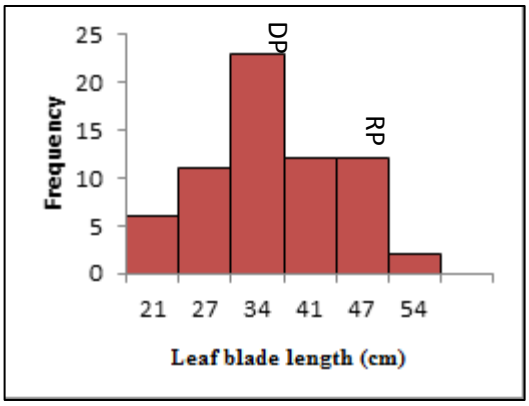
Sl. No	Plant character	Recurrent parent (Uma)			Donor parent (ISM)			BC_1F_1s		
		Range		Mean	Range		Mean	Range		Mean
		Min	Max		Min	Max		Min	Max	
1	Plant height (cm)	56.97	73.04	66.29	42.07	52.53	47.98	39.60	71.20	53.08
2	Days to 50% flowering	123.40	132.50	127.54	144.4	151.6	147.78	126.00	234.00	137.22
3	Leaf width (cm)	0.92	1.15	1.03	0.85	1.09	0.94	0.67	1.60	1.00
4	Leaf blade length (cm)	35.81	55.34	48.39	28.31	42.75	36.69	20.66	50.60	32.40
5	Productive tillers/plant	7.20	9.80	8.27	7.60	10.4	8.65	5.00	12.00	7.31
6	Panicle length (cm)	16.81	19.63	17.88	21.37	23.06	22.16	13.57	21.63	17.88
7	Spikelets/panicle	93.80	101.56	97.16	85.85	120.21	98.86	86.40	112.50	98.03
8	Grains/panicle	79.02	90.53	84.35	72.27	95.06	84.49	66.57	99.8	85.03
9	1000 grain weight (g)	17.20	21.06	18.69	9.12	11.20	10.32	12.32	24.20	18.73
10	Grain length (mm)	6.12	6.91	6.15	6.32	6.79	6.58	6.12	7.00	6.73
11	Grain width (mm)	2.70	2.86	2.79	1.73	1.96	1.78	2.40	2.90	2.75
12	Decorticated grain length (mm)	5.32	6.07	5.85	4.88	5.05	4.96	5.10	6.50	5.77
13	Decorticated grain width (mm)	2.22	2.46	2.34	1.34	1.62	1.47	2.10	2.60	2.36
14	Grain yield /plant (g)	14.31	17.67	15.79	7.82	9.46	8.69	11.91	19.88	16.01
15	Straw yield /plant (g)	18.86	21.18	19.69	10.44	14.14	12.76	16.49	23.19	19.92



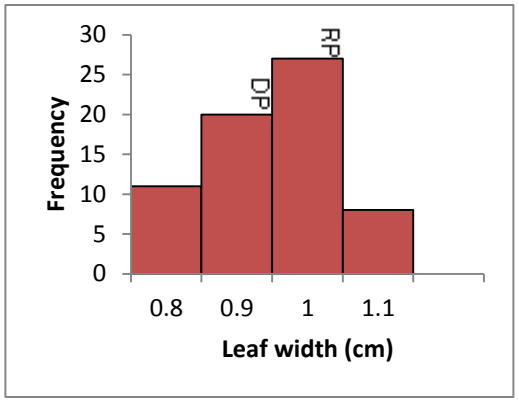
A) Plant height



B) Days to 50 per cent flowering

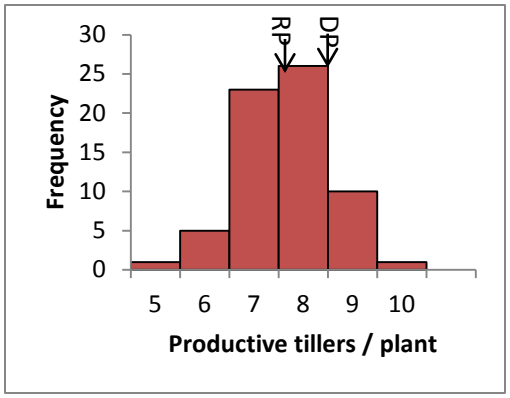


C) Leaf blade length

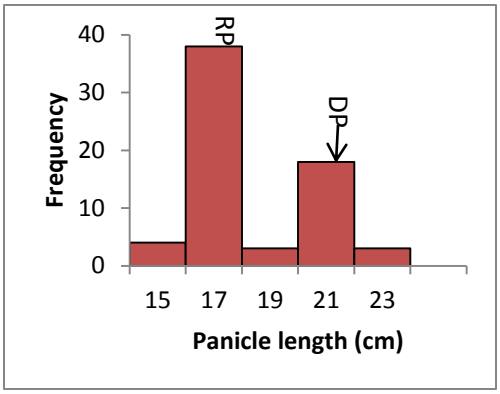


D) Leaf width

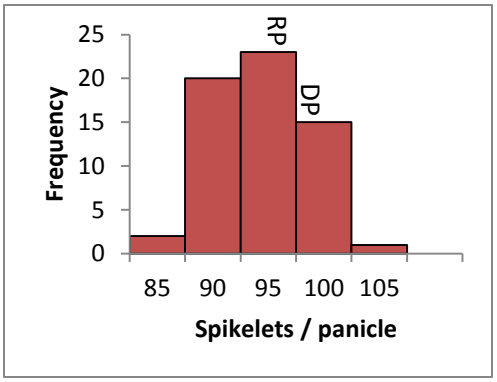
Figure 6 (A) to 6 (D). Frequency distribution of the BC_1F_1s and parents for morphological characters



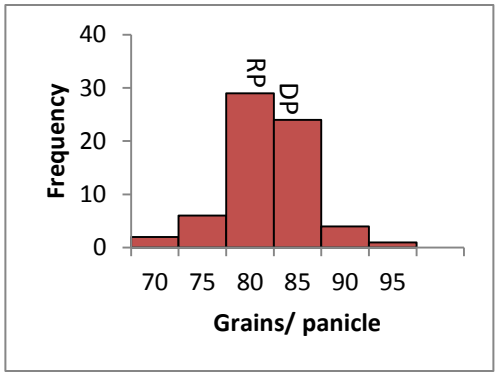
E) No. of productive tillers



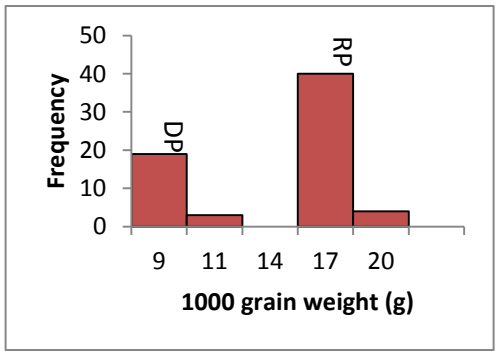
F) Panicle length



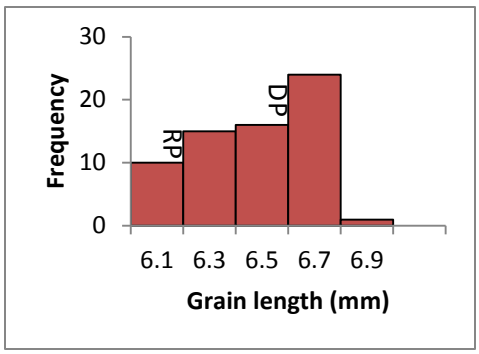
G) Spikelets/ panicle



H) Grains/panicle

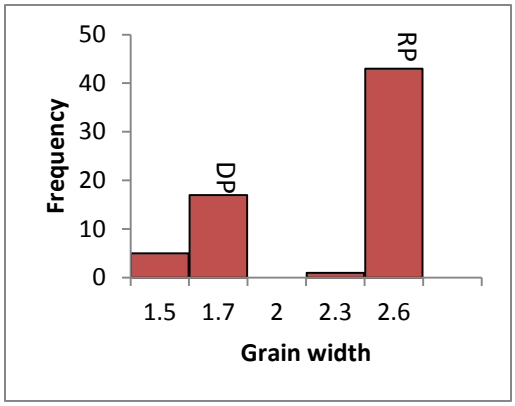


I)1000 grain weight

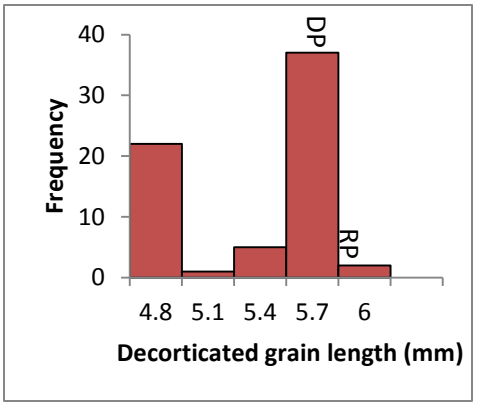


J) Grain length

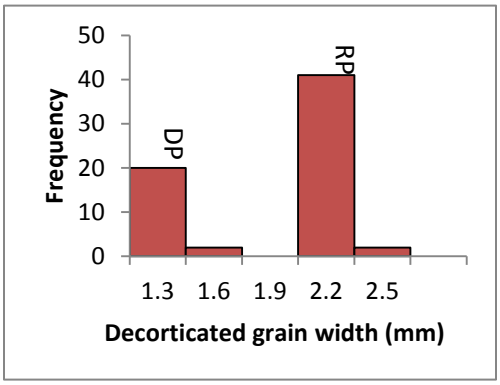
Figure 6 (E) to 6 (J). Frequency distribution of the BC_1F_1s and parents for morphological characters



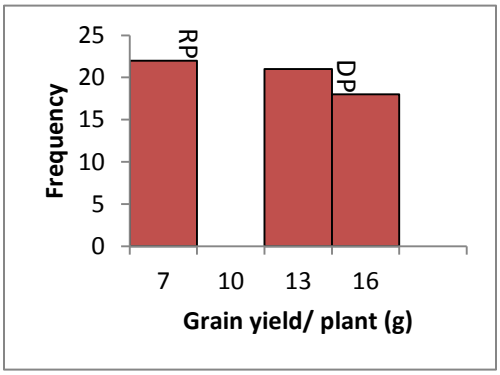
K) Grain width



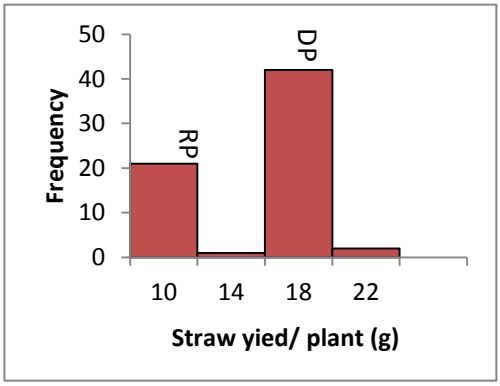
L) Decorticated grain length



M) Decorticated grain width

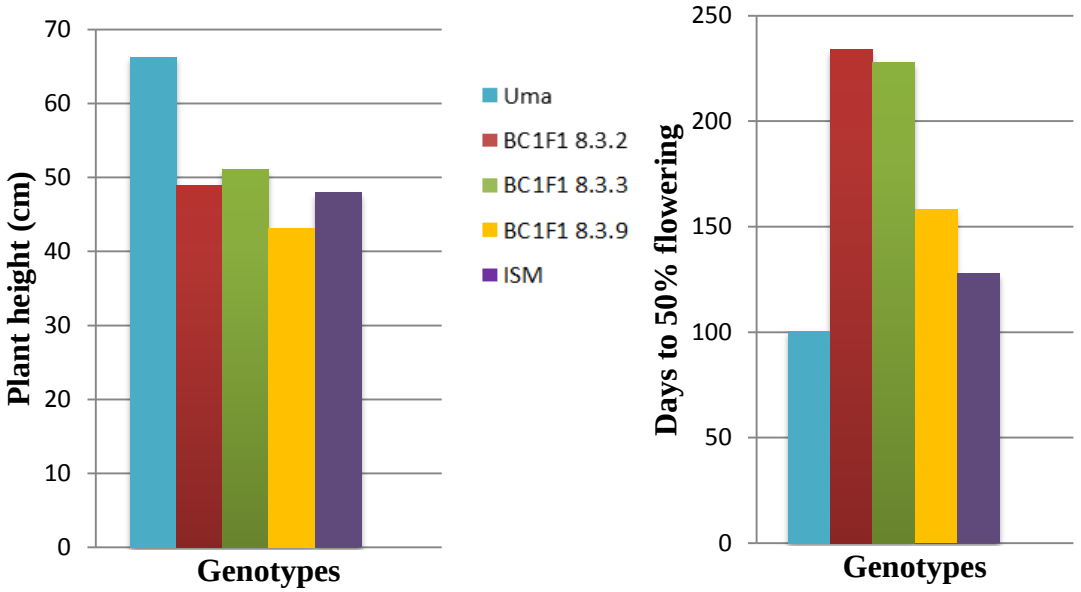


N) Grain yield/plant



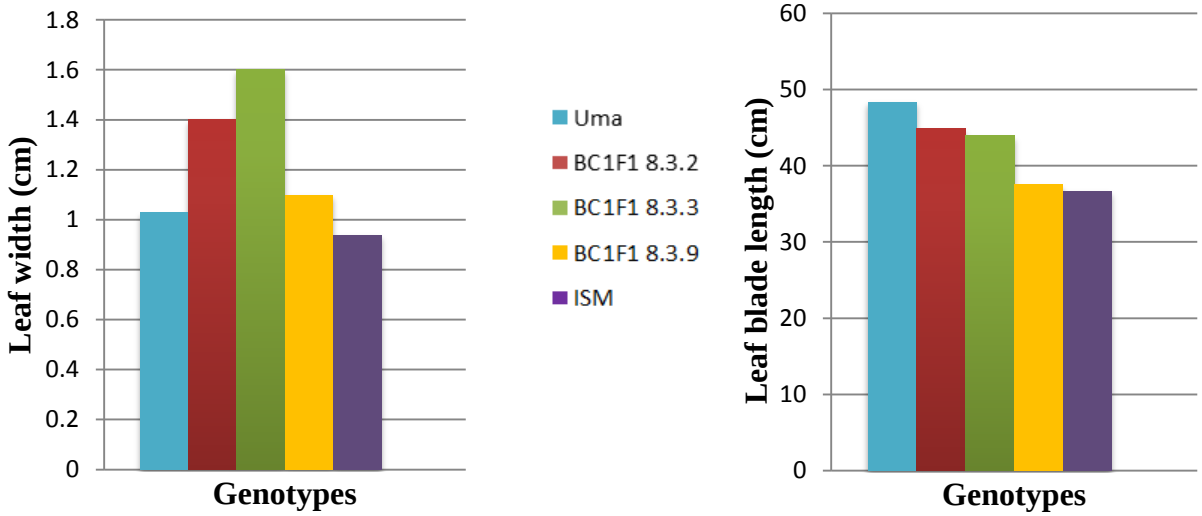
O) Straw yield/plant

Figure 6 (F) to 6 (O). Frequency distribution of the BC_1F_1s and parents for morphological characters



A) Plant height

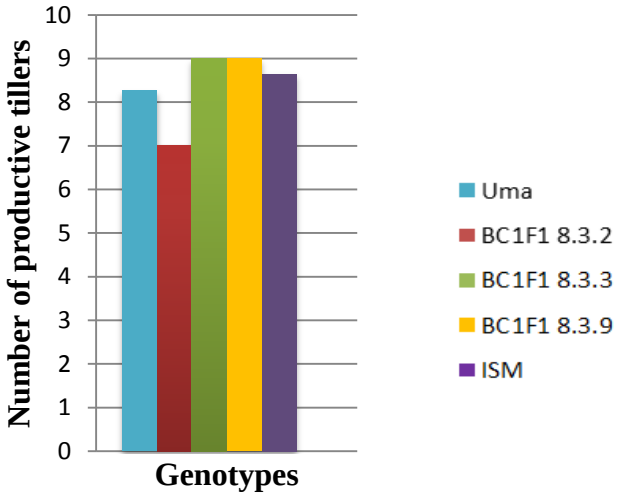
B) Days to 50 per cent flowering



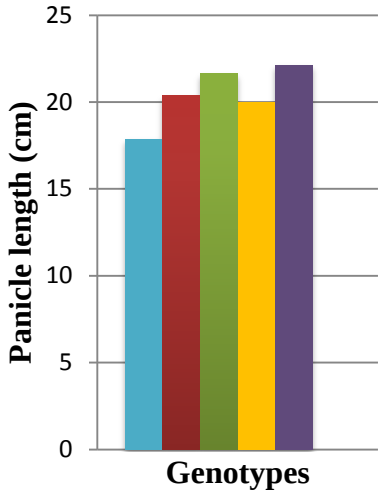
C)Leaf width

D) Leaf blade length

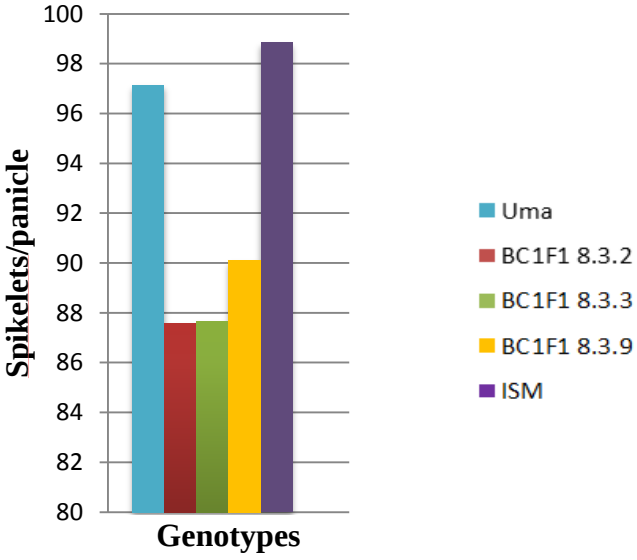
Figure 7 (A) to 7 (D). Morphological characteristics of R-genes introgressed BC_1F_1s and parents



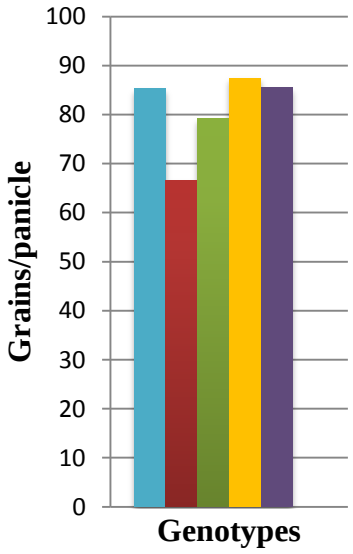
E) No. of productive tillers



F) panicle length

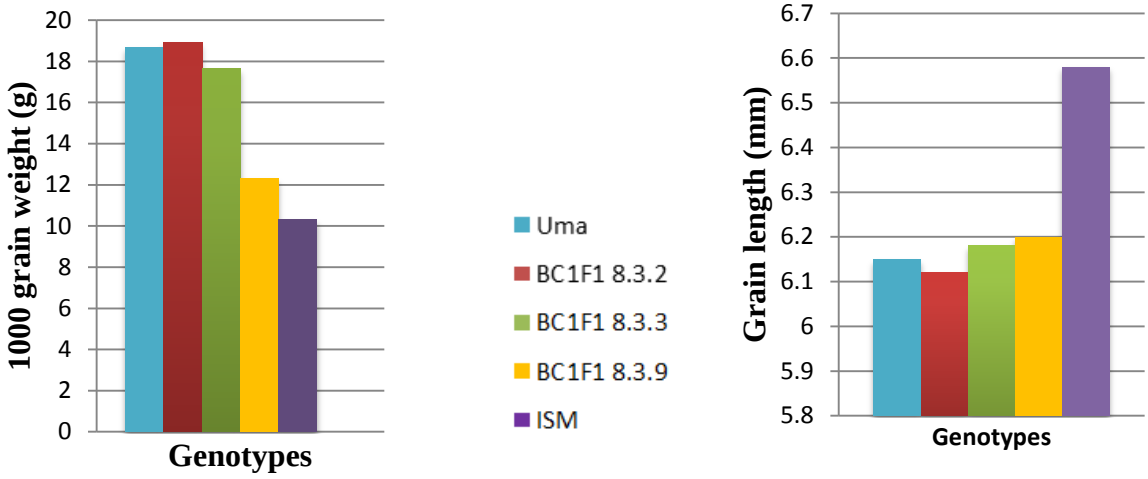


G) Spikelets/panicle

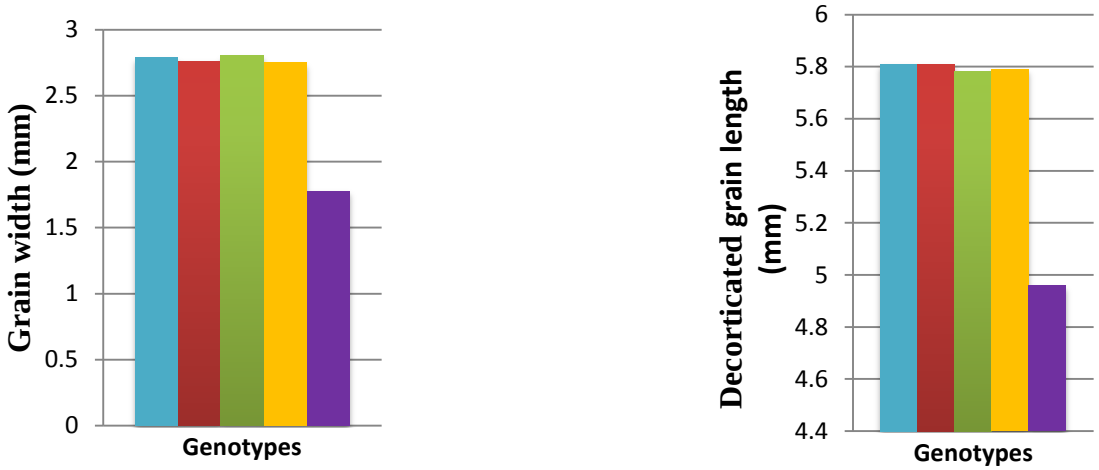


H) Grains/panicle

Figure 7 (E) to 7 (H). Morphological characteristics of R-genes introgressed BC_1F_1s and parents

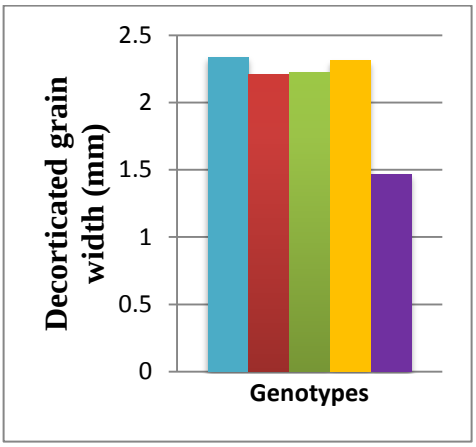


I) 1000 grain weight **J) Grain length**



K) Grain width **L) Decorticated grain length**

Figure 7 (I) to 7 (L). Morphological characteristics of R-genes introgressed BC_1F_1s and parents



M) Decorticated grain width

Figure 7 (M). Morphological characteristics of R-genes introgressed BC_1F_1s and parents

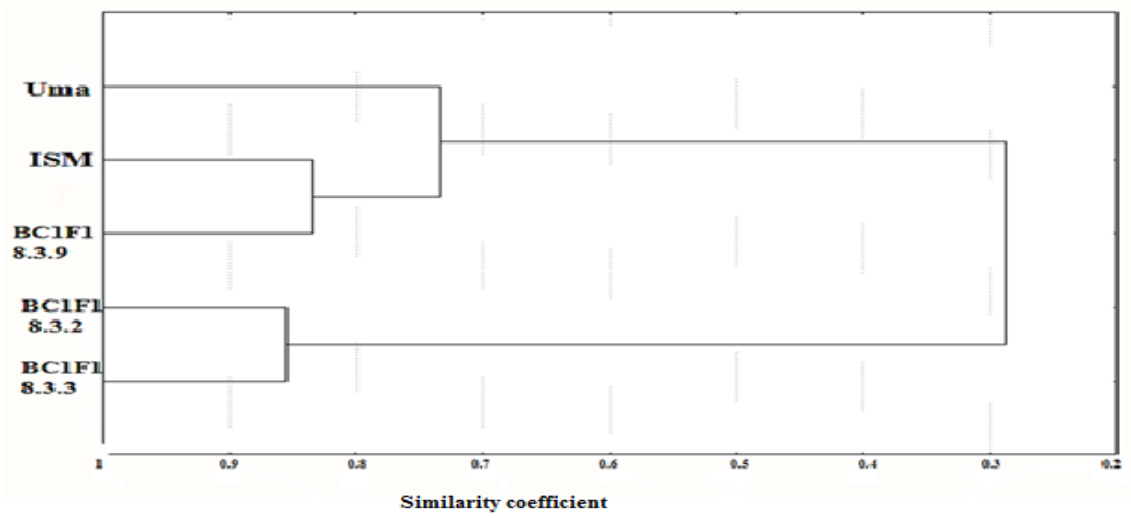


Figure 8. Clustering of BC_1F_1s and parental genotypes based on morphological characters

4.2.15. Straw yield / plant

The BC_1F_1s recorded an average straw yield per plant of 19.92 g. It ranged between 16.49 g (Plant no.1.1.2) and 23.19 g (Plant no.21.3.3). An average straw yield of 19.69 g was recorded in the recurrent parent Uma while it was 12.76 g in donor parent ISM.

4.3. Clustering of R-genes introgressed BC_1F_1s and parents based on morphological characters

Clustering of the three R gene introgressed BC_1F_1s and parents based on morphological characters grouped the recurrent parent Uma, ISM and BC_1F_1plant no. 8.3.9 into a single cluster and the other two BC_1F_1s *i.e.,* plant no. 8.3.2 and plant no. 8.3.3 into a separate cluster at 70 per cent similarity (Figure 8).

4.4. Production of BC_2F_1s

The three R-genes introgressed BC_1F_1s *i.e.,* plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9 were backcrossed to the recurrent parent Uma to obtain BC_2F_1s (Table 15). Of the seven panicles produced by BC_1F_1 8.3.2, four of them with 67, 89, 98 and 86 spikelets were backcrossed with the recurrent parent Uma. This resulted in six BC_2F_1s.

The plant no.8.3.3 produced twelve panicles. Nine of these were backcrossed to recurrent parent Uma which resulted in 12 seeds.

Spikelets on six out of nine panicles in plant no.8.3.9 were backcrossed to parent Uma resulting in ten BC_2F_1s.

4.5. Production of BC_1F_2s

Selfing three panicles out of the seven panicles in BC_1F_1 plant no. 8.3.2, resulted in the production of 273 BC_1F_2 seeds while in plant 8.3.3, 284 BC_1F_2 seeds were obtained. Selfing in plant 8.3.9 produced 293 BC_1F_2 seeds (Table 15).

Table 15. BC_2F_1s and BC_1F_2s produced from the R genes introgressed BC_1F_1s

Sl. No.	**BC_1F_1**	**BC_2F_1s (nos.)**	**BC_1F_2s (nos.)**
1	Plant no. 8.3.2	6	273
2	Plant no 8.3.3	12	284
3	Plant no. 8.3.9	10	293
Total		**28**	**850**

Discussion

V. Discussion

Rice is the staple food and major source of carbohydrate for the people of Kerala. However, the supply of rice in the state is far less than the existing demand. Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae (Xoo)* is one of the most devastating diseases that occurs recurrently in the rice growing belts of the state. The widely grown elite variety Mo 16 (Uma) is found to be highly susceptible to BB pathogen. Yield reduction due to the disease is reported to vary from 74 per cent to 81 per cent (Srinivasan and Gnanamanickam, 2005). Considering the impact of the disease on food security and economy of the state, an attempt was made to introgress three R-genes (*xa5, xa13* and *Xa21*) into variety Uma from donor parent Improved Samba Mahsuri (ISM) through Marker Assisted Selection (MAS) under the project 'Rice-Gene pyramiding to develop cultivars with durable resistance to Bacterial Leaf Blight through Marker Assisted Selection'.The resultant F_1s were backcrossed to Uma (recurrent parent) to produce BC_1F_1s. The present study aimed to identify the R gene introgressed lines from among the BC_1F_1s thus produced and backcross the R gene introgressed BC_1F_1s to the recurrent parent (Uma) to produce BC_2F_1s. In addition, production of selfed generation *i.e.*, BC_1F_2s of the R gene pyramided BC_1F_1s were also envisaged. The results obtained are discussed in detail below.

5.1. Genotyping of the BC_1F_1s

The spread of the BB pathogen is rapid through water. Owing to the highly fragmented/terraced nature of the rice ecosystems in Kerala, the disease spreads quickly as the irrigation water flows in and out of the BB affected field into the neighboring healthy rice fields. Disease forecasting and disease management are the advocated options to check the spread of BB pathogen and to prevent crop loss. However, as the total chemical control is unavailable or impractical, disease forecasting is found to have limited utility (Murty and Devadath, 1982). Biological

control is found to be a cost effective and eco-friendly alternative to chemical control. The genus *Streptomyces* a gram-positive bacteria of Actinomycetes group is said to be a source of antibiotic active against *X. oryzae* (Phuong-Hoa *et al.,* 2012). Exploiting the antibacterial activity of *Streptomyces* is an effective control strategy widely used against all the races of BB pathogen without any negative impact on the crop. Spraying cowdung extract @ 20 g/1 was found to be a substitute for the costly phyto-antibiotic preparations (Mary *et al*., 2001).

The rice growing seasons in the state coincides with the monsoon showers. Spraying of antibiotics *e.g.* Streptocycline and cowdung extract recommended against the BB pathogen usually becomes ineffective due to washing off of the spray fluid. Moreover, neither antibiotics nor cowdung extract provide complete insulation of the crop from BB pathogen. Hence, although prophylactic measures and chemical control of the disease are recommended, durable resistance of varieties is a much better option to combat this biotic stress (Chen *et al*., 2011).

Many genes that exhibit complete resistance to this pathogen have been reported (Chen *et al*., 2011). Peng *et al.* (2015) reported that till date, the most effective and economic means to control BB disease is to introduce disease resistance genes into rice plants. Pyramiding of resistance genes into rice genotypes is advocated as an efficient strategy to ensure durable resistance against BB pathogen (Pink, 2002; McDowell and Woffenden, 2003). However, accumulating major R-genes for resistance into elite cultivars is laborious, time consuming and may prove difficult in case of existence of epistasis or involvement of many genes (Rao*et al.*, 2002; Akhtar *et al.*, 2010). Marker assisted selection has been suggested as an effective alternative to conventional breeding to pyramid R-genes (Joshi and Nayak, 2010). Marker assisted backcross (MAB) breeding approach has been successfully attempted for introgression of bacterial blight resistance genes into rice crop

(Sanchez *et al*., 2000; Singh *et al*., 2001; Narayanan *et al*., 2002). This strategy ensures precise identification of genotypes possessing the genes of interest and also makes sure that it resembles the recurrent parent in all other aspects except for the resistance.

5.1.1. Foreground selection

Sufficient quantity of good quality total genomic DNA extracted from 95 BC_1F_1 plants, were subjected to foreground selection along with the donor parent ISM and recurrent parent Uma using rice microsatellites.

STS marker RG 556 is found to be tightly linked to R gene *xa5* at a distance of 0.1cM. The PCR analysis of the genomic DNA of the 95 BC_1F_1s and the two parents using the STS marker after restriction digestion of the PCR product with Dra1 restriction enzyme did not produce any polymorphism. In all the BC_1F_1s and the parents, alleles of size 128 bp, 514 bp, 587 bp, 624 bp, 650 bp and 836 bp were present indicating that all the BC_1F_1 individuals and the parents carried the R gene *xa5* (Table 16). Similarly, when the DNA of BC_1F_1s were resolved on agarose gels using the functional marker xa5 SR and the banding pattern scored with reference to the parents, the BC_1F_1 individuals could be classified into homozygotes for both ISM and Uma alleles (186 bp). This further confirmed the presence of *xa5* gene in both the parents as well as in all the BC_1F_1s studied. Similar reports were reported by Tuyen and Lang (2004). They had also found monomorphic banding pattern on using the xa5 SR marker for BB resistance in several local rice cultivars.

R gene *xa5* is reported to provide only moderate resistance against the *Xoo* pathogen (Sundaram *et al.*, 2008). Studies by Bharathkumar *et al.*, 2008 revealed that resistance in rice cultivars with single BB resistance gene broke down in the field and

a R gene pyramid was found to be more durable. Higher level of resistance to the *Xoo* pathogen, than would be expected from the sum of the parental levels has been reported in multiple BB resistance gene pyramided lines compared to those with single resistance gene (Yoshimura *et al*., 1996, Huang *et al*., 1997 and Sundaram *et al*., 2008).

The PCR products on amplification with STS marker RG 136 followed by restriction digestion with Hinf1 enzyme produced homozygous alleles for the gene *xa13* in three BC_1F_1individuals (Plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9) as in the resistant donor parent ISM. Hence the BC_1F_1 plants, no. 8.3.2, no. 8.3.3 and no. 8.3.9 were also inferred to possess R gene *xa13*.

The 560 bp amplicon (Table 16) associated with the resistant allele in homozygous state from donor parent ISM was observed to be present in the above three BC_1F_1 individuals on using the functional marker *xa13* promoter. This confirmed that out of the 95 BC_1F_1individuals screened, the three BC_1F_1 plants have been introgressed with the two R-genes *xa5* and *xa13* in the homozygous state. All other BC_1F_1s possessed a single R gene (*xa5*). Swamy *et al*., (2006) reported that stacking of multiple BB resistance genes by MAS into the susceptible variety Pusa Basmati 1 background was most effective in imparting durable resistance. The lines which carried two or more BB resistant genes were reported to show a higher degree of resistance over lines containing single BB resistant genes. The single *Xa* gene containing lines IRBB 4 (*Xa4*), IRBB21 (*Xa21*), MH2R (*xa5*) were susceptible or only moderately resistant to the *Xoo* isolates. However, marker aided introgression of *xa13* and *Xa21* into MH2R showed clear resistance against the same *Xoo* isolates. The result indicated that combinations of R gene provided a broader spectrum of resistance to the disease. Such observations were also reported by Singh *et al*. (2001).

The STS marker pTA 248 is reported to be tightly linked to dominant R gene *Xa21*. It is reported to be located at a distance of 0.2cM from *Xa21* (Dokku *et al*., 2013). Hence, the marker pTA 248 has been widely used for precise and early detection of genotypes carrying the R gene *Xa21*. Out of the 95 BC_1F_1 individuals scored with the STS marker pTA 248, only the three, 2-R gene pyramided BC_1F_1s individuals namely plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9 were found to possess alleles (855 bp) similar to the donor parent ISM (Table 16). These were also found to be homozygous with the donor parent allele.

Foreground selection of the 95 BC_1F_1 individuals therefore revealed that only three BC_1F_1 plants (plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9) (Plate 7) were introgressed with the three R-genes *xa5, xa13* and *Xa21*. The study also pointed out the presence of R gene *xa5* in both parents ISM and Uma, as well as in the BC_1F_1s. The alleles of the R-genes in each of the three 3-R gene pyramids thus obtained were also found to be in the homozygous state as in the donor parent and therefore expected to show a higher degree of resistance to the BB pathogen. Gnanamanickam *et al*. (1999) had observed a sub-population of *Xoo* virulent to rice line IRBB21 from a pathogen isolate from Kerala. According to Priyadarsini and Gnanamanickam (1999), rice line NH56 carrying four R-genes, (*Xa4 + xa5 + xa13 + Xa21*) was found to be resistant to Kerala isolates of the *Xoo* pathogen. However, breakdown of resistance of cultivars has *Xa4* withbeen reported earlier by *et a* Mew*l.* (1992). Therefore introgression of the three R-genes (*xa5, xa13*and *Xa 21*) into a rice genotype would be most ideal to impart durable resistance against the BB pathogen.

The three gene combination (*xa5+xa13+Xa21*) of pyramiding was also reported to be highly effective in providing resistance to BB pathogen by several earlier workers (Sanchez *et al*., 2000; Singh *et al*., 2001; Nguyen *et al*., 2008; Sundaram *et al*., 2008; Shanti *et al*., 2010; Bharani *et al*., 2010; Salgotra *et al*., 2012;

Recurrent parent Uma

Donor parent Improved Samba Mahsuri

R-genes introgressed BC_1F_1s

Plate 7. R genes introgressed BC_1F_1s and parents

Pradhan *et al*., 2015b). Sundaram *et al.* (2008), suggested that a quantitative complementation through which the multiple genes have an additive effect on the overall resistance may be the reason for higher resistance offered by the multiple gene pyramided lines.

5.2. Background selection

Background selection in the BC_1F_1 generation may greatly help in enhancing the efficiency of marker assisted backcross breeding and help release a cultivar with enhanced BB resistance (Joseph *et al*., 2004). The background profiling of the three R gene introgressed BC_1F_1 plants (plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9) was done along with the donor parent ISM and the recurrent parent Uma using 22 rice microsatellite markers that were reported to exhibit polymorphism between the two parents Uma and ISM. All the three BC_1F_1s when analysed with the RM markers were found to exhibit the allele in the homozygous state as found in the donor parent ISM (Table 16). Results indicated that the alleles of the donor parent were present in the three R-genes pyramided BC_1F_1s in the homozygous state for the all other 21 markers analysed. Such higher proportion of donor fragments can be expected in the early backcross generations such as BC_1F_1. Predominance of donor alleles in the BC_1F_1s may be attributed to linkage drag from the donor. Higher linkage drag from the donor parent responsible mainly for the reduced background recovery has been earlier reported by Rajpurohit *et al*. (2011). The contribution of the recurrent parent increases by one-half with each generation of backcrossing while the undesirable donor allele contribution reduces considerably (Singh *et al.*, 2001). Repeated backcrossing followed by rigorous background selection through MAS is preferable to enhance the recurrent parent genome at a faster rate. Higher recovery of recurrent parent genome have been reported in the later generations of backcrossing *viz*., BC_2

and further generations (Sundaram *et al*., 2008; Salgotra *et al*., 2012; Suh *et al*., 2013).

However background profiling of the BC_1F_1 plant no. 8.3.2 with marker RM 307, revealed the presence of alleles from both the parents, ISM and Uma. This pointed out that plant no. 8.3.2 was heterozygous at RM 307 locus unlike the two R-gene pyramided BC_1F_1s (plants no. 8.3.3 and plant no. 8.3.9). Hence, the BC_1F_1 plant no. 8.3.2 could be expected to segregate for the allele in subsequent generation.

The use of rice microsatellite markers for background selection has been successfully used to estimate the recovery of recurrent parent genome. The expected recovery of background of recurrent parent in BC_1F_1 generation is 75 per cent (Meksem *et al*., 2009). In the present study, considering the segregation of the 22 markers that were reported to be polymorphic between the parents in the earlier study, the recurrent parent genome contribution among the three R gene pyramided BC_1F_1s was estimated. Among the three R-genes pyramided BC_1F_1 individuals, the recovery of recurrent parent genome was found to be high in plant no. 8.3.2 (23.90 %) while it was 21.80 per cent each in plant no. 8.3.3 and 8.3.9 (Table 13) but lower than the expected recovery (75 %). The contribution of the recurrent parent to the genome of the backcross progeny was less than the expected at each background generations of the cross between Samba Mahsuri and SS1113. Similar results were also reported in the transfer of the three resistance gene *xa5, xa13* and *Xa21*from SS1113 into a rice cultivar, Triguna and this low recovery of recurrent parent genome has been attributed to a 'pull', through still unknown mechanism which results in the inheritance of additional undesirable loci from the donor parent genome (Sundaram *et al*., 2008).

The lower recovery of recurrent parent genome may also be due to the extremely lower number of rice microsatellite markers used. The rice genome of 400-

450 Mbp size has around 50,000 gene loci. In the present study, only a small fraction of the marker loci has been covered (22 RM markers) and this might be the reason for the low recovery obtained. Repeated backcrossing of the pyramided lines to the recurrent parent has been found essential as in conventional breeding to obtain pyramided genotypes with maximum background recovery of recurrent parent. Pradhan *et al*. (2015a), Cuc *et al*. (2012), and Dash *et al*. (2016) have reported high recurrent parent genome contribution in advanced backcross generations. Rajpurohit *et al*. (2011) had observed 81.57 per cent to 92.10 per cent background recovery in BC_2F_2 progenies while Sundaram *et al.*, 2008 were able to pyramid three BB resistance genes *xa5, xa13* and *Xa21* using MAB breeding in an elite rice variety Samba Mahsuri along with nearly 97 per cent background recovery by BC_4F_1 generation through foreground and background selection during each backcross generations. Pradhan *et al*. (2015a), suggested that higher recovery of the recurrent parent genome in the later backcross generation may be attributed to a reduced linkage drag in the fragments flanking the three resistance genes (*xa5, xa13* and *Xa21*) and the use of more number of polymorphic microsatellite markers. Similar results were obtained earlier by Suh *et al.* (2013) and Dokku *et al*. (2013).

The dendrogram generated out of the marker data resulted in two clusters, one with the three R gene introgressed individuals along with the donor parent ISM. This indicated that the R-gene introgressed plants were genetically more similar to the donor parent ISM. Similar to the present study, the dendrogram generated by Dokku *et al*. (2008) grouped the 10 pyramided lines analysed and two parents into two clusters. Cluster I had the donor IRBB60 and four pyramided lines while cluster II had all the remaining lines and the recurrent parent Tapaswini.

According to Suh *et al.* (2013) and Dokku*et al*. (2013) production of further backcross and selfed generations of the R-genes introgressed lines will ensure the

Table 16. Segregation of molecular markers in R gene introgressed BC_1F_1s and parents

Sl. No.	Markers	Nature of amplification	Number of amplicon	Size of amplicon (bp)		
				Polymorphic		
				Donor parent (ISM)	R-gene introgressed BC_1F_1 plants	Recurrent parent (Uma)
Markers employed in the foreground selection						
1	xa5	Monomorphic	1	186		
2	RG 556	Monomorphic	6	836, 650, 624, 587, 514 and 128		
3	xa13	Polymorphic	2	560		328
4	RG 136	Polymorphic	4	625,388 and 206		868,388 and 206
5	pTA 248	Polymorphic	2	855		734
Markers employed in the background selection						
6	RM 1	Polymorphic	2	412		374
7	RM16	Polymorphic	2	497		410
8	RM 205	Monomorphic	1	327		
9	RM 214	Polymorphic	2	206		299
10	RM 252	Polymorphic	2	497		410
11	RM 254	Polymorphic	2	374		353
12	RM 307	Polymorphic	2	386	386 and 206 (Plant no. 8.3.2)	206
13	RM 5586	Polymorphic	2	327		206
14	RM 10871	Polymorphic	2	409		393
15	RM 11554	Polymorphic	3	467 and 220		453
16	RM 13910	Polymorphic	2	393		254
17	RM 14725	Polymorphic	2	508		476
18	RM 15026	Polymorphic	2	443		528
19	RM 15303	Polymorphic	2	443		528
20	RM 15561	Polymorphic	2	357		284

Segregation of molecular markers in R gene introgressed BC_1F_1s and parents

Sl. No.	Markers	Nature of amplification	Number of amplicon	Size of amplicon (bp)		
				Polymorphic		
				Donor parent (ISM)	R-gene introgressed BC_1F_1 plants	Recurrent parent (Uma)
21	RM 15583	Monomorphic	1	255		
22	RM 17182	Polymorphic	2	357		284
23	RM 19199	Polymorphic	2	476		532
24	RM 24616	Polymorphic	2	476		524
25	RM 26213	Polymorphic	2	458		504,
26	RM 26868	Polymorphic	2	410		443
27	RM 28267	Polymorphic	2	524		504

release of a cultivar with durable resistance to the *Xoo* pathogen. Considering this, backcrossing of the identified three R gene pyramided BC_1F_1s *i.e*., plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9, has yielded 28 BC_2F_1s (Table 16). These BC_2F_1s are to be subjected to further foreground and background profiling to identify and recover the 3-R gene pyramided lines with maximum recovery of recurrent parent (Uma).

Selfing would help achieve homozygosity at the various loci in an individual. Joseph *et al*. (2004) were able to recover 80.40 to 86.70 per cent recurrent parent background 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 by a combination of phenotypic and molecular marker aided selection. Hence, selfing of the three R gene pyramids were attempted. This has resulted in the production of 850 BC_1F_2s (Table 16). These may also be subjected to

foreground and background profiling to identify the stable 3-R gene pyramided lines and incorporate in further breeding programmes.

5.4. Morphological characterization of BC_1F_1 individuals

Morphological characterization of each backcross individuals (BC_1F_1s**)** and the parents (donor parent ISM and recurrent parent Uma) was done to assess the variability existing in the population. Backcross breeding programme aims to integrate one or few desirable traits into an otherwise desirable cultivar. In the present study, transfer of resistance genes to BB pathogen was envisaged into elite highly acceptable susceptible cultivar Uma. As opined by Sundaram *et al*. (2008), the complete recovery of yield and other morphological characters of recurrent parent must be ensured in the three gene introgressed lines as pyramiding the R-genes imparting resistance to the BB pathogen without recovery of yield and other characters would be futile as the developed lines may not be accepted by the farmers. Hence, it is imperative to ensure the recovery of the agro-morphological characters in the gene introgressed lines

Results indicated (Table 15) the presence of wide variability for the morphological characters across the BC_1F_1population. The agronomic evaluation of the BC_1F_1 population for plant height, days to 50% flowering, leaf width, leaf blade length, number of productive tillers, panicle length, number of spikelets and grains per panicle, 1000 grain weight, grain length and width, decorticated grain length and width, grain and straw yield (Figure 6) indicated the occurrence of segregants that were better than the parental genotypes while in a few genotypes the magnitude for the traits studied was found to be lower than that of the parental genotypes. However, the variation for characters plant height, leaf width, number of productive tillers, panicle length, number of spikelets and grains/ panicle, 1000 grain weight, decorticated and grain width and grain yield was comparatively lower with very few genotypes outperforming the recurrent and donor parents. For the traits like days to 50 per cent flowering, leaf blade length, grain and decorticated grain length, higher

frequency of BC_1F_1 individuals resembling the donor parent ISM was observed while for most of the traits the population tended towards the recurrent parent.

The three R-genes pyramided BC_1F_1s was found to be intermediate between the two parents for characters like plant height, leaf blade length, panicle length, spikelets/panicle, grains/panicle and 1000 grain weight (Figure 7). However, all the three pyramided individuals were late in flowering compared to the recurrent parent Uma. Two of these genotypes *i.e*., 8.3.2 (234days) and 8.3.3 (228 days) flowered later than the donor parent. The length of the leaf blade in these two genotypes was also found to be greater than that of the donor parent. However all the three introgressed individuals produced longer panicles than both the parents. Spikelets/ panicle and grains/panicle were very less for the three individuals. However, 1000 grain weight, grain length and width and decorticated grain length and width were almost similar to that of the recurrent parent Uma. The shape of the grain and kernel of the R-gene pyramids as per IRRI (1996) and DUS (Rani *et al*. 2004) respectively was also medium as in Uma (Plate 8). These plants also possessed red kernels unlike the white kernels of donor parent ISM. Hence it can be concluded that the three R-genes introgressed BC_1F_1s resembled the recurrent parent Uma with respect to grain and kernel characteristics. Similar findings on inheritance of grain and kernel characteristics of R-gene pyramids were reported by Joseph *et al*. (2004) and Sundaram *et al*. (2008).

The dendrogram generated out of the morphological characters (Figure 8) grouped the two R-genes pyramids, plant no. 8.3.2 and plant no. 8.3.3 into a single cluster while the recurrent parent Uma, donor parent ISM and the BC_1F_1plant no. 8.3.9 were grouped into another cluster. The difference in clustering of genotypes based on morphological and molecular data may be because the number of marker loci analysed were a few, as well as, the BC_1F_1 plant no. 8.3.2 and plant no. 8.3.3 had also registered longer days to flowering and higher leaf width than both the parents. Such wide variation in the BC_1F_1 generation is expected when the parental genotypes

Plate 8. Grains of BC_1F_1s and parents

involved are distinctly different from each other. The donor line ISM is a tall, long duration variety with medium slender grains and white kernels while the recurrent parent Uma is a medium tall variety of 115 -140 days duration with short bold grains and red kernels. Joseph *et al,* 2004 had reported low background recovery in a few pyramided lines which according to him could be improved by an addition of one more round of background selection in the further backcross generations. However, they had also recovered a few segregants with favourable characteristics of Pusa Basmati 1 with two BB resistant genes through MAS just in BC_1 due to stringent phenotypic selection. Vasal *et al*. (1993) and Babu *et al.* (2005) had however reported low frequency of 100% opaque hard kernelled quality protein maize segregants in the BC_2F_2 families. Sundaram *et al*. (2008) had obtained promising three R gene pyramided lines in the BC_4F_6 generation of the cross between Samba Mahsuri and donor line SS1113 yielding EDV, Improved Samba Mahsuri. Hence, it can be concluded that rapid line conversion strategy requires application of a combination of both marker aided and phenotypic selection approaches in handling segregating generation to fix target locus, reduction of linkage drag and recovery of maximum amount of recurrent parent genome. Higher success would be achieved through this approach by advancing the backcross population.

Use of molecular markers closely linked to BB resistance along with phenotype based-selection in the present study has resulted in identification of three 3-R gene pyramided BC_1F_1s (plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9) from among a population of 95 BC_1F_1s. This has greatly reduced the number of BC_1F_1individuals that are required to be backcrossed to the recurrent parent Uma to accelerate the recovery of recurrent parent genome. According to Dwivedi *et al.* (2007), efficient and precise identification of desired multigene pyramided genotypes is possible using the molecular marker assisted selection compared to the conventional breeding method.

Development of advanced lines with resistance gene combinations have practical breeding value by providing a wider spectrum of resistance against most of the existing isolates of BB in the region and will have a high impact on yield stability and sustainability of rice crop in the region (Singh *et al,* 2001). The three R-genes introgressed BC_1F_1s plants are expected to show resistance to the *Xoo* pathotypes prevalent in Kerala. Further backcrossing and selfing of the identified backcrossed individuals in combination with marker assisted evaluation of these lines for BB resistance will result in the production of advanced lines of Uma. Moreover, this will enable pathogen inoculation and disease scoring studies to ensure the presence of the three R-genes and also to assess the extent of resistance offered by the pyramided BC_1F_1plants to various strains of *Xoo* pathogen.


Summary

VI. Summary

The study 'Gene pyramiding for bacterial blight resistance in rice variety Uma (Mo 16)' was carried out at Kerala Agricultural University (KAU), Vellanikkara during 2014-2016. The BC_1F_1 generation plants developed under the DBT project: 'Rice-Gene pyramiding to develop cultivars with durable resistance to Bacterial Leaf Blight through Marker Assisted Selection' constituted the study material. The study aimed to identify BC_1F_1 plants pyramided with bacterial blight resistance genes (*xa5, xa13* and *Xa21*) using molecular markers and also the production of BC_1F_2 and BC_2F_1 generations of the genotypes thus identified. The study was executed in four phases *viz*., I) Genotyping of BC_1F_1 population, II), Morphological characterization of BC_1F_1s III) Production of BC_2F_1s and IV) Production of BC_1F_2s. The salient findings of the study are summarized below.

Foreground selection of BC_1F_1s

1. Sufficient quantity of good quality total genomic DNA was extracted from 95 BC_1F_1 plants, the donor parent ISM and recurrent parent Uma
2. The PCR analysis of the genomic DNA of the 95 BC_1F_1s and the two parents using the STS marker RG 556 did not produce any polymorphism.
3. Restriction digestion of the PCR product of STS marker RG 556 with Dra1 restriction enzyme resulted in production of alleles of size 128 bp, 514 bp, 587 bp, 624 bp, 650 bp and 836 bp. This indicated that all the BC_1F_1 individuals and the parents carried the R gene *xa5*.
4. Analysis of 95 BC_1F_1s on amplification with the functional marker xa5 SR produced alleles of size 186 bp in all the BC_1F_1 plants and the parents, which confirmed the presence of R gene *xa5* in both the parents as well as all the BC_1F_1s studied.
5. The PCR products of the 95 BC_1F_1s and the two parents on amplification with STS marker RG 136 linked to R gene *xa13* also did not generate polymorphic amplicons.

6. However, restriction digestion of the PCR product of STS marker RG 136with Hinf1 produced alleles similar to that of the donor parent ISM in three BC_1F_1s namely, plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9, indicating the presence of R gene *xa13*in these plants.
7. The use of functional marker xa13 pro resulted in the production of 560bp allele associated with the resistant allele of gene *xa 13* in homozygous state from donor parent ISM in the three BC_1F_1s mentioned above. This confirmed that the three BC_1F_1s namely, plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9 possessed two R-genes *xa5* and *xa13*.
8. Out of the 95 BC_1F_1 individuals scored with the STS marker pTA 248 linked to R gene *Xa 21*, only the three 2 R-genes pyramided BC_1F_1s individuals namely no. 8.3.2, no. 8.3.3 and no. 8.3.9 were found to possess alleles (855 bp) similar to the donor parent ISM. These were also found to be homozygous with the donor parent allele.
9. Through foreground selection using R gene linked PCR based markers, three BC_1F_1s individuals (plant no. 8.3.2, no. 8.3.3 and no. 8.3.9) were identified to be pyramided with the three R-genes *xa 5, xa 13* and *Xa 21*.

Background selection of the 3-R gene introgressed BC_1F_1s

1. Background selection of the three R-genes introgressed BC_1F_1s using 22 rice microsatellite markers located on the chromosomes 1, 2, 3, 4, 5, 9, 11 and 12 were found to exhibit the allele in the homozygous state as found in the donor parent ISM.
2. PCR analysis with the marker RM 307 however, revealed the presence of alleles from both the parents, ISM and Uma in the BC_1F_1 plant no. 8.3.2 indicating that the plant was heterozygous at the marker locus and can be expected to segregate for the alleles at this locus in subsequent generation.

3. Considering the segregation of the 22 rice microsatellite markers that were reported to be monomorphic between the parents, the per cent recurrent parent genome in the three R gene introgressed $BC_1F_1$was estimated to be higher in plant 8.3.2 (23.90%) and 21.80 per cent each in $BC_1F_1$8.3.3 and BC_1F_1 8.3.9.
4. The dendrogram generated out of the marker data grouped the R-gene introgressed BC_1F_1 into two clusters. Cluster I was a monogenic cluster with only the recurrent parent Uma. The three R-genes introgressed BC_1F_1s namely plant 8.3.2, plant 8.3.3 and plant 8.3.9 and donor parent ISM in Cluster II. This indicated that the three R gene introgressed BC_1F_1s exhibited similarity with the donor parent Uma.

Morphological characterization of BC_1F_1s

1. Wide variability in morphological characters was observed among the BC_1F_1individuals studied.
2. The agronomic evaluation of the BC_1F_1 population for plant height, days to 50 per cent flowering, leaf width, leaf blade length, number of productive tillers, panicle length, number of spikelets and grains per panicle, 1000 grain weight, grain length and width, decorticated grain length and width, grain and straw yield indicated the occurrence of segregants that were better than the parental genotypes (Uma and ISM) while in a few genotypes the magnitude for the traits was found to be lower than that of the parental genotypes.
3. The variation for characters plant height, leaf width, number of productive tillers, panicle length, number of spikelets and grains/ panicle, 1000 grain weight, decorticated and grain width and grain yield was comparatively

lower with very few genotypes outperforming the recurrent parent and donor parent.

4. For the traits like days to 50 per cent flowering, leaf blade length, grain and decorticated grain length, higher frequency of BC_1F_1 individuals resembling the donor parent ISM was observed while for most of the traits the population tended towards the recurrent parent.
5. The three R gene pyramided BC_1F_1s was found to be intermediate between the two parents for characters like plant height, leaf blade length, panicle length, spikelets/panicle, grains/panicle and 1000 grain weight.
6. All the three pyramided individuals were late in flowering compared to the recurrent parent Uma. Two of these genotypes *i.e.,* 8.3.2 (234days) and 8.3.3 (228 days) flowered later than the donor parent. The length of the leaf blade in these two genotypes was also found to be greater than that of the donor parent.
7. All the three introgressed individuals produced longer panicles than both the parents.
8. The grain length and shape as well as the decorticated grain length and shape of R-genes introgressed BC_1F_1 plants were medium as in recurrent parent Uma.
9. These R-genes introgressed plants also possessed red, medium sized kernels as observed in the recurrent parent Uma.
10. The dendrogram generated out of the morphological characters grouped the two R-genes pyramids, plant no. 8.3.2 and plant no. 8.3.3 into a single cluster while the recurrent parent Uma, donor parent ISM and the BC_1F_1plant no. 8.3.9 were grouped into another cluster.

Production of BC_2F_1s and BC_1F_2s

1. The three R-genes introgressed BC_1F_1s *i.e.,* plant no. 8.3.2, no. 8.3.3 and no. 8.3.9 were backcrossed to the recurrent parent Uma to obtain BC_2F_1s. This has resulted in 28 BC_2F_1s.
2. A total of 850 BC_1F_2 seeds were obtained by selfing of the three R gene introgressed BC_1F_1s (plant no. 8.3.2, 8.3.3 and 8.3.9).
3. Marker assisted evaluation of the backcrossed and selfed individuals for BB resistance coupled will help identify 3-R gene introgressed individuals. Further backcrossing of the identified R gene introgressed individuals will result in the advanced lines with maximum genome recovery of recurrent parent Uma.

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Appendices

Appendix I. Quantity and quality of DNA of the BC_1F_1s

Sl. No	Genotype	Quantity (µg/ml)	Quality	Sl. No.	Genotype	Quantity (µg/ml)	Quality
1	1.1.1	95	1.82	49	5.3.5	87	1.92
2	1.1.2	70	1.84	50	6.1.1	93	1.83
3	1.1.3	80	1.90	51	6.1.2	72	1.81
4	1.1.4	91	1.99	52	6.1.3	64	1.84
5	1.1.5	92	1.86	53	6.1.4	80	1.82
6	1.1.6	59	1.92	54	6.1.5	65	1.87
7	1.1.7	75	1.94	55	6.2.1	70	1.85
8	1.1.8	70	1.93	56	6.2.2	60	1.93
9	1.1.9	58	1.92	57	7.1.1	80	1.80
10	1.1.10	90	1.98	58	7.1.2	66	1.87
11	1.1.11	80	1.82	59	7.1.3	80	1.81
12	1.1.12	64	1.91	60	7.1.4	63	1.87
13	1.1.13	82	1.82	61	7.4.1	55	1.86
14	1.1.14	83	1.91	62	7.4.2	75	1.84
15	1.1.15	85	1.97	63	7.4.3	62	1.90
16	1.1.16	72	1.82	64	8.1.1	75	1.88
17	1.1.17	80	1.96	65	8.1.2	95	1.86
18	2.1.1	90	1.81	66	8.1.3	72	1.86
19	2.1.2	75	1.83	67	8.3.1	80	1.89
20	2.1.3	59	1.91	68	8.3.2	76	1.83
21	2.2.1	90	1.88	69	8.3.3	84	1.87
22	2.2.2	93	1.98	70	8.3.4	80	1.89
23	2.2.3	84	1.95	71	8.3.5	65	1.93
24	3.1.1	65	1.88	72	8.3.6	95	1.97
25	3.1.2	80	1.97	73	8.3.7	87	1.87
26	3.1.3	90	1.87	74	8.3.9	91	1.95
27	3.1.4	87	1.98	75	12.1.1	92	1.86
28	3.1.5	80	1.92	76	12.1.2	97	1.82
29	3.1.6	67	1.82	77	12.1.3	78	1.89
30	3.2.1	83	1.82	78	14.2.1	84	1.80
31	3.2.2	75	1.89	79	14.2.2	66	1.92
32	3.2.3	80	1.91	80	14.2.3	90	1.90
33	3.2.4	96	1.91	81	14.3.1	67	1.80
34	3.2.5	85	1.92	82	14.3.2	55	1.90

Quantity and quality of DNA of the BC_1F_1s

Sl. No	Genotype	Quantity (µg/ml)	Quality	Sl. No.	Genotype	Quantity (µg/ml)	Quality
35	4.1.1	63	1.86	83	21.1.1	72	1.60
36	4.1.2	90	1.80	84	21.1.2	65	1.80
37	4.1.3	80	1.92	85	21.1.3	69	1.90
38	4.2.1	70	1.88	86	21.1.4	80	1.90
39	4.2.2	65	1.86	87	21.1.5	90	1.82
40	4.3.1	95	1.95	88	21.2.1	58	1.86
41	4.3.2	98	1.88	89	21.2.2	82	1.82
42	5.1.1	70	1.86	90	21.2.3	96	1.93
43	5.1.2	62	1.81	91	21.2.4	67	1.87
44	5.1.3	60	1.88	92	21.2.5	97	1.89
45	5.3.1	55	1.97	93	21.3.1	92	1.90
46	5.3.2	53	1.94	94	21.3.2	68	1.92
47	5.3.3	95	1.88	95	21.3.3	84	1.97
48	5.3.4	83	1.93				

Appendix II. List of markers used for parental polymorphism survey

Sl. No.	Chromosome no.	Number of markers analysed	Number of polymorphic markers identified	Name of the polymorphic markers
1	**1**	42	6	RM 1 RM 11554 RM 10871 RM 11342 RM 12253 RM 3340
2	**2**	30	4	RM 485 RM 561 RM 13910 RM 13599
3	**3**	30	10	RM 16 RM 15016 RM 15026 RM 15843 RM 15861 RM 7324 RM 14725 RM 14487 RM 15583 RM 15561
4	**4**	41	11	RM 214 RM 252 RM 307 RM 5586 RM 6679 RM 470 RM 5270 RM 17182 RM 17377 RM 6089 RM 17669
5	**5**	35	2	RM 19199 RM 13
6	**6**	26	3	RM 589 RM 402 RM 439
7	**7**	29	3	RM 21345 RM 22171 RM 248

List of markers used for parental polymorphism survey

Sl. No.	Chromosome no.	Number of markers analysed	Number of polymorphic markers identified	Name of the polymorphic markers
8	**8**	33	3	RM 23087 RM 339
9	**9**	22	3	RM 205 RM 24616 RM 434
10	**10**	22	1	RM 25217
11	**11**	29	4	RM 254 RM 27172 RM 26213 RM 26868
12	**12**	25	2	RM 28267 RM 27863
	Total	**364**	**52**	

Appendix III (a). Morphological characterization of BC_1F_1s

Plant no.	Plant height (cm)	Days to 50% flowering	Leaf width (cm)	Leaf blade length (cm)	Productive tillers / plant	Panicle length (cm)	Spikelets / panicle	Grains / panicle
1.1.1	40.80	131	0.84	23.14	7.00	16.18	106.50	92.50
1.1.2	42.10	132	0.83	22.35	8.00	18.49	108.10	86.80
1.1.3	40.10	131	0.88	21.69	8.00	16.75	110.60	95.60
1.1.4	41.80	134	0.86	22.41	7.00	19.24	92.40	87.60
1.1.5	46.40	133	0.98	26.73	8.00	18.91	103.90	90.20
1.1.6	50.20	130	0.99	23.99	6.00	19.54	112.50	90.10
1.1.7	56.30	134	0.87	23.37	7.00	19.64	97.50	86.50
1.1.8	48.90	126	0.72	26.21	7.00	19.24	102.10	92.10
1.1.9	40.50	132	0.72	24.12	8.00	19.81	96.40	79.20
1.1.10	48.30	131	0.85	23.14	7.00	18.24	89.60	76.40
1.1.11	45.30	137	0.79	24.79	5.00	16.94	94.50	80.40
1.1.12	47.20	127	0.82	24.72	6.00	15.62	88.50	78.60
1.1.13	43.60	134	0.86	23.70	7.00	18.92	102.90	90.30
1.1.14	49.50	126	0.88	29.40	8.00	16.28	88.60	76.90
1.1.15	50.20	128	0.67	24.06	7.00	17.54	105.70	92.40
1.1.16	48.20	129	0.99	30.64	8.00	19.61	104.60	91.80
1.1.17	51.20	130	0.74	23.63	6.00	15.28	97.60	87.60
2.1.1	50.60	135	0.85	22.81	6.00	16.94	95.60	85.20
2.1.2	48.90	134	0.78	26.30	5.00	18.27	93.80	80.60
2.1.3	62.30	131	0.82	28.98	6.00	19.47	102.70	90.70
2.2.1	51.30	135	1.18	29.43	7.00	18.19	92.50	81.60
2.2.2	53.60	130	0.99	26.42	8.00	19.82	86.70	79.40
2.2.3	49.60	134	1.22	30.12	6.00	16.27	105.80	86.80
3.1.1	50.10	140	1.24	30.60	5.00	17.14	104.50	90.90
3.1.2	59.40	137	1.35	29.34	5.00	16.94	98.80	86.90
3.1.3	50.60	134	0.99	42.62	7.00	18.27	95.30	82.60
3.1.4	49.10	132	0.86	43.50	6.00	19.76	89.60	79.10
3.1.5	51.40	138	1.11	47.60	8.00	16.49	103.40	90.70
3.1.6	49.60	138	1.26	26.88	5.00	18.91	96.80	85.90
3.2.1	41.40	134	0.96	20.66	5.00	14.78	103.50	92.50
3.2.2	56.20	132	1.04	26.78	6.00	19.64	95.40	84.20
3.2.3	51.30	131	1.24	27.88	7.00	19.60	96.70	82.60
3.2.4	56.70	135	1.27	26.95	7.00	18.25	92.40	83.90
3.2.5	48.90	132	1.07	25.88	6.00	16.48	94.80	80.50
4.1.1	50.40	132	0.88	21.17	6.00	19.54	95.60	89.50
4.1.2	59.10	136	0.94	20.91	6.00	16.08	92.80	86.40
4.1.3	62.10	140	0.91	22.15	7.00	18.27	101.80	90.40
4.2.1	68.40	135	1.04	23.53	8.00	18.61	98.30	86.20
4.2.2	65.10	132	0.92	25.71	8.00	19.64	91.40	80.40
4.3.1	68.20	137	0.91	35.25	8.00	17.28	101.80	92.50
4.3.2	57.40	135	1.12	46.80	7.00	19.54	106.50	94.80
5.1.1	53.70	134	0.90	37.03	7.00	16.91	105.60	99.80
5.1.2	67.40	137	0.92	46.50	8.00	17.64	92.50	86.70
5.1.3	57.80	135	1.06	41.30	7.00	18.92	98.80	81.50

Appendix III (a). Morphological characterization of BC_1F_1s

Plant no.	Plant height (cm)	Days to 50% flowering	Leaf width (cm)	Leaf blade length (cm)	Productive tillers / plant	Panicle length (cm)	Spikelets / panicle	Grains / panicle
5.3.1	49.50	136	0.92	29.13	7.00	17.84	96.80	82.60
5.3.2	42.60	129	0.99	32.45	8.00	19.64	98.90	81.70
5.3.3	39.60	137	0.87	31.86	9.00	16.07	86.40	76.90
5.3.4	44.80	138	1.06	28.88	7.00	15.23	87.30	72.40
5.3.5	50.60	138	1.12	42.61	8.00	16.21	102.10	84.90
6.1.1	48.70	132	0.99	26.78	8.00	17.94	103.50	89.90
6.1.2	46.50	135	0.98	38.65	8.00	15.67	105.80	92.80
6.1.3	50.60	132	1.02	47.25	7.00	16.27	104.90	88.90
6.1.4	46.30	129	1.22	39.25	8.00	18.08	99.80	80.70
6.1.5	51.20	134	0.98	27.86	7.00	19.42	104.60	91.20
6.2.1	57.08	135	1.16	27.85	7.00	18.26	96.50	81.40
6.2.2	67.20	135	1.04	26.47	8.00	18.91	91.20	80.70
7.1.1	62.10	132	1.04	27.64	8.00	16.94	86.50	79.80
7.1.2	60.90	135	0.93	28.48	7.00	18.23	88.90	78.20
7.1.3	56.30	134	1.11	24.53	5.00	19.89	92.80	81.60
7.1.4	55.60	136	0.92	26.94	8.00	18.76	93.70	83.60
7.4.1	54.20	132	1.01	22.57	6.00	13.57	102.50	92.80
7.4.2	67.30	135	1.06	29.38	8.00	16.27	92.40	80.70
7.4.3	62.20	139	1.2	27.06	5.00	16.94	86.4	71.3
8.1.1	60.80	140	1.05	29.65	7.00	18.91	98.6	82.4
8.1.2	60.60	139	1.01	33.88	8.00	18.27	89.5	75.6
8.1.3	63.10	138	1.05	32.79	8.00	17.93	99.4	79.5
8.3.1	58.90	140	0.89	34.70	7.00	17.92	106.5	93.5
8.3.2	48.97	234	1.40	44.88	7.00	20.38	87.57	66.57
8.3.3	51.12	228	1.60	43.96	12.00	21.63	87.66	79.25
8.3.4	59.20	132	0.94	35.46	7.00	19.57	104.90	92.40
8.3.5	68.30	138	1.02	29.82	6.00	16.58	98.90	86.90
8.3.6	71.20	140	1.02	30.14	8.00	17.82	110.50	90.10
8.3.7	66.40	139	0.92	29.12	9.00	16.24	97.50	89.90
8.3.8	58.40	140	0.84	37.17	8.00	18.46	99.20	87.60
8.3.9	43.21	158	1.10	37.51	9.00	19.98	90.11	87.44
12.1.1	53.30	132	1.02	36.50	8.00	19.51	98.70	84.60
12.1.2	46.80	128	1.12	35.70	7.00	16.27	92.50	80.90
12.1.3	48.60	134	0.99	38.70	8.00	14.27	93.60	79.80
14.2.1	42.80	134	0.98	32.10	8.00	16.49	92.50	82.60
14.2.2	56.30	136	1.02	35.20	10.00	18.72	91.40	78.90
14.2.3	55.90	141	1.01	36.40	9.00	19.25	102.50	90.50
14.3.1	41.20	142	0.94	32.10	7.00	16.48	105.90	90.50
14.3.2	51.60	147	1.01	39.70	9.00	19.64	98.80	84.60
21.1.1	62.30	138	0.96	50.60	9.00	16.57	99.80	87.50
21.1.2	58.90	140	1.02	49.30	8.00	18.64	102.80	92.80
21.1.3	52.60	137	0.99	45.50	8.00	16.19	108.90	91.70
21.1.4	48.70	139	1.02	39.80	7.00	18.93	97.40	84.90
21.1.5	52.90	140	1.22	46.50	8.00	16.23	103.50	90.20
21.2.1	56.40	135	1.01	42.60	9.00	16.94	96.50	82.60
21.2.2	49.80	142	1.22	39.80	9.00	18.46	99.40	81.40

Appendix III (a). Morphological characterization of BC_1F_1s

Plant no.	Plant height (cm)	Days to 50% flowering	Leaf width (cm)	Leaf blade length (cm)	Productive tillers / plant	Panicle length (cm)	Spikelets / panicle	Grains / panicle
21.2.3	52.50	148	0.98	41.40	8.00	17.68	89.70	76.40
21.2.4	58.80	136	0.87	47.40	9.00	16.58	98.70	81.20
21.2.5	49.70	148	0.99	41.60	7.00	19.24	99.30	80.30
21.3.1	53.60	137	1.04	48.30	9.00	19.24	110.50	82.90
21.3.2	49.60	128	0.99	38.70	7.00	17.32	98.90	84.60
21.3.3	48.70	132	1.02	36.80	6.00	16.91	102.50	83.70
Mean	54.75	136.62	1.02	33.00	7.31	17.88	97.63	84.75

Appendix III (b). Grain characters of BC_1F_1s

Plant no.	1000 grain weight (g)	Grain length (mm)	Grain width (mm)	Decorticated grain length (mm)	Decorticated grain width (mm)	Grain yield/ plant (g)	Straw yield / plant (g)
1.1.1	15.20	6.80	2.70	5.60	2.40	14.06	18.91
1.1.2	15.60	6.50	2.50	5.10	2.20	13.54	16.49
1.1.3	18.90	7.00	2.90	6.20	2.50	18.06	20.34
1.1.4	19.60	6.90	2.80	5.30	2.30	17.17	22.53
1.1.5	15.90	6.50	2.70	5.80	2.40	14.34	17.81
1.1.6	18.40	6.80	2.60	5.70	2.20	16.57	19.24
1.1.7	20.50	6.80	2.80	5.20	2.50	17.73	20.27
1.1.8	16.70	7.00	2.70	6.40	2.10	15.38	19.57
1.1.9	22.60	6.90	2.90	5.80	2.30	17.89	20.53
1.1.10	23.40	6.80	2.40	5.60	2.10	17.87	21.34
1.1.11	17.90	6.80	2.80	5.70	2.30	14.39	19.54
1.1.12	21.80	6.30	2.80	5.90	2.40	17.13	22.48
1.1.13	18.60	6.90	2.90	5.80	2.50	16.79	20.12
1.1.14	24.20	6.50	2.60	5.70	2.20	18.61	22.34
1.1.15	17.80	7.00	2.50	6.10	2.10	16.44	20.47
1.1.16	19.10	6.80	2.80	5.60	2.40	17.53	22.51
1.1.17	18.20	6.90	2.90	5.80	2.60	15.94	20.13
2.1.1	18.90	6.50	2.90	5.90	2.50	16.11	19.28
2.1.2	16.20	6.90	2.70	5.60	2.40	13.05	17.19
2.1.3	17.90	6.60	2.80	5.70	2.50	16.23	19.81
2.2.1	19.50	6.70	2.60	5.60	2.40	15.91	19.81
2.2.2	16.20	6.90	2.60	5.40	2.60	12.86	17.54
2.2.3	17.50	6.80	2.90	5.90	2.50	15.19	20.14
3.1.1	19.50	6.50	2.80	5.60	2.20	17.72	21.3
3.1.2	16.50	6.60	2.90	5.30	2.40	14.33	18.24
3.1.3	17.30	6.90	2.60	5.80	2.30	14.29	19.46
3.1.4	20.80	7.00	2.90	6.40	2.10	16.45	19.24
3.1.5	21.40	6.90	2.80	5.90	2.30	19.41	23.15
3.1.6	18.60	6.80	2.90	5.70	2.10	15.97	19.24
3.2.1	18.60	6.80	2.60	5.80	2.20	17.20	21.34
3.2.2	15.30	6.90	2.70	5.90	2.10	12.88	16.57
3.2.3	19.10	6.80	2.80	5.60	2.30	15.78	19.62
3.2.4	17.80	6.90	2.90	5.80	2.50	14.93	19.24
3.2.5	18.90	7.00	2.80	6.50	2.40	15.21	18.91
4.1.1	18.90	6.80	2.60	5.70	2.20	16.91	20.18
4.1.2	20.30	6.50	2.90	5.60	2.30	17.54	21.16
4.1.3	18.90	6.30	2.80	5.90	2.10	17.09	22.56
4.2.1	17.80	6.90	2.90	5.80	2.30	15.34	19.24
4.2.2	16.50	6.40	2.80	5.90	2.40	13.26	18.16
4.3.1	21.50	6.80	2.90	5.90	2.40	19.88	23.14
4.3.2	16.80	6.70	2.70	5.60	2.50	15.92	20.14
5.1.1	19.50	6.90	2.50	5.70	2.10	19.46	22.16
5.1.2	18.70	6.80	2.60	5.80	2.40	16.21	21.18
5.1.3	15.60	7.00	2.90	6.40	2.50	12.71	16.81

Appendix III (b). Grain characters of BC_1F_1s

Plant no.	1000 grain weight (g)	Grain length (mm)	Grain width (mm)	Decorticated grain length (mm)	Decorticated grain width (mm)	Grain yield/ plant (g)	Straw yield / plant (g)
5.3.1	18.60	6.80	2.80	5.60	2.20	15.36	19.24
5.3.2	17.30	6.50	2.90	5.70	2.30	14.13	18.24
5.3.3	19.10	6.90	2.60	5.90	2.40	14.69	19.42
5.3.4	20.50	6.70	2.80	5.60	2.50	14.84	17.82
5.3.5	16.90	6.60	2.70	5.80	2.60	14.34	18.12
6.1.1	18.60	6.30	2.80	5.90	2.40	16.72	19.34
6.1.2	14.60	6.90	2.90	5.80	2.30	13.52	17.51
6.1.3	18.90	6.80	2.60	5.60	2.20	16.80	19.24
6.1.4	20.50	7.00	2.70	6.20	2.30	16.54	20.13
6.1.5	19.50	6.80	2.80	5.70	2.40	17.78	22.18
6.2.1	20.50	6.70	2.80	5.90	2.40	16.68	19.24
6.2.2	22.90	6.80	2.60	5.80	2.30	18.48	22.13
7.1.1	20.10	6.60	2.80	5.60	2.30	16.03	20.14
7.1.2	16.90	6.50	2.90	5.80	2.50	13.21	18.42
7.1.3	18.30	6.90	2.60	5.80	2.40	14.93	18.16
7.1.4	20.50	7.00	2.80	6.20	2.50	17.13	20.43
7.4.1	20.30	6.50	2.80	5.90	2.40	18.83	22.15
7.4.2	16.90	6.90	2.90	5.70	2.30	13.63	18.24
7.4.3	16.70	6.80	2.80	5.30	2.50	11.91	16.54
8.1.1	19.20	6.90	2.60	5.80	2.20	15.82	19.24
8.1.2	20.60	6.80	2.90	5.90	2.60	15.57	20.43
8.1.3	18.90	6.50	2.70	5.60	2.20	15.02	19.81
8.3.1	18.20	6.70	2.80	5.80	2.50	17.01	21.16
8.3.2	18.90	6.12	2.76	5.81	2.21	-	-
8.3.3	17.65	6.18	2.80	5.78	2.22	-	-
8.3.4	19.40	7.00	2.60	6.30	2.20	17.92	20.43
8.3.5	20.50	6.90	2.80	5.70	2.30	17.81	20.43
8.3.6	18.60	6.80	2.50	5.90	2.10	16.76	20.16
8.3.7	20.30	6.30	2.90	5.40	2.50	18.25	22.46
8.3.8	15.90	6.90	2.80	5.60	2.40	13.92	18.52
8.3.9	12.32	6.20	2.75	5.79	2.31	-	-
12.1.1	18.60	6.90	2.80	5.60	2.40	15.73	19.24
12.1.2	17.10	6.80	2.90	5.90	2.50	13.83	18.52
12.1.3	20.30	6.60	2.70	5.80	2.60	16.19	19.24
14.2.1	20.50	6.50	2.80	5.80	2.50	16.93	19.24
14.2.2	19.20	6.80	2.60	5.90	2.30	15.15	20.18
14.2.3	16.10	6.90	2.90	5.70	2.20	14.57	18.42
14.3.1	18.90	6.20	2.50	5.70	2.10	17.12	22.43
14.3.2	20.30	6.80	2.60	5.60	2.30	17.17	21.61
21.1.1	20.50	6.50	2.80	5.90	2.50	17.94	20.35
21.1.2	18.70	6.90	2.60	5.80	2.30	17.35	20.42
21.1.3	19.60	6.80	2.90	5.60	2.60	17.97	22.14
21.1.4	20.40	6.90	2.80	5.80	2.40	17.32	20.18
21.1.5	16.80	6.50	2.70	5.70	2.50	15.15	19.24
21.2.1	18.90	6.90	2.80	5.40	2.50	15.61	19.23
21.2.2	20.40	6.50	2.90	5.80	2.60	16.60	19.24

Appendix III (b). Grain characters of BC_1F_1s

Plant no.	1000 grain weight (g)	Grain length (mm)	Grain width (mm)	Decorticated grain length (mm)	Decorticated grain width (mm)	Grain yield/ plant (g)	Straw yield / plant (g)
21.2.3	18.90	6.80	2.60	5.70	2.40	14.43	19.17
21.2.4	19.40	6.70	2.80	5.80	2.30	15.75	19.37
21.2.5	20.70	6.60	2.70	5.90	2.50	16.62	19.48
21.3.1	20.60	6.90	2.80	5.80	2.40	17.07	20.14
21.3.2	19.80	6.80	2.60	5.90	2.40	16.75	22.34
21.3.3	15.60	6.90	2.90	5.70	2.60	13.05	23.19
Mean	18.75	6.73	2.75	5.77	2.37	15.95	19.94

Appendix IV (a). Morphological characterization of recurrent parent Uma

Plant no.	Plant height (cm)	Days to 50% flowering	Leaf width (cm)	Leaf blade length (cm)	Productive tillers / plant	Panicle length (cm)	Spikelets / panicle	Grains / panicle
1.1	67.51	125.80	1.12	38.37	7.40	17.16	101.56	90.52
2.1	70.93	127.50	1.10	35.81	7.70	17.62	101.43	85.25
2.2	69.20	127.00	1.10	45.25	7.20	17.49	98.67	82.59
3.1	68.20	127.70	0.99	45.11	7.60	19.63	96.38	82.51
3.2	69.95	127.10	1.00	45.88	9.10	18.17	99.61	87.00
4.1	69.86	127.20	0.97	51.07	7.90	16.81	97.66	83.89
4.2	66.82	124.30	0.92	50.17	8.40	18.01	98.24	85.82
4.3	69.34	124.20	1.04	48.09	8.90	17.76	98.98	86.34
5.1	73.04	129.30	0.99	55.34	8.90	17.61	94.24	82.56
5.2	66.79	131.80	1.08	47.62	7.90	17.71	97.97	85.69
6.1	66.73	123.40	1.05	46.83	8.40	18.14	93.8	79.02
6.2	68.57	130.10	1.05	52.09	8.00	18.24	95.07	83.48
7.1	70.90	124.10	1.01	54.39	8.50	17.78	90.66	79.29
7.4	67.75	130.90	0.89	53.08	7.20	17.99	100.34	89.96
8.1	67.02	128.20	1.04	45.92	8.40	17.42	97.00	84.37
8.3	67.02	132.50	0.99	47.29	8.10	18.37	94.20	82.59
12.1	56.97	130.00	1.08	46.95	7.80	17.98	100.84	90.53
14.2	60.17	128.00	1.08	50.22	8.90	17.53	96.24	83.01
14.3	60.82	127.00	1.15	53.24	8.50	17.89	96.24	83.86
21.1	57.92	126.80	1.05	48.33	9.80	17.62	96.70	82.93
21.2	59.31	127.80	0.96	50.74	8.80	18.55	94.79	80.57
21.3	63.62	125.30	1.09	52.96	8.60	17.88	96.94	84.00
Mean	66.29	127.54	1.03	48.39	8.27	17.88	97.16	84.35

Appendix IV (b). Grain characters of recurrent parent Uma

Plant no.	1000 grain weight (g)	Grain length (mm)	Grain width (mm)	Decorticated grain length (mm)	Decorticated grain width (mm)	Grain yield/ plant (g)	Straw yield / plant (g)
1.1	18.52	6.21	2.75	5.32	2.46	16.83	19.93
2.1	17.96	6.46	2.70	5.68	2.22	15.44	19.93
2.2	17.74	6.38	2.78	5.60	2.36	14.79	18.71
3.1	17.43	6.18	2.76	5.90	2.42	14.31	19.21
3.2	18.14	6.24	2.79	5.87	2.41	15.93	20.21
4.1	18.60	6.19	2.78	5.84	2.26	15.63	19.67
4.2	17.50	6.24	2.76	5.83	2.42	15.01	19.53
4.3	17.72	6.34	2.76	5.79	2.29	15.33	19.88
5.1	18.62	6.17	2.79	5.72	2.29	15.35	18.88
5.2	17.70	6.19	2.79	5.96	2.41	15.11	19.30
6.1	18.94	6.91	2.83	6.07	2.34	14.98	19.10
6.2	18.04	2.24	2.83	5.95	2.38	14.96	18.86
7.1	19.24	6.35	2.81	5.90	2.40	15.32	19.44
7.4	19.42	6.12	2.77	5.85	2.30	17.50	19.46
8.1	17.20	6.24	2.79	5.92	2.34	14.52	20.11
8.3	17.68	6.22	2.81	5.83	2.36	14.65	19.02
12.1	19.17	6.13	2.70	5.99	2.25	17.33	20.73
14.2	21.03	6.35	2.80	5.96	2.24	17.44	21.18
14.3	19.44	6.59	2.83	5.94	2.31	16.31	19.57
21.1	19.98	6.72	2.85	5.87	2.33	16.88	19.95
21.2	20.06	6.35	2.86	5.83	2.34	16.11	19.33
21.3	21.04	6.58	2.82	6.03	2.35	17.67	21.18
Mean	18.69	6.15	2.79	5.85	2.34	15.79	19.69

Appendix V (a). Morphological characterization of donor parent ISM

Plant	Plant height (cm)	Days to 50% flowering	Leaf width (cm)	Leaf blade length (cm)	Productive tillers / plant	Panicle length (cm)	Spikelets / panicle	Grains / panicle
1.1	44.04	144.4	0.88	28.31	6.60	21.75	108.51	72.27
2.1	46.25	147.8	0.86	31.00	8.60	21.95	92.11	79.37
2.2	47.19	146.1	0.92	31.45	9.10	21.88	97.89	81.78
3.1	49.39	150.3	0.94	38.10	10.40	22.11	98.27	85.05
3.2	52.53	146.7	0.85	40.67	8.40	22.49	93.67	83.24
4.1	49.16	145.8	0.94	37.69	8.50	22.53	94.29	83.09
4.2	49.53	151.6	0.86	38.74	9.80	22.84	109.23	79.88
4.3	44.96	148.2	0.85	32.72	8.00	23.03	99.97	82.09
5.1	50.70	149.9	0.91	36.84	8.30	23.04	93.66	84.95
5.2	51.30	149.8	0.88	37.78	9.70	22.32	85.85	74.57
6.1	48.26	146.8	0.91	38.59	9.60	21.58	93.03	83.11
6.2	52.43	149.1	0.88	42.75	9.00	22.32	91.84	83.87
7.1	50.99	147.3	0.90	37.10	9.20	22.14	98.6	87.28
7.4	48.89	151.5	0.89	38.81	8.80	21.93	120.21	95.06
8.1	48.88	146.8	0.90	38.72	8.70	20.82	101.41	89.05
8.3	48.55	147.3	0.98	40.21	9.60	21.58	97.27	86.66
12.1	45.72	146.7	1.06	34.38	7.90	21.62	101.65	88.90
14.2	48.57	148.0	1.09	40.26	8.10	21.37	103.38	88.66
14.3	45.03	146.0	1.04	37.62	7.60	23.06	95.35	88.02
21.1	46.59	147.2	1.03	38.51	7.90	22.89	99.95	87.78
21.2	42.07	145.9	1.03	33.03	8.00	21.93	98.63	87.28
21.3	44.62	148.0	1.06	33.85	8.50	22.28	100.28	86.76
Mean	**47.98**	**147.78**	**0.94**	**36.69**	**8.65**	**22.16**	**98.86**	**84.49**

Appendix V (b). Grain characters of donor parent ISM

Plant no.	1000 grain weight (g)	Grain length (mm)	Grain width (mm)	Decorticated grain length (mm)	Decorticated grain width (mm)	Grain yield/ plant (g)	Straw yield / plant (g)
1.1	10.84	6.39	1.86	5.23	1.57	7.82	10.44
2.1	10.50	6.35	1.86	5.31	1.54	8.32	11.54
2.2	10.36	6.51	1.85	5.03	1.55	8.46	11.69
3.1	10.66	6.60	1.79	5.16	1.52	9.07	13.10
3.2	10.82	6.39	1.88	5.21	1.59	8.91	11.44
4.1	10.60	6.67	1.82	5.38	1.45	8.82	13.02
4.2	11.20	6.43	1.88	5.46	1.56	8.94	13.27
4.3	10.48	6.47	1.96	5.67	1.61	8.62	12.34
5.1	10.76	6.38	1.92	5.81	1.62	9.12	12.51
5.2	11.12	6.42	1.78	5.37	1.47	8.28	11.55
6.1	10.08	6.32	1.85	5.94	1.55	8.40	12.67
6.2	11.18	6.62	1.83	5.26	1.40	9.37	13.91
7.1	10.16	6.68	1.66	5.34	1.42	8.85	13.38
7.4	9.12	6.68	1.64	4.93	1.34	8.71	13.23
8.1	10.62	6.64	1.65	5.00	1.41	9.46	14.14
8.3	10.14	6.61	1.58	5.76	1.46	8.79	13.05
12.1	9.53	6.75	1.69	5.91	1.34	8.48	12.22
14.2	9.75	6.8	1.74	5.91	1.37	8.65	13.37
14.3	9.72	6.74	1.78	5.91	1.45	8.56	13.63
21.1	9.66	6.74	1.73	5.82	1.40	8.48	12.96
21.2	9.72	6.79	1.75	5.95	1.42	8.47	13.23
21.3	10.06	6.75	1.73	5.95	1.35	8.74	14.14
Mean	**10.32**	**6.58**	**1.78**	**5.51**	**1.47**	**8.69**	**12.76**

GENE PYRAMIDING FOR BACTERIAL BLIGHT RESISTANCE IN RICE VARIETY UMA (Mo 16)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Exploiting host-plant resistance through pyramiding of resistance genes have been recommended as the best approach to impart durable resistance to rice varieties in order to combat the bacterial blight (BB) disease caused by *Xanthomonas oryzae* pv.*oryzae* (*Xoo*). In lieu of this, F_1s were produced by hybridizing the susceptible elite rice variety Uma with resistant donor parent Improved Samba Mahsuri (ISM) harbouring three R-genes *xa5, xa13* and *Xa21*. BC_1F_1 individuals were generated by backcrossing the F_1s using variety Uma as the recurrent parent. The present study aimed to identify the R-genes introgressed individuals in the BC_1F_1 population as well as to produce BC_2F_1s and BC_1F_2s of the identified R-genes introgressed BC_1F_1s.

Foreground selection of the BC_1F_1 individuals was done using the R gene linked molecular markers. Restriction digestion of the PCR product of STS marker RG 556, linked to R gene *xa5,* with Dra1 restriction enzyme, resulted in production of alleles of size 128 bp, 514 bp, 587 bp, 624 bp, 650 bp and 836 bp in all the BC_1F_1 individuals as well as the parents indicating the presence of R gene *xa5* in all the individuals studied. Amplification of DNA of the individuals with the functional marker xa5 SR further confirmed the presence of R gene *xa5* in both the parents as well as in all the BC_1F_1s.

Restriction digestion of the PCR product of STS marker RG 136, linked to R gene *xa13,* with Hinf1, produced alleles similar to that of the donor parent ISM in three BC_1F_1s namely, plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9, indicating the presence of R gene *xa13* in these plants. The presence of gene *xa13* in the identified BC_1F_1s was further affirmed by using the functional marker xa13 promoter. The analysis had resulted in the production of 560bp allele associated with the resistant allele of gene *xa13* in homozygous state from donor parent ISM in the three BC_1F_1s mentioned above.

Out of the 95 BC_1F_1 individuals scored with the STS marker pTA 248 linked to R gene *Xa21,* only BC_1F_1s plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9 were found to possess *Xa 21*. Results thus obtained revealed thatBC_1F_1plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9 were R gene pyramids (*xa 5+xa 13+ Xa 21*).

Background profiling of the three R-genes introgressed BC_1F_1s using 22 rice microsatellite markers, revealed presence of the donor parent allele in the homozygous state. PCR analysis with the marker RM 307, however, revealed the presence of alleles from both the parents, ISM and Uma in the BC_1F_1 plant no. 8.3.2. This indicated that the plant was heterozygous at the marker locus and can be expected to segregate for the alleles at this locus in subsequent generations.

Considering the segregation of the 22 markers the per cent recurrent parent genome recovery in the R-genes introgressed BC_1F_1s was estimated to be higher in BC_1F_1 plant no.8.3.2 but lower than the expected estimate of 75 per cent. This was also confirmed by graphical genotyping. The dendrogram thus generated out of the marker data, grouped the R-genes introgressed BC_1F_1s with ISM indicating that the three R-genes introgressed BC_1F_1s exhibited greater similarity with donor parent parent ISM at the genome level.

Evaluation of BC_1F_1 individuals for morphological traits revealed presence of wide variability. The three R-genes introgressed BC_1F_1s were late in flowering compared to the recurrent parent Uma. Two of these genotypes *i.e.*,plant no. 8.3.2 (234 days) and plant no. 8.3.3 (228 days) flowered later than the donor parent. However, the three R-genes introgressed BC_1F_1s resembled the recurrent parent Uma with respect to grain and kernel characteristics.

Backcrossing the three R-genes introgressed BC_1F_1s *i.e.,* plant no. 8.3.2, plant no. 8.3.3 and plant no. 8.3.9 to the recurrent parent Uma resulted in 28 BC_2F_1s. Simultaneously, selfing of these individuals produced 850 BC_1F_2 seeds. Foreground and background profiling of these generations can ensure precise identification of genotypes that resembles the recurrent parent Uma possessing the resistance genes of interest with maximum recovery of recurrent parent genome.