MARKER ASSISTED SELECTION FOR BACTERIAL LEAF BLIGHT RESISTANCE GENES IN THE BACKCROSS PROGENIES OF PRATHYASA VARIETY OF RICE (*Oryza sativa* L.)

by

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COLLEGE OF AGRICULTURE

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2019

DECLARATION

I, hereby declare that this thesis entitled **"Marker assisted selection for bacterial leaf blight resistance genes in the backcross progenies of Prathyasa variety of rice (*Oryza sativa* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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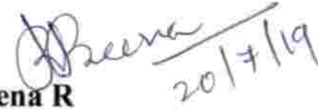

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LIST OF ABBREVIATIONS AND SYMBOLS USED

°C	Degree Celsius
μ	Mean
μl	Micro litre
μg	Micro gram
μM	Micro molar
%	Per cent
BC_1F_1	First filial backcross generation
BC_2F_1	Second filial backcross generation
BC_2F_2	Second filial second backcross generation
BLB	Bacterial leaf blight
bp	base pair
CAPS	Cleaved Amplified Polymorphic Sequences
cm	Centimetre
DNA	Deoxy ribonucleic acid
dNTP	Deoxy ribonucleoside triphosphate
dsDNA	Double stranded DNA
CTAB	Cetyl trimethyl ammonium bromide
ED	Euclidean distance
EDTA	Ethylene diamine tetra acetic acid
et al.	And others

F_2	Second filial generation
FM	Functional Marker
Fig.	Figure
g	Gram
ha	Hectare
HCl	Hydrochloric acid
i.e.	that is
ISSR	Inter-simple sequence repeats
KCl	Potassium chloride
kg	Kilo gram
m	Metre
mm	Millimetre
M	Molar
MAS	Marker Assisted Selection
MAB	Marker assisted backcross
MABB	Marker assisted backcross breeding
mg	Milligram
$MgCl_2$	Magnesium chloride
min	Minutes
mM	Milli molar
NaCl	Sodium chloride
ng	Nanogram

nm	Nanometre
No.	Number
OD	Optical Density
PCR	Polymerase Chain Reaction
$Plant^{-1}$	Per plant
$Panicle^{-1}$	Per panicle
pM	Pico Molar
pv.	Pathovar
PVP	Poly Vinyl Pyrrolidone
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic marker
RCF	Relative centrifugal force
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
RNase	Ribonuclease
RPG	Recurrent parent genome
rpm	Revolutions per minute
S. D	Standard deviation
S. E	Standard Error
SCAR	Sequence Characterized Amplified Region
sec	Seconds
sp. or spp.	Species (Singular and Plural)

SSR	Simple sequence repeats
TAE	Tris-Acetate-EDTA
TE	Tris EDTA
Taq	Thermus aquaticus
Tm	Annealing Temperature
U	Unit
UV	Ultra Violet
v/v	Volume / Volume
viz.	Namely
w/v	Weight / Volume
Xoo	*Xanthomonas oryzae* pv *oryzae*

Introduction

1. INTRODUCTION

Rice (*Oryza sativa* L.) is agronomically and nutritionally, most important food crop of the world serving as the staple food of nearly 3.2 billion people. More than 90 per cent of the world's rice is grown and consumed in Asia (Khush, 2005). In Indian scenario, 41% (104.32 million tonnes) of the total food grain production (252.22 million tonnes) and 35% (43.39 million hectares) of the total area under food grains is rice (122.65 million hectares, GOI, 2016). However, the national productivity of crop is merely 2400 kg ha^{-1}, which is far behind the potential productivity of several released varieties. In Kerala, the area and production of this crop is diminishing year by year. In 2017-18, the area and production of rice were 194235 hectares and 521310 tonnes holding average productivity of 2684 kg ha^{-1}. The area and production of rice in Kerala had decreased by 47% and 38% respectively with respect to that in 2001-02 (GOK, 2018). Several reasons may be listed for this scenario.

Rice plays a pivotal role in the Indian economy and is the staple food for two-thirds of the population. To meet increasing food demands, concrete efforts are required to boost rice productivity by minimizing production losses due to pests and diseases.

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a devastating disease of rice resulting in yield losses of up to 80% depending on the stage of the crop, cultivar susceptibility and the environmental conditions (Srinivasan and Gnanamanickam, 2005). The disease was not reported only from Asian countries, but also from Africa, Latin America, Carribean and USA (Chu *et al.*, 2006). In India, the disease reached epidemic proportions throughout the country by 1975 (Rangaswami, 1975). In Kerala, it was reported to be severe in additional crop season of June-October. After the great flood of Kerala in 2018, the rice fields were substantially affected by this disease (Satish, 2018).

Management measures of BLB include the application of chemicals, biocontrol, disease forecasting and enhancing host plant resistance. However, to

establish permanent yield stability by economical and eco-friendly means host plant resistance is considered as the best. BLB resistant varieties are developed by introgressing resistance genes from diverse gene sources within the germplasm (Khush *et al.*, 1989)

The longterm cultivation of the monogenic resistant varieties resulted in a significant shift in the virulence pattern of the pathogen causing break down of resistance (Mew *et al.*, 1992). The best available solution for this problem is to pyramid multiple resistance genes into a single varietal background. The chance of pathogen population to overcome the pyramided resistance is much rare than that of single gene resistance (Mundt, 1990). Conventional breeding methods are inefficient for gene pyramiding as they cannot manage the evaluation of multiple resistance genes having similar expression and also the inheritance of the recessive resistance genes.

Marker assisted selection (MAS) addresses these limitations of conventional breeding and boosts up the pace of gene pyramiding. The availability of molecular markers closely linked to or located within the resistance genes (i.e. functional markers) makes the task of gene pyramiding easier (Sanchez *et al.*, 2000).

Functional markers have already been developed for the important BLB resistance genes *xa13*, *Xa21* and *xa5* namely, xa13pro, pTA248 and xa5FM respectively (Ronald *et al.*, 1992; Rao *et al.*, 2002; Sundaram *et al.*, 2011; Hajira *et al.*, 2016) and these can be used for marker assisted pyramiding of these genes.

The recovery of essential plant characteristics and agro-morphological traits of the recurrent parent is essential in gene pyramiding. The repeated backcrossing is employed to improve the recurrent parent genome recovery. However a strict selection for the morphometric traits and quality characters in breeding lines improves the RPG recovery and it could reduce the number of backcrosses (Joesph *et al.*, 2004).

Prathyasa is a semi dwarf, short duration, medium tillering variety with medium bold red kernelled grains and good cooking quality. However, this variety which was released specifically to suit the additional crop season of Kuttanadu due to its short duration, is highly susceptible to BLB.

Under these circumstances a project entitled "Development of rice varieties for Kerala with pyramided genes for resistance to BLB by Marker Assisted Selection" supported by Department of Biotechnology (DBT), Government of India, was undertaken at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani to pyramid the BLB resistance genes *xa13*, *Xa21* and *xa5* from donor line Improved Samba Mahsuri into the varieties Aiswarya and Prathyasa through marker assisted selection.

The present study was a part of the above project to identify the introgression of BLB resistance genes *xa13*, *Xa21* and *xa5* in the backcross progenies of Prathyasa variety of rice through marker assisted selection and to evaluate these lines morphologically to assess Prathyasa phenome recovery.

Review of Literature

2. REVIEW OF LITERATURE

Rice, the principal staple crop of India, feeds more than 60% of the country's population. The current production of the crop in India is around 104 million tonnes (GOI, 2016), but the demand for the produce is expected to increase significantly in the near future due to ever growing population of the country. Even though, green revolution and hybrid rice technologies had increased rice production and productivity tremendously in the past three decades, the production and productivity gains have to be protected from major biotic and abiotic stresses. Among these stresses, insect pests and diseases are major constraints of rice production. Increasing productivity by managing these curbs through conventional means of chemical application not only result in high cost but also cause serious environmental threats. In such a context genetic enhancement of host-plant resistance is the prime and best solution till date.

Host plant resistance is depicted as an active and dynamic response of the plants to a pest either by resisting infection, infestation or colonization. This includes all the inherent mechanisms of the plant that enables it to resist, withstand, lessen and overcome the attacks of pests and pathogen. The gene for gene relationships between pest and host, as well as knowledge of vertical and horizontal resistance, provided genetic validity for developing varieties with resistance genes. However, the varieties with mono resistance genes will not be effective because the long term monoculture of such resistant varieties may result in significant variation in the virulence pattern of pathogen population leading to breakdown of resistance and evolution of new races of pathogen (Khush, 1971; Mew *et al.*, 1992). Thus an efficient strategy to delay the breakdown of resistance is pyramiding multiple resistance genes for obtaining enhanced durable resistance. Thus a valid, fruitful, durable and convenient method to enhance host plant resistance is gene pyramiding. Studies substantiated this and reported that the degree, as well as the spectrum of resistance, would be enhanced by pyramiding major resistance genes in elite cultivars (Singh *et al.*, 2001; Kottapalli *et al.*, 2010; Suh *et al.*, 2013).

The rice genome project and advances in genomics assisted in mapping a series of commercially important genes using linkage and DNA markers. Among them, a wide variety of disease and pest resistance genes having consistent expression against these stresses and their linked markers were also identified. Forty-two major genes against bacterial leaf blight (Vikal and Bhatia, 2017), ninety-nine major genes encoding resistance against rice blast (Wang *et al.*, 2014), thirty-six major genes and sixty-three QTLs of resistance to brown plant hopper, fifteen major genes and seventy-two QTLs of resistance against white backed plant hopper, fourteen major genes and seventy two QTLs against green leaf hopper (Fujita *et al.*, 2013), eleven major genes of resistance against gall midge (Himabindu *et al.*, 2010), and fifty QTLs against sheath blight (Vidya and Ramalingam, 2018) have been identified till date. Such a spectrum of resistance available within the germplasm of rice made a revolutionary advancement of resistance breeding of the crop. It also urged breeding for durable pest resistance through gene pyramiding.

The commercial acceptance, the genetic importance and consumer importance of the crop urged faster breeding programmes in gene pyramiding. The sequence data of numerous markers housed in world genome databases made it faster through marker assisted selection. This review of literature covers details of Bacterial Leaf Blight (BLB) disease and notable advances in breeding for BLB resistance through Marker Assisted Selection (MAS).

2.1 BACTERIAL LEAF BLIGHT DISEASE AND DISTRIBUTION

Bacterial leaf blight is one of the oldest recorded rice disease. It was first reported by farmers in 1884 in the southern region of Japan and was called "white withering disease" (Tagami and Mizukami, 1962). Its bacterial nature was established and the bacterium was described by Ishiyama in 1922. The disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Swing *et al.,* 1990), is one of the important disease of rice globally in both rainfed and irrigated agro-ecosystem. It is the most devastating disease of rice causing significant yield reduction under serious infestations in many rice growing countries. The incidence of this disease

was first reported from India by Srinivasan *et al.,* (1959) in Maharashtra. The disease is prevalent in almost all paddy growing regions in Maharashtra (Mizukami, 1964; Srivastava 1967). During 1975, the disease had reached epidemic proportions in Bihar and other neighbouring states (Rangaswami, 1975) and it is known to result in yield losses ranging from 74 to 81% in susceptible cultivars (Veena *et al*., 1996). The disease incidence ranges from 70 to 80% leading to significant crop damages (Sere *et al.,* 2005; Basso *et al.,* 2011).

The introduction and large scale commercial cultivation of high yielding but susceptible rice cultivars lead to the widespread of disease throughout the Indian subcontinent. The disease was also reported from China, Taiwan, Korea, Malaysia, Indonesia, Australia, Vietnam, and the Philippines. By now, it is recorded in almost all the rice-growing countries in Asia except those in the Middle East (Ou, 1985). It has also been reported in Africa: in Mali (Buddenhagen *et al*., 1979), the Cameroons (Notteghem and Baudin, 1981), and Senegal (Trinh, 1980). The disease has also been found in Latin America and the Caribbean (Mew *et al.,* 1993).

Although *X. oryzae* is not historically considered indigenous to the United States, strains of a yellow bacterium causing mild BLB-like symptoms were collected from rice fields in Texas and Louisiana in 1987 (Jones *et al*., 1989). The bacterium was classified as *X. oryzae* based on serological tests and fatty acid profiling but they were divergent from the identified pathovars from Asia and Africa causing BLB (Triplett *et al.,* 2011). Till date pathogen and the respective symptoms were not found in Europe except in USSR (Vzoroff, 1938).

In Kerala, the disease was first reported in rice growing tracts of Palakkad district in 1976. Later it was found to be endemic to the major rice bowls of Kerala *viz.*, Palakkad and Kuttanad (Mary, 1980). According to reports (Nair *et al*., 1990; Sreekumar, 1991), the disease was found to recur in epiphytotic proportion in the Kuttanad tract almost every year during the additional crop season from June-July to September-October. After the flood in 2018, the disease was encountered in both these areas in a substantial factor (Satish, 2018).

2.2 CAUSAL ORGANISM AND SYMPTOMS OF DISEASE

The *Xanthomonas oryzae* pv. *oryzae*, (*Xoo*) is a gram-negative, non-spore forming short rod with round ends 0.55 x 3.5-2.17µm and monotrichous flagellate bacterium. The pathogen is a member of the c-subdivision of the gram-negative γ-proteobacteria. It is aerobic and grows best at a temperature and pH of 25-30°C and 6.5-7.5 respectively (Ishiyama 1922). The isolate of bacterium varied in the rate of utilization of different carbon and nitrogen sources (Ou, 1985). Iron has been found to enhance the virulence of *X. oryzae* pv. *oryzae* (Ansari and Sridhar, 2001). *Xoo* produces typical circular convex, whitish yellow to straw yellow colonies with a smooth surface, entire margin, and opaque against transmitted light. The pathogen survives primarily in/on infected seeds, stubbles, straw, ratoons, self-sown plants and rhizosphere of winter crops and perennial wild plants, especially *Leersia oryzoides*, *Zizania latifolia*, *Leptochola chinensis*, *L. panacea*. and *Cyperus rotundus* and wild *Oryza* species O.*rufipogon* and *O. australiensis* (Singh *et al*., 1980; Thrimurthy and Devadath, 1981; Devadath, 1982; Sunder and Dodan, 1989). The inactive pathogen in the stubbles arises into growth form after receiving moisture when conditions are favourable.

BLB is a vascular disease whereby *Xanthomonas oryzae* pv. *oyzae* continues to grow until the xylem vessels are clogged with bacterial cells and xanthomonadins, which is a yellow soluble pigment and extracellular polysaccharide important in the protection of bacteria from desiccation. The bacterium invades through wounds caused by the root development or any other injuries occurred during handling, insect attack or through natural openings like hydathodes and stomata on leaves and becomes systemic in the xylem of rice plant (Devadath and Rao, 1975; Nada *et al*., 1981). Infection is favoured by a temperature of 25-30°C, high humidity, shading, heavy dose of nitrogenous fertilizers, rain, flooding, and severe winds. The bacterium can be disseminated by irrigation water, by splashing or windblown rain, by the plant to plant contact, by trimming tools used in transplanting, or by handling during transplanting (Devadath, 1982).

There are three main symptoms caused by bacterial blight *viz.*, wilt or 'Kresek', leaf blight, and yellow leaf or pale yellow. The wilt syndrome, known as 'Kresek' is the most destructive manifestation of the disease found between the temperature 28°C and 34°C. It occurs in tropics, affects the crop from the seedlings to the early tillering stage. The lesions are usually initiated at the edge of the upper part of leaves where hydathodes, through which the bacterium can invade, are distributed more frequently (Ou, 1985). Infected areas of the leaves can be detected before symptom appearance by means of immersing the cut ends in a diluted solution of basic fuchsin dye for one to two days (Goto, 1965). Leaves of infected plants wilt and roll up, turning greyish green. The leaves then turn yellow to straw-coloured and wither, and the entire plant generally dies. Plants that do survive are stunted and yellowish. Total crop failure is not uncommon with Kresek.

Leaf blight, the most common syndrome, generally occurs from the maximum tillering stage onwards. It begins as water-soaked stripes on the leaf blades. The stripes increase in length and width, become yellow and then white, and may coalesce to cover the entire leaf blade. Drops of bacterial exudates may be observed on young lesions forming beads or strands of exudate on the leaf surface, a characteristic sign of the disease and a source of secondary inoculum (Mew *et al.,* 1993). Older infected leaves may appear greyish from the growth of saprophytic fungi. Small, circular lesions with water-soaked margins may also form on the glumes with severe infections. If panicles are produced, the number of immature grains and sterility percentage will increase as the photosynthate production and accumulation is reduced due to the disease symptoms. Besides these typical symptoms, the leaves sometimes roll and wither, following infection. Infected plants produce fewer and lighter grains and the grain is of poor quality.

Yellow leaf or pale yellow syndrome in tropics is associated with bacterial leaf blight. Uniform pale yellow or a broad yellow stripe can be observed on the youngest leaf of the plant (Saha *et al.,* 2015). With the yellow leaf symptom, the bacteria are not present in the leaf itself but can be found in the internodes and crowns of affected stems.

2.3 HOST PLANT RESISTANCE FOR BACTERIAL LEAF BLIGHT RESISTANCE

Even though the pathogen infects, perpetuates and causes losses to several cultivars of the crop, many of the rice cultivars or germplasm have been found to exhibit varying genetic resistance against *X. oryzae* pv. *oryzae* strains. Thus, host plant resistance can be used as a tool for disease management (Nino-Liu *et al.*, 2005). As chemical and biological practices are inefficient in providing a substantial result, development of disease resistant cultivars through different breeding approaches seems to be the most important strategy to control this disease.

Genetic analysis of many plant-pathogen interactions has demonstrated that plants often contain a single locus that confers resistance against a complementary avirulence gene (Flor, 1951). Since BLB is the most destructive disease of rice, its disease diagnosis, management and control have been widely studied and scrutinized. However, enhancing genetic resistance has proven to be the most effective method of controlling BLB disease. In rice, the genetics of resistance to the pathogen has been well characterized and studied. The genetics of resistance to bacterial blight was first carried out by Japan and IRRI, subsequently, followed by Sri Lanka, India, China, and so on.

Effector (*avr*) genes and insertion sequences are the factors found to be playing a major role in generating a high degree of genetic diversity and race differentiation, evident from the genome structure analysis of *Xoo* (Ochiai *et al.*, 2005). There are several races of *Xoo*, which secrete race-specific effectors into the xylem to trigger individualized response and cause infection. The resistance genes (R genes) are activated by transcriptional factors released by the bacteria which bind and activate transcription of these genes resulting in resistance response (Hummel *et al.,* 2012). Avirulence factors are responsible to activate *Xoo* resistance genes that determine host specificity *via* gene-for-gene interactions. Avirulence factors are recognized by the host plant by which reducing the virulence of the pathogen. Since unique virulence and avirulence factors are being produced by each

race of *Xoo*, R genes have evolved to provide resistance to individual races of the pathogen (Nino-Liu *et al.*, 2006).

As there is a diversity of *Xoo* strains in different countries, scientists found that it was difficult to characterize and distinguish the resistance genes. In order to compare the identified genes, the identical differential standard was set up (Ogawa, 1993). Till date, about 42 BLB resistance genes, designated from *Xa1* to *xa42* (Cheema *et al.*, 2008; Kim *et al.*, 2015; Busungu *et al.*, 2016; Vikal and Bhatia, 2017), conferring resistance against various strains of *Xoo*, have been identified from the rice germplasm collections worldwide. Among these identified genes, approximately 64% are dominant genes including incomplete dominant genes and 15 genes (*xa5, xa8, xa13, xa15, xa19, xa20, xa24, xa25, xa26b, xa28, xa31, xa32, xa33, xa34* and *xa42*) are recessive (Chen *et al.*, 2011; Liang *et al.*, 2017; Vikal and Bhatia, 2017).

2.4 MECHANISM OF R-GENE MEDIATED RESISTANCE

To date, only nine R-genes have been isolated and cloned, including *Xa1, Xa3/Xa26, xa5, Xa10, xa13, Xa21, Xa23, xa25,* and *Xa27*, and five types of proteins are encoded by these genes suggesting multiple mechanisms of R-gene–mediated *Xoo* resistance. The five types of proteins are LRR receptor kinase protein, MtN3/saliva class protein, Transcription Activator-Like (TAL) effector-dependent class protein, NBS-LRR class proteins and a typical gamma subunit of transcription factor IIA (TFIIAγ) (Vikal and Bhatia, 2017).

Xa21 and *Xa3/Xa26* genes codes for LRR receptor kinase protein (Song *et al.,* 1995) which mediates almost relative immunity towards the *Xoo* strains. *Xa3/Xa26* codes for a plasma membrane-localized LRR kinase protein (Sun *et al.*, 2004). The interaction of E3 ubiquitin ligase with the kinase domain of *Xa21* protein provides a substrate for the *Xa21* serine and threonine kinase activity, leading to *Xa21*-mediated immunity by the full accumulation of *Xa21* protein (Wang *et al.*, 2006). MtN3/saliva class protein is produced by two R genes *viz.*, *xa13* and *xa25* both of which are recessive and confers race-specific resistance to

Philippine *Xoo* strain PXO339 (Chu *et al*., 2006; Liu *et al*., 2011). The TAL effector-dependent class proteins in the BLB resistance mechanism are the products of genes *Xa27, Xa10,* and *Xa23* (Gu *et al*., 2005; Tian *et al*., 2014; Wang *et al*., 2014; Wang *et al*., 2015). *Xa1* is the only gene so far identified to produce NBS-LRR class protein among BLB Resistance genes (Yoshimura *et al*., 1998; Vikal and Bhatia, 2017). The *xa5,* which is recessive belongs to the group which encodes a typical gamma subunit of transcription factor i.e. TFIIAγ, which is a common transcription factor essential for the transcription by RNA polymerase II (Iyer and McCouch, 2004).

2.5 MOLECULAR MAPPING OF BACTERIAL BLIGHT RESISTANCE GENES

The success of resistance breeding programs lies in the effort for the identification and characterization of major genes for resistance. The widespread massive cultivation of resistant varieties with a single R gene enabled the pathogen to evolve virulence against the resistance. Pyramiding multiple genes into the elite cultivars can delay this simultaneous evolution of pathovars. The possibility of the concurrent evolution of virulence in the pathogen against two or more resistance genes is much lower than for a single gene. The dominance and epistasis effects of putative R genes result in difficulty in gene pyramiding using conventional breeding methods. Moreover, the identification of genes with similar reactions to two or more races and their transfer through conventional approaches are inconvenient. However, closely linked molecular markers with each R-genes facilitate easier identification of plants with two or three genes. All the needed resistance genes in any breeding program can be traced using the dominant and co-dominant expression of linked molecular markers. Among the identified 42 resistance genes, gene-specific markers are available for *xa5*, *xa13,* and *Xa21* (Huang *et al*., 1997; Chunwongse *et al*., 1993).

The reported BLB R-genes are evenly distributed throughout the 12 rice chromosomes. Among these genes, 13 genes (*Xa3/Xa26*, *Xa4*, *Xa6*, *xa9*, *Xa10*,

Xa21, *Xa22*, *Xa23*, *Xa30*, *Xa32*, *Xa35*, *Xa39* and *Xa40*) are clustered on chromosome 11, whereas chromosomes 1, 9, and 10 are devoid of any BLB R-genes (Kou and Wang, 2013; Vikal and Bhatia, 2017).

Three molecular markers *viz.*, RAPD248, RAPD818, and RG103 had been identified by Ronald *et al.* (1992) were found to co-segregate with the gene *Xa21*. Yoshimura *et al.* (1995) developed detailed linkage map of chromosome 11 by integrating the conventional maps (Yoshimura, 1983; Ogawa *et al.*, 1986) with two RFLP maps (McCouch and Tanksley, 1991; Saito *et al.*, 1991). They reported that *Xa3* and *Xa4* loci were tagged with RFLP marker XNpb181 at the top of chromosome 11, at map distances of 2.3 cM and 1.7 cM, respectively. *Xa10* was also tagged with an RAPD locus $OO7_{2000}$ with the proximity of 5.3 cM to the gene.

In a mapping attempt for gene *Xa33,* fourty nine SSR markers were identified flanking the gene on both sides the gene on chromosome 7. The gene then was fine mapped between two SSR markers (RMWR7.1 and RMWR7.6) located at a genetic distance of 0.9 and 1.2 cM, respectively, from the gene and flanking it (Natrajkumar *et al.*, 2012).

The dominant resistance gene *Xa38* identified from *Oryza nivara* accession IRGC 81825 was mapped on long arm of chromosome 4. Based on gene annotation, cloning and sequencing of three NBS LRR loci identified in the target region *O. nivara* resulted in polymorphic marker LOC_Os04g53050, between the gene source (*O. nivara* accession IRGC 81825) and the cultivated rice. The polymorphism reported was due to a 48 bp deletion of the locus in *O. nivara* accession (Bhasin *et al.*, 2011). Such several works have been carried out and many linked molecular markers are available for each of the resistance genes.

2.5.1 Molecular Genetics of *xa13*

The completely recessive gene *xa13* was first identified by Ogawa *et al.* (1987) in the cultivar BJ1 and had been transferred into IR24 background and provided accession number as IRBB13. The gene specifically offers resistance to

Philippine *Xoo* race 6 and many Indian isolates. In RAPD and RFLP mapping by Zhang *et al.* (1996), the gene was tagged to an RAPD marker OPAC5-900 and three RFLP markers namely RZ28, CD0116 and RG136 on the telomeric region of chromosome 8 with a mapping distance 5.3 cM, 5.1 cM, 4.8 cM, and 3.7 cM respectively from the gene. Sanchez *et al.* (1999) fine mapped the gene on the long arm of chromosome 8 flanked by two RFLP markers, RG136, and R2027. Among those RG136 have been extensively utilized for marker assisted breeding programmes (Huang *et al.*, 1997; Singh *et al.*, 2001). Sundaram *et al.* (2011) developed a functional marker for *xa13* namely xa13pro targeting the nucleotide polymorphism associated with the gene. This co-dominant PCR based marker amplifies a ~500bp fragment in resistant genotypes and a ~250 bp fragment in susceptible genotypes.

Chu *et al.* (2006) carried out sequence analysis of the 14.8 kb fragment carrying *xa13* gene. This fragment possesses only two apparently intact candidate gene, i.e. an extensin-like gene and a homologue of nodulin (MtN3) and the 5′ end of a predicted hypothetical protein. In the same study PCR-based markers (E6a, SR6, ST9, SR11) that were tightly linked to *xa13* were also identified, which were user-friendly than the previously identified RFLP markers. According to the study of Yang *et al.* (2006), infection by *Xoo* strain PXO99A elevated the expression of the rice gene *Os8N3*, a member of the MtN3 gene family regulated by type III effector gene *PthXo-1* of *Xoo* strain PXO99A. *Os8N3* was located near *xa13* and found inactive in lines with *xa13*. According to the study, PXO99A resistant plants were obtained when *Os8N3* was silenced by means of inhibitory RNA silencing. In nutshell, it was understood that *Os8N3* was a susceptibility gene, which could be the dominant allele *Xa13,* driven by the same promoter resulting in susceptibility reaction upon pathogen infection.

2.5.2 Molecular Genetics of *Xa21*

Xa21 was originally identified in an African wild species *O. longistaminata* and confers broad-spectrum resistance to many Indian isolates of *Xoo*. The

accession was originally retained in India in CRRI, Cuttack. Later it was transferred to IRRI, Los Banos, Manila and found that it was resistant to all six known races of bacterial blight in the Philippines. The gene was then introgressed into the *O.sativa* cultivar IR24 (Khush *et al.*, 1989; Khush *et al.*, 1991).

Three molecular markers *viz.*, RAPD248, RAPD818, and RG103 had been identified by Ronald *et al.* (1992) and he found that they co-segregate with the gene *Xa21* on the long arm of chromosome 11. Later, RG103 was found tightly linked with the gene *Xa21* with a map distance of 1.2 cM. Then, pTA248, a PCR-based STS marker which was very closely linked (~0.1 cM) to the gene was developed. Eventually, it was clarified that the marker is within the gene (Rao *et al.*, 2002). Hence the marker pTA248, specific for the dominant gene *Xa21* was identified as a functional marker.

Bacterial artificial chromosome (BAC) libraries containing *Xa21* locus were constructed by adopting a map based strategy which later facilitated cloning of the gene (Wang *et al.*, 1994). Song *et al.* (1995) cloned, characterized and reported that a receptor Kinase-like protein with serine-threonine specificity (LRR kinase protein) was encoded by the gene *Xa21*, and it was the first BLB R-gene characterization. The *Xa21* gene activity observed was very minimal at the initial stages of crop growth and gradually increase with the crop growth. A complete resistance to the pathogen by the gene was reported at the adult stage (Century *et al.*, 1999). However, The broad spectrum resistance offered by the gene as well as the availability of tightly linked marker pTA248 enabled several breeders to deploy the gene singly or in combination in several cultivars worldwide. pTA248 amplifies a 950bp fragment in resistant and 660bp fragment in susceptible genotypes in a codominant fashion. Hence, it offers high utility for marker assisted introgression of the gene (Ronald *et al.*, 1992; Huang *et al.*, 1997).

2.5.3 Molecular Genetics of *xa5*

The recessive gene *xa5* identified by Petpisit *et al.* (1977) that was mapped by Yoshimura *et al.* (1995) using a set of NIL and RFLP markers. In the experimental population, no recombinants were observed among the gene and the three RFLP markers namely RZ390, RG556 and RG207 employed by them. *xa5* region was fine mapped between RG556 and RZ390 by Blair and McCouch (1997) on chromosome 5. Yang *et al.* (1998) constructed BAC contig having the *xa5* locus and identified BAC clone 44B4, hybridized to both RG207 and RG556 which suggests that BAC clone 44B4 carried the *xa5* locus.

According to Huang *et al.* (1997), RG556 was located ~0.1 cM close to the gene and a CAPS marker was developed with restriction enzyme digestion using *DraI.* The marker provided a specific amplicon polymorphism in a co-dominant fashion. Similarly, Iyer and McCouch (2007) produced a CAPS marker which also requires a PCR amplification followed by restriction enzyme digestion. The xa5FM, a solely PCR based twin marker targeting the 2bp functional nucleotide polymorphism was suggested later by Sundaram *et al.* (2011) and techniques for it have been refined by Hajira *et al.* (2016). The functional marker xa5FM is the most adopted marker for *xa5* gene introgression recently as it requires PCR amplification only.

The *xa5* gene offers resistance to Japanese races and Philippine *Xoo* races 1, 2, 3, and 5 by restricting the bacterial movement rather than their multiplication. The gene *Xa5* codes for a transcription factor subunit (TFIIAγ) which is involved in the recruitment of the basal transcription machinery of RNA polymerase II by eukaryotic transcription factors (Iyer and McCouch, 2004). It is postulated that the TAL effectors encoded by *Xoo avr* factors usurp parts of the eukaryotic transcription machinery to regulate rice gene expression, The *Xoo* gene, *avrXa5* codes for such a TAL protein corresponding to *Xa5*. However, the recessive *xa5* was reported to have a missense mutation which doesn't compromise its general

function in transcription (Jiang *et al.*, 2006) but may evade the TAL protein mediated virulence (Boch and Bonas, 2010).

2.6 DNA MARKERS AND MARKER ASSISTED SELECTION IN RICE

DNA markers are DNA sequence having a known location on a chromosome and associated with a particular gene or trait. They can be utilized to identify the inheritance of linked trait of interest as they are co-inherited with the trait. The rice as a model monocot crop and its small genome size of 430 Mb (Arumuganathan and Earle, 1991) accelerated the mapping of genes with respect to these molecular markers. The Rice Genome Project (Sasaki and Burr, 2000) was intended with such a target. The DNA markers thus identified can be used in the breeding programmes to improve several agronomic traits of rice including insect pest resistance, disease resistance, salt tolerance, submergence tolerance, grain aroma, temperature-sensitive male sterility, amylose content, semi-dwarf stature, shattering resistance photoperiod sensitivity and wide compatibility (Babu *et al.*, 2004).

Several DNA markers are available throughout the rice genome namely RFLP, RAPD, AFLP, SSR, SCAR, CAPS, ISSR, STS, SNPs and so on. Among those RAPD, RFLP, SSR, STS and SCAR markers are widely used in rice breeding. SNPs are getting popular now for genome-wide screening. RAPD and AFLP markers are having dominant nature, low reproducibility, as well as restriction enzyme digestion and use of radiochemicals, limits their usage in several aspects. In that case, SSR markers are advantageous due to their co-dominant nature, high polymorphism, reproducibility, and reliability (Babu *et al.*, 2004).

RFLP marker was the first molecular marker system developed which is the only marker based on hybridization. The co-dominant marker was once widely used for genome mapping and ruled out due to its high DNA requirement, inconvenient use of radiochemicals, the cost, and time-consuming procedure (Jena and Mackill 2008). RFLP markers have been used to map blast resistance genes *Pi1, Piz-5* and *Pita* using NILs. The identified RFLP markers were utilized to inrogress these

genes into a breeding line (Hittalmani *et al.*, 2000). The RFLP marker RG556 and RG136 linked with *xa5* and *xa13* respectively were used for MAS by Huang *et al.* (1997). RFLP markers were reported for some essential traits of rice including submergence tolerance, salt tolerance, gall midge resistance, rice tungro spherical virus resistance and drought tolerance (Jena and Mackill, 2008).

RAPD markers were developed by Williams *et al.* (1990). RAPD markers were seldom used for specific gene transfers even though the initial mapping of genes involve them. RAPD markers linked with the BLB R gene *Xa10* were identified by Yoshimura *et al.* (1995). The dominant nature and low reproducibility of the marker was its drawback. However, they were widely used for diversity analysis among rice genotypes. (Mackill 1995; Choudhury *et al.*, 2001; Davierwala *et al.*, 2000). RAPD and RFLP markers were once widely used for linkage mapping and several traits of interest and resistance genes have been tagged with it.

SSR markers or simple sequence repeats are the most depended markers of recent time for breeding. The SSR markers or otherwise microsatellites were identified to have linkage with several stress tolerance characters of rice including salt tolerance (*saltol* QTL, Thomson *et al.*, 2010), drought tolerance (*DRO1* QTL, Uga *et al.*, 2011) and submergence tolerance (*Sub1* QTL, Neeraja *et al.*, 2007).

When a specific marker locus is amplified using unique sequences flanking the locus as forward and reverse primers, the marker is called Sequence Tagged Sites (STS) (Beckmann and Soller, 1990). This marker with locus identity provides an easier selection of linked major genes and can be multiplexed for higher throughput (Mitchell *et al.*, 1997). Both STS and SSR markers are co-dominant. The STS markers of genes linked with RAPD, RFLP and SSR markers can be successfully developed by sequencing. The RFLP clones RG556 and RG136 specific for BLB R genes *xa5* and *xa13* were used as STS markers to develop Improved Samba Mahsuri by Sundaram *et al.* (2008). Similarly, STS markers based on RAPD locus linked to brown plant hopper resistance gene *Bph 13(t)* was used to introgress the gene into popular rice cultivar (Renganayaki *et al.*, 2002)

Another eminent DNA markers are CAPS (Cleaved Amplified Polymorphic Sequence) and SCAR (Sequence-characterized amplified region). CAPS is the combination of the PCR-RFLP technique whereas SCAR is solely based on PCR and the sequence of DNA. Both these codominant markers can be developed from polymorphic RAPD clones (Paran and Michelmore, 1993; Maeda *et al.*, 1990). However, CAPS require an additional restriction enzyme digestion (Maeda *et al.*, 1990). The co-dominant nature and high reproducibility of both these markers over RAPD and RFLP provides them much importance. Neeraja *et al.* (2007) designed a CAPS marker, GnS2, based on an SSR in the *Sub1A* gene, and this marker amplified the specific band linked to submergence tolerance.

The use of molecular or DNA markers in the selection of plants carrying genomic regions that are responsible for the traits of interest can be called as Marker Assisted Selection (MAS). With the advent of numerous molecular markers and dense marker genetic maps facilitated the selection of major genes as well as Quantitative Trait Loci's through MAS. In a plant breeding programme, the essential requirements for marker-assisted selection are: marker(s) (preferably DNA markers) should be closely linked (1 cM or less) with the desired trait; availability of efficient means of screening markers in a large breeding population, at present, this means, PCR technology which facilitates easier analysis; highly reproducible, economically feasible and user-friendly screening techniques. However, the above requirements are more or less the essential characteristics of molecular or DNA markers under concern (Collard and Mackill, 2008). Random DNA markers have a main drawback that their predictive value is depended over the linkage with the trait of interest (Lubberstedt *et al.*, 1998). Any recombination within them can break the linkage. Even in the case of flanking markers for a gene double crossing over can cause the loss of targeted locus (Toojinda *et al.*, 1998). Thus the need for faster breeding urged the reliability of markers. Thus a new variant of molecular markers was developed called Functional Molecular Markers (FMM) or simply functional markers. The molecular markers derived from the functional variants present in the genic region, or the markers that exist within a

genic region which causally governs the trait variation, are known as FMMs (Kage *et al.*, 2016). Functional markers are more reliable because the chance of recombination between the gene of interest and marker as in the case of random markers is nil.

The advantages of marker assisted selection are: time saving as DNA can be isolated at any stage of crop growth; consistency attained as environmental factors do not influence DNA markers; biosafety offered in the case of resistance trait evaluation; efficiency achieved through marker assisted early generation evaluation of breeding population; accurate selection of complex traits in the case of polygenic characters. The utilization of markers spread throughout the genome facilitates an increase in percentage recovery of the recurrent parent genome in backcross breeding programmes (Jena and Mackill, 2008). Thus DNA markers simultaneously facilitate efficient foreground and background selection. The markers of foreground selection will be trait specific whereas the markers for background selection will be genome specific.

According to several reports (Singh *et al.*, 2001; Narayanan *et al.*, 2002; Joseph *et al.*, 2004; Hittalmani *et al.*, 2000), introgressing multiple R genes against a single pest or disease provided durable resistance in host plants since it lead to the consecutive expression without any breakdown of resistance. It also limited the co-evolution of the target pest. The introgressions of multiple R genes into the susceptible elite cultivar through conventional breeding poses several difficulties especially in the identification of inheritance of these genes having a similar expression. In this context linked molecular markers helps the breeder to select the genes at the molecular level rather than at complex phenotypic level. So there comes the importance of marker assisted selection. In order to improve an elite cultivar by introgressions of multiple resistance genes marker assisted selection is made use of and the scheme is called Marker assisted backcross breeding (MABB) or Marker assisted backcrossing (MAB).

MABB had been attempted to transfer blast resistance genes, brown plant hopper resistance, gall midge resistance genes and submergence tolerance to elite Indian rice cultivars (Sharma *et al.*, 2004; Neeraja *et al.*, 2007; Khan *et al.*, 2018; Venkanna *et al.*, 2018).

2.7 GENE PYRAMIDING THROUGH MARKER ASSISTED BACKCROSS BREEDING FOR BACTERIAL LEAF BLIGHT RESISTANCE

Gene pyramiding is depicted as a breeding strategy aimed at integrating multiple genes showing varying resistance to a single pathogen or pest from multiple parents into an elite cultivar or genotype. It is mainly used in improving existing elite cultivars and developing Essentially Derived Varieties (EDVs) to meet specific situations of biotic stress. The pyramiding concept is based on horizontal resistance that, cumulating major genes of resistance provide a spectrum of resistance to a number of pathogenic races or insect biotypes specific to the genes pyramided. Based on the gene for gene relationship between hosts and pests, the horizontal resistance offered by pyramiding restricts co-evolution of the pathogen (Mundt, 1990). According to the proven concept of the gene for gene relationship, when a specific race of pathogen is able to match all the resistance genes of the host with appropriate virulence genes, then only the pathogen will be virulent over the host mechanism to produce disease or susceptible reaction (Flor, 1951; Person, 1959). So in such a context, the co-evolution of the pathogen in gene pyramided systems is limited compared to single gene resistance introgressions. So as the number of genes in pyramiding increases the spectrum of resistance increases due to the race specificity of genes through horizontal resistance, while the durability of resistance increases as per gene for gene relation between host and pathogen. So gene pyramiding was undertaken as the best alternative for conventional disease management. The purview of gene pyramiding was accelerated by the integration of molecular techniques with conventional breeding approaches. When multiple genes from donor genotype(s) are to be introgressed into an elite genotype, the necessity is to maintain the qualitative and quantitative attributes of the elite genotype as such along with the genes of resistance from the donor(s). Such an

objective can be achieved through marker assisted backcross breeding (MABB). MABB is an important tool for gene pyramiding.

Marker assisted backcrossing involves introgression of the gene(s) controlling a trait of interest while retaining all the essential characteristics of the recurrent parent (Collard and Mackill, 2008). The objective of marker assisted backcross breeding strategy is to integrate one or more traits or characters from a donor genotype(s) into a recipient genotype and selecting against the simultaneous donor introgressions across the rest of the recipient genome. The advent of DNA markers throughout the genome and its polymorphism across both the parents facilitates the objective of MABB. In other words, they increase the genetic gain per unit time or unit backcross (Tanksley *et al.*, 1989; Hospital, 2003).

The advantages of the use of markers in backcross breeding are the main assets of MABB. They are (1) systematic foreground selection of the target gene, (2) effective background selection for the genome of recurrent parent, (3) minimization of linkage drag associated with the target locus (4) rapid breeding of new genotypes with agronomically important traits. Thus the success of MABB depends on the linked markers for the target locus as well as genome spread polymorphic markers for both genomes, the size of the population, the number of backcrosses and the position and number of markers for background selection (Frisch *et al.*, 1999; Frisch and Melchinger, 2005).

The number of backcrosses in a MABB can be restricted to four instead of six even with limited population and a limited number of markers (Frisch *et al.*, 1999). This justified that MABB is advantageous even when the resources in a breeding programme are limited. Based on their simulation studies it was evident that the recovery of recurrent parent genome in BC_3 of MAB is equivalent to the gain of recurrent parent genome in BC_7 without markers. According to Servin and Hospital (2002), the proportion of recurrent parent genome obtained per backcross increases with the number of optimally positioned DNA markers increases per chromosome.

The MAB and MAS have been utilized in several breeding programmes to enhance BLB resistance through gene pyramiding. Huang *et al*., (1997) pyramided four bacterial leaf blight resistance genes, *Xa4*, *xa5*, *xa13,* and *Xa21,* with the aid of RFLP and PCR markers into IR24 and they designed STS markers for gene *xa5* and *xa13* from RFLP markers RG556 and RG136 respectively. The pyramided line from the above study was used as donor parent (IRBB59) to introgress multiple genes, *xa5*, *xa13*, and *Xa21* into new plat type line, IR65598-112 and the two sister lines, IR65600-42 and IR65600-96 (Sanchez *et al.*, 2000). The same STS markers were utilized for the study. Both studies reported that the pyramided lines displayed higher levels of resistance than single gene lines against BLB.

Marker assisted selection was employed by Singh *et al.* (2001) to develop three gene pyramided lines of PR106 at Punjab Agricultural University using IRBB62 with *xa5*, *xa13*, and *Xa2l* genes as donor parent. The pyramided lines provided resistance to 17 *Xoo* isolates from Punjab and six *Xoo* races from the Philippines. The inoculation study concluded that gene combinations provided broad-spectrum resistance to the pathogen population; *Xa21* was found to be more effective than recessive genes *xa5* and *xa13.*

A hybrid line Shuhui527 introgressed with BLB resistance genes *Xa21* and *Xa4* from IRBB60 using marker assisted selection expressed a high level of resistance to the *Xoo* strain CI-C VIII (Huang *et al.*, 2003).

The high yielding scented variety Pusa Basmati 1 was made BLB resistant by introgressing *xa13* and *Xa21* by Joseph *et al.* (2004). Background analysis using 252 polymorphic AFLP markers signified recurrent parent genome recovery of 80.4 to 86.7% in BC_1F_3 selections. Gopalakrishnan *et al*. (2008) integrated an association mapping strategy with the above study and identified some beneficial characters from the donor segments in BC_1F_5 selections. This integrated strategy with backcross pedigree method provided an 11.9% yield advantage over the recurrent parent. The improved selection in BC_1F_5 was released as Improved Pusa Basmati 1.

A temperature sensitive genetic male sterile line (TGMS) was introgressed with *Xa4*, *Xa7*, and *Xa21* by employing MAS. The F_2 plants having three gene combinations (*Xa4+Xa7+Xa21*) provided promising resistance to all *Xoo* races inoculated (Perez *et al.,* 2008)

Samba Mahsuri was improved by introgressing three major BLB resistance genes, *xa5*, *xa13*, and *Xa2l* from a donor line (SS111 3) having all the three genes in a homozygous condition. The background selection with polymorphic microsatellite markers reported the recovery of 97% of the recurrent parent Samba Mahsuri genome in the three gene pyramided lines of BC_4F_1 generation (Sundaram *et al*., 2008).

The genes *xa13* and *Xa21* were introgressed into the genetic background of Triguna, a mid-early duration variety at Indian Institute of Rice Research (Sundaram *et al*., 2009). The donor parent was SS1113 with *xa13, xa21* and *xa5* (Singh *et al*., 2001).

Basavaraj *et al*. (2010) carried out the introgression of BB resistance genes *xa13* and *Xa21* into Pusa6B and PRR78 (the maintainer parent and the restorer parent of the hybrid Pusa RH10) using a marker assisted backcross breeding program. Analysis of quantitative characters and six grain quality characters made them to recover the recurrent parent genome ranging from 85.14 to 97.30% and 87.04 to 92.81% in the 10 best BC_2F_5 families of Pusa 6B and PRR78, respectively. They had selected characters such as days to 50% flowering, plant height, number of tillers, length of panicle, number of grains per panicle, spikelet fertility, thousand grain weight and yield per plant for recurrent parent genome (RPG) recovery analysis.

Bacterial blight resistance genes *Xa4*, *xa5*, *xa13*, and *Xa21* were pyramided into the hybrid rice restorer parent, KMR3 and maintainer lines *viz*., PRR78, IR58025B, Pusa 6B and another the popular rice cultivar Mahsuri. The pyramided

lines conferred promising durable resistance against 10 highly virulent isolates of *Xanthomonas oryzae* pv. *oryzae* (Shanti *et al.,* 2010).

In TamilNadu Agricultural University, Coimbatore, three BLB resistance genes (*xa5*, *xa13*, and *Xa21*) were pyramided by utilizing marker assisted backcross breeding into ADT43, ADT47 and ASD16, popular high yielding rice cultivars of South India (Perumalsamy *et al.*, 2010; Bharani *et al.*, 2010). They reported that the combination of genes was promising in providing complete resistance than single genes. The quantitative characters like plant height, number of effective tillers, number of grains panicle^{-1}, 1000 grain weight and grain yield plant^{-1} were selected to assess the recurrent parent phenome recovery in breeding lines.

In order to improve the rice hybrid Pusa RH10, Rajpurohit *et al.* (2010) introgressed the genes *xa13* and *Xa21* from donor Pusa1460 into the parental lines of the hybrid Pusa6B and PRR78 which were susceptible to the disease. STS markers RG136 and pTA248 were used for foreground selection whereas STMS markers were used for background selection. In a similar attempt, gene *Xa21* was introduced into the restorer line KMR 3R, and thereby its derived hybrid KRH2. Markers were used to select the resistance gene as well as fertility restorers (Hari *et al.*, 2011).

SSR and ISSR based marker assisted selection was utilized to introgress two genes for BLB resistance, *xa13* and *Xa21* along with sd-1, a semi-dwarfing gene in the traditional Indian basmati rice Type 3 Basmati. The donor parent in the programme was PR106-P2 (Rajpurohit *et al.*, 2010). The selections in BC_2F_3 progenies possessing genes were highly resistant to the disease. Similarly, two BLB susceptible Basmati varieties were made BLB resistant by introgressing *xa13* and *Xa21* from Improved Samba Mahsuri through marker assisted foreground selection along with a phenotypic selection for high yield and short stature (Pandey *et al.*, 2012). The selected plants with both genes were forwarded up to BC_1F_5 generation.

Introgression of four dominant BLB R genes *Xa7, Xa21, Xa22,* and *Xa23* into restorer line Huahui 1035 was attempted by Huang *et al.* (2012). 10 promising lines with Huahui 1035 background was obtained with all the four genes.

A BLB resistant donor line, IRBB59 having 3 R-genes *xa5*, *xa13* and *Xa21*, was used to introgress *xa13* and *Xa21* along with an aroma gene (*fgr*) into a rice variety IRS 5441-2. The BC_1F_3 generation lines with both genes as well as with basmati quality were most effective against the virulent isolate of BLB (Salgotra *et al.*, 2012). Functional markers were used to identify the presence of genes.

Suh *et al.* (2013) developed three elite advanced backcross breeding lines of japonica cultivar Mangeumbyeo introgressed with *Xa4, xa5* and *Xa21* from an indica donor IRBB57. According to him, pyramided lines with multiple genes exhibited higher resistance to *Xoo* than the lines having single resistance genes. 92.1% genome recovery of the variety was reported when SSR markers were used for background selection.

According to the study by Win *et al.* (2013), the triple and double gene introgressed lines of MK-75, developed by pyramiding BLB genes *xa5, Xa21,* and *xa33* had increased level of resistance against Thai and Myanmar *Xoo* strains than normal MK-75.

At Acharya N. G. Ranga Agricultural University, Rajendranagar, Hyderabad Magar *et al.* (2014) pyramided *xa13* and *Xa21* from B95-1 to a high yielding rice variety, MTU1010 (Cottondora Sannalu). Linked STS markers, xa13pro and pTA248 for *xa13* and *Xa21* respectively were used for foreground selection. Mendelian inheritance was identified for the genes (*xa13* and *Xa21*) when F_2 populations of the cross were analysed.

BC_3F_2 plants of the backcross breeding programme conducted were analysed to transfer three major BLB resistance genes (*Xa21*, *xa13*, and *xa5*) into Jalmagna variety by Pradhan *et al.* (2015). The lines showed a maximum recipient parent genome recovery of 95% and three gene pyramided lines of the generation were

reported to have superior resistance character to the disease. He employed 120 SSR markers to assess the RPG recovery. Besides this, fourteen quantitative characters and two qualitative characters were studied at each stage of breeding. He clustered the R gene pyramided lines using Jaccard's similarity coefficient

Abhilash *et al.* (2016) used RPBio Patho-1 (possessing *Xa21+Pi2*), RPBio Patho-2 (possessing *Xa21+Pi54*) and FBR1 15EM (possessing *Xa33*) as the donors to transfer *Xa21* and *Xa33* genes for BLB resistance and *Pi2* and *Pi54* for Blast resistance to RPHR-1005. They reported that the plants having the gene combination *Xa21+Pi2*, *Xa21+Pi54* and *Xa33* in BC_2F_2 generation in homozygous condition possessed >92% recovery of the RPG.

In the joint project of Acharya N. G. Ranga Agricultural University, Guntur, Andhra Pradesh, and Prof. Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, two major bacterial blight resistance genes (*Xa21* and *xa13*) and a major gene for blast resistance (*Pi54*) were introgressed into an Indian rice variety MTU1010 through marker-assisted backcross breeding. (Arunakumari *et al.*, 2016).

Ellur *et al.* (2016a) introgressed BLB R-genes, *xa13* and *Xa21*, and the blast R genes, *Pi2* and *Pi54* into the background genome of Pusa Basmati 1121 (PB1121) and Pusa Basmati 6. Pusa 1728-23-33-31-56, one of the near-isogenic line showed 95.8% recovery of RPG in background analysis. A High degree of resemblance to PB6 was also shown in the phenotypic analysis.

Ellur *et al.* (2016b) developed a near isogenic line (NIL) of PB1121 by introgressing *Xa38* gene from PR114-Xa38 using a modified marker-assisted backcross breeding (MABB) scheme. The NILs of PB1121, carrying the gene for resistance *Xa38* alone and the other line carrying *xa13* + *Xa21* were effectively resistant against *Xoo* races 1, 2, 3 and 6. In addition, line with *Xa38* shown resistance to race 5 of *Xoo* to which *xa13+Xa21* combination was susceptible. They claimed that this strategy was very effective in reducing the linkage drag.

BLB R-genes *Xa21*, *xa13* and *xa5* were deployed in Safri-17 variety using pTA248, xa13pro and xa5FM markers respectively through marker assisted selection by Kadu *et al.* (2016). Their study also confirmed that breeding lines with two or three genes provided effective durable resistance than lines with single genes.

Vallabh Basmati 22, a variety susceptible to bacterial leaf blight and blast was made resistant by introgressing *xa13* and *Xa21* for BLB resistance whereas *Pi54* for blast resistance by Srikanth *et al.* (2016). In this study, Improved Samba Mahsuri served as the donor parent of BLB resistance genes while Tetep, a Vietnamese cultivar served as a donor of *Pi54* gene. Four three gene pyramided lines of the recurrent parent with high yield and basmati grain type have been identified in the ICF_4 generation. and they showed significant resistance to bacterial blight and blast in inoculation study (Srikanth *et al.*, 2016).

The functional marker xa5FM and STS markers, xa13pro and Xa21F/R were utilized to introduce genes *xa5, xa13*, and *Xa21* respectively from IRBB59, a donor parent into Karma Mahsuri, a popular high yielding rice variety susceptible to BLB (Deshmukh *et al.*, 2017). In BC_2F_3 population 22 lines were confirmed with the presence of the three genes.

Recently, Baliyan *et al.* (2018) carried out MAS and stringent phenotypic selection to transfer *Xa21, xa13* and *xa5* into CSR-30, a salt-tolerant Basmati variety, without compromising the basmati characters in selection. IRBB-60 having *Xa21, xa13* and *xa5* was the donor parent in the breeding scheme. 131 polymorphic SSR markers reported to have assisted in recovering up to 97.1% of RPG in the three-gene-pyramided genotypes of BC_3F_1 generation.

Recently, Dubraj and Safri-17 varieties of Chhattisgarh have also been improved with gene pyramiding approach against BLB using genes *xa5, xa13* and *Xa21* (Dnyaneshwar *et al.*, 2018).

Uma, a popular high yielding rice variety of Kerala have been made BLB resistant by introgressing three BLB genes namely *xa5, xa13*, and *Xa21* from Improved Samba Mahsuri as donor parent. STS markers RG556, RG136 and pTA248 were used for MAS of the genes *xa5, xa13*, and *Xa21* respectively. Genome recovery of 81.82% of the recurrent parent was reported in the triple gene pyramided line, plant No. 8.3.9.10 in BC_2F_1 generation. The morphological similarity of the line with Uma satisfied this result (Tintumol, 2016; Megha *et al*., 2019).

Materials and Methods

3. MATERIALS AND METHODS

The research work named "Marker assisted selection for bacterial leaf blight resistance genes in the backcross progenies of Prathyasa variety of rice (*Oryza sativa* L.)." was undertaken at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during the period 2017-2019.

The experiment involved the utilization of both biometrical analysis as well as modern molecular tools. The materials and methods employed for the study are listed below.

3.1 PLANT MATERIAL

This project was a part of the ongoing DBT project "Development of rice varieties for Kerala with pyramided genes for resistance to BLB by Marker Assisted Selection" at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani. BC_2F_2 seeds were collected from BC_2F_1 parents with two or three gene combinations and greater than or equal to 80% recurrent parent genome recovery located in the above project. Details of BC_2F_1 plants selected are given in Table 1. Seeds of recurrent parent Prathyasa and donor parent Improved Samba Mahsuri (pyramided with *xa13*, *Xa21* and *xa5*) were also raised for comparison on salient features of the recurrent parent and donor parent given in Table 2. Plate 1 shows the different BC_2F_2 seeds.

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Table 1. Details of BC_2F_1 lines

Sl. No.	Progeny no. (BC_2F_1)	Sample no. (BC_2F_1)	Recurrent parent Genome recovery (%)	Gene combination
1	ICDE 28-7/23/2	PR-6	91.30	*xa13* and *Xa21*
2	ICDE 12-3/13	PR-556	85.71	*xa13* and *Xa21*
3	ICDE 12-3/6	PR-549	85.57	*xa13* and *Xa21*
4	ICDE 12-3/14	PR-557	84.90	*xa13* and *Xa21*
5	ICDE 13-3/46/4	PR-4	84.37	*xa5, xa13* and *Xa21*
6	ICDE 12-3/1	PR-544	83.96	*xa13* and *Xa21*
7	ICDE 12-3/4	PR-547	81.73	*xa13* and *Xa21*
8	ICDE 28-7/23/5	PR-9	79.41	*xa13* and *Xa21*
9	ICDE 8-4/38/9	PR-20	78.12	*xa13* and *Xa21*
10	ICDE 13-3/46/3	PR-3	74.32	*xa13* and *Xa21*

Table 2. The salient features of the recurrent parent and donor parent

No.	Parent	Variety	Source	Feature
A	Recurrent parent	Prathyasa (MO21)	Rice Research Station KAU, Moncompu, Alappuzha, Kerala.	Medium duration, medium tillering semi dwarf variety with red long bold kernels. Susceptible to bacterial blight.
B	Donor parent	Improved Samba Mahsuri (RPBio-226)	Indian Institute of Rice Research, Hyderabad, India.	Long duration, high tillering semi dwarf variety with medium slender white kernels. Resistant to bacterial blight with *xa5*, *xa13* and *Xa21* resistance genes.

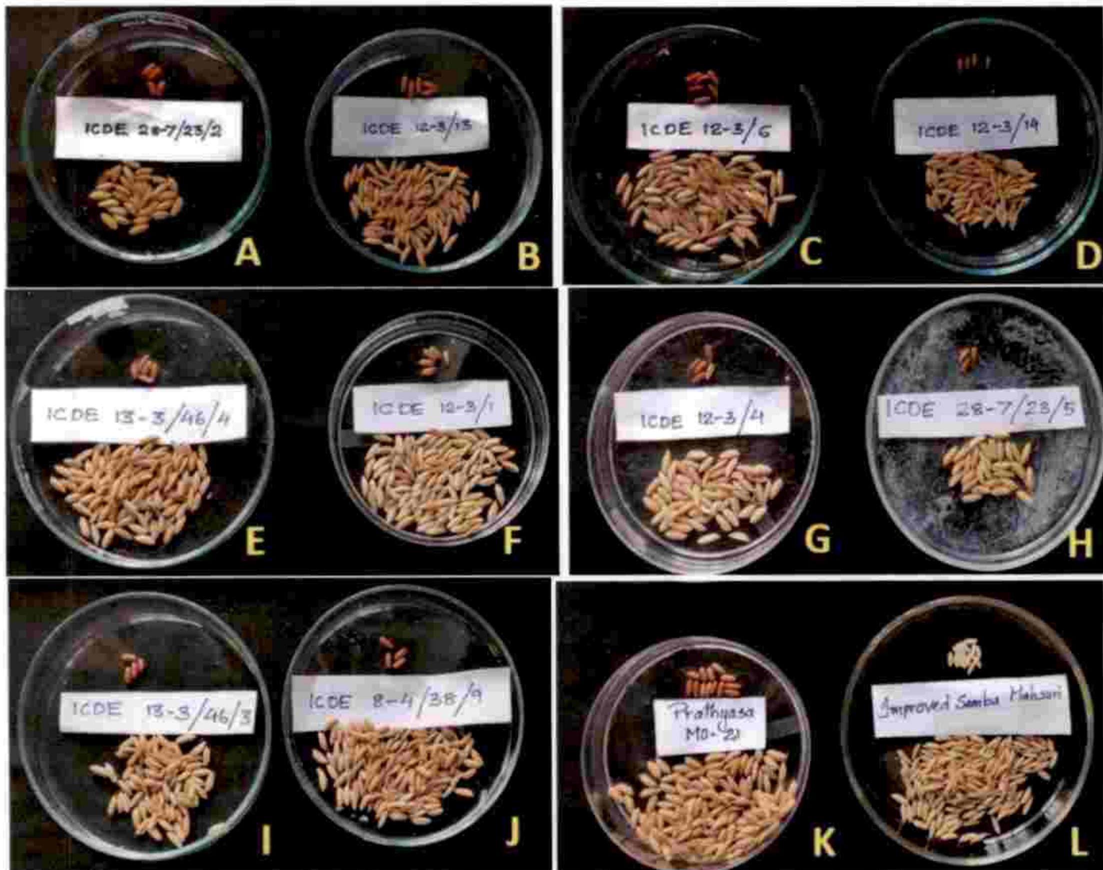

Plate 1. BC_2F_2 seeds

A: BC_2F_2 seeds of ICDE/28-7/23/2;
B: BC_2F_2 seeds of ICDE/12-3/13;
C: BC_2F_2 seeds of ICDE/ 12-3/6;
D: BC_2F_2 seeds of ICDE/12-3/14;
E: BC_2F_2 seeds of ICDE/13-3/46/4;
F: BC_2F_2 seeds of ICDE/12-3/1;
G: BC_2F_2 seeds of ICDE/12-3/4;
H: BC_2F_2 seeds of ICDE/28-7/23/5;
I: BC_2F_2 seeds of ICDE/13-3/46/3;
J: BC_2F_2 seeds of ICDE/8-3/38/9;
K: Prathyasa(MO 21, Recurrent parent);
L: Improved Samba Mahsuri (RPBio-226, Donor parent).

3.2 FOREGROUND SELECTION IN THE BC_2F_2 PLANTS

The BC_2F_2 and parent seeds were germinated in petri dishes or glass bottles, sown in plastic pots and maintained as a nursery for 21-25 days. The seedlings were transplanted in well-puddled lowland field provided with a basal dose of NPK of medium duration variety of rice (KAU,2017) (Plate 2). The spacing was 25x25 cm^2. A top dressing was given for all the plants in between tillering and maximum tillering stage.

Each F_2 family was grown as an individual block separated from other family blocks and with field borders by 40 cm. The experiment was done during the period from September 2018 to March 2019. Necessary plant protection measures were undertaken during the entire stretch to protect the plants from insects and diseases.

The young leaves from individual plants at the tillering stage were collected for DNA isolation.

3.2.1 Isolation of Genomic DNA

Stock Solutions

CTAB buffer: 100ml

CTAB	2.0 g
1 M Tris pH 8.0	10.0 mL
0.5 M EDTA pH 8.0	4.0 mL
5 M NaCl	28.0 mL
Distilled water	40.0 mL
PVP 40	1.0 g

Adjusted to pH 5.0 with HCl and made up to 100 mL with distilled water.

1x TE buffer:100mL

1 M Tris-Cl (pH 8.0)	1.0 mL
0.5 M EDTA (pH 8.0)	0.2 mL
Distilled water	98.8 mL

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Plate 2: The main field

Mixed the solution gently and autoclaved for sterilization.

Both stocks were kept at room temperature (25°C)

DNA isolation was carried out using the standard Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1990) in addition to which an RNase treatment step was included for improving the purity of DNA. Young leaf bits (200-300 mg fresh weight) were ground using ample volume of liquid nitrogen in chilled mortar with pestles. 500 µL of prewarmed CTAB was added directly to the mortars to scrape the powder and swirled well. The mixture was then transferred to 1.5-2 mL centrifuge tubes and was incubated at 60°C for 30 minutes in a water bath with gentle swirling at intervals. After incubation, in order to spindown the plant debris, the tubes with CTAB/plant extract mixture were spun at 12000 g (RCF) for 5 minutes. The green viscous supernatant was again transferred to clean microfuge tubes and 5 µL RNase A (10 µg mL^{-1}) was added in each tube. An incubation period of 1 hour was allowed for RNA degradation. The tubes were then spun at 13000 rpm for 1-2 minutes after adding and mixing an equal volume of Chloroform: Isoamyl alcohol (v/v:24:1). The mixture was mixed gently but thoroughly before centrifuging. The coloured or clear supernatant from each tube was carefully transferred into new fresh microfuge tubes without disturbing the middle and bottom layers. This aqueous phase was kept at -20°C after adding an equal amount of ice-cold Isopropanol. This was the precipitation step of DNA. Generally, the tubes were incubated at -20°C in the deep freezer overnight. Later after spinning the tubes at 13000 rpm for 1 minute, the aqueous phase was drained off by keeping the precipitated pellet at the bottom. After washing the pellet 1-2 times in cool absolute alcohol for 7-15 minutes, the pellets were resuspended in extra pure sterile water or 1x TE buffer. Tubes were centrifuged for pelleting the DNA dispersed while washing. Generally, 110-150 µL of TE buffer was added per tube for the DNA from 200 mg of plant sample. The tubes with dissolved pellets in 1x TE buffer were kept at -20°C deep freezer.

3.2.2 Agarose Gel Electrophoresis

Stock Solutions or Reagents

50X TAE Buffer

Tris base	240.00 g
Acetic acid	57.1 mL
0.5 M EDTA pH 8.0	186.12 g
Distilled water	942.9 mL

Final volume was 1000 mL. The solution was stored at 4°C.

6X loading dye

Sucrose	4.0 g
Bromophenol blue	0.025 g
Distilled water	10 mL

Loading dye solution was stored at 4°C.

Ethidium bromide

Ethidium bromide	100 mg
Distilled water	10 mL

100 mg powder well dissolved in 5-6 mL distilled water and made up to 10 mL. Dye solution was stored at 4°C.

Agarose gel electrophoresis was carried out to identify the presence and quality of the isolated DNA. Horizontal gel electrophoresis was carried out using 0.8% gel slabs. The slabs were prepared by melting Agarose (0.8 g) in 100 mL 1x TAE buffer. Ethidium bromide was added for fluorescence to the solution at the rate of 3 μL (10 mg mL^{-1}) per 100 mL gel after cooling the solution to 42-45°C. The solution was then poured to a height of 3 mm-5 mm into a sealed gel casting tray with combs fixed in position. The gel was left at room temperature for 15-20 minutes for solidification. Prior to solidification, the electrophoresis tank was filled

with 1x TAE buffer enough to submerge the gel. Ensuring a height of 1mm of buffer over the gel, the solidified gel tray was submerged into the tank after removing the combs and tapes. It was ensured that the wells of gel were near to the negative terminal of the tank. The required volume (10-20 µL) of DNA samples were loaded into the wells of gel using a micropipette. Prior to loading, each DNA sample was mixed well with loading dye (Bromophenol blue) in a ratio of 5:1 (v/v). A constant 60V power supply was provided for the run through attached anode and cathode. Biorad powerpack was used for the power supply. The power was turned off when the loading dye moved about $3/4^{th}$ of the gel. The gel was documented using the G-Box gel documentation system.

3.2.3 Quantification of DNA

The quantity of DNA in the sample was analysed using UV spectrophotometer reading. In a UV spectrophotometer, the absorbance of diluted DNA was determined at 260 nm UV light. Since the optical density (OD) of pure dsDNA at A_{260} corresponds to 1.0 (for 50 µg mL^{-1} solution of dsDNA) the amount of DNA was calculated using the formula;

$$\text{Amount of DNA } (\mu g/ml) = A_{260} \times \text{dilution factor} \times 50$$

$$\text{where Dilution factor} = \frac{\text{Volume of water in } \mu L}{\text{Volume of DNA in } \mu L}$$

and A_{260} = Absorbance at 260 nm.

The purity or quality of DNA was determined from the ratio of absorbance values at 260 nm and 280 nm i.e. A_{260}/A_{280}. The best quality of DNA was identified from the ratio values ranging between 1.8 and 2.0.

In the study, 3 µL of DNA dissolved in TE buffer was diluted with 3 mL of sterile distilled water. The diluted DNA samples were read against distilled water as blank at 260 nm and 280 nm for absorbance. The purity and quantity of DNA were determined using the above given formulae.

3.2.4 Molecular Markers

Closely linked DNA markers specific to the BLB resistance genes *viz.*, *Xa21, xa13* and *xa5* were used. To identify *Xa21* gene, the only dominant gene in the study, the STS marker pTA248 reported by Ronald *et al.* (1992) was used. The functional marker xa13pro developed by Sundaram *et al.* (2011) whose primers based on promoter sequence producing functional variations of the gene was used to identify the recessive gene *xa13*. The multiplex PCR based marker xa5FM developed and refined by Sundaram *et al.* (2011) and Hajira *et al.* (2016) was used for the recessive gene, *xa5*.

The markers and their sequence details are given in Table 3. Primers were synthesized by Sigma-Aldrich Chemicals, Bangalore.

Table 3. Details of markers specific to bacterial blight resistance genes

Gene	Marker	Sequence	Tm °C
Xa21	pTA248F	5'AGACGCGGAAGGGTGGTTCCCGGA3'	55.3°C
	pTA248R	5'AGACGCGGTAATCGAAAGATGAAA3'	
xa13	xa13proF	5'GGCCATGGCTCAGTGTTTAT3'	55.3°C
	xa13proR	5'GAGCTCCAGCTCTCCAAATG3'	
xa5	xa5FM-SF	5'GTCTGGAATTTGCTCGCGTTCG3'	57°C
	xa5FM-SR	5'TGGTAAAGTAGATACCTTATCAAACTGGA3'	
	xa5FM-RF	5'AGCTCGCCATTCAAGTTCTTGAG3'	
	xa5FM-RR	5'TGACTTGGTTCTCCAAGGCTT3'	

Tm: Annealing temperature of primers.

3.2.5 Polymerase Chain Reaction

The DNA isolated from BC_2F_2 plants were used for PCR amplification using the three gene specific primers i.e. STS marker pTA248, functional marker xa13pro and functional marker xa5FM were amplified using their respective primers.

The PCR was performed in a 25 µl reaction mixture. In the case of pTA248 and xa13pro markers, each 25 µL of PCR mixture was constituted by

1 μL of 45-50 ng genomic DNA,

2.5 μL of 10x PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl and 0.01 mg mL^{-1} gelatin),

2.5 μL of 25 mM $MgCl_2$,

2 μL of 10 mM dNTPs (2.5 mM each),

1 μL of 10 pM each of forward-reverse primers and

0.2 μL of 5 U μL^{-1} of Taq DNA polymerase.

The remaining volume was constituted by sterile distilled water. An Eppendorf master thermal cycler nexus gradient was utilized to carry out the PCR amplification.

In the case of xa5FM marker, the same Eppendorf master thermal cycler nexus gradient was utilized to carry out the multiplex PCR amplification. Here a pair of forward and reverse primers amplify bands specifying resistant alleles of the gene, whereas another forward-reverse primer pair amplifies bands specific to the susceptible allele. Hence the PCR reaction mixture used slightly varied from the former two markers. The 25 μL of PCR mixture was constituted by

1 μL of 45-50 ng genomic DNA,

3 μL 10X PCR buffer, (10 mM Tris, pH 8.4, 50 mM KCl and 0.01 mg/ml gelatin)

2.7 μL of 25mM $MgCl_2$

3 μL 10mM dNTPs (2.5 mM each)

1 μL of 10 pM each of forward reverse primers and

0.2 μL of 5 U/μL of Taq DNA polymerase.

The reaction volume was made up to 25 μL using sterile distilled water.

The primers amplified their respective bands when an initial denaturation of template DNA at 94°C for 5 min was followed by 35 cycles of amplification with the following reaction conditions: a 30 sec to 1 min denaturation at 94°C, a 30 sec to 1 min annealing at 55.3°C for xa13pro and pTA248, and 57°C for xa5FM, 72°C for 1 min of primer extension. After 35 cycles of amplification a final extension of

5-7 min at 72°C (5 min for pTA248 and xa13pro, 7 min for xa5FM). Amplified products were resolved in 2% agarose gel with ethidium bromide. Bands were visualized under SYNGENE G-Box F3 gel documentation unit. A 100 bp ladder was added in the first well of the gel to provide a standard reference to score molecular weight of the products.

3.3 MORPHOMETRIC EVALUATION OF THE BC_2F_2 PROGENIES

The lines having gene combinations were morphometrically evaluated using the following characters.

3.3.1 Plant Height (cm)

The height from the base of the main tiller to the tip of the panicle (excluding awn) was measured as plant height at the time of harvest and expressed in centimetre (cm).

3.3.2 Days to Maturity

The Number of days taken from germination to complete maturity of the plant was recorded.

3.3.3 Number of Productive Tillers Plant^{-1}

The productive tillers with healthy panicles were counted at the stage of physiological maturity.

3.3.4 Length of Panicle (cm)

The length of the best five panicles was measured at the time of harvest, the average of the values taken and expressed in centimeter (cm).

3.3.5 Number of Grains Panicle^{-1}

The filled grains of best five panicles were counted, the average of the values taken and expressed in number.

3.3.6 1000 Grain Weight

The weight of a random sample of 1000 whole grains was taken using a precision balance and the mean was computed and expressed in grams (g).

3.3.7 Length/Breadth (L/B) Ratio of Grain

The Length/Breadth ratio of the grain was determined by dividing the length of grain by its corresponding breadth, both of which have been measured by using the dial caliper.

3.3.8 Kernel Colour

Rice seeds were manually dehusked and visual observation was taken based on the following table (Table 4):

Table 4. Kernel colour characterization

Sl. No	Colour
1	White
2	Light brown
3	Variegated brown
4	Dark brown
5	Light red
6	Red
7	Variegated purple
8	Purple
9	Dark purple

3.4 STATISTICAL ANALYSIS

The data recorded on different experiments were subjected to the following statistical analysis.

3.4.1 Descriptive Statistics

Observations on the plant height (cm), days to maturity, number of productive tillers plant^{-1}, length of panicle, number of grains panicle^{-1}, 1000 grain weight, length/breadth ratio of grain were recorded on the recurrent parent, donors and selected plants in the segregating populations. The descriptive statistical analysis was carried out for each character and it was calculated as follows:

3.4.1.1 Range

It records the highest and lowest value in the observed value for each character in parents and the segregating populations.

3.4.1.2 Arithmetic Mean

It is calculated by the following formula:

$$\bar{X} = \Sigma X / N$$

Where, $\bar{X}$ = Mean, ΣX = Sum of all the observations, N = Total number of observations.

3.4.1.3 Variance

$$V = \Sigma(\bar{X} - X)^2 / N$$

Where, X = Individual reading, $\bar{X}$ = Mean, N = Sample size

3.4.1.4 Standard Deviation

$$S.D = \sqrt{\Sigma(\bar{X} - X)^2 / N}$$

Where, X = Individual reading, $\bar{X}$ = Mean, N = Sample size

3.4.1.5 Standard Error

$$S.E = S.D/\sqrt{N}$$

Where, S.D = Standard Deviation, N = total number of observations.

3.4.2 Euclidean Distance

The proximity dissimilarity matrix was constructed using euclidean distance method for seven morphological characters by estimating euclidean distance using the formula suggested by Shifriss and Sacks (1980).

$$\text{Euclidean Distance} = \sum_{k-1}^{7}\left(\frac{Xik - Xjk}{Sk}\right)^2$$

Where,

X_{ik} =Performance of the ith individual for k^{th} character

X_{jk} = Performance of the jth individual for k^{th} character

S_k = Standard deviation of the kth character

Genetic divergence (genetic distance) of pyramided lines from the recurrent parent was measured by euclidean distance method using Statistical Package for the Social Sciences (SPSS version 22.0). The dendrogram was constructed based on the euclidean distance using the same software (SPSS version 22.0).

Results

4. RESULTS

Bacterial leaf blight disease of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a major disease of rice causing significant economic loss to farmers. As chemical control of the disease is ineffective, exploitation of host plant resistance is the most suitable practical strategy for the disease management in an eco-friendly manner. As many as 42 BLB resistance genes, conferring resistance against various strains of *Xoo*, have been identified from the rice germplasm collections worldwide and utilized for resistance breeding programmes. However, the longterm cultivation of the monogenic resistant varieties resulted in significant shift in the virulence pattern of the pathogen causing break down of resistance. As a solution to this, pyramiding multiple resistance genes into different cultivars was carried out. Gene pyramiding is now faster and easier due to the advent of linked molecular markers for each gene. The present study was a part of gene pyramiding programme to introgress the BLB resistance genes *xa13*, *Xa21,* and *xa5* to a popular rice variety Prathyasa through marker assisted selection. The study was undertaken to identify the introgressions of resistance genes in the BC_2F_2 population and to evaluate these lines morphometrically to assess Prathyasa phenome recovery.

4.1 FOREGROUND SELECTION IN THE BC_2F_2 PLANTS

4.1.1 Quantitative and Qualitative Estimation of DNA

The quantity of genomic DNA was estimated by reading the absorbance of DNA samples at 260 nm of UV (A_{260}). The purity of DNA samples was also accounted by measuring the absorbance at 280 nm (A_{280}). The ratio of A_{260} upon A_{280} ranged from 1.80 to 1.83 in both the parents which indicated pure DNA. The DNA yield per microlitre of the parental sample was 84 ng and 75 ng for Prathyasa and Improved Samba Mahsuri respectively. The average content of DNA from BC_2F_2 lines was 76 ng μL^{-1} The quantity and quality of DNA of the parental lines and F_2 segregants averaged over the samples is given in Table 5.

Table 5. Quantity and quality of genomic DNA of parents and BC_2F_2 individuals

Individuals	Quantity of DNA (ng μL^{-1})			Quality of DNA (A_{260}/A_{280} ratio)		
	Mean	Range		Mean	Range	
		Maximum	Minimum		Maximum	Minimum
Prathyasa (Recurrent Parent)	84	91	66	1.8	1.89	1.79
ISM (Donor parent)	75	92	71	1.83	1.92	1.80
BC_2F_2 individuals	76	88	54	1.89	1.99	1.79

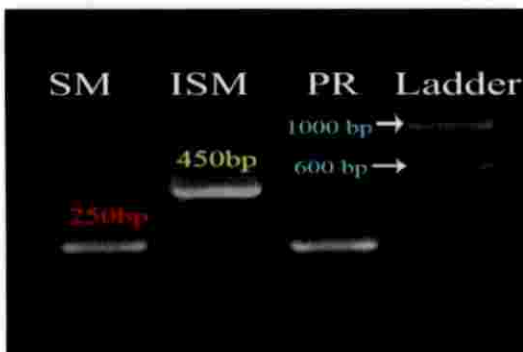
The quality of DNA was visually ensured through electrophoretic resolution in 0.8% (w/v) agarose gel. The bright intact bands of DNA was obtained for all samples (Plate 3).

4.1.2 Polymerase Chain Reaction (PCR)

The isolated parental DNA was amplified using gene specific markers through polymerase chain reaction and electrophoretically resolved in 2% gel. The amplification pattern of xa13pro produced a 450 bp (~500 bp) amplified product in the donor parent with resistance gene *xa13* in homozygous form. The DNA segment amplified in sample DNA of Prathyasa was a 250 bp product (Plate 4a). Thus, the amplification pattern indicates presence of gene *xa13* (homozygous resistance allele) will produce 450 bp PCR product and absence of the gene (susceptible allele) will produce a 250 bp PCR product.

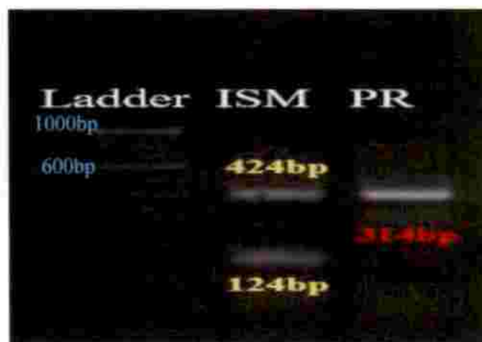
The marker pTA248, which is specific for the gene *Xa21* amplified a 950 bp (~1000 bp) fragment in donor parent ISM, whereas a 660 bp fragment in the susceptible parent Prathyasa. Thus, the presence of the *Xa21* gene can be identified with the presence of 950 bp amplicon in the PCR reaction, whereas its absence can be indicated with the presence of amplicons of size 660 bp (Plate 4b).

Plate 3. Gel Electrophoresis for visualization of presence and purity of DNA; Samples 1-20: DNA of Prathyasa, Improved Samba Mahsuri and ICDE/13-3/46/4 progenies 1-18 respectively.



4a. xa13pro marker amplification

4b. pTA248 marker amplification



4c. xa5FM marker amplification

Plate 4. PCR amplification of parental genomic DNA using gene linked markers

SM: Samba Mahsuri; ISM; Improved Samba Mahsuri; PR: Prathyasa

The multiplex functional marker xa5FM specific for the identification of *xa5* gene amplified a 424 bp (~450 bp) and 124 bp (~150 bp) products in the donor parent sample, whereas 424 bp and 313 bp (~300 bp) products in recurrent parent sample (Plate 4c). Thus, the resistance allele of *xa5* gene was indicated with 424 bp and 124 bp amplicons in a polymerase chain reaction of the sample DNA with xa5FM marker, while the susceptible allele of the gene or otherwise the absence of the gene was indicated through the 424 bp and 313 bp amplified products.

All these three markers are co-dominant markers, hence the heterozygous condition of their respective genes can be identified with the presence of both resistance and susceptible products in the same DNA sample. Thus, this validation confirmed the presence and absence of genes in donor and recurrent parents and also provided the amplification pattern of each marker in the presence and absence of each gene. The amplification products of each of the markers are given in Table 6.

Table 6. Amplification pattern of gene linked markers of the resistance genes

Sl. No.	Gene	DNA Marker	Amplification products (in bp) in:		
			Presence of gene (donor parent)	Absence of gene (recurrent parent)	Genes in heterozygous condition
1	*Xa21*	pTA248	950 (~1000)	660 (~600)	950 and 660
2	*xa13*	xa13pro	450	250	450 and 250
3	*xa5*	xa5FM	424 (~450), 124 (~150)	424, 313 (~300)	424, 313 and 124

4.1.3 Marker Assisted Foreground Analysis

A total of 289 BC_2F_2 individuals (Table 7) were screened for genes *xa13*, *Xa21* and *xa5* using functional markers xa13pro, pTA248, and xa5FM respectively.

Table 7. BC_2F_2 progenies used in foreground selection

Sl. No.	BC_2F_1 parent accession no.	Sample no. of the parent (BC_2F_1)	Total no. of Progenies maintained (BC_2F_2)
1	ICDE 28-7/23/2	PR-6	11
2	ICDE 12-3/13	PR-556	27
3	ICDE 12-3/6	PR-549	43
4	ICDE 12-3/14	PR-557	17
5	ICDE 13-3/46/4	PR-4	53
6	ICDE 12-3/1	PR-544	25
7	ICDE 12-3/4	PR-547	22
8	ICDE 28-7/23/5	PR-9	8
9	ICDE 8-4/38/9	PR-20	58
10	ICDE 13-3/46/3	PR-3	25
			Total = 289

All the individual samples were amplified for *xa13* and *Xa21* screening, while only the samples identified with either *xa13*, *xa21* or both were amplified for *xa5* screening. Among the 289 plants subjected to molecular analysis, 155 plants (Table 8) were found to have resistance genes in combination.

Table 8. BC_2F_2 progenies identified with the presence of R-genes in combination

Sl. No.	BC_2F_1 parent accession no.	Sample no. of the parent (BC_2F_1)	Progenies identified with R gene combination (BC_2F_2)
1	ICDE 13-3/46/4	PR-4	21
2	ICDE 12-3/1	PR-544	25
3	ICDE 12-3/4	PR-547	22
4	ICDE 12-3/13	PR-556	27
5	ICDE 12-3/6	PR-549	43
6	ICDE 12-3/14	PR-557	17
			Total = 155

Besides this, thirty-one plants with heterozygous *xa13*, thirty-two plants with homozygous *xa13*, two plants with heterozygous *Xa21* and three plants with homozygous *Xa21* were also identified. The graphical representation of the plants is given in Figure 1.

Among the 289 BC_2F_2 plants analyzed, 153 plants showed the presence of both *xa13* and *Xa21* genes when amplified with their respective markers xa13pro and pTA248, whereas two plants showed the presence of *xa13* and *xa5,* identified with the markers specific for them. None of the plants showed the combination of *Xa21* and *xa5,* as well as triple gene combination (Table 9, Figure 2).

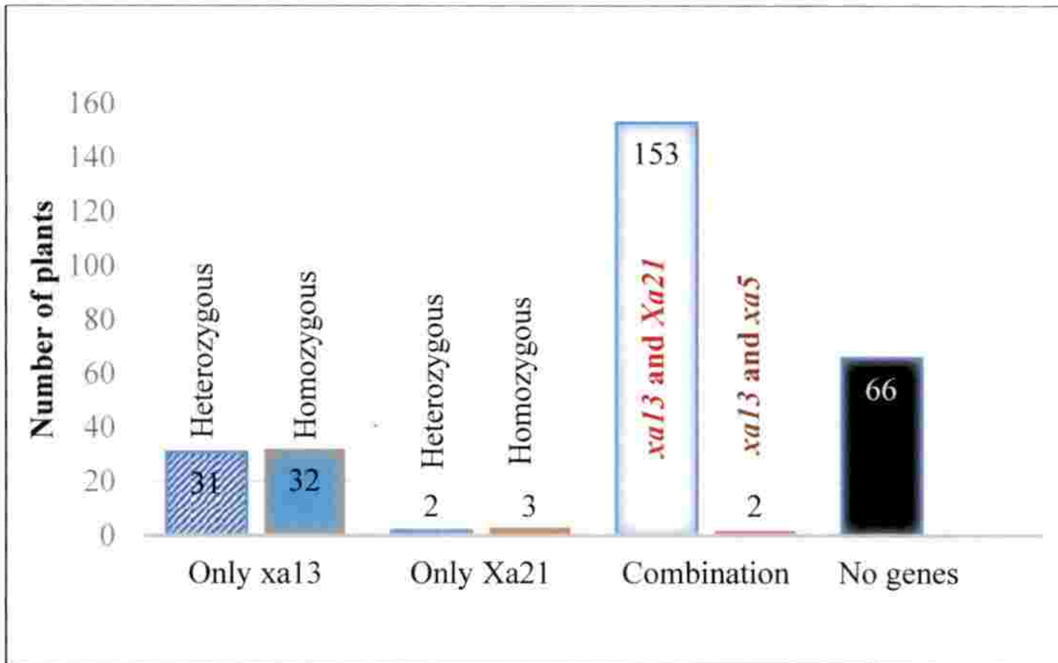
4.1.3.1 The Gene Combination of xa13 and Xa21

The presence of genes *xa13* and *xa21* in combination was identified in 153 plants among which, 136 plants showed resistant allele in homozygous condition for both genes.

4.1.3.1.1 Resistance Gene Distribution in ICDE 13-3/46/4 Progenies

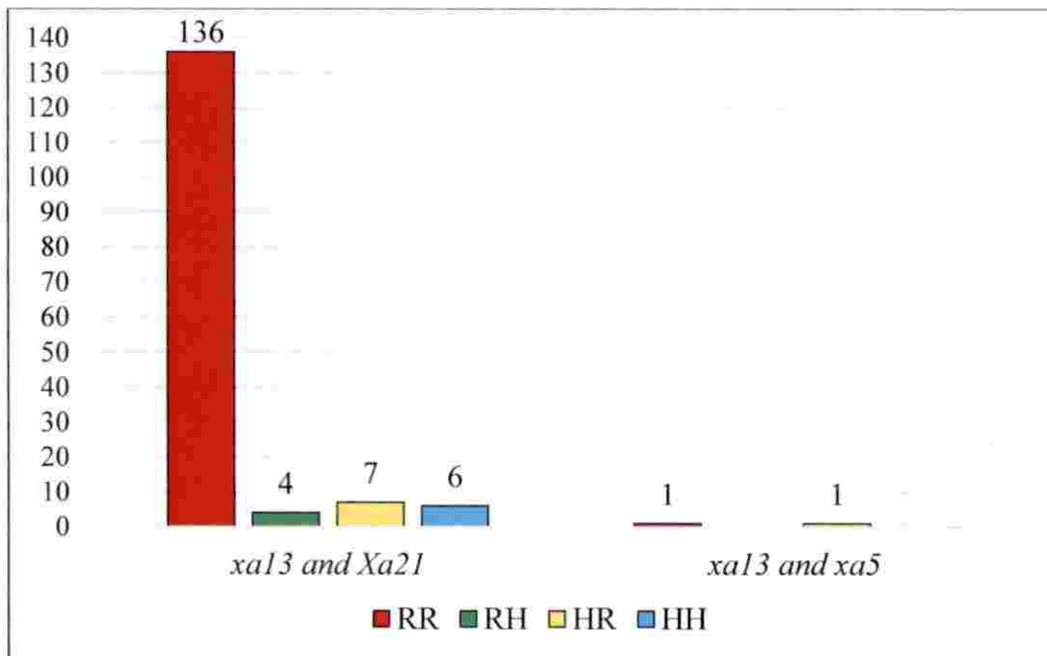
F_2 progeny population of the backcross progeny ICDE 13-3/46/4 (BC_2F_1 Parent, PR-4) with 53 members showed segregation of genes *xa13* and *Xa21*. Single resistance allele in homozygous combination of both genes was identified in the 7th plant (ICDE 13-3/46/4/7, Plate 5a and 5b) as well as the 48th plant (ICDE 13-3/46/4/48, Plate 5i and 5j).

Seven plants namely, ICDE 13-3/46/4/11 (11th, Plate 5c and 5d), ICDE 13-3/46/4/12 (12th, Plate 5c and 5d), ICDE 13-3/46/4/18 (18th, Plate 5c and 5d), ICDE 13-3/46/4/22 (22nd, Plate 5c and 5d), ICDE 13-3/46/4/24 (24th, Plate 5e and 5f), ICDE 13-3/46/4/50 (50th, Plate 5i and 5j) and ICDE 13-3/46/4/53 (53rd, Plate 5i and 5j) had the heterozygous allele for xa13pro while having single resistance allele for pTA248. Hence they were identified with a combination of the heterozygous *xa13* gene along with homozygous *Xa21* gene.



R-gene: Resistance gene status

Figure 1. Distribution of BLB resistance genes in the BC_2F_2 population



R-resistance allele in homozygous state; H: Resistance allele in heterozygous state

Figure 2. BC_2F_2 plants identified with presence of genes in combination

Table 9. BC_2F_2 progenies of Prathyasa identified with a combination of BLB resistance genes

Sl. No.	Progeny no.	Sample no.	*xa13* (xa13pro)	*Xa21* (pTA248)	*xa5* (xa5FM)
Parents of the breeding programme					
A	MO 21	Prathyasa	- -	- -	- -
B	RPBio-226	ISM	+ +	+ +	+ +
ICDE 13-3/46/4 (PR 4) progenies					
1	ICDE 13-3/46/4/7	PR 4-07	+ +	+ +	- -
2	ICDE 13-3/46/4/10	PR 4-10	+ -	+ -	- -
3	ICDE 13-3/46/4/11	PR 4-11	+ -	+ +	- -
4	ICDE 13-3/46/4/12	PR 4-12	+ -	+ +	- -
5	ICDE 13-3/46/4/13	PR 4-13	+ +	+ -	- -
6	ICDE 13-3/46/4/14	PR 4-14	+ +	+ -	- -
7	ICDE 13-3/46/4/15	PR 4-15	+ -	+ -	- -
8	ICDE 13-3/46/4/16	PR 4-16	+ -	+ -	- -
9	ICDE 13-3/46/4/17	PR 4-17	+ -	+ -	- -
10	ICDE 13-3/46/4/18	PR 4-18	+ -	+ +	- -
11	ICDE 13-3/46/4/22	PR 4-22	+ -	+ +	- -
12	ICDE 13-3/46/4/24	PR 4-24	+ -	+ +	- -
13	ICDE 13-3/46/4/31	PR 4-31	+ -	+ -	- -
14	ICDE 13-3/46/4/33	PR 4-33	+ -	+ -	- -
15	ICDE 13-3/46/4/34	PR 4-34	+ +	+ -	- -
16	ICDE 13-3/46/4/41	PR 4-41	+ -	- -	+ +
17	ICDE 13-3/46/4/44	PR 4-44	+ +	+ -	- -
18	ICDE 13-3/46/4/46	PR 4-46	+ +	- -	+ +
19	ICDE 13-3/46/4/48	PR 4-48	+ +	+ +	- -
20	ICDE 13-3/46/4/50	PR 4-50	+ -	+ +	- -
21	ICDE 13-3/46/4/53	PR 4-53	+ -	+ +	- -
ICDE 12-3/1 (PR 544) progenies					
22	ICDE 12-3/1/1	PR 544-1	+ +	+ +	- -
23	ICDE 12-3/1/2	PR 544-2	+ +	+ +	- -
24	ICDE 12-3/1/3	PR 544-3	+ +	+ +	- -
25	ICDE 12-3/1/4	PR 544-4	+ +	+ +	- -

+ + : homozygous resistance gene; + - : heterozygous resistance gene; - - : homozygous susceptible gene

Table 9 Continued.

Sl. No.	Progeny No.	Sample no.	*xa13* (xa13pro)	*Xa21* (pTA248)	*xa5* (xa5FM)
26	ICDE 12-3/1/5	PR 544-5	+ +	+ +	- -
27	ICDE 12-3/1/6	PR 544-6	+ +	+ +	- -
28	ICDE 12-3/1/7	PR 544-7	+ +	+ +	- -
29	ICDE 12-3/1/8	PR 544-8	+ +	+ +	- -
30	ICDE 12-3/1/9	PR 544-9	+ +	+ +	- -
31	ICDE 12-3/1/10	PR 544-10	+ +	+ +	- -
32	ICDE 12-3/1/11	PR 544-11	+ +	+ +	- -
33	ICDE 12-3/1/12	PR 544-12	+ +	+ +	- -
34	ICDE 12-3/1/13	PR 544-13	+ +	+ +	- -
35	ICDE 12-3/1/14	PR 544-14	+ +	+ +	- -
36	ICDE 12-3/1/15	PR 544-15	+ +	+ +	- -
37	ICDE 12-3/1/16	PR 544-16	+ +	+ +	- -
38	ICDE 12-3/1/17	PR 544-17	+ +	+ +	- -
39	ICDE 12-3/1/18	PR 544-18	+ +	+ +	- -
40	ICDE 12-3/1/19	PR 544-19	+ +	+ +	- -
41	ICDE 12-3/1/20	PR 544-20	+ +	+ +	- -
42	ICDE 12-3/1/21	PR 544-21	+ +	+ +	- -
43	ICDE 12-3/1/22	PR 544-22	+ +	+ +	- -
44	ICDE 12-3/1/23	PR 544-23	+ +	+ +	- -
45	ICDE 12-3/1/24	PR 544-24	+ +	+ +	- -
46	ICDE 12-3/1/25	PR 544-25	+ +	+ +	- -
ICDE 12-3/4 (PR 547) progenies					
47	ICDE 12-3/4/1	PR 547-1	+ +	+ +	- -
48	ICDE 12-3/4/2	PR 547-2	+ +	+ +	- -
49	ICDE 12-3/4/3	PR 547-3	+ +	+ +	- -
50	ICDE 12-3/4/4	PR 547-4	+ +	+ +	- -
51	ICDE 12-3/4/5	PR 547-5	+ +	+ +	- -
52	ICDE 12-3/4/6	PR 547-6	+ +	+ +	- -
53	ICDE 12-3/4/7	PR 547-7	+ +	+ +	- -
54	ICDE 12-3/4/8	PR 547-8	+ +	+ +	- -
55	ICDE 12-3/4/9	PR 547-9	+ +	+ +	- -
56	ICDE 12-3/4/10	PR 547-10	+ +	+ +	- -

+ + : homozygous resistance gene; + - : heterozygous resistance gene; - - : homozygous susceptible gene

Table 9 Continued.

Sl. No.	Progeny No.	Sample no.	*xa13* (xa13pro)	*Xa21* (pTA248)	*xa5* (xa5FM)
57	ICDE 12-3/4/11	PR 547-11	+ +	+ +	- -
58	ICDE 12-3/4/12	PR 547-12	+ +	+ +	- -
59	ICDE 12-3/4/13	PR 547-13	+ +	+ +	- -
60	ICDE 12-3/4/14	PR 547-14	+ +	+ +	- -
61	ICDE 12-3/4/15	PR 547-15	+ +	+ +	- -
62	ICDE 12-3/4/16	PR 547-16	+ +	+ +	- -
63	ICDE 12-3/4/17	PR 547-17	+ +	+ +	- -
64	ICDE 12-3/4/18	PR 547-18	+ +	+ +	- -
65	ICDE 12-3/4/19	PR 547-19	+ +	+ +	- -
66	ICDE 12-3/4/20	PR 547-20	+ +	+ +	- -
67	ICDE 12-3/4/21	PR 547-21	+ +	+ +	- -
68	ICDE 12-3/4/22	PR 547-22	+ +	+ +	- -
ICDE 12-3/6 (PR 549) progenies					
69	ICDE 12-3/6/1	PR 549-1	+ +	+ +	- -
70	ICDE 12-3/6/2	PR 549-2	+ +	+ +	- -
71	ICDE 12-3/6/3	PR 549-3	+ +	+ +	- -
72	ICDE 12-3/6/4	PR 549-4	+ +	+ +	- -
73	ICDE 12-3/6/5	PR 549-5	+ +	+ +	- -
74	ICDE 12-3/6/6	PR 549-6	+ +	+ +	- -
75	ICDE 12-3/6/7	PR 549-7	+ +	+ +	- -
76	ICDE 12-3/6/8	PR 549-8	+ +	+ +	- -
77	ICDE 12-3/6/9	PR 549-9	+ +	+ +	- -
78	ICDE 12-3/6/10	PR 549-10	+ +	+ +	- -
79	ICDE 12-3/6/11	PR 549-11	+ +	+ +	- -
80	ICDE 12-3/6/12	PR 549-12	+ +	+ +	- -
81	ICDE 12-3/6/13	PR 549-13	+ +	+ +	- -
82	ICDE 12-3/6/14	PR 549-14	+ +	+ +	- -
83	ICDE 12-3/6/15	PR 549-15	+ +	+ +	- -
84	ICDE 12-3/6/16	PR 549-16	+ +	+ +	- -
85	ICDE 12-3/6/17	PR 549-17	+ +	+ +	- -

+ + : homozygous resistance gene; + - : heterozygous resistance gene; - - : homozygous susceptible gene

Table 9 Continued.

Sl. No.	Progeny No.	Sample no.	*xa13* (xa13pro)	*Xa21* (pTA248)	*xa5* (xa5FM)
86	ICDE 12-3/6/18	PR 549-18	+ +	+ +	- -
87	ICDE 12-3/6/19	PR 549-19	+ +	+ +	- -
88	ICDE 12-3/6/20	PR 549-20	+ +	+ +	- -
89	ICDE 12-3/6/21	PR 549-21	+ +	+ +	- -
90	ICDE 12-3/6/22	PR 549-22	+ +	+ +	- -
91	ICDE 12-3/6/23	PR 549-23	+ +	+ +	- -
92	ICDE 12-3/6/24	PR 549-24	+ +	+ +	- -
93	ICDE 12-3/6/25	PR 549-25	+ +	+ +	- -
94	ICDE 12-3/6/26	PR 549-26	+ +	+ +	- -
95	ICDE 12-3/6/27	PR 549-27	+ +	+ +	- -
96	ICDE 12-3/6/28	PR 549-28	+ +	+ +	- -
97	ICDE 12-3/6/29	PR 549-29	+ +	+ +	- -
98	ICDE 12-3/6/30	PR 549-30	+ +	+ +	- -
99	ICDE 12-3/6/31	PR 549-31	+ +	+ +	- -
100	ICDE 12-3/6/32	PR 549-32	+ +	+ +	- -
101	ICDE 12-3/6/33	PR 549-33	+ +	+ +	- -
102	ICDE 12-3/6/34	PR 549-34	+ +	+ +	- -
103	ICDE 12-3/6/35	PR 549-35	+ +	+ +	- -
104	ICDE 12-3/6/36	PR 549-36	+ +	+ +	- -
105	ICDE 12-3/6/37	PR 549-37	+ +	+ +	- -
106	ICDE 12-3/6/38	PR 549-38	+ +	+ +	- -
107	ICDE 12-3/6/39	PR 549-39	+ +	+ +	- -
108	ICDE 12-3/6/40	PR 549-40	+ +	+ +	- -
109	ICDE 12-3/6/41	PR 549-41	+ +	+ +	- -
110	ICDE 12-3/6/42	PR 549-42	+ +	+ +	- -
111	ICDE 12-3/6/43	PR 549-43	+ +	+ +	- -
ICDE 12-3/13 (PR 556) progenies					
112	ICDE 12-3/13/1	PR 556-1	+ +	+ +	- -
113	ICDE 12-3/13/2	PR 556-2	+ +	+ +	- -
114	ICDE 12-3/13/3	PR 556-3	+ +	+ +	- -
115	ICDE 12-3/13/4	PR 556-4	+ +	+ +	- -
116	ICDE 12-3/13/5	PR 556-5	+ +	+ +	- -

+ + : homozygous resistance gene; + - : heterozygous resistance gene; - - : homozygous susceptible gene

Table 9 Continued.

Sl. No.	Progeny No.	Sample no.	*xa13* (xa13pro)	*Xa21* (pTA248)	*xa5* (xa5FM)
117	ICDE 12-3/13/6	PR 556-6	+ +	+ +	- -
118	ICDE 12-3/13/7	PR 556-7	+ +	+ +	- -
119	ICDE 12-3/13/8	PR 556-8	+ +	+ +	- -
120	ICDE 12-3/13/9	PR 556-9	+ +	+ +	- -
121	ICDE 12-3/13/10	PR 556-10	+ +	+ +	- -
122	ICDE 12-3/13/11	PR 556-11	+ +	+ +	- -
123	ICDE 12-3/13/12	PR 556-12	+ +	+ +	- -
124	ICDE 12-3/13/13	PR 556-13	+ +	+ +	- -
125	ICDE 12-3/13/14	PR 556-14	+ +	+ +	- -
126	ICDE 12-3/13/15	PR 556-15	+ +	+ +	- -
127	ICDE 12-3/13/16	PR 556-16	+ +	+ +	- -
128	ICDE 12-3/13/17	PR 556-17	+ +	+ +	- -
129	ICDE 12-3/13/18	PR 556-18	+ +	+ +	- -
130	ICDE 12-3/13/19	PR 556-19	+ +	+ +	- -
131	ICDE 12-3/13/20	PR 556-20	+ +	+ +	- -
132	ICDE 12-3/13/21	PR 556-21	+ +	+ +	- -
133	ICDE 12-3/13/22	PR 556-22	+ +	+ +	- -
134	ICDE 12-3/13/23	PR 556-23	+ +	+ +	- -
135	ICDE 12-3/13/24	PR 556-24	+ +	+ +	- -
136	ICDE 12-3/13/25	PR 556-25	+ +	+ +	- -
137	ICDE 12-3/13/26	PR 556-26	+ +	+ +	- -
138	ICDE 12-3/13/27	PR 556-27	+ +	+ +	- -
ICDE 12-3/14 (PR 557) progenies					
139	ICDE 12-3/14/1	PR 557-1	+ +	+ +	- -
140	ICDE 12-3/14/2	PR 557-2	+ +	+ +	- -
141	ICDE 12-3/14/3	PR 557-3	+ +	+ +	- -
142	ICDE 12-3/14/4	PR 557-4	+ +	+ +	- -
143	ICDE 12-3/14/5	PR 557-5	+ +	+ +	- -
144	ICDE 12-3/14/6	PR 557-6	+ +	+ +	- -
145	ICDE 12-3/14/7	PR 557-7	+ +	+ +	- -
146	ICDE 12-3/14/8	PR 557-8	+ +	+ +	- -
147	ICDE 12-3/14/9	PR 557-9	+ +	+ +	- -
148	ICDE 12-3/14/10	PR 557-10	+ +	+ +	- -
149	ICDE 12-3/14/11	PR 557-11	+ +	+ +	- -

+ + : homozygous resistance gene; + - : heterozygous resistance gene; - - : homozygous susceptible gene

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Table 9 Continued.

Sl. No.	Progeny No.	Sample no.	*xa13* (xa13pro)	*Xa21* (pTA248)	*xa5* (xa5FM)
150	ICDE 12-3/14/12	PR 557-12	+ +	+ +	- -
151	ICDE 12-3/14/13	PR 557-13	+ +	+ +	- -
152	ICDE 12-3/14/14	PR 557-14	+ +	+ +	- -
153	ICDE 12-3/14/15	PR 557-15	+ +	+ +	- -
154	ICDE 12-3/14/16	PR 557-16	+ +	+ +	- -
155	ICDE 12-3/14/17	PR 557-17	+ +	+ +	- -

+ + : homozygous resistance gene; + - : heterozygous resistance gene; - - : homozygous susceptible gene

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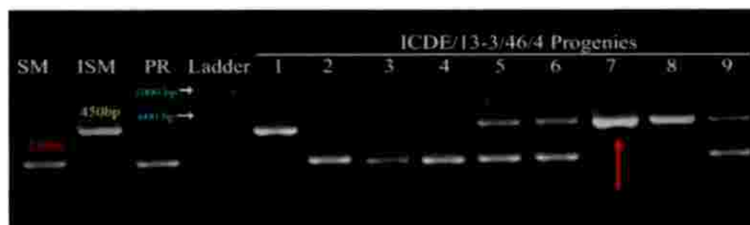
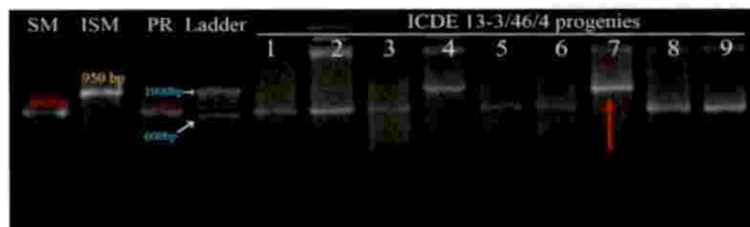
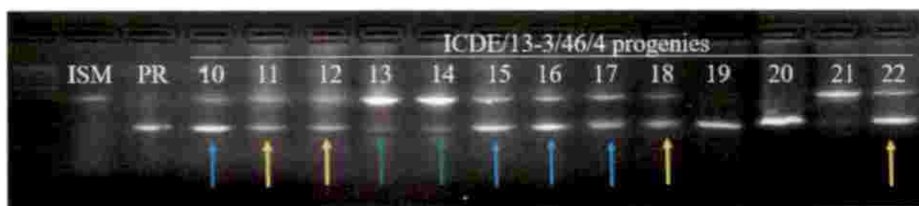


5a. xa13pro amplification of progenies 1-9



5b. pTA248 amplification of progenies 1-9



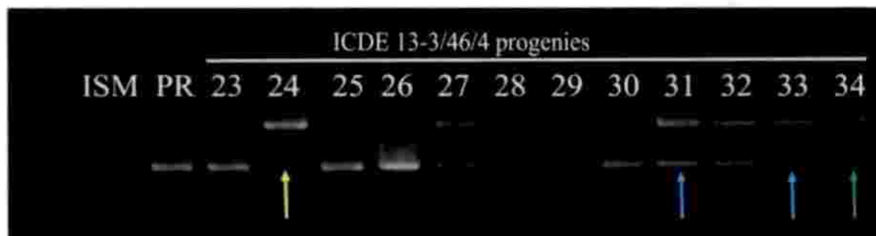
5c. xa13pro amplification of progenies 10-22

5d. pTA248 amplification of progenies 10-22

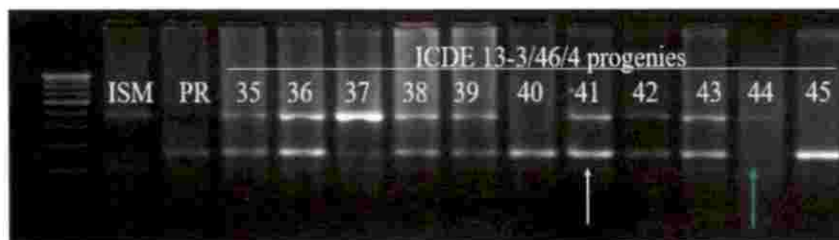
Plate 5. The amplification profile of ICDE 13-3/46/4 progenies 1 to 53 using gene linked markers

ISM: Improved Samba Mahsuri; PR: Prathyasa; red arrow – samples with homozygous *xa13* and *Xa21*; yellow arrow – samples with heterozygous *xa13* and homozygous *Xa21*; green arrow - samples with homozygous *xa13* and heterozygous *Xa21*; blue arrow - samples with of heterozygous *xa13* and heterozygous *Xa21*.

5e. xa13pro amplification of progenies 23-34



5f. pTA248 amplification of progenies 23-34

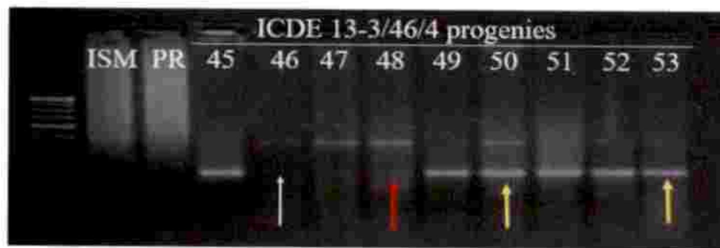


5g. xa13pro amplification of progenies 35-45

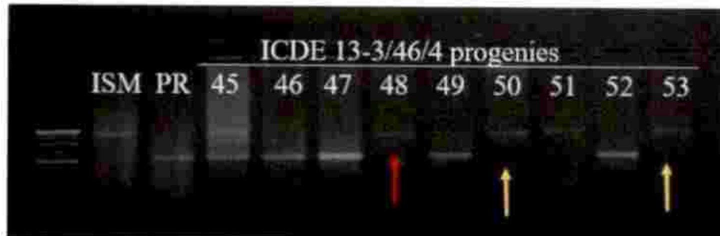
5h. pTA248 amplification of progenies 35-45

Plate 5. The amplification profile of ICDE 13-3/46/4 progenies 1 to 53 using gene linked markers

ISM: Improved Samba Mahsuri; PR: Prathyasa; yellow arrow – samples with heterozygous *xa13* and homozygous *Xa21*; green arrow - samples with homozygous *xa13* and heterozygous *Xa21*; blue arrow - samples with of heterozygous *xa13* and heterozygous *Xa21*; white arrow- samples with heterozygous *xa13* and homozygous *xa5*



5i. xa13pro amplification of progenies 45-53



5j. pTA248 amplification of progenies 45-53

Plate 5. The amplification profile of ICDE 13-3/46/4 progenies 1 to 53 using gene linked markers

ISM: Improved Samba Mahsuri; PR: Prathyasa; white arrow- samples with heterozygous *xa13* and homozygous *xa5*; red arrow – samples with homozygous *xa13* and *Xa21*; yellow arrow – samples with heterozygous *xa13* and homozygous *Xa21*.

The BC_2F_2 plants, ICDE 13-3/46/4/13 (13th plant, plate 5c and 5d), ICDE 13-3/46/4/14 (14th, Plate 5c and 5d), ICDE 13-3/46/4/34 (34th, Plate 5e and 5f) and ICDE 13-3/46/4/44 (44th, Plate 5g and 5h) showed homozygous *xa13* and heterozygous *Xa21* gene combination. Similarly, six F_2 plants namely, ICDE 13-3/46/4/10 (10th plant, Plate 5c and 5d), ICDE 13-3/46/4/15 (15th, Plate 5c and 5d), ICDE 13-3/46/4/16 (16th, Plate 5c and 5d), ICDE 13-3/46/4/17 (17th, Plate 5c and 5d), ICDE 13-3/46/4/31 (31st, Plate 5e and 5f) and ICDE 13-3/46/4/33 (33rd, Plate 5e and 5f) were having the heterozygous allele for both genes, *xa13* and *Xa21*. The amplification profile of these plants with the respective markers showed both resistance allele (450 bp for xa13pro and 950 bp for pTA248) and susceptible allele (250 bp for xa13pro and 660 bp for pTA248) in each amplification.

4.1.3.1.2 Resistance Gene Distribution in Progenies of ICDE 12-3/1, ICDE 12-3/4, ICDE 12-3/6, ICDE 12-3/13 and ICDE 12-3/14

The F_2 progenies of each individual ICDE 12-3/1, ICDE 12-3/4, ICDE 12-3/6, ICDE 12-3/13 and ICDE 12-3/14 showed no segregation for genes *xa13* and *Xa21*. The common noticeable fact among the amplification profile of every individual in these BC_2F_2 progenies was that the markers xa13pro and pTA248 showed only the resistance allele (450 bp allele and 950 bp respectively; Plate 6-10). Hence a uniform result of homozygous *xa13* and homozygous *Xa21* gene combination was inferred in all the individuals of each BC_2F_2 family.

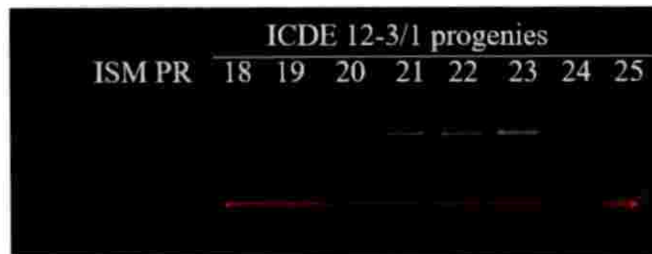
4.1.3.2 The Gene Combination of xa13 and xa5

The F_2 progeny of ICDE 13-3/46/4 showed the presence of genes *xa13* and *xa5* in combination in two plants. The plants identified with the presence of either *xa13*, *Xa21* or both were subjected to PCR amplification using xa5FM functional marker. The marker amplified alleles specific to the presence of *xa5* in the homozygous condition in plants identified with *xa13* gene, i.e. ICDE 13-3/46/4/41 and ICDE 13-3/46/4/46. The ICDE 13-3/46/4/41 plant showed heterozygous loci for *xa13* (41st, Plate 5g) and homozygous recessive *xa5* (41st, Plate 11) gene. A combination of homozygous loci was observed for both *xa13* and *xa5* genes in

6a. xa13pro amplification of progenies 1-17

6b. pTA248 amplification of progenies 1-17

6c. xa13pro amplification of progenies 18-25



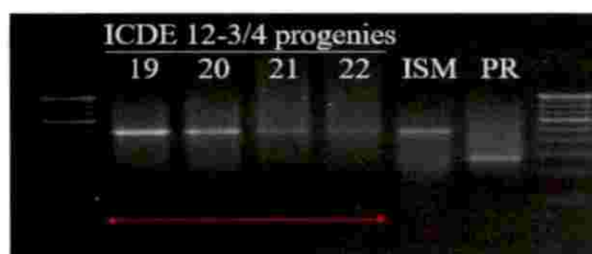
6d. pTA248 amplification of progenies 18-25

Plate 6. The amplification profile of ICDE 12-3/1 progenies 1 to 25 using gene linked markers

ISM: Improved Samba Mahsuri; PR: Prathyasa; red arrow – samples with homozygous *xa13* and *Xa21.*

7a. xa13pro amplification of progenies 1-18

7b. pTA248 amplification of progenies 1-18

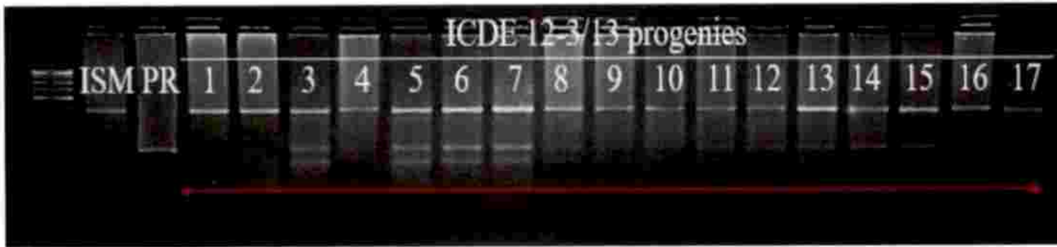


7c. xa13pro amplification of progenies 19-22

7d. pTA248 amplification of progenies 19-22

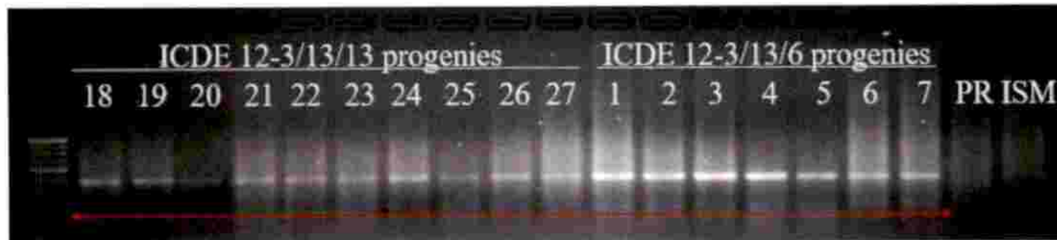
Plate 7. The amplification profile of ICDE 12-3/4 progenies 1 to 22 using gene linked markers

ISM: Improved Samba Mahsuri; PR: Prathyasa; red arrow – samples with homozygous *xa13* and *Xa21.*

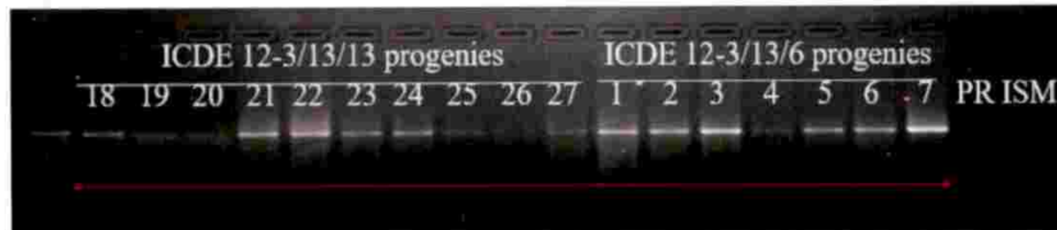


8a. xa13pro amplification of progenies ICDE 12-3/13 progenies 1-17

8b. pTA248 amplification of progenies ICDE 12-3/13 progenies 1-17



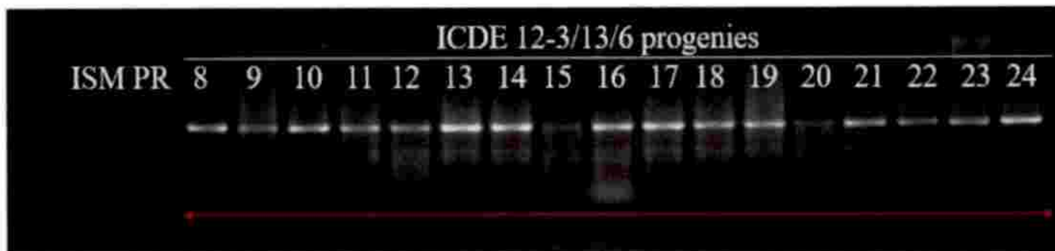
8c. xa13pro amplification of progenies ICDE 12-3/13 progenies 18-27 and ICDE 12-3/6 progenies 1-7



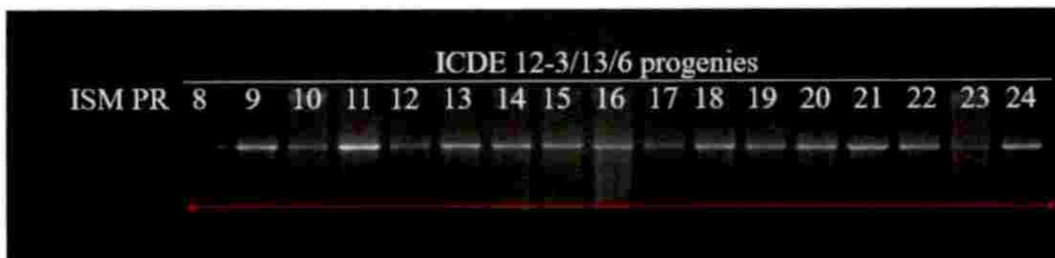
8d. pTA248 amplification of progenies ICDE 12-3/13 progenies 18-27 and ICDE 12-3/6 progenies 1-7

Plate 8. The amplification profile of ICDE 12-3/13 progenies 1 to 27 and ICDE 12-3/6 progenies 1-7 using gene linked markers

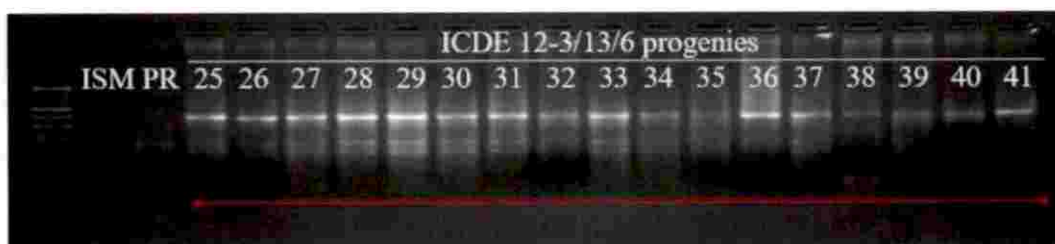
ISM: Improved Samba Mahsuri; PR: Prathyasa; red arrow – samples with homozygous *xa13* and *Xa21*.



9a. xa13pro amplification of progenies progenies 8-24



9b. pTA248 amplification of progenies 8-24



9c. xa13pro amplification of progenies progenies 25-41

9d. pTA248 amplification of progenies 25-41

Plate 9. The amplification profile of ICDE 12-3/6 progenies 8 to 41 using gene linked markers

ISM: Improved Samba Mahsuri; PR: Prathyasa; red arrow – samples with homozygous *xa13* and *Xa21*.

10a. xa13pro amplification of progenies progenies 1-17

10b. pTA248 amplification of progenies progenies 1-17

Plate 10. The amplification profile of ICDE 12-3/14 progenies 1 to 17 using gene linked markers

ISM: Improved Samba Mahsuri; PR: Prathyasa; red arrow – samples with homozygous *xa13* and *Xa21.*

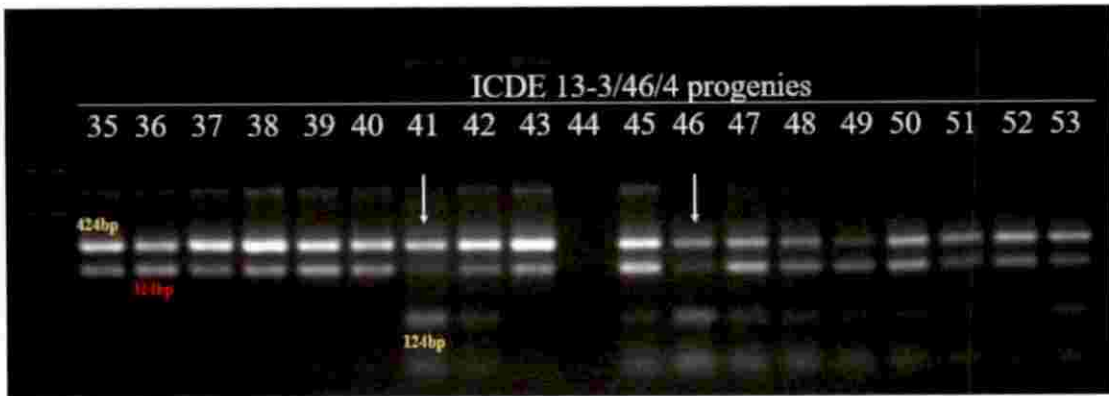


Plate 11. The amplification profile of ICDE 13-3/46/4 progenies 35 to 53 using gene linked marker xa5FM

White arrow – samples with combination of *xa13* (either heterozygous or homozygous) and homozygous *xa5.*

plant ICDE 13-3/46/4/46 (Plate 5i and Plate 11). No other plants were identified with a combination of *xa5* with any other gene.

4.2 MORPHOMETRIC EVALUATION OF BC_2F_2 PROGENIES

All the BC_2F_2 plants identified with gene combination were subjected to morphometric evaluation prior to harvest. The plant characters such as plant height (cm), days to maturity, number of productive tillers plant^{-1}, length of panicle (cm), number of grains panicle^{-1}, 1000 grain weight (g) and length/breadth ratio of grains were recorded. The qualitative trait, kernel colour was also recorded along with the quantitative traits. These phenotypic data were recorded on a family basis and represented in table 10 to table 16.

4.2.1 Plant Height

Almost all the BC_2F_2 plants had a plant height of more than 100 cm except a few plants with height less than 100 cm. The average plant height of Prathyasa and Improved Samba Mahsuri was 98.09 and 110.25 cm respectively (Table 10).

4.2.1.1 ICDE 13-3/46/4 Progenies

The progenies manifested a varying height ranging from 89 cm to 119 cm. The BC_2F_2 individual ICDE 13-3/46/18 recorded the maximum height of 119 cm, whereas ICDE 13-3/46/16 showed the minimum height of 89 cm. The plants had a height similar to parental varieties except for ICDE 13-3/46/14 (111 cm), ICDE 13-3/46/15 (111 cm) and ICDE 13-3/46/18 (119 cm) which marked a height superior to the better parent ISM. Three plants ICDE 13-3/46/11 (97 cm), ICDE 13-3/46/12 (96 cm) and ICDE 13-3/46/16 (89 cm) recorded a height less than the shorter parent Prathyasa. The 21 progenies (Table 11) showed an average height of 103.76 cm with a standard error of 1.52.

Table 10. Morphometric data of parents

Sl. No.	Accession Name	Sample no.	Plant height (cm)	Days to maturity	Number of productive tillers plant^{-1}	Length of panicle (cm)	Number of grains panicle^{-1}	1000 grain weight (g)	Length/Breadth (L/B) ratio of grain	Kernel Colour
A	MO 21	Prathyasa	98.09	108.28	11.98	23.16	126.12	26.67	2.77	Red
B	RPBio-226	ISM	110.25	145.33	15.87	21.41	116.10	15.15	4.03	White

Table 11. Morphometric data of progenies of ICDE 13-3/46/4 identified with resistance genes

Sl. No.	Progeny No.	Sample no.	Plant height (cm)	Days to maturity	Number of productive tillers plant^{-1}	Length of panicle (cm)	Number of grains panicle^{-1}	1000 grain weight (g)	Length/Breadth (L/B) ratio of grain	Kernel colour
1	ICDE 13-3/46/4/7	PR 4-07	98	110	12	23.18	118	24.86	2.63	Red
2	ICDE 13-3/46/4/10	PR 4-10	106	124	18	24.70	125	24.60	2.72	Red
3	ICDE 13-3/46/4/11	PR 4-11	97	122	8	20.88	132	24.37	2.70	Red
4	ICDE 13-3/46/4/12	PR 4-12	96	110	5	21.48	130	24.70	2.81	Red
5	ICDE 13-3/46/4/13	PR 4-13	103	118	3	18.71	128	25.66	2.76	Red
6	ICDE 13-3/46/4/14	PR 4-14	111	126	11	22.34	130	25.24	2.76	Red
7	ICDE 13-3/46/4/15	PR 4-15	111	132	8	22.74	122	24.92	2.71	Light Red
8	ICDE 13-3/46/4/16	PR 4-16	89	132	4	20.78	124	24.74	2.67	Red
9	ICDE 13-3/46/4/17	PR 4-17	107	124	9	21.38	122	25.38	2.70	Light Red
10	ICDE 13-3/46/4/18	PR 4-18	119	117	9	26.38	129	25.85	2.83	Red
11	ICDE 13-3/46/4/22	PR 4-22	110	115	12	24.02	132	26.00	2.65	Red
12	ICDE 13-3/46/4/24	PR 4-24	110	119	11	21.86	124	25.95	2.82	Red
13	ICDE 13-3/46/4/31	PR 4-31	98	112	12	23.60	129	25.54	2.83	Red

Table 11 Continued.

Sl. No.	Progeny No.	Sample no.	Plant height (cm)	Days to maturity	Number of productive tillers plant^{-1}	Length of panicle (cm)	Number of grains panicle^{-1}	1000 grain weight (g)	Length/Breadth (L/B) ratio of grain	Kernel colour
14	ICDE 13-3/46/4/33	PR 4-33	100	124	11	22.88	136	25.00	2.88	Red
15	ICDE 13-3/46/4/34	PR 4-34	109	111	19	23.78	130	24.72	2.84	Red
16	ICDE 13-3/46/4/41	PR 4-41	105	122	11	22.35	129	25.16	2.79	Red
17	ICDE 13-3/46/4/44	PR 4-44	101	122	11	22.82	131	24.96	2.89	Red
18	ICDE 13-3/46/4/46	PR 4-46	108	118	12	26.79	134	24.98	2.73	Red
19	ICDE 13-3/46/4/48	PR 4-48	96	112	13	21.09	119	25.58	2.76	Red
20	ICDE 13-3/46/4/50	PR 4-50	100	118	10	22.17	125	26.16	2.80	Red
21	ICDE 13-3/46/4/53	PR 4-53	105	117	16	25.14	131	27.02	2.84	Red
Maximum Value of sample			119	132	19	26.79	136	27.02	2.89	-
Minimum Value of sample			89	110	3	18.71	118	24.37	2.63	-
Average			103.76	119.29	10.71	22.81	127.60	25.30	2.77	-
Standard Deviation			6.95	6.47	4.00	1.92	4.82	0.64	0.07	-
Variance			48.29	41.91	16.01	3.70	23.25	0.41	0.01	-
Standard Error of Mean			1.52	1.41	0.87	0.42	1.05	0.14	0.02	-

4.2.1.2 ICDE 12-3/1 progenies

All the 24 plants of ICDE 12-3/1 progeny identified with a homozygous combination of resistance gene *xa13* and *xa21* marked a height more than the donor parent ISM. The exception to this was the plant ICDE 12-3/1/17, which was the only progeny with a height equal to the donor parent. The average plant height of the family was 118.44 cm with the minimum being 110 cm and maximum being 124 cm. The standard deviation of the trait for the family was 6.95. The height data of individuals are given in Table 12.

4.2.1.3 ICDE 12-3/4 progenies

The average height of the family (ICDE 12-3/4 progenies) was 117.40 cm ranging from 124 cm to 110 cm in height (Table 13) with a standard error of 0.84. Except ICDE 12-3/4/17 (110 cm) all the plants in the family recorded a height more than the donor parent. All the plants of the family were identified to have both *xa13* and *Xa21* genes in homozygous condition. Even though, the common fact evident from the nature of plant height of the family was the plants were similar to the donor parent but they have to be similar to the recurrent parent.

4.2.1.4 ICDE 12-3/6 progenies

The BC_2F_2 plants had an average height of 117.31 cm ranging from 109 cm 124 cm (table 14) with a standard error of 0.59. The variance of trait was 14.90. The plants ICDE 12-3/6/13 and ICDE 12-3/6/15 were the only BC_2F_2 progenies of the family to have a height similar to the donor parent. Although all of the F_2 progenies of the family were having the combination of the resistance genes in a homozygous condition with no segregation, none of them was similar to the recurrent parent.

4.2.1.5 ICDE 12-3/13 progenies

The ICDE 12-3/13 progenies recorded an average height of 115.44 cm with minimum and a maximum height of 105 cm (ICDE 12-3/13/22) and 129 cm (ICDE 12-3/13/21) respectively. Six individuals (ICDE 12-3/13/1 (109 cm), ICDE 12-

Table 12. Morphometric data of progenies of ICDE 12-3/1 identified with resistance genes

Sl. No	Progeny No.	Sample no.	Plant height (cm)	Days to maturity	Number of productive tillers $plant^{-1}$	Length of panicle (cm)	Number of grains $panicle^{-1}$	1000 grain weight (g)	Length/Breadth (L/B) ratio of grain	Kernel Colour
1	ICDE 12-3/1/1	PR 544-1	117	129	14	22.94	128	23.32	3.27	Light red
2	ICDE 12-3/1/2	PR 544-2	116	120	13	22.68	123	23.80	3.17	Dark brown
3	ICDE 12-3/1/3	PR 544-3	118	126	15	22.98	124	25.56	2.90	Dark brown
4	ICDE 12-3/1/4	PR 544-4	113	120	16	23.48	126	24.28	3.11	Dark brown
5	ICDE 12-3/1/5	PR 544-5	119	124	13	25.00	123	22.40	3.25	Dark brown
6	ICDE 12-3/1/6	PR 544-6	115	121	17	24.08	132	22.92	3.32	Light red
7	ICDE 12-3/1/7	PR 544-7	115	124	11	23.88	110	22.58	3.38	Dark brown
8	ICDE 12-3/1/8	PR 544-8	124	120	11	23.74	124	22.00	3.29	Dark brown
9	ICDE 12-3/1/9	PR 544-9	121	131	18	25.30	125	22.72	3.21	Red
10	ICDE 12-3/1/10	PR 544-10	121	127	16	23.66	119	25.03	3.04	Dark brown
11	ICDE 12-3/1/11	PR 544-11	115	120	17	22.90	102	22.04	3.34	Dark brown
12	ICDE 12-3/1/12	PR 544-12	116	132	13	24.90	120	25.52	3.02	Dark brown
13	ICDE 12-3/1/13	PR 544-13	123	127	12	25.34	130	25.20	3.01	Light red
14	ICDE 12-3/1/14	PR 544-14	119	126	15	22.02	124	22.44	3.36	Light red
15	ICDE 12-3/1/15	PR 544-15	121	130	15	22.58	123	21.68	3.71	Red
16	ICDE 12-3/1/16	PR 544-16	121	122	14	24.30	117	24.08	2.99	Light red
17	ICDE 12-3/1/17	PR 544-17	110	120	19	23.10	111	21.34	3.60	Light brown
18	ICDE 12-3/1/18	PR 544-18	121	118	18	22.22	120	24.36	2.92	Light red
19	ICDE 12-3/1/19	PR 544-19	119	129	13	23.48	130	25.20	2.95	Light red
20	ICDE 12-3/1/20	PR 544-20	121	133	16	23.14	132	24.20	3.00	Dark brown

Table 12 Continued.

Sl. No.	Progeny No.	Sample no.	Plant height (cm)	Days to maturity	Number of productive tillers plant^{-1}	Length of panicle (cm)	Number of grains panicle^{-1}	1000 grain weight (g)	Length/Breadth (L/B) ratio of grain	Kernel Colour
21	ICDE 12-3/1/21	PR 544-21	122	125	16	22.32	125	23.20	3.14	Light red
22	ICDE 12-3/1/22	PR 544-22	123	126	11	26.34	139	23.08	3.15	Light red
23	ICDE 12-3/1/23	PR 544-23	116	121	10	23.50	121	21.32	3.24	Light red
24	ICDE 12-3/1/24	PR 544-24	113	124	16	24.30	125	24.12	3.10	Light red
25	ICDE 12-3/1/25	PR 544-25	122	129	15	25.42	130	23.56	3.08	Light red
Maximum Value of sample			124	133	19	26.34	139	25.56	3.71	-
Minimum Value of sample			110	118	10	22.02	102	21.32	2.90	-
Average			118.44	124.96	14.56	23.74	123.32	23.44	3.18	-
Standard Deviation			3.66	4.34	2.43	1.14	7.74	1.31	0.20	-
Variance			13.51	18.79	5.92	1.29	59.98	1.69	0.04	-
Standard Error of Mean			0.74	0.87	0.49	0.23	1.55	0.26	0.04	-

Table 13. Morphometric data of progenies of ICDE 12-3/4 identified with resistance genes

Sl. No.	Progeny No.	Sample no.	Plant height (cm)	Days to maturity	Number of productive tillers $plant^{-1}$	Length of panicle (cm)	Number of grains $panicle^{-1}$	1000 grain weight (g)	Length/Breadth (L/B) ratio of grain	Kernel Colour
1	ICDE 12-3/4/1	PR 547-1	111	118	14	23.68	119	21.6	3.24	Dark brown
2	ICDE 12-3/4/2	PR 547-2	124	121	13	26.48	122	22.94	3.29	Dark brown
3	ICDE 12-3/4/3	PR 547-3	113	119	16	22.70	100	23.76	3.57	Light brown
4	ICDE 12-3/4/4	PR 547-4	116	124	14	23.02	114	23.46	3.35	Light red
5	ICDE 12-3/4/5	PR 547-5	120	124	16	24.76	114	23.60	3.24	Red
6	ICDE 12-3/4/6	PR 547-6	113	122	13	22.80	105	22.86	3.12	Dark brown
7	ICDE 12-3/4/7	PR 547-7	118	124	10	23.20	109	20.13	3.45	Dark brown
8	ICDE 12-3/4/8	PR 547-8	121	124	10	23.62	114	24.72	2.93	Dark brown
9	ICDE 12-3/4/9	PR 547-9	122	126	17	23.82	120	21.62	3.24	Dark brown
10	ICDE 12-3/4/10	PR 547-10	113	122	15	20.66	113	23.72	3.13	Light red
11	ICDE 12-3/4/11	PR 547-11	116	120	14	21.36	113	23.96	3.22	Light red
12	ICDE 12-3/4/12	PR 547-12	113	128	16	22.44	119	24.24	3.14	Light red
13	ICDE 12-3/4/13	PR 547-13	121	131	17	24.74	126	23.64	3.11	Red
14	ICDE 12-3/4/14	PR 547-14	119	128	13	25.68	128	23.66	3.19	Light red
15	ICDE 12-3/4/15	PR 547-15	119	131	18	26.40	133	25.66	2.97	Dark brown
16	ICDE 12-3/4/16	PR 547-16	118	119	14	22.78	111	23.64	3.15	Light red
17	ICDE 12-3/4/17	PR 547-17	110	124	13	21.84	114	22.86	3.10	Dark brown
18	ICDE 12-3/4/18	PR 547-18	121	128	10	23.58	126	24.62	2.93	Red
19	ICDE 12-3/4/19	PR 547-19	117	120	11	22.92	122	25.83	2.99	Dark brown
20	ICDE 12-3/4/20	PR 547-20	115	122	12	21.20	118	22.70	3.24	Light red

Table 13 Continued.

Sl. No.	Progeny No.	Sample no.	Plant height (cm)	Days to maturity	Number of productive tillers plant^{-1}	Length of panicle (cm)	Number of grains panicle^{-1}	1000 grain weight (g)	Length/Breadth (L/B) ratio of grain	Kernel Colour
21	ICDE 12-3/4/21	PR 547-21	119	128	10	21.86	117	23.43	3.09	Light brown
22	ICDE 12-3/4/22	PR 547-22	123	120	15	23.86	125	24.04	3.00	Light red
Maximum Value of sample			124	131	18	26.48	133	25.83	3.57	-
Minimum Value of sample			110	118	10	20.66	100	20.13	2.93	-
Average			117.37	123.78	13.69	23.33	117.40	23.49	3.17	-
Standard Deviation			3.99	3.91	2.46	1.57	7.83	1.28	0.16	-
Variance			15.86	15.24	6.03	2.47	61.45	1.64	0.03	-
Standard Error of Mean			0.84	0.83	0.528	0.33	1.67	0.28	0.03	-

Table 14. Morphometric data of progenies of ICDE 12-3/6 identified with resistance genes

Sl. No.	Progeny No.	Sample no.	Plant height (cm)	Days to maturity	Number of productive tillers $plant^{-1}$	Length of panicle (cm)	Number of grains $panicle^{-1}$	1000 grain weight (g)	Length/Breadth (L/B) ratio of grain	Kernel Colour
1	ICDE 12-3/6/1	PR 549-1	112	113	21	22.40	119	21.68	4.42	Light red
2	ICDE 12-3/6/2	PR 549-2	115	120	14	23.32	112	23.55	3.07	Light brown
3	ICDE 12-3/6/3	PR 549-3	116	116	13	22.62	108	22.48	3.10	Light red
4	ICDE 12-3/6/4	PR 549-4	114	119	16	24.32	94	23.58	3.07	Dark brown
5	ICDE 12-3/6/5	PR 549-5	113	113	13	23.38	103	23.15	3.18	Dark brown
6	ICDE 12-3/6/6	PR 549-6	121	122	18	23.64	95	21.60	3.45	Light red
7	ICDE 12-3/6/7	PR 549-7	124	123	12	26.36	111	21.83	3.71	Red
8	ICDE 12-3/6/8	PR 549-8	118	118	16	23.24	106	23.06	3.15	Light red
9	ICDE 12-3/6/9	PR 549-9	123	117	14	23.10	110	24.20	3.07	Dark brown
10	ICDE 12-3/6/10	PR 549-10	114	120	17	23.54	116	23.48	3.09	Dark brown
11	ICDE 12-3/6/11	PR 549-11	118	119	15	23.50	120	24.45	2.98	Dark brown
12	ICDE 12-3/6/12	PR 549-12	113	114	15	21.46	113	23.53	3.25	Dark brown
13	ICDE 12-3/6/13	PR 549-13	109	116	16	29.16	135	24.08	3.38	Light red
14	ICDE 12-3/6/14	PR 549-14	119	120	10	28.62	121	23.80	3.53	Dark brown
15	ICDE 12-3/6/15	PR 549-15	110	124	12	27.42	112	22.35	4.13	Light red
16	ICDE 12-3/6/16	PR 549-16	119	113	13	28.30	113	22.63	3.95	Dark brown
17	ICDE 12-3/6/17	PR 549-17	114	124	15	22.24	114	23.70	3.22	Light red
18	ICDE 12-3/6/18	PR 549-18	118	120	17	23.76	85	22.30	3.66	Light brown
19	ICDE 12-3/6/19	PR 549-19	119	124	16	22.92	112	22.53	3.32	Light red
20	ICDE 12-3/6/20	PR 549-20	121	120	15	21.20	111	23.70	3.05	Light red
21	ICDE 12-3/6/21	PR 549-21	110	114	18	21.86	118	23.73	3.20	Dark brown

Table 14 Continued.

Sl. No.	Progeny No.	Sample no.	Plant height (cm)	Days to maturity	Number of productive tillers plant^{-1}	Length of panicle (cm)	Number of grains panicle^{-1}	1000 grain weight (g)	Length/Breadth (L/B) ratio of grain	Kernel Colour
22	ICDE 12-3/6/22	PR 549-22	118	118	14	23.86	110	24.68	3.13	Dark brown
23	ICDE 12-3/6/23	PR 549-23	114	117	12	23.16	119	23.75	3.35	Dark brown
24	ICDE 12-3/6/24	PR 549-24	113	119	15	21.41	105	24.30	3.09	Light red
25	ICDE 12-3/6/25	PR 549-25	119	121	18	23.16	117	23.50	3.04	Dark brown
26	ICDE 12-3/6/26	PR 549-26	121	120	13	22.32	105	21.65	3.73	Dark brown
27	ICDE 12-3/6/27	PR 549-27	119	127	16	22.56	120	21.83	3.42	Dark brown
28	ICDE 12-3/6/28	PR 549-28	121	131	18	23.86	118	23.10	3.26	Dark brown
29	ICDE 12-3/6/29	PR 549-29	123	122	13	22.82	120	22.65	3.50	Red
30	ICDE 12-3/6/30	PR 549-30	118	122	11	22.14	117	22.75	3.22	Dark brown
31	ICDE 12-3/6/31	PR 549-31	115	130	11	24.02	120	23.33	3.01	Dark brown
32	ICDE 12-3/6/32	PR 549-32	121	121	13	25.80	105	23.75	3.01	Dark brown
33	ICDE 12-3/6/33	PR 549-33	119	117	14	21.86	114	24.30	3.47	Dark brown
34	ICDE 12-3/6/34	PR 549-34	118	120	19	23.46	122	23.88	3.16	Dark brown
35	ICDE 12-3/6/35	PR 549-35	114	122	16	23.16	138	25.15	2.92	Dark brown
36	ICDE 12-3/6/36	PR 549-36	115	128	15	23.74	125	23.60	3.26	Dark brown
37	ICDE 12-3/6/37	PR 549-37	122	118	17	26.12	112	23.98	3.25	Dark brown
38	ICDE 12-3/6/38	PR 549-38	119	113	12	26.74	114	24.18	3.37	Light brown
39	ICDE 12-3/6/39	PR 549-39	124	121	12	28.14	110	23.65	3.69	Dark brown
40	ICDE 12-3/6/40	PR 549-40	121	110	13	23.86	127	23.63	3.01	Dark brown
41	ICDE 12-3/6/41	PR 549-41	120	132	14	24.42	123	22.65	3.28	Dark brown
42	ICDE 12-3/6/42	PR 549-42	114	124	13	24.28	116	21.60	3.42	Dark brown
43	ICDE 12-3/6/43	PR 549-43	116	126	19	23.32	126	23.62	3.05	Dark brown

Table 14 Continued.

	Plant height (cm)	Days to maturity	Number of productive tillers plant^{-1}	Length of panicle (cm)	Number of grains panicle^{-1}	1000 grain weight (g)	Length/Breadth (L/B) ratio of grain	Kernel colour
Maximum Value of sample	124	132	21	29.16	138	25.15	4.42	-
Minimum Value of sample	109	110	10	21.20	85	21.60	2.92	-
Average	117.31	120.19	14.74	23.97	114.21	23.28	3.32	-
Standard Deviation	3.86	4.99	2.48	2.02	9.81	0.90	0.32	-
Variance	14.90	24.92	6.15	4.07	96.31	0.81	0.10	-
Standard Error of Mean	0.59	0.76	0.38	0.31	1.50	0.14	0.05	-

3/13/5 (110 cm), ICDE 12-3/13/6 (109 cm), ICDE 12-3/13/17 (109 cm), ICDE 12-3/13/22 (105 cm) and ICDE 12-3/13/27 (110 cm)) were having a height less than or equal to the better parent ISM. None of the individuals was similar to Prathyasa in plant height. The Table 15 provides the phenotypic data of ICDE 12-3/13 progenies. The standard deviation and standard error of mean of the trait for the family was 5.33 and 1.03 respectively.

4.2.1.6 ICDE 12-3/14 progenies

The progenies of ICDE 12-3/14 plant marked a varying height ranging from 123 cm (ICDE 12-3/14/15) to 108 cm (ICDE 12-3/14/9) with an average of 114.35 cm and a standard error of 1.17 (Table 16). In the progenies of ICDE 12-3/14 also, six plants (ICDE 12-3/14/1 (108 cm), ICDE 12-3/14/2 (109 cm), ICDE 12-3/14/3 (109 cm), ICDE 12-3/14/9 (108 cm), ICDE 12-3/14/10 (109 cm) and ICDE 12-3/14/17 (110 cm)) were having a height less than or equal to the donor parent, Improved Samba Mahsuri. Remaining 11 plants were taller than the better parent ISM. Hence from the phenotypic observation of plant height, none of the ICDE 12-3/14 progenies resembled the recurrent parent exactly.

4.2.2 Days to Maturity

The number of days to maturity of each BC_2F_2 individual in family wise is depicted in the Tables 11-16. The parents Prathyasa and Improved Samba Mahsuri were harvested in mean 108 and 145 days respectively (Table 10). The values of each BC_2F_2 individual ranged between these values with some exceptions.

4.2.2.1 ICDE 13-3/46/4 Progenies

Generally, the plants of ICDE 13-3/46/4 progenies were more or less similar to the recurrent parent for the trait, number of days to maturity. The progenies took an average of 119.29 days to reach maturity ranging from 132 days to 110 days (ICDE 13-3/46/4/12). Except for ICDE 13-3/46/4/15 and ICDE 13-3/46/4/16 (both at 132 days), all other plants were harvested with full maturity before 130 days. The recurrent parent Prathyasa matured early in 108 days, while 145 days were needed

Table 15. Morphometric data of progenies of ICDE 12-3/13 identified with resistance genes

Sl. No.	Progeny No.	Sample no.	Plant height (cm)	Days to maturity	Number of productive tillers $plant^{-1}$	Length of panicle (cm)	Number of grains $panicle^{-1}$	1000 grain weight (g)	Length/Breadth (L/B) ratio of grain	Kernel Colour
1	ICDE 12-3/13/1	PR 556-1	109	125	18	24.12	115	19.72	3.26	Dark brown
2	ICDE 12-3/13/2	PR 556-2	116	126	12	22.46	106	21.32	3.88	Dark brown
3	ICDE 12-3/13/3	PR 556-3	114	121	10	22.28	118	22.40	3.46	Light red
4	ICDE 12-3/13/4	PR 556-4	115	124	10	24.60	129	23.32	3.35	Light brown
5	ICDE 12-3/13/5	PR 556-5	110	129	13	24.23	115	22.04	3.32	Dark brown
6	ICDE 12-3/13/6	PR 556-6	109	123	14	23.50	115	22.58	3.34	Dark brown
7	ICDE 12-3/13/7	PR 556-7	119	131	10	25.72	109	21.68	2.93	Dark brown
8	ICDE 12-3/13/8	PR 556-8	125	127	11	24.40	115	23.56	3.17	Red
9	ICDE 12-3/13/9	PR 556-9	120	120	19	28.25	148	25.20	2.96	Dark brown
10	ICDE 12-3/13/10	PR 556-10	116	132	14	24.76	132	22.44	3.13	Dark brown
11	ICDE 12-3/13/11	PR 556-11	116	127	17	24.52	130	22.72	3.09	Light brown
12	ICDE 12-3/13/12	PR 556-12	115	126	19	24.36	123	22.00	3.06	Light red
13	ICDE 12-3/13/13	PR 556-13	118	130	13	24.22	124	24.08	3.05	Dark brown
14	ICDE 12-3/13/14	PR 556-14	114	122	17	25.68	126	22.92	3.55	Dark brown
15	ICDE 12-3/13/15	PR 556-15	124	123	14	26.40	115	25.56	3.02	Red
16	ICDE 12-3/13/16	PR 556-16	117	119	16	22.78	102	24.28	3.00	Dark brown
17	ICDE 12-3/13/17	PR 556-17	109	129	11	21.84	116	24.20	2.95	Dark brown
18	ICDE 12-3/13/18	PR 556-18	120	136	15	24.78	118	24.36	2.93	Red
19	ICDE 12-3/13/19	PR 556-19	118	138	14	24.68	120	25.03	3.01	Light red

Table 15 Continued.

Sl. No.	Progeny No.	Sample no.	Plant height (cm)	Days to maturity	Number of productive tillers plant^{-1}	Length of panicle (cm)	Number of grains panicle^{-1}	1000 grain weight (g)	Length/Breadth (L/B) ratio of grain	Kernel colour
20	ICDE 12-3/13/20	PR 556-20	114	120	17	24.50	122	25.92	2.99	Light red
21	ICDE 12-3/13/21	PR 556-21	129	126	14b	21.44	125	25.20	3.14	Dark brown
22	ICDE 12-3/13/22	PR 556-22	105	120	13	28.32	129	23.08	3.85	Light red
23	ICDE 12-3/13/23	PR 556-23	114	124	12	24.60	121	24.12	3.19	Light red
24	ICDE 12-3/13/24	PR 556-24	116	121	10	24.70	123	23.20	3.10	Light red
25	ICDE 12-3/13/25	PR 556-25	112	124	12	24.30	118	23.80	3.07	Dark brown
26	ICDE 12-3/13/26	PR 556-26	113	123	14	25.38	126	23.85	3.24	Light red
27	ICDE 12-3/13/27	PR 556-27	110	132	16	23.92	125	24.05	3.16	Dark brown
Maximum Value of sample			129	138	19	28.32	148	25.92	3.88	-
Minimum Value of sample			105	119	10	21.44	102	19.72	2.93	-
Average			115.44	125.86	13.89	24.48	121.02	23.44	3.20	-
Standard Deviation			5.33	4.95	2.76	1.59	8.99	1.42	0.26	-
Variance			28.41	24.59	7.64	2.55	80.89	2.02	0.06	-
Standard Error of Mean			1.03	0.95	0.54	0.30	1.74	0.27	0.04	-

Table 16. Morphometric data of progenies of ICDE 12-3/14 identified with resistance genes

Sl. No.	Progeny No.	Sample no	Plant height (cm)	Days to maturity	Number of productive tillers $plant^{-1}$	Length of panicle (cm)	Number of grains $panicle^{-1}$	1000 grain weight (g)	Length/Breadth (L/B) ratio of grain	Kernel Colour
1	ICDE 12-3/14/1	PR 557-1	108	121	16	23.82	123	23.45	3.03	Light red
2	ICDE 12-3/14/2	PR 557-2	109	121	14	23.24	117	21.63	3.31	Light red
3	ICDE 12-3/14/3	PR 557-3	109	126	16	24.08	124	23.42	3.17	Dark brown
4	ICDE 12-3/14/4	PR 557-4	116	124	15	25.92	130	24.15	3.16	Light red
5	ICDE 12-3/14/5	PR 557-5	116	120	13	23.36	126	23.48	3.18	Light red
6	ICDE 12-3/14/6	PR 557-6	119	120	15	24.53	117	23.85	3.02	Dark brown
7	ICDE 12-3/14/7	PR 557-7	118	124	16	26.32	96	21.20	3.34	Dark brown
8	ICDE 12-3/14/8	PR 557-8	121	128	15	25.28	119	23.70	3.10	Red
9	ICDE 12-3/14/9	PR 557-9	108	120	10	24.88	124	25.55	2.95	Dark brown
10	ICDE 12-3/14/10	PR 557-10	109	124	14	27.20	112	22.34	3.37	Light red
11	ICDE 12-3/14/11	PR 557-11	113	126	16	25.04	127	24.88	2.98	Light brown
12	ICDE 12-3/14/12	PR 557-12	117	126	11	23.68	80	21.35	3.39	Dark brown
13	ICDE 12-3/14/13	PR 557-13	118	126	17	23.36	76	23.13	3.28	Dark brown
14	ICDE 12-3/14/14	PR 557-14	114	122	10	23.20	96	22.15	4.39	Light brown
15	ICDE 12-3/14/15	PR 557-15	123	118	11	25.90	100	23.40	3.09	Dark brown
16	ICDE 12-3/14/16	PR 557-16	116	118	16	24.50	102	23.20	3.01	Dark brown
17	ICDE 12-3/14/17	PR 557-17	110	114	19	24.10	128	25.38	3.00	Light red
Maximum Value of sample			123	128	19	27.20	130	25.55	4.39	-
Minimum Value of sample			108	114	10	23.20	76	21.20	2.95	-
Average			114.35	122.24	14.35	24.61	111.66	23.31	3.22	-
Standard Deviation			4.81	3.72	2.57	1.19	5.35	1.29	0.33	-
Variance			23.12	13.82	6.62	1.42	28.63	1.65	0.11	-
Standard Error of Mean			1.17	0.90	0.62	0.29	1.29	0.31	0.08	-

for Improved Samba Mahsuri. Hence ICDE 13-3/46/4/15 and ICDE 13-3/46/4/16 were more proximal to ISM for this trait. The standard error of the trait for the family was 1.41 with a variance of 41.91. The data is given in Table 11.

4.2.2.2 ICDE 12-3/1 Progenies

The ICDE 12-3/1 progenies had an average number of days to maturity of 124.96 days with almost all individuals having the trait values above 120 days ranging from 118 days to 133 days. The only exception was ICDE 12-3/1/18 which matured in 118 days. As the Prathyasa scored 108 days for the trait the individuals were distant enough from the recurrent parent for the trait with an average of 125 days and a standard deviation of 4.34 (Table 12).

4.2.2.3 ICDE 12-3/4 Progenies

These progenies showed an average for the trait, days to maturity with a value of 123.78. Like ICDE 12-3/1 progenies, almost all progenies of ICDE 12-3/4 progenies matured and harvested after reaching 120 days or more after sowing. The exceptions were ICDE 12-3/4/1 (118 days), ICDE 12-3/4/3 (119 days) and ICDE 12-3/4/16 (119 days). The plants ICDE 12-3/4/13 and ICDE 12-3/4/15 were harvested at 131 days with full maturity, the maximum value for the trait. Thus the range of values for the trait was from 118 days to 131 days. The twenty two membered family showed a standard deviation and standard error of 3.91 and 0.83 respectively (Table 13).

4.2.2.4 ICDE 12-3/6 Progenies

The mean days to maturity of ICDE 12-3/6 progenies was 120.19 days ranging from the minimum value of 110 days (ICDE 12-3/6/40) to the maximum value of 132 days (ICDE 12-3/6/41). There were plants which matured in 5 days after the recurrent parent got completely matured, the plants were ICDE 12-3/6/1, ICDE 12-3/6/5, ICDE 12-3/6/16 and ICDE 12-3/6/38. The plant with least value for the trait was ICDE 12-3/6/40 having only two days difference for maturity with

the recurrent parent. The standard deviation and standard error of the trait for the family was 4.99 and 0.76 respectively (Table 14).

4.2.2.5 ICDE 12-3/13 Progenies

Among ICDE 12-3/13 progenies, ICDE 12-3/13/19 had maximum delay in maturity of 138 days after sowing. The progeny ICDE 12-3/13/16 took only 119 days to mature, the minimum value for the trait in the progenies. The average value of the family for the trait was 125.86 days with a standard deviation and standard error of 4.95 and 0.95 respectively (Table 15). Thus, almost all plants of the family were very less similar to the recurrent parent than expected.

4.2.2.6 ICDE 12-3/14 Progenies

The average number of days to maturity for the family consisting of 17 plants was 122.24 days ranging from minimum value of 114 days (ICDE 12-3/1/17) to a maximum value of 128 days (ICDE 12-3/14/8). The plant ICDE 12-3/14/17 was somewhat similar to the recurrent parent when days to maturity was considered. The standard error was 0.90 (Table 16).

4.2.3 Number of Productive Tillers Plant^{-1}

The number of productive tillers plant^{-1} is an important trait determining the varietal characteristics as well as the yield of a rice plant. The number of tillers was counted at the stage of physiological maturity and tabulated with respect to each individual (Tables 10-16). The average count for recurrent parent Prathyasa and donor parent Improved Samba Mahsuri was 11.98 and 15.87 tillers respectively (Table 10).

4.2.3.1 ICDE 13-3/46/4 Progenies

The average number of productive tillers per plant among the progenies ICDE 13-3/46/4 was 10.71. The number of productive tillers for Prathyasa was twelve. So the average value of the family is almost proximal to the recurrent parent. There

were four plants, ICDE 13-3/46/4/1, ICDE 13-3/46/4/22, ICDE 13-3/46/4/31 and ICDE 13-3/46/4/46 with the count equal to that of the recurrent parent. Five plants of the family with gene combination were superior to the donor parent. The maximum value for the trait was nineteen (ICDE 13-3/46/4/34), whereas the minimum was three tillers (ICDE 13-3/46/4/13) among the twety-one progenies with gene combination. The variance for the trait was 16.01 (Table 11).

4.2.3.2 ICDE 12-3/1 Progenies

The 25 membered F_2 progenies of ICDE 12-3/1 had an average number of productive tillers, 14.56 (S.E=0.49), which is 1.5 less than the value of donor parent. the maximum value was nineteen tillers (ICDE 12-3/1/17) and a minimum value was ten tillers (ICDE 12-3/1/23). The plant ICDE 12-3/1/13 had a value equal to that of recurrent parent Prathyasa. The Table 12 included the data of number productive tillers of the progenies.

4.2.3.3 ICDE 12-3/4 Progenies

The ICDE 12-3/4 progenies had an average of 13.69 for the trait number of productive tillers ranging from ten (4 individuals- ICDE 12-3/4/7, ICDE 12-3/4/8, ICDE 12-3/4/18 and ICDE 12-3/4/21) to eighteen tillers (ICDE 12-3/4/15). The standard deviation of the trait for the family was 2.46 (Table 13). The plant ICDE 12-3/4/20 had an equal value for the trait as that of the recurrent parent. Some of the plants were similar to Prathyasa while some were not, while considering the trait productive tillers.

4.2.3.4 ICDE 12-3/6 Progenies

The average count of productive tillers of the ICDE 12-3/6 progenies was 14.74 ranging from a minimum of ten tillers in ICDE 12-3/6/14 to a maximum of twenty-one tillers in ICDE 12-3/6/1 plant. The standard error was 0.38. The plants ICDE 12-3/6/7, ICDE 12-3/6/15, ICDE 12-3/6/23, ICDE 12-3/6/38 and ICDE 12-3/6/39 had twelve productive tillers and were similar to the recurrent parent which is having same number of productive tillers (Table 14).

4.2.3.5 ICDE 12-3/13 Progenies

The maximum number of productive tillers in the ICDE 12-3/13 progenies was nineteen and the minimum number was ten. ICDE 12-3/13/12 was the plant with maximum productive tillers, whereas ICDE 12-3/13/3, ICDE 12-3/13/4, ICDE 12-3/13/7, and ICDE 12-3/13/24 were the plants with a minimum number of productive tillers. The average of the family was 13.88 productive tillers (S.E=0.54; Table 15), which is the intermediate value between both parents for the trait. ICDE 12-3/13/2, ICDE 12-3/13/23 and ICDE 12-3/13/25 were the plants having twelve productive tillers exactly same as that of Prathyasa. The standard deviation of the character was 2.76.

4.2.3.6 ICDE 12-3/14 Progenies

The trait value of each individual having gene combination in ICDE 12-3/14 progeny family ranges from ten to nineteen tillers with ICDE 12-3/14/9 and ICDE 12-3/14/14 plants with least count of productive tillers and ICDE 12-3/14/17 with the maximum count. The average of the family was 14.35 with a standard error of 0.62 (Table 16). No individual was observed with the same number of productive tillers as that of the recurrent parent. Five plants were having sixteen productive tillers same as that of donor parent while the individuals ICDE 12-3/14/13 and ICDE 12-3/14/17 outnumbered the donor parent for the trait.

4.2.4 Length of Panicle (cm)

The length of panicle of each BC_2F_2 individual identified with genes of resistance was calculated by taking the average of length of five best panicles of the plant. Thus, the panicle length of Prathyasa and Improved Samba Mahsuri was 23.16 cm and 21.41 cm respectively (Table 10). Hence it can be said that an identifiable significant difference between the parents was not there. The average panicle length of each BC_2F_2 individual with resistance gene combination was measured and tabulated family wise (Tables 11-16).

4.2.4.1 ICDE 13-3/46/4 Progenies

The average length of a panicle in ICDE 13-3/46/4 progenies was 22.81 cm (S.E=0.42) which was slightly less than that of the recurrent parent (Table 11). The longest panicle among them was found in ICDE 13-3/46/4/46 with 26.79 cm. The shortest was 18.71 cm found in ICDE 13-3/46/4/13 plant. In the family, there were plants with panicle length longer than the better parent and shorter than the other parent. The plant ICDE 13-3/46/4/7 was found to have an average panicle length of 23.18 cm similar to the recurrent parent Prathyasa.

4.2.4.2 ICDE 12-3/1 Progenies

The ICDE 12-3/1 progenies were having an average length of panicle 23.74 cm ranging from the longest panicle size, 26.34 cm of ICDE 12-3/1/22 and shortest, 22.02 cm of ICDE 12-3/1/14. The standard error was 0.23 (Table 12). There were plants with in the panicle length clustering around the recurrent parent mean. Only a few plants were having a much higher difference with the recurrent parent for the trait.

4.2.4.3 ICDE12-3/4 Progenies

The mean length of panicle calculated cumulating mean length of five best panicles of each individual in the ICDE 12-3/4 progenies was 23.33 cm. The highest value of the trait in the progeny was 26.48 cm, whereas the least value of the trait was 20.66 cm. ICDE 12-3/4/2 and ICDE 12-3/4/10 were the plants that showed the longest and shortest panicles respectively (Table 13). The variance of the character was 2.47.

4.2.4.4 ICDE 12-3/6 Progenies

The average value calculated over forty-three BC_2F_2 progenies of the family was 23.97 cm. The highest mean of panicle length was for ICDE 12-3/6/13 plant and the lowest mean value was for ICDE 12-3/6/20. The highest and lowest values were 29.16 cm and 21.20 cm respectively(Table 14). There were several plants with

a mean length of panicle similar to the mean of Prathyasa parent. However the variance of the data was 4.07.

4.2.4.5 ICDE 12-3/13 Progenies

Among ICDE 12-3/13 progenies identified with gene combination, the BC_2F_2 plant ICDE 12-3/13/22 showed the highest mean for length of the panicle. The value was 28.32 cm. Similarly, the shortest panicle length among the progeny was found in ICDE 12-3/13/21 with 21.44 cm (Table 15). The average value of panicle length for the entire family was 24.48 cm with a standard error of 0.30. The mean of the family itself deviates from recurrent parent value by 1.32 cm.

4.2.4.6 ICDE 12-3/14 Progenies

The shortest and longest length of panicle among ICDE 12-3/14 progenies was found in ICDE 12-3/14/14 and ICDE 12-3/14/10 respectively in the seventeen membered family each of which having *xa13* and *Xa21* genes in combination. The shortest length was 23.20 cm and it was the same as that of Prathyasa. The length of the longest panicle was 27.20 cm. The family was observed to have an average panicle length of 24.61 cm (S.E=0.29) calculated from the mean of each individual.

4.2.5 Number of Grains Panicle^{-1}

The number of grains per panicle of a rice plant is essentially the yield determining character of the plant. Definitely, each variety has characteristic yield determined by these traits such as number of grains per panicle, grain weight and so on. The mean number of grains panicle^{-1} of Prathysa and Improved Samba Mahsuri was 126.12 and 116.10 respectively (Table 10). The value was calculated by taking the mean of number of grains of five best panicles of each individual. The values of each BC_2F_2 individual family wise is given in the Tables 11-16.

4.2.5.1 ICDE 13-3/46/4 Progenies

The average value of number of grains per panicle of twenty-one progenies (ICDE 13-3/46/4 progenies) identified with resistance gene combination was 127.60 or otherwise 128 grains with a standard error and variance of 1.05 and 23.25 respectively (Table 11). The maximum grains per panicle was observed in the progeny ICDE 13-3/46/4/33 (136 grains) and the minimum was observed in ICDE 13-3/46/4/7 (118 grains). Even the progeny with least number of grains was superior to the donor parent for the trait.

4.2.5.2 ICDE 12-3/1 Progenies

The progeny ICDE 12-3/1/22 was found to have the highest number of grains per panicle among the family with 139 grains. The plant ICDE 12-3/1/11 was having the least number of grains with 102 grains per panicle. The average number of grains per each panicle of the family was calculated as 123.32 with a standard error of 1.55. Table 12 provides the morphometric data of the progenies.

4.2.5.3 ICDE 12-3/4 Progenies

The twenty-two membered family had an average number of grains per panicle of 117.40 grains (S.E=1.67). Among the progenies, the maximum number of grains per panicle was observed in the progeny ICDE 12-3/4/15 and it was 133 grains. The minimum value was 100 grains per panicle which was found in the plant ICDE 12-3/4/3 (Table 13). The average of the family itself deviates from donor parent only by one or two grains which may not be significant.

4.2.5.4 ICDE 12-3/6 Progenies

The mean value of number of grains per panicle over the forty-three progenies was calculated as 114.21 grains ranging from the maximum of 138 grains in ICDE 12-3/6/35 to a minimum value of 85 in ICDE 12-3/6/18. There were several plants with their mean values of number of grains per panicle clustering around the value of donor parent. ICDE 12-3/6/43 had exactly same mean value for grains per panicle

(126) as the recurrent parent Prathyasa (Table 14). The variance of the character was 1.50.

4.2.5.5 ICDE 12-3/13 Progenies

The mean value of ICDE 12-3/13 progenies for the trait of number grains per panicle was 121.02 grains which were averaged over the twenty-seven progenies identified with presence BLB R-genes in combination. Highest value was 148 grains in ICDE 12-3/13/9 and lowest value was 102 grains in ICDE 12-3/4/16. While having a glimpse over the tabulated data (Table 15) there are plants with their values superior to the recurrent parent as well as inferior to the donor parent. The progeny ICDE 12-3/13/26 was exactly having the same number of grains per panicle as the recurrent parent Prathyasa.

4.2.5.6 ICDE 12-3/14 Progenies

The trait number of grains panicle^{-1} of ICDE 12-3/14 progenies ranged from a minimum value of 76 grains in ICDE 12-3/14/13 to a maximum of 130 grains in ICDE 12-3/14/4 (Table16) . The mean value of the trait calculated over the seventeen membered family was 111.66 grains. Except four plants (ICDE 12-3/14/4 (130 grains), ICDE 12-3/14/5 (126 grains), ICDE 12-3/14/11 (127 grains) and ICDE 12-3/14/17 (128 grains)), none of the plants were found superior to the better parent, Prathyasa. The number of grains panicle^{-1} of the Prathyasa parent was 126 grains (Table 10).

4.2.6 1000 Grain Weight (g)

1000 grain weight is a peculiar character of each variety. Hence it is used to compare the similarity of a segregating population or backcross population with its parents. The 1000 grain weight of parents Prathyasa and Improved Samba Mahsuri was 26.67 g and 15.15 g respectively (Table 10). The 1000 grain weight of each individual reported with presence of genes in combination was tabulated family wise in Tables 11-16.

4.2.6.1 ICDE 13-3/46/4 Progenies

The 1000 grain weight of twenty one ICDE 13-3/46/4 progenies was determined and tabulated. The average value of the family was 25.30 g ranging from 24.37 g (ICDE 13-3/46/4/11) to 27.02 g (ICDE 13-3/46/4/53). All the plants in the family were having 1000 grain weight more than or equal to 24 g (Table 11). Except the plant ICDE 13-3/46/4/53, none among the twenty-one progenies were found superior to the better parent for the trait. The better parent was Prathyasa with 1000 grain weight 26.67 g.

4.2.6.2 ICDE 12-3/1 Progenies

The ICDE 12-3/1 progenies marked a mean 1000 grain weight of 23.44 g. The twenty-five membered BC_2F_2 family reported to have gene combination showed the highest value of 1000 grain weight, 25.56 g. The lowest value was 21.32 g. The progenies, ICDE 12-3/1/3 and ICDE 12-3/23 were the plants with the highest and the lowest values for the trait respectively (Table 12). With a few plants as exception, the progenies had 1000 grain weight below 24 g.

4.2.6.3 ICDE 12-3/4 Progenies

Among the ICDE 12-3/4 progenies, except few plants the 1000 grain weight of individuals was below 24 g with an average of 23.49 g and standard error of 0.28 (Table 13). Among twenty-two plants reported to have gene combination, none of them had an equal 1000 grain weight as the recurrent parent had. The maximum value of the trait among the progeny was 25.83 g (ICDE 12-3/4/19) and the minimum value of the trait was 20.13 g (ICDE 12-3/4/7), whereas the trait value of recurrent parent was 26.67 g.

4.2.6.4 ICDE 12-3/6 Progenies

The mean 1000 grain weight of ICDE 12-3/6 progenies was 23.28 g ranging from a minimum of 21.60 g to a maximum value of 25.15 g. The Table 14 shows the data of 1000 grain weight of the progeny. ICDE 12-3/6/42 and ICDE 12-3/6/35

were the plants having the minimum and maximum grain weight respectively. Among the forty-three plants having gene combination, only about nine plants were having 1000 grain weight more than or equal to 24 g.

4.2.6.5 ICDE 12-3/13 Progenies

The BC_2F_2 family with twenty-seven progenies with gene combination had 23.44 g as the mean 1000 grain weight with a standard error of 0.27 (Table 15). The BC_2F_2 plant with the highest 1000 grain weight was ICDE 12-3/13/20. The plant was found to have a weight of 25.92 g for 1000 whole grains. Similarly, the progeny ICDE 12-3/13/1 was found to have the lowest 1000 grain weight, i.e. 19.72 g.

4.2.6.6 ICDE 12-3/14 Progenies

Thousand grains of ICDE 12-3/14 progenies weighed an average of 23.31 g (S.E=0.31), whereas the maximum weight was observed in ICDE 12-3/14/9 (25.55 g). The minimum weight of 1000 grains among the progenies was 21.20 g, when the grains of ICDE 12-3/14/7 was weighed. Among the seventeen plants reported with a combination of resistance genes, only four plants were having a 1000 grain weight more than 24 g, whereas the trait value of Prathyasa parent was 26.67 g.

4.2.7 Length/Breadth Ratio of Grain

The length and breadth of the grains were measured using vernier caliper. The ratio was then calculated and tabulated. The L/B ratio of recurrent parent Prathyasa was 2.77 and of Improved Samba Mahsuri was 4.03 (Table 10). The length/breadth ratio of each individual is tabularized in Tables 11-16.

4.2.7.1 ICDE 13-3/46/4 Progenies

The ICDE 13-3/46/4 progenies showed only less variation in length/breadth ratio of the grain ranging from 2.63 (ICDE 13-3/46/4/7) to 2.89 (ICDE 13-3/46/4/44). The mean L/B ratio of the family was 2.77 which is the same as that of recurrent parent (Table 11). Hence all the plants were more similar to recurrent

parent in grain size expressed quantitatively as its L/B ratio. The standard error of mean of the trait was 0.02.

4.2.7.2 ICDE 12-3/1 Progenies

The difference between the maximum and minimum values of L/B ratio in the ICDE 12-3/1 progenies was 0.81. Hence a significant variation can be observed among the plants with a maximum value of 3.71 (ICDE 12-3/1/15) to a minimum value of 2.90 (ICDE 12-3/1/3). The average value of the BC_2F_2 family was 3.18 (Table 12).

4.2.7.3 ICDE 12-3/4 Progenies

The lowest L/B ratio of grain in the family was observed in ICDE 12-3/4/18 and ICDE 12-3/4/10 with a value of 2.93. The highest ratio of 3.57 was observed in ICDE 12-3/4/3. The mean ratio of the family was 3.17 (Table 13). The higher ratio of the progenies proclaims its similarity towards donor parent rather than to the recipient one. Higher value was due to longer grains with narrow breadth.

4.2.7.4 ICDE 12-3/6 Progenies

The forty-three membered family manifested a wide variation for the trait grain size, estimated quantitatively as L/B ratio. The mean ratio of the family was 3.32 (S.E=0.05) ranging from 2.92 (ICDE 12-3/6/35) to 4.42 (ICDE 12-3/6/1). The ratio of majority of the progenies was between the 3.0 and 3.4 (Table 14). Hence there was no plant that had similarity with the recurrent parent for the trait.

4.2.7.5 ICDE 12-3/13 Progenies

The mean L/B ratio of grains in ICDE 12-3/13 progenies was 3.20. The maximum value of the trait was 3.88 observed in ICDE 12-3/13/2, whereas the minimum value was 2.93 showed by ICDE 12-3/13/18 (Table 15). Considering a few plants as exceptions, all the plants were significantly differed from the recurrent parent for the trait than expected.

4.2.7.6 ICDE 12-3/14 Progenies

The mean value of the family for the trait was found to be 3.22. The progenies ICDE 12-3/14/9 and ICDE 12-3/14/14 recorded the minimum and maximum value of length/breadth ratio of the grain respectively. The lowest was 2.95 and the highest value was 4.39 (Table 16). Hence the progeny ICDE 12-3/14/14 was found superior to the donor parent for the trait. The L/B ratio recorded in donor parent was 4.03. Considering the values and the size of the grains, it can be concluded that no plants were found to have an equivalent L/B ratio of grain as that of the recurrent parent.

4.2.8 Kernel Colour

The kernel colour of each individual as well as parents were recorded visually. The kernel colour of recurrent parent Prathyasa was red where as that of donor parent Improved Samba Mahsuri was white (Table 10). The kernel colour of each individual identified with resistance gene combination was recorded and shown in the Tables 11-16.

4.2.8.1 ICDE 13-3/46/4 Progenies

Among the ICDE 13-3/46/4 progenies, all the plants inherited the red kernel colour of Prathyasa with some exceptions. The exceptions were plants ICDE 13-3/46/4/15 and ICDE 13-3/46/4/17, which had light red kernels (Table 11).

4.2.8.2 ICDE 12-3/1 Progenies

The plants ICDE 12-3/1/9 and ICDE 12-3/1/15 were the only plants observed with red kernels (Table 12). All other plants had varying kernel colour of light red, dark brown and light brown. Twelve plants were observed with light red colour of kernel.

4.2.8.3 ICDE 12-3/4 Progenies

Among ICDE 12-3/4 progenies, three plants namely, ICDE 12-3/4/5, ICDE 12-3/4/13 and ICDE 12-3/4/18 were found with red colour for the kernels (Table13). The remaining plants had a discrete variation for kernel colour from light red to light brown. Among the progenies, eight plants were found to have light kernels.

4.2.8.4 ICDE 12-3/6 Progenies

Among the forty-three plants identified with gene combination, two plants had red kernel, ten plants had light red kernels, twenty-eight plants had dark brown kernels and three plants had light brown kernels. The plants found with red kernels were ICDE 12-3/6/7 and ICDE 12-3/6/29 (Table 14).

4.2.8.5 ICDE 12-3/13 Progenies

Three progenies of ICDE 12-3/13 namely, ICDE 12-3/13/8, ICDE 12-3/13/15 and ICDE 12-3/13/18 were observed with red kernels (Table 15). Eight plants had light red kernels, fourteen plants had dark brown kernels and the remaining had light brown kernels.

4.2.8.6 ICDE 12-3/14 Progenies

Among the ICDE 12-3/14 progenies the 8th plant was the only plant observed with red kernel colour. Other kernel colour such as light red (six plants), dark brown (eight plants) and light brown (two plants) were also observed in the progenies.

4.3 EUCLIDEAN DISTANCE FROM RECURRENT PARENT

The 155 BC_2F_2 plants identified with the combination of R-genes were subjected to proximity dissimilarity matrix analysis using Euclidean distance method by Shifriss and Sacks, (1980). The quantitative trait data of each individual was used to calculate the euclidean distance. The euclidean distance was a measure of proximity towards recurrent parent for each individual. For an individual,

minimum the euclidean distance from the recurrent parent, maximum will be proximity to the recurrent parent.

4.3.1 Euclidean Distance Analysis of Each BC_2F_2 Family

The mean values of quantitative characters of each BC_2F_2 family was tabulated (Table 17) and the euclidean distance of the family from Prathyasa was calculated by considering each family as an individual. The euclidean distance analysis revealed that family of ICDE 13-3/46/4 progenies was the most proximal to the recurrent parent Prathyasa with a euclidean distance of 2.73. The euclidean distance of each family from Prathyasa is provided in Table 17.

4.3.2 Euclidean Distance Analysis of Each BC_2F_2 progeny

The euclidean distance of all the individuals identified with gene combination from the recurrent parent Prathyasa was calculated based upon the proximity dissimilarity matrix of the individuals and the parents. It resulted in a 157 x 157 size proximity dissimilarity matrix. The Tables 18-23 represent the euclidean distance of each individual from Prathyasa in family wise. The progenies with minimum euclidean distance from the parent Prathyasa were ICDE 13-3/46/4/31 (1.25), ICDE 13-3/46/4/7 (2.35), ICDE 13-3/46/4/48 (3.06), ICDE 13-3/46/4/50 (4.01), ICDE 13-3/46/4/22 (5.60), ICDE 13-3/46/4/53 (6.92) and ICDE 13-3/46/4/24 (7.83) (Table 18). The maximum euclidean distance among the BC_2F_2 progenies was 57.20 of the progeny ICDE 12-3/14/14 (Table 23). The genetic distance between the parents was 125.10 (Table 24).

The plants identified with the presence of *xa13* and *xa5* genes i.e. ICDE 13-3/46/4/41 and ICDE 13-3/46/4/46 had a euclidean distance of 8.45 and 11.46 respectively (Table 18).

Table 17. Mean morphometric data of BC_2F_2 progenies identified with resistance genes

Sl. No.	F_2 family	Sample no.	Plant height (cm)	Days to maturity	Number of productive tillers plant^{-1}	Length of panicle (cm)	Number of grains panicle^{-1}	1000 grain weight (g)	Length/Breadth (L/B) ratio of grain	ED value from Prathyasa
1	ICDE 12-3/1 progenies	PR 544	118.44	125	15	23.74	123.3	23.44	3.18	15.34
2	ICDE 12-3/4 progenies	PR 547	117.37	124	14	23.33	117.4	23.49	3.17	14.60
3	ICDE 12-3/6 progenies	PR 549	117.31	120	15	23.97	114.2	23.28	3.32	18.98
4	ICDE 12-3/13 progenies	PR 556	115.44	126	14	24.48	121.0	23.44	3.20	14.46
5	ICDE 12-3/14 progenies	PR 557	114.35	122	14	24.61	111.7	23.31	3.22	19.63
6	ICDE 13-3/46/4 progenies	PR 4	103.76	119	11	22.81	127.6	25.30	2.77	2.73
7	MO 21	Prathyasa	98.09	108.28	11.98	23.16	126.12	26.67	2.77	0.00
8	RPBio-226	ISM	110.25	145.33	15.87	21.41	116.10	15.15	4.03	49.04
Mean			114.446	122.717	13.658	23.824	119.205	23.709	3.142	
Standard deviation			7.402	10.285	1.664	1.030	5.771	3.405	0.390	

ED: Euclidean distance

Table 18. Genetic distance of ICDE 13-3/46/4 progenies from Prathyasa

Sl. No.	Progeny No.	Sample no.	Euclidean distance value from recurrent parent Prathyasa
1	ICDE 13-3/46/4/7	PR 4-07	2.35
2	ICDE 13-3/46/4/10	PR 4-10	15.87
3	ICDE 13-3/46/4/11	PR 4-11	12.16
4	ICDE 13-3/46/4/12	PR 4-12	8.44
5	ICDE 13-3/46/4/13	PR 4-13	19.55
6	ICDE 13-3/46/4/14	PR 4-14	14.95
7	ICDE 13-3/46/4/15	PR 4-15	24.53
8	ICDE 13-3/46/4/16	PR 4-16	29.93
9	ICDE 13-3/46/4/17	PR 4-17	12.51
10	ICDE 13-3/46/4/18	PR 4-18	17.12
11	ICDE 13-3/46/4/22	PR 4-22	5.60
12	ICDE 13-3/46/4/24	PR 4-24	7.83
13	ICDE 13-3/46/4/31	PR 4-31	1.25
14	ICDE 13-3/46/4/33	PR 4-33	10.19
15	ICDE 13-3/46/4/34	PR 4-34	10.40
16	ICDE 13-3/46/4/41	PR 4-41	8.45
17	ICDE 13-3/46/4/44	PR 4-44	7.96
18	ICDE 13-3/46/4/46	PR 4-46	11.46
19	ICDE 13-3/46/4/48	PR 4-48	3.06
20	ICDE 13-3/46/4/50	PR 4-50	4.01
21	ICDE 13-3/46/4/53	PR 4-53	6.92

Table 19. Genetic distance of ICDE 12-3/1 progenies from Prathyasa

Sl. No.	Progeny No.	Sample no.	Euclidean distance value from recurrent parent Prathyasa
1	ICDE 12-3/1/1	PR 544-1	29.61
2	ICDE 12-3/1/2	PR 544-2	17.28
3	ICDE 12-3/1/3	PR 544-3	20.49
4	ICDE 12-3/1/4	PR 544-4	14.98
5	ICDE 12-3/1/5	PR 544-5	29.58
6	ICDE 12-3/1/6	PR 544-6	24.48
7	ICDE 12-3/1/7	PR 544-7	28.21
8	ICDE 12-3/1/8	PR 544-8	32.45
9	ICDE 12-3/1/9	PR 544-9	42.38
10	ICDE 12-3/1/10	PR 544-10	26.97
11	ICDE 12-3/1/11	PR 544-11	31.70
12	ICDE 12-3/1/12	PR 544-12	27.31
13	ICDE 12-3/1/13	PR 544-13	28.16
14	ICDE 12-3/1/14	PR 544-14	32.89
15	ICDE 12-3/1/15	PR 544-15	48.29
16	ICDE 12-3/1/16	PR 544-16	22.85
17	ICDE 12-3/1/17	PR 544-17	35.09
18	ICDE 12-3/1/18	PR 544-18	22.05
19	ICDE 12-3/1/19	PR 544-19	24.77
20	ICDE 12-3/1/20	PR 544-20	36.07
21	ICDE 12-3/1/21	PR 544-21	30.50
22	ICDE 12-3/1/22	PR 544-22	36.01
23	ICDE 12-3/1/23	PR 544-23	28.33
24	ICDE 12-3/1/24	PR 544-24	19.05
25	ICDE 12-3/1/25	PR 544-25	34.39

Table 20. Genetic distance of ICDE 12-3/4 progenies from Prathyasa

Sl. No.	Progeny No.	Sample no.	Euclidean distance value from recurrent parent Prathyasa
1	ICDE 12-3/4/1	PR 547-1	21.75
2	ICDE 12-3/4/2	PR 547-2	33.29
3	ICDE 12-3/4/3	PR 547-3	27.18
4	ICDE 12-3/4/4	PR 547-4	24.86
5	ICDE 12-3/4/5	PR 547-5	28.92
6	ICDE 12-3/4/6	PR 547-6	22.62
7	ICDE 12-3/4/7	PR 547-7	44.04
8	ICDE 12-3/4/8	PR 547-8	23.33
9	ICDE 12-3/4/9	PR 547-9	39.72
10	ICDE 12-3/4/10	PR 547-10	20.86
11	ICDE 12-3/4/11	PR 547-11	19.95
12	ICDE 12-3/4/12	PR 547-12	23.61
13	ICDE 12-3/4/13	PR 547-13	36.74
14	ICDE 12-3/4/14	PR 547-14	30.15
15	ICDE 12-3/4/15	PR 547-15	34.53
16	ICDE 12-3/4/16	PR 547-16	20.79
17	ICDE 12-3/4/17	PR 547-17	20.55
18	ICDE 12-3/4/18	PR 547-18	26.56
19	ICDE 12-3/4/19	PR 547-19	13.49
20	ICDE 12-3/4/20	PR 547-20	23.67
21	ICDE 12-3/4/21	PR 547-21	29.40
22	ICDE 12-3/4/22	PR 547-22	23.16

Table 21. Genetic distance of ICDE 12-3/6 progenies from Prathyasa

Sl. No.	Progeny No.	Sample no.	Euclidean distance value from recurrent parent Prathyasa
1	ICDE 12-3/6/1	PR 549-1	55.24
2	ICDE 12-3/6/2	PR 549-2	18.30
3	ICDE 12-3/6/3	PR 549-3	21.27
4	ICDE 12-3/6/4	PR 549-4	25.69
5	ICDE 12-3/6/5	PR 549-5	17.87
6	ICDE 12-3/6/6	PR 549-6	46.62
7	ICDE 12-3/6/7	PR 549-7	47.24
8	ICDE 12-3/6/8	PR 549-8	24.60
9	ICDE 12-3/6/9	PR 549-9	22.78
10	ICDE 12-3/6/10	PR 549-10	19.42
11	ICDE 12-3/6/11	PR 549-11	16.63
12	ICDE 12-3/6/12	PR 549-12	16.43
13	ICDE 12-3/6/13	PR 549-13	25.98
14	ICDE 12-3/6/14	PR 549-14	34.12
15	ICDE 12-3/6/15	PR 549-15	46.81
16	ICDE 12-3/6/16	PR 549-16	43.14
17	ICDE 12-3/6/17	PR 549-17	22.32
18	ICDE 12-3/6/18	PR 549-18	47.74
19	ICDE 12-3/6/19	PR 549-19	32.03
20	ICDE 12-3/6/20	PR 549-20	25.19
21	ICDE 12-3/6/21	PR 549-21	15.25
22	ICDE 12-3/6/22	PR 549-22	18.12
23	ICDE 12-3/6/23	PR 549-23	16.03
24	ICDE 12-3/6/24	PR 549-24	18.12
25	ICDE 12-3/6/25	PR 549-25	24.93
26	ICDE 12-3/6/26	PR 549-26	41.54
27	ICDE 12-3/6/27	PR 549-27	38.04
28	ICDE 12-3/6/28	PR 549-28	40.78
29	ICDE 12-3/6/29	PR 549-29	33.33
30	ICDE 12-3/6/30	PR 549-30	25.11
31	ICDE 12-3/6/31	PR 549-31	27.38
32	ICDE 12-3/6/32	PR 549-32	27.60
33	ICDE 12-3/6/33	PR 549-33	22.31
34	ICDE 12-3/6/34	PR 549-34	23.98
35	ICDE 12-3/6/35	PR 549-35	15.92
36	ICDE 12-3/6/36	PR 549-36	26.46
37	ICDE 12-3/6/37	PR 549-37	28.95
38	ICDE 12-3/6/38	PR 549-38	22.73
39	ICDE 12-3/6/39	PR 549-39	43.76
40	ICDE 12-3/6/40	PR 549-40	17.01
41	ICDE 12-3/6/41	PR 549-41	39.32
42	ICDE 12-3/6/42	PR 549-42	30.96
43	ICDE 12-3/6/43	PR 549-43	27.46

Table 22. Genetic distance of ICDE 12-3/13 progenies from Prathyasa

Sl. No.	Progeny No.	Sample no	Euclidean distance value from recurrent parent Prathyasa
1	ICDE 12-3/13/1	PR 556-1	41.05
2	ICDE 12-3/13/2	PR 556-2	46.72
3	ICDE 12-3/13/3	PR 556-3	25.39
4	ICDE 12-3/13/4	PR 556-4	24.06
5	ICDE 12-3/13/5	PR 556-5	30.90
6	ICDE 12-3/13/6	PR 556-6	21.97
7	ICDE 12-3/13/7	PR 556-7	42.59
8	ICDE 12-3/13/8	PR 556-8	34.85
9	ICDE 12-3/13/9	PR 556-9	34.64
10	ICDE 12-3/13/10	PR 556-10	35.67
11	ICDE 12-3/13/11	PR 556-11	29.78
12	ICDE 12-3/13/12	PR 556-12	32.95
13	ICDE 12-3/13/13	PR 556-13	27.92
14	ICDE 12-3/13/14	PR 556-14	29.33
15	ICDE 12-3/13/15	PR 556-15	27.99
16	ICDE 12-3/13/16	PR 556-16	21.64
17	ICDE 12-3/13/17	PR 556-17	20.63
18	ICDE 12-3/13/18	PR 556-18	39.51
19	ICDE 12-3/13/19	PR 556-19	39.40
20	ICDE 12-3/13/20	PR 556-20	14.32
21	ICDE 12-3/13/21	PR 556-21	35.11
22	ICDE 12-3/13/22	PR 556-22	32.68
23	ICDE 12-3/13/23	PR 556-23	19.15
24	ICDE 12-3/13/24	PR 556-24	20.27
25	ICDE 12-3/13/25	PR 556-25	17.74
26	ICDE 12-3/13/26	PR 556-26	19.84
27	ICDE 12-3/13/27	PR 556-27	27.28

Table 23. Genetic distance of ICDE 12-3/14 progenies from Prathyasa

Sl. No.	Progeny No.	Sample no.	Euclidean distance value from recurrent parent Prathyasa
1	ICDE 12-3/14/1	PR 557-1	14.70
2	ICDE 12-3/14/2	PR 557-2	23.51
3	ICDE 12-3/14/3	PR 557-3	21.03
4	ICDE 12-3/14/4	PR 557-4	23.07
5	ICDE 12-3/14/5	PR 557-5	18.10
6	ICDE 12-3/14/6	PR 557-6	20.74
7	ICDE 12-3/14/7	PR 557-7	46.48
8	ICDE 12-3/14/8	PR 557-8	31.91
9	ICDE 12-3/14/9	PR 557-9	8.94
10	ICDE 12-3/14/10	PR 557-10	30.17
11	ICDE 12-3/14/11	PR 557-11	19.65
12	ICDE 12-3/14/12	PR 557-12	52.88
13	ICDE 12-3/14/13	PR 557-13	51.83
14	ICDE 12-3/14/14	PR 557-14	57.20
15	ICDE 12-3/14/15	PR 557-15	31.15
16	ICDE 12-3/14/16	PR 557-16	23.64
17	ICDE 12-3/14/17	PR 557-17	11.34

4.3.3 Clustering of Resistance Genes Introgressed BC_2F_2 Lines and Parents Based on Proximity Dissimilarity Matrix.

Based on the euclidean distance values of each BC_2F_2 individuals from Prathyasa, the individuals with euclidean distance less than 15 units were selected and the proximity dissimialrity matrix of only those individuals was reconstituted from the original matrix. It included twenty-two individuals with gene combination and the parents (Table 24). The clustering of these individuals based on the dissimilarity matrix resulted in a dendrogram as shown in Figure 3. The plants ICDE 13-3/46/4/31, ICDE 13-3/46/4/7 and ICDE 13-3/46/4/48 were in the same cluster along with the recurrent parent Prathyasa. This indicated that these plants had maximum similiarity with the recurrent parent when compared with others.

Table 24. Proximity dissimilarity matrix of resistance genes introgressed BC_2F_2 individuals and parents

	Prathyasa	4-31	4-07	4-48	4-50	4-22	4-53	4-24	4-44	4-12	4-41	557-9	4-33	4-34	557-17	4-46	4-11	4-17	547-19	556-20	557-1	4-14	544-4	ISM
Prathyasa	0																							
PR 4-31	1.25	0																						
PR 4-07	2.35	1.87	0																					
PR 4-48	3.06	3.21	2.17	0																				
PR 4-50	4.01	2.61	4.29	3.31	0																			
PR 4-22	5.60	4.07	6.49	9.25	4.75	0																		
PR 4-53	6.92	5.42	9.65	11.23	8.12	3.73	0																	
PR 4-24	7.83	6.10	7.57	6.82	2.44	2.98	7.93	0																
PR 4-44	7.96	3.75	6.88	6.62	1.80	4.98	7.58	3.31	0															
PR 4-12	8.44	7.42	8.00	8.63	6.38	13.70	23.48	11.85	9.58	0														
PR 4-41	8.45	4.82	7.14	6.75	1.75	3.68	7.70	1.41	0.59	10.47	0													
PR 557-9	8.94	5.50	8.25	11.50	4.38	3.11	5.97	3.49	3.34	13.66	3.09	0												
PR 4-33	10.19	5.29	9.58	9.00	3.07	6.34	8.54	5.02	0.36	11.28	1.28	4.78	0											
PR 4-34	10.40	8.45	9.94	11.60	14.22	7.05	5.45	11.21	12.49	27.12	11.84	12.53	14.58	0										
PR 557-17	11.34	9.12	11.83	12.83	13.63	7.01	3.72	10.10	11.59	29.33	11.02	10.48	13.54	0.84	0									
PR 4-46	11.46	7.16	10.78	17.25	10.30	3.46	4.99	9.63	7.25	20.08	7.58	3.29	7.94	10.17	9.49	0								
PR 4-11	12.16	8.13	9.72	7.96	3.65	11.50	18.62	7.18	3.14	5.67	3.58	10.38	3.27	23.24	23.97	16.69	0							
PR 4-17	12.51	9.40	10.09	8.96	2.96	7.00	13.76	1.80	3.22	11.26	1.50	5.37	4.36	19.04	17.95	13.08	3.87	0						
PR 547-19	13.49	10.93	13.17	14.04	7.35	4.38	9.37	1.83	6.93	18.96	4.43	3.26	8.99	12.46	10.06	9.19	14.07	4.93	0					
PR 556-20	14.32	11.41	14.07	15.48	12.16	6.02	3.80	6.99	10.01	30.70	8.51	6.36	11.90	5.07	2.40	7.77	22.65	12.85	4.99	0				
PR 557-1	14.70	9.11	10.61	12.13	10.45	8.04	7.88	7.53	5.94	23.61	5.88	6.43	7.45	5.53	4.48	7.93	14.80	10.51	7.44	3.89	0			
PR 4-14	14.95	10.44	13.31	12.99	5.38	5.12	10.11	2.16	3.04	17.11	1.31	4.28	3.41	14.80	13.10	8.96	6.96	1.82	3.24	8.13	7.05	0		
PR 544-4	14.98	10.33	12.97	13.79	11.04	6.69	6.97	6.04	7.16	25.32	6.22	6.20	8.81	4.84	3.12	8.37	17.20	10.60	4.56	1.98	1.07	6.25	0	
ISM	125.10	104.92	106.47	104.84	99.95	106.83	109.43	92.12	80.45	113.59	82.62	88.78	80.53	96.04	92.29	97.92	86.34	85.37	86.92	86.77	61.68	78.99	68.05	0

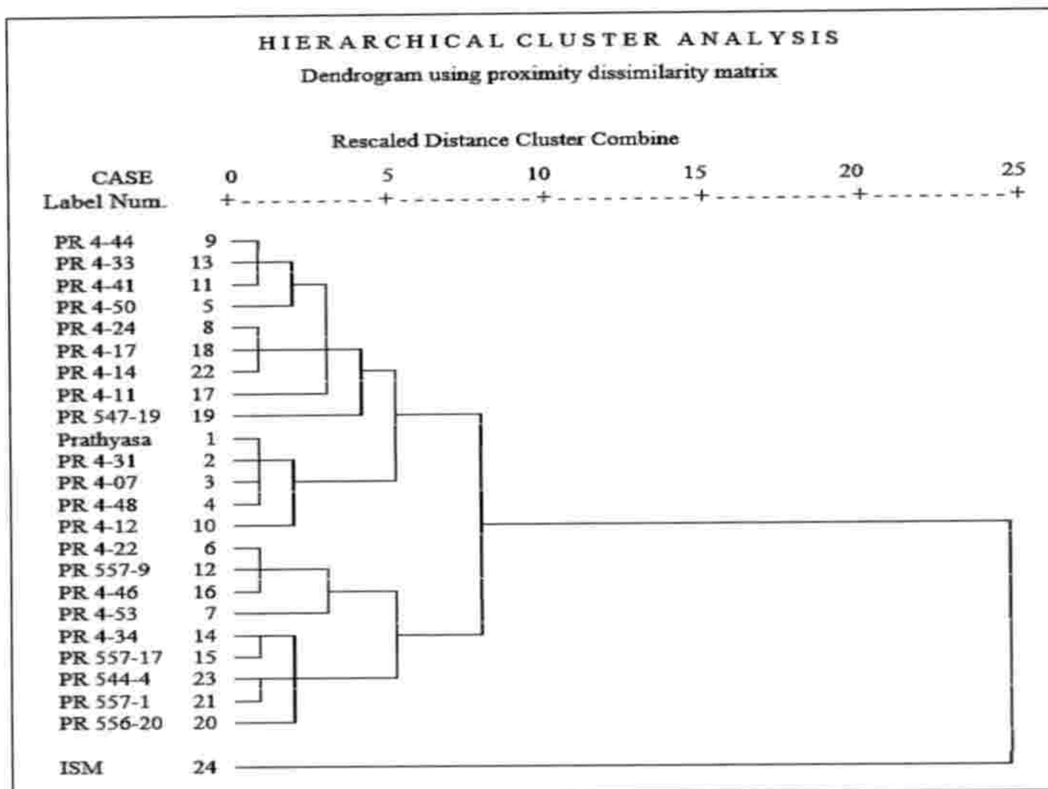


Figure 3. Clustering of pyramided BC_2F_2 lines and parental lines based on quantitative characters

Discussion

5. DISCUSSION

Rice is the important staple food of Kerala. The customs and culture of Kerala are interlinked with paddy cultivation and practices. Rice germplasm of Kerala is already enriched with numerous landraces and high yielding varieties. The high yielding rice varieties of Kerala are threatened by various stresses including biotic stresses. Among them, the recurring disease bacterial leaf blight is a major menace. The popular short duration red kernelled variety, Prathyasa released from the Rice Research Station, Mancombu is also affected by this devastating disease. Even though the variety has inherent capacity to tolerate several stresses including diseases, significant crop loss is caused by bacterial leaf blight. The potential yield loss caused by the disease ranges from 71 per cent to 84 per cent (Srinivasan and Gnanamanickam, 2005). Owing to the highly fragmented terrace nature of rice ecosystems in Kerala as well as the lowland submerged paddy field clusters of Kuttanadu, the spread of disease is very rapid through water. Hence a reliable and economical control through chemical agents is not advisable and also the chemical control for BLB is not effective (Devadath 1989). Continuous usage of chemicals in a sensitive ecosystem poses hazards. Host plant resistance of the rice genome offers the most effective, economical and eco-friendly management of BLB for any ecological situation.

To develop such resistant varieties a breeding programme entitled "Development of rice varieties for Kerala with pyramided genes for resistance to BLB by Marker Assisted Selection" was undertaken. It was aimed at introgressing the BLB resistance genes *xa13*, *Xa21* and *xa5* from the donor parent Improved Samba Mahsuri to the susceptible variety Prathyasa, the recurrent parent. The programme resulted in BC_2F_1 lines with pyramided genes and having about 80 per cent or more recurrent parent genome recovery identified through Marker Assisted Selection. The present study was to identify homozygous resistance gene combinations (*xa13*, *Xa21* and *xa5*) from the segregating BC_2F_2. Morphological characterization of the lines was also undertaken to trace the genetic similarity to the recurrent parent. The results of the study are discussed in detail below.

5.1 GENOTYPING OF BC_2F_2 LINES

While enhancing the host plant resistance through the deployment of single resistance genes, monocropping of these lines exerts strong selection pressure on the pathogen for matching virulence (Pink and Puddephat, 1999). Several genes that exhibit complete resistance to the BLB pathogen are identified till date (Kim *et al.*, 2015; Busungu *et al.*, 2016; Vikal and Bhatia, 2017). Pyramiding of resistance genes into the rice genotypes is advocated as an efficient strategy of durable resistance against bacterial leaf blight. However, the introgression of resistance genes into elite cultivars at a time is laborious time consuming and may prove difficult in case of existence of epistasis or involvement of many genes (Rao *et al.*, 2002). Hence Marker Assisted Selection was advocated as an alternative to conventional breeding approaches (Joshi and Nayak, 2010). The Marker Assisted Backcross breeding is an efficient strategy to develop durable resistance in elite cultivars without losing its inherent qualitative and quantitative characters.

Pyramiding for disease resistance has been reported in several situations such as wheat powdery mildew resistance (Wang *et al.*, 2001; Zhang *et al.*, 2002), rust resistance (Singh *et al.*, 2004), cyst nematode resistance (Barloy *et al.*, 2006) and common bean anthracnose resistance (Garzon *et al.*, 2008).

Gene pyramiding has been successfully employed in resistance breeding programmes of rice for diseases such as bacterial leaf blight (Huang *et.al.*, 1997; Singh *et al.*, 2001; Sundaram *et al.*, 2008), blast (Hittalmani *et al.*, 2000; Narayanan *et al.*, 2004; Wang *et al.*, 2004), sheath blight (Vidya and Ramalingam, 2018) and insect pests such as brown plant hopper (Sharma *et al.*, 2004; Fujita *et al.* 2009) and gall midge (Katiyar *et al.*, 2001; Venkanna *et al.*, 2018). These breeding programmes were strictly employed with MAS for the selection of target genes as well as genomes.

The breeding programmes traditionally employ anonymous molecular markers to establish a genetic linkage with a phenotype. However, the effectiveness of MAS diminishes by the occasional uncoupling of the genes and markers.

According to Iyer and McCouch (2007), this occasional uncoupling could result in errors in the identification of the target traits or resistance genes. Hence it signifies the usage of functional markers based on the functional polymorphism within the gene sequences causing phenotypic variation for MAS as it is more efficient in gene identification and selection (Andersen and Lubberstedt, 2003).

The present study utilized the functional marker xa13pro for the gene *xa13*. The primers of marker were originally developed by Sundaram *et al.* (2011). The marker for gene *Xa21* used was pTA248 developed by Ronald *et al.* (1992). It was also a functional marker (Rao *et al.*, 2002). For *xa5*, a functional marker xa5FM (Sundaram *et al.*, 2011; Hajira *et al.*, 2016) was employed. E xperimental evidence on reliability of the functional markers of BLB resistance genes *Xa21* (pTA248) and *xa5* (xa5FM) had been depicted by Pradhan *et al.* (2015).

5.1.1 Foreground Selection

Foreground selection of plants was carried out using good quality DNA isolated from the parents and BC_2F_2 individuals. When the genomic DNA of these individuals were amplified by means of PCR using marker xa13pro and resolved on two per cent agarose gel and scored on banding pattern with reference to parents, 218 BC_2F_2 plants and the donor parent amplified the 450 bp allele. Some of the plants also had a 250 bp allele as same as the recurrent parent along with the resistance allele. So the samples that had only the resistance allele (173 plants; Figure 1 and Figure 2) indicated the presence of homozygous resistance gene *xa13* (Sundaram *et al.*, 2008; Hajira *et al.*, 2016; Arunakumari *et al.*, 2016). The co-dominant alleles of the marker in individuals (45 individuals) indicated the presence of heterozygous gene *xa13*.

As the marker employed was a codominant functional marker (Sundaram *et al.*, 2011) amplifying the InDel polymorphism in the promoter region of *Os8N3*, the candidate gene for *xa13* (Chu *et al.*, 2006), it can be inferred that all the 218 individuals were introgressed with gene *xa13*. The codominance of the marker indicated heterozygous alleles of the gene. As it was a functional marker,

phenotypic evaluation for disease resistance is not required to identify the gene. It was because no recombination was reported between the marker and the gene as the marker sequence is within the promoter sequence of the gene (Sundaram *et al.*, 2011).

The 950 bp allele associated with the resistance allele of *Xa21* of donor parent ISM was observed in 146 BC_2F_2 individuals when their sample DNAs were amplified using the pTA248 marker (Figure 1 and Figure 2). Hence they are having the gene in the homozygous state. Twelve individuals were also identified with both 950 bp allele of the ISM and 660 bp allele of Prathyasa. This indicates the presence of the gene in the heterozygous state. Among these 146 individuals, 136 individuals had already identified with homozygous recessive gene *xa13* (Figure 2,). Seven plants were also identified with heterozygous *xa13*. Thus a gene combination of *xa13* and *Xa21* (*xa13xa13Xa21Xa21* and *Xa13xa13Xa21Xa21*) was established in the individuals. Similar reports of backcross individuals with *xa13* and *Xa21* genes were reported earlier (Sundaram *et al.*, 2008; Gopalakrishnan *et al.*, 2008; Pradhan *et al.*, 2015). The functional marker quality of pTA248 marker ensures cent per cent assurance for the presence or absence of gene *Xa21* (Hajira *et al.*, 2016, Rao *et al.*,2002). Among the twelve individuals with heterozygous gene *Xa21*, four were identified homozygous *xa13*, and six were identified with heterozygous *xa13*.

On amplifying the DNA of individuals with either genes *xa13*, *Xa21* or both using xa5FM marker, none of the individuals were identified with the triple gene. However, two plants were identified for a combination of *xa13* and *xa5*. Among them, one had homozygous *xa13* and homozygous *xa5* (ICDE 13-3/46/4/46) and the other had heterozygous *xa13* and homozygous *xa5* (ICDE 13-3/46/4/41). The xa5FM was a functional marker targeting the 2-bp polymorphism in the second exon of the gene. The 2-bp polymorphism encodes a transcription factor 2A (TFIIA) which was earlier characterized and found responsible for xa5 conferred resistance (Iyer and McCouch, 2004). The marker amplifies a 424 bp product and 124 bp product in association with resistance allele of the gene in the donor parent.

A product of 424 bp and 313 bp was amplified in the recurrent parent genome. All the 3 alleles were present when the gene was heterozygous.

5.1.2 Pyramiding of Genes

Foreground selection of 289 BC_2F_2 revealed 136 individuals that were identified for the homozygous combination of *xa13* and *Xa21* (*xa13xa13Xa21Xa21*), four individuals identified with homozygous *xa13* and heterozygous *Xa21* (*xa13xa13Xa21xa21*), seven individuals with heterozygous *xa13* and homozygous *Xa21* (*Xa13xa13Xa21Xa21*) and six individuals with both heterozygous gene (*Xa13xa13Xa21xa21*). One individual with homozygous *xa13* and homozygous *xa5* (*xa13xa13xa5xa5*, ICDE 13-3/46/4/46) and one individual with heterozygous *xa13* and homozygous *xa5* (*Xa13xa13xa5xa5*, ICDE 13-3/46/4/41) were also identified (Figure 2).

The recessive gene *xa13* offers moderate resistance to Indian *Xoo* isolates and specific resistance to Phillippine *Xoo* race 6. Hence the gene independently will not be effective for durable resistance to BLB in India (Singh *et al.*, 2001). The gene *Xa21* confers broad-spectrum race specific resistance to Indian isolates of *Xoo* and is the most widespread BLB resistance gene in the rice cultivated area. The *Xa21* gene resistance gradually increases from the seedling stage to the subsequent stages reaching 100 per cent at the adult stage (Century *et al.*, 1999), while the *xa5* gene mediated resistance is not dose dependent (Iyer and McCouch 2004). *xa5* offers partial or moderate resistance to Indian races of the *Xoo*.

According to Singh *et al.* (2001), for Indian context *Xa21* was the most effective gene followed by *xa13* and *xa5*. However, he reported that the combination of *Xa21* with other genes possess the most durable and complete resistance. According to the study, the combination of *xa5* and *xa13* individually with *Xa21* was reported more effective than the combination itself, while the triple gene pyramided line was the best and provided complete resistance with least lesion length. In the present study, as no triple gene pyramided line had been identified, the lines with *xa13* and *Xa21* genes are expected to provide the durable resistance

for Kerala scenario. Similar resistance expression was reported for this combination in earlier studies (Sanchez *et al.*, 2000; Gopalakrishnan *et al.*, 2008; Sundaram *et al.*, 2008; Perumalsamy *et al.*, 2010; Bharani *et al.*, 2010; Rajpurohit *et al.*, 2010; Pradhan *et al.*, 2015; Kadu *et al.*, 2016).

The additive effect of multiple resistance genes producing a horizontal resistance might be the reason for durable broad spectrum resistance of pyramided lines (Sundaram *et al.,* 2008). Such an observation urges the introgressions of more than two genes for a long, durable, complete resistance. In the present study, although no triple gene pyramided lines were available, the lines with *xa13* and *xa5* can be utilized to develop the triple gene pyramided line by crossing with the lines of *xa13* and *Xa21*.

5.2 MORPHOMETRIC EVALUATION OF BC_2F_2 PROGENIES

Morphometric evaluation of all the BC_2F_2 plants introgressed with R genes was carried out prior to harvest to determine the existing variability within the population. The plant characteristics of parents (ISM and Prathyasa) was also recorded. In a backcross breeding programme for improving elite cultivars by integrating one or few desirable traits, it is essential to reconstitute all the important agronomic characteristics of the original variety except for the traits of interest. In this study, the transfer of BLB resistance genes from the donor parent Improved Samba Mahsuri into the cultivar Prathyasa was undertaken to impart BLB resistance to the cultivar. Hence it was important to identify the similarity of morphological characters between the recurrent parent and the backcross progenies to have a selection of genotypes having maximum proximity with the recurrent parent.

According to the reports (Joseph *et al.*, 2004; Gopalakrishnan *et al.*, 2008; Pradhan *et al.*, 2015), the stringent observation of agro-morphological characters in the backcross progeny could yield faster recovery of recurrent parent characters within limited backcrosses. When both the parents have elite characteristics the agro-morphological characterization (in backcross progeny) could yield

transgressive segregants for some of the characters including yield. Such a transgressive segregant for yield and yield related characters of MTU1010 was obtained while introgressing genes from Improved Samba Mahsuri (Arunakumari *et al.*, 2016). The pyramiding attempts of Joseph *et al.* (2004) Gopalakrishnan *et al.* (2008), Pradhan *et al.* (2015) and Arunakumari *et al.* (2016) were strictly followed by agro-morphological characterization at different stages of breeding.

Sundaram *et al.* (2008) opined that the complete recovery of recurrent parent characters including yield, grain type and colour of the grain must be ensured in a gene pyramiding programme to produce an essentially derived variety with same acceptance as that of original variety among farmers. Hence it is always advocated to practice morphological selection along with MAB.

In the purview of the above opinions and observations, the present study selected the quantitative characters such as plant height (cm), days to maturity, number of productive tillers plant^{-1}, length of panicle (cm), number of grains panicle^{-1}, 1000 grain weight (g) and length/breadth ratio of grains to assess the similarity of derived progenies with the recurrent parent. The essential qualitative trait kernel colour was also recorded along with the quantitative traits.

5.2.1 Plant Height

When all the pyramided BC_2F_2 lines (155 plants) were considered at a time, wide variability was observed for the trait. The frequency distribution of plant height is shown in Figure 4. The plant height of the recurrent parent was 98 cm. Here selection was targeted to identify short-statured plants as same as Prathyasa. A few plants had the same height as the recurrent parent had. The progenies with homozygous *xa13* and *Xa21* genes (*xa13xa13Xa21Xa21*), ICDE 13-3/46/4/7 and ICDE 13-3/46/4/48 had a plant height of 98 and 96 cm respectively. The majority of ICD E 13-3/46/4 progenies had plant height near to that of the recurrent parent. The average height of ICDE 13-3/46/4 progenies was also proximal to the recurrent parent when compared to other progeny families.

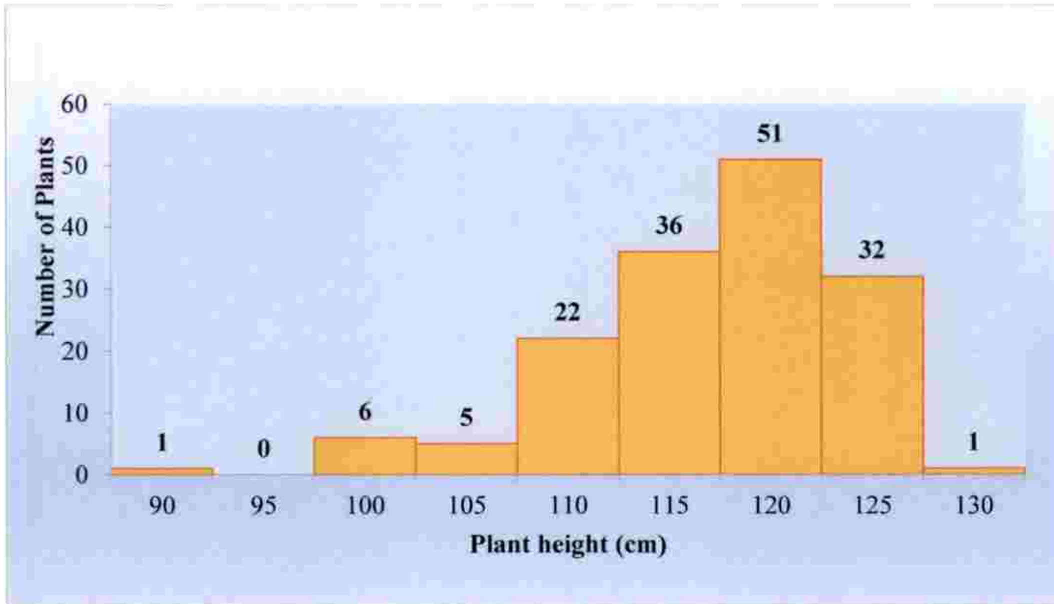


Figure 4. Frequency distribution of pyarmided BC_2F_2 lines for plant height (cm)

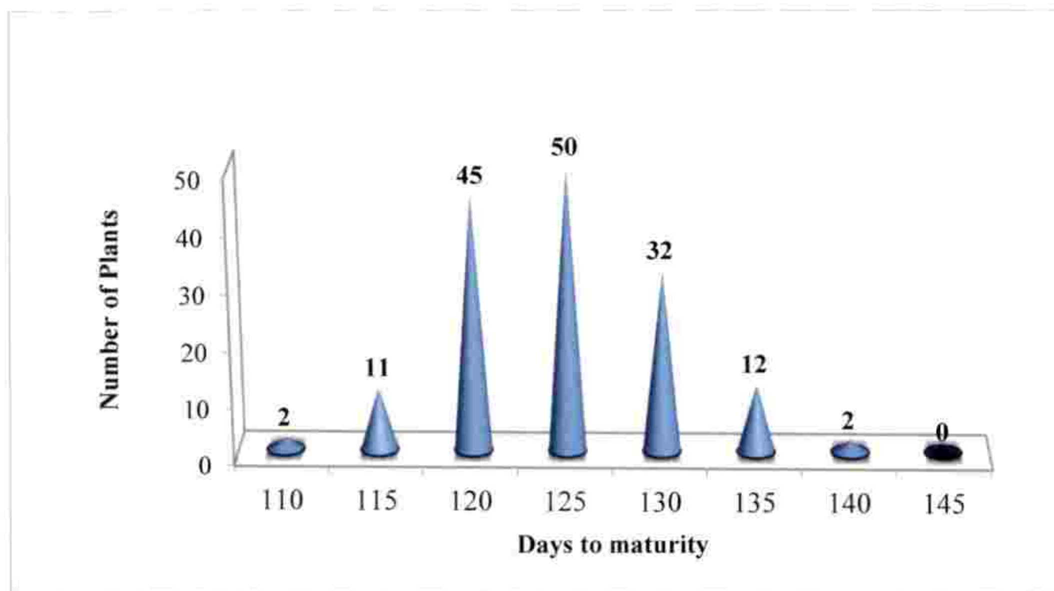


Figure 5. Frequency distribution of pyarmided BC_2F_2 lines for days to maturity

Several plants had a height more than the better parent (ISM-110 cm) for the trait. Almost all the F_2 progenies of ICDE 12-3/1, ICDE 12-3/4, ICDE 12-3/6, ICDE 12-3/13 had a height even more than the better parent. The average height of these progenies was also higher than the donor parental value. The reason for this tall stature in the majority of progenies may be due to the hybridity existing within the genome or due to segregation/transgressive segregation observed in the F_2 generation (Arunakumari *et al.*, 2016). Because Hundekar (2017) reported that the BC_1F_1 parent of these progenies ICDE 12-3 had 110 cm plant height for the trait. When these parents were further backcrossed, accidental selfing may have resulted causing transgressive segregation in the proceeding progenies. It may also be due to the effect of treated gibberellin (GA_3) for retrieving germination in the seeds.

5.2.2 Days to maturity

The pyramided BC_2F_2 lines were found to be intermediate between the recurrent parent and donor parent for the trait days to maturity. The objective was to identify pyramided lines maturing early as similar to Prathyasa. None of the progeny matured before the early maturing recurrent parent (108 days) and after the late maturing donor parent (145 days). However, a wide variation for the trait was observed within these limits (Figure 5). The variation in this trait may be due to the segregation in the F_2 progeny. The progenies ICDE 13-3/46/4/7 (110 days), ICDE 12-3/6/40 (110 days) and ICDE 13-3/46/4/48 (112 days) were the plants with homozygous R genes *xa13* and *Xa21* that matured early like the recurrent parent. Gopalakrishnan *et al.* (2008) reported a similar finding. According to him the majority of BC_1F_5 lines of 'PB 1' introgressed with *xa13* and *Xa21* were similar to the recurrent parent for days to maturity. Pradhan *et al.* (2015) observed intermediate values between the recurrent parent (Jalmagna) and donor parent (CRMAS2232-85- 'Improved Swarna') for days to maturity in the BC_3F_3 lines.

5.2.3 Number of Productive Tillers Plant^{-1}

Among the pyramided BC_2F_2 lines the variation for the number of productive tillers plant^{-1} was from three (ICDE 13-3/46/4/13) to twenty-one. (ICDE 12-3/6/1).

The frequency graph illustrating the variation is given (Figure 6). The variation was observed in progeny family of each BC_2F_1 individual. The variation among each progeny family was as expected in an F_2 progeny. The recurrent parent was observed with twelve productive tillers while the donor parent was observed with sixteen productive tillers. The progenies, ICDE 13-3/46/4/7 and ICDE 13-3/46/4/48 (*xa13xa13Xa21Xa21*), which had maximum similarity for plant height and days to maturity with the recurrent parent, had 12 and 13 productive tillers respectively. Thus, these plants were more similar to the recurrent parent for the trait. Similar findings on inheritance of number of productive tillers of R-gene pyramided lines was reported by Joseph *et al.* (2004) in BC_1F_3 lines, Gopalakrishnan *et al.* (2008) in advanced selections.

5.2.4 Length of Panicle

The variation in the trait length of panicle was beyond the parental limits. The minimum length 18.7 cm was observed in ICDE 13-3/46/4/13 and maximum length 29.16 cm was observed in ICDE 12-3/6/13. However the parents had only a little difference among them with 21.41 cm in donor parent and 23.16 cm in recurrent parent. The progeny ICDE 13-3/46/4/7 individual with homozygous R-gene combination (*xa13xa13Xa21Xa21*) had on par length for panicle with the recurrent parent. The frequency of individuals with varying length for the trait is illustrated (Figure 7). The segregating backcross population always portray such a continuous wide and significant variation for the quantitative character, length of panicle (Gopalakrishnan *et al.*, 2008; Arunakumari *et al.*, 2016).

5.2.5 Number of Grains Panicle^{-1}

The trait had been utilized as an important observation to validate the recovery of recurrent parent genome in the backcross individuals by Joseph *et al.* (2004), Gopalakrishnan *et al.* (2008), Basavaraj *et al.* (2010) Pradhan *et al.* (2015), and Arunakumari *et al.* (2016). They have evaluated the trait characteristics in advanced generations, however, strict selection based upon trait was practised in each generation.

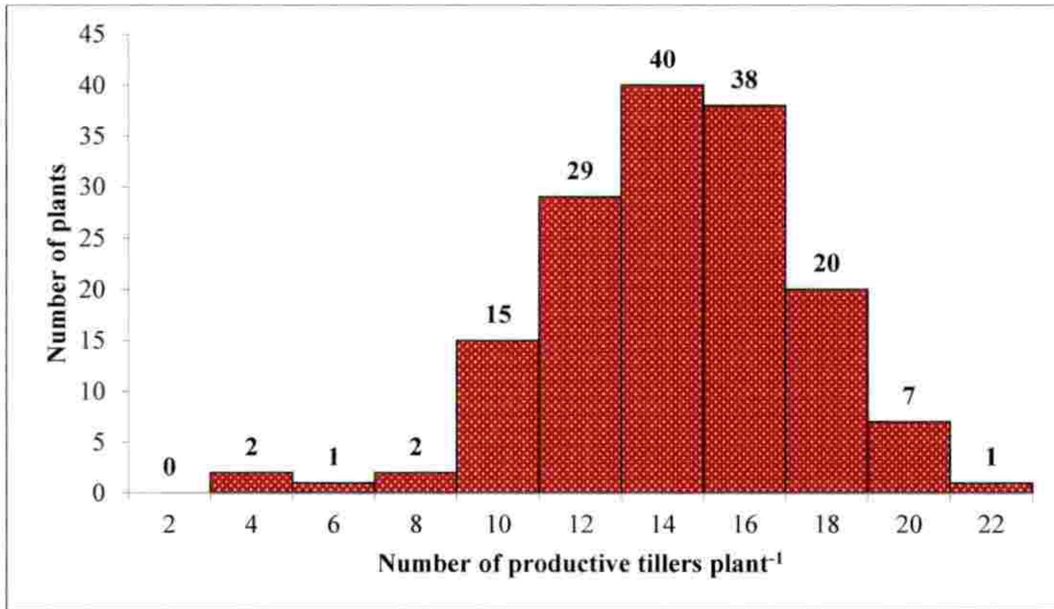


Figure 6. Frequency distribution of pyarmided BC_2F_2 lines for number of productive tillers plant^{-1}

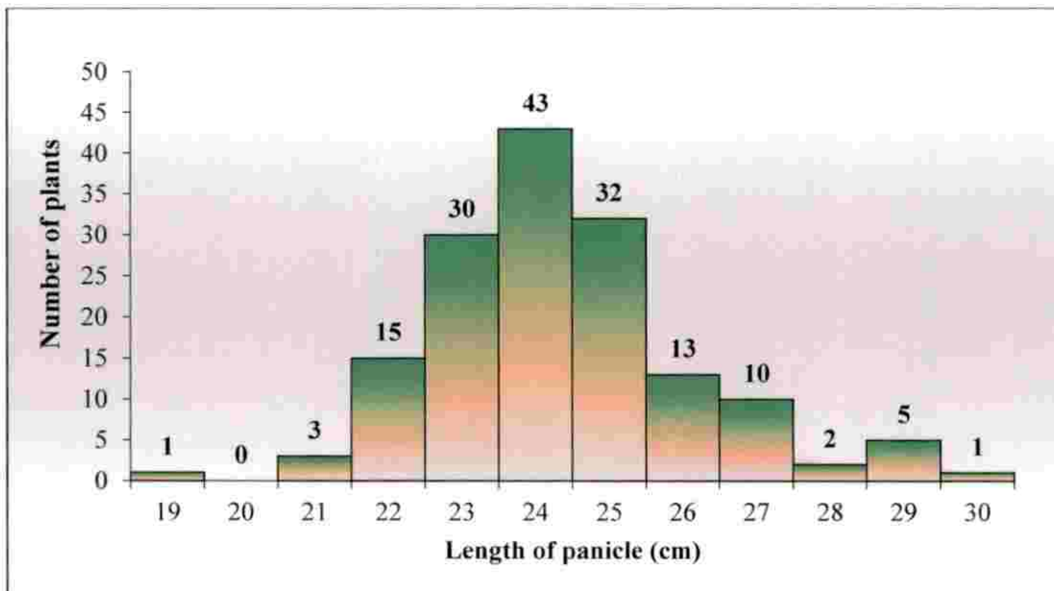


Figure 7. Frequency distribution of pyarmided BC_2F_2 lines for length of panicle (cm)

The number of grains per panicle of a rice plant is essentially the yield determining character of the plant. Definitely, each variety has characteristic yield determined by these traits such as number of grains per panicle, grain weight and so on. Hence in the BC_2F_2 population, the trait was studied to differentiate the individuals with respect to its similarity to the recurrent parent. The variation observed for number of grains panicle^{-1} in the R-gene pyramided BC_2F_2 individuals ranges from 76 grains in ICDE 12-3/14/13 to 148 filled grains in ICDE 12-3/13/9 plant. The variation observed was beyond the parental values for this trait. However such a wide variation was due to segregation in F_2 generation. The pyramided lines with significant difference for the trait than both the parents may be due to additive effect for the character. A frequency curve showing the variation number of productive tillers is shown in Figure 8. The Figure 8 indicates that majority of individuals had mean grains panicle^{-1} in between 115 to 130 grains. However the value for recurrent parent was 126 grains. Several plants had similarity with the recurrent parent for the trait.

5.2.6 1000 grain weight

The characteristics of grains were important in any pyramiding programme to improve resistance. Grain is the economical character determining consumer acceptance. Hence a deviation from original variety for grain characteristics might lead to poor acceptance from farmers for improved variety. Hence observations on grain characteristics of pyramided individuals were undertaken along with the backcross breeding (Joseph *et al.*, 2004; Sundaram *et al.*, 2008; Hari *et al.*, 2011; Arunakumari *et al.*, 2016; Hundekar, 2017).

The peculiar trait of 1000 grain weight is a unique factor of each variety. The variation in this trait is determined by several factors including grain filling, size of the grain, length/breadth ratio of the grain, grain type and so on. The yield of a plant can be determined from its yield determining traits when 1000 grain weight is available. Also as it is a peculiar character for each variety, it was used to compare the similarity of a segregating population or backcross population with its parents.

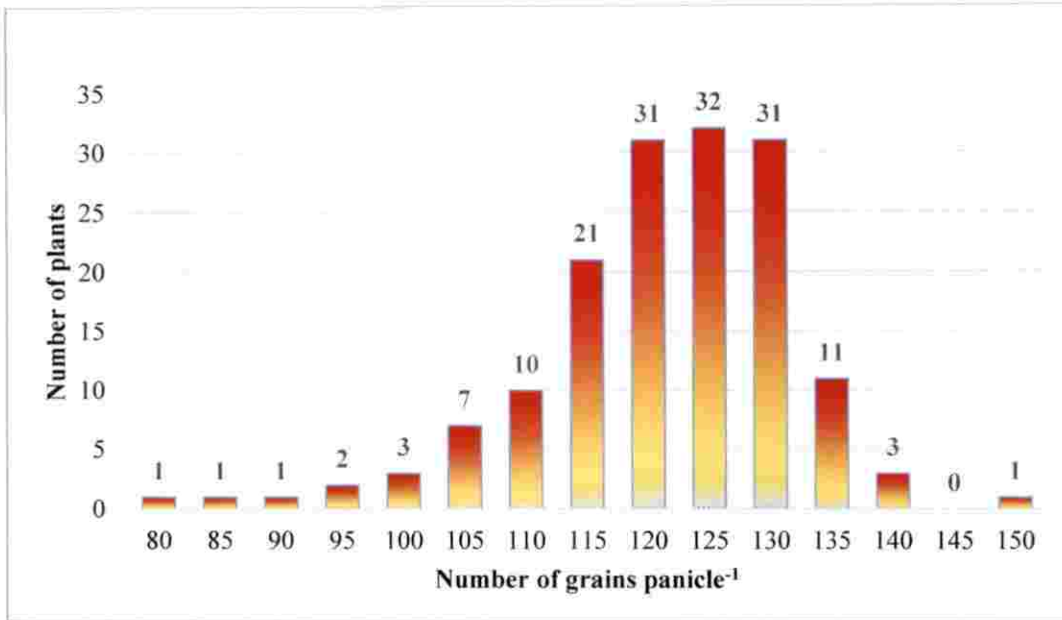


Figure 8. Frequency distribution of pyarmided BC_2F_2 lines for number of grains panicle^{-1}

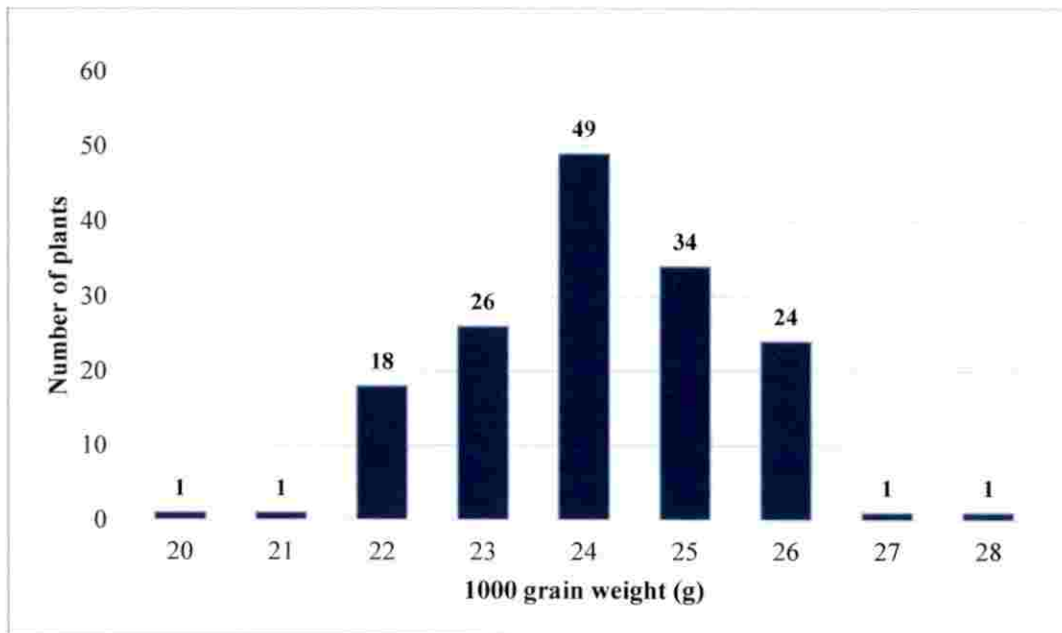


Figure 9. Frequency distribution of pyarmided BC_2F_2 lines for 1000 grain weight (g)

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The variation for 1000 grain weight in R-gene pyramided BC_2F_2 individuals was intermediate between the donor parent and recurrent parent (Figure 9). The donor parent had 15.15 g, 1000 grain weight, whereas the recurrent parent had 26.67 g weight for 1000 grains. The majority of BC_2F_2 individuals had 1000 grain weight within 21 g and 26 g (Figure 9). Among BC_2F_2 individuals, 19.72 g was the minimum weight (ICDE 12-3/13/1) and maximum weight was 27.02 g (ICDE 13-3/46/4/53) which was the only weight higher than recurrent parent among individuals. A similar finding for the trait was reported by Basavaraj *et al.*, (2010) in improved lines of Pusa 6B, a parental line of hybrid PusaRH10.

5.2.7 Length/Breadth (L/B) Ratio of Grain

Head rice recovery, kernel length, kernel breadth, length/breadth ratio, kernel length after cooking, elongation ratio, alkali spreading value and amylose content are the grain qualities studied in advanced pyramided lines of basmati varieties (Gopalakrishnan *et al.*, 2008; Basavaraj et al 2010). The number of grain characters studied signifies the importance of reconstitution of the essential characteristics of the grain during pyramiding. The donor parent in the present study had medium slender grains while recurrent parent had short bold grain. Hence the character length/breadth ratio of grain was studied to select the pyramided lines most similar to recurrent parent to advance from BC_2F_2 population.

The BC_2F_2 lines introgressed with R-genes showed a variation for the trait within the values of parents with some exceptions. Anyhow majority of the progenies had L/B ratio values within 2.6 and 3.5 (Figure 10). The maximum value among the individuals was 4.42 (ICDE 12-3/6/1) and the minimum value was 2.63 (ICDE 13-3/46/4/7). The selection criteria was to identify lines with same trait values as the recurrent parent. The recurrent parent had 2.77 L/B ratio whereas donor parent had 4.03. The L/B ratio was used as a significant criterion while improving Samba Mahsuri for BLB resistance (Sundaram *et al.*, 2008).

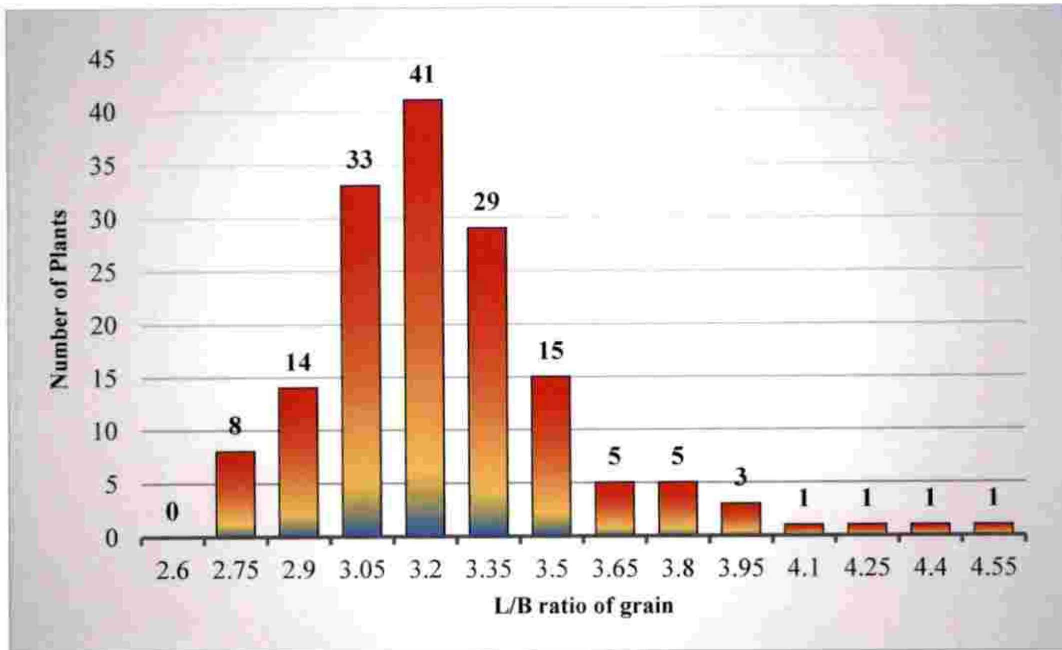

Figure 10. Frequency distribution of pyarmided BC_2F_2 lines for Length/Breadth (L/B) ratio of grain

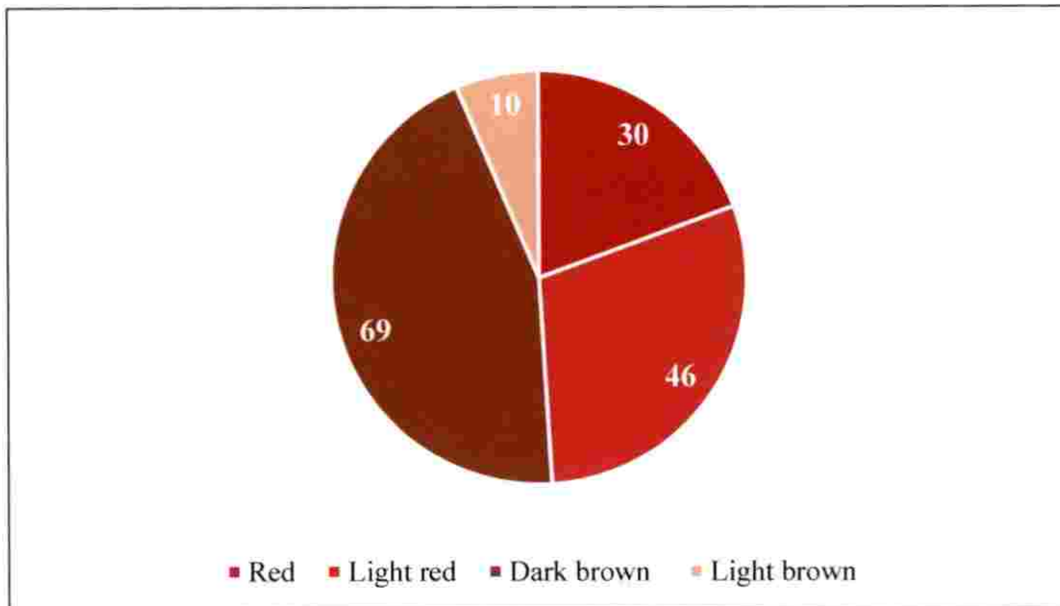

Figure 11. Frequency distribution of pyarmided BC_2F_2 lines for kernel colour

5.2.8 Kernel Colour

The trait kernel colour should always be included as an important morphological observation in MABB programmes of imparting genes to elite cultivars from distinct cultivars of different kernel colour (Collard and Mackill 2008). The trait is important in the present study because the donor parent had white kernels and recurrent parent had red kernels. In the present study, individuals having maximum similarity with Prathyasa for other characters will only be selected if they have inherited the same kernel colour of Prathyasa. Among the BC_2F_2 individuals, thirty individuals had red kernels, forty six had light red kernels, sixty nine had dark brown kernels and ten lines had light brown kernels (Figure 11). The character was well studied during similar breeding attempts of pyramiding BLB R-genes by Bharani *et al.* (2010) and Perumalsamy *et al.* (2010). Even though Kernel colour is not a quantity trait it is as important as yield for consumer acceptance (Sundaram *et al.*, 2011).

5.3 EUCLIDEAN DISTANCE ANALYSIS

In order to select the best lines from R-gene pyramided BC_2F_2 population, a similarity coefficient was to be selected. Pradhan *et al.* (2015) worked out a genetic distance coefficient (Jaccard's Coefficient) based on 14 agro-morphological traits to select best pyramided lines from 20 pyramids and the parents. In the present study euclidean distance (Shifriss and Sacks, 1980) was used to mark the genetic distance between BC_2F_2 progenies and the recurrent parent.

The euclidean distance analysis of seven quantitative characters revealed that the progenies ICDE 13-3/46/4/31 (Plate 12), ICDE 13-3/46/4/7 (Plate 13), ICDE 13-3/46/4/48 (Plate 14), ICDE 13-3/46/4/50 (Plate 15), ICDE 13-3/46/4/22 (Plate 16), ICDE 13-3/46/4/53 (Plate 17) and ICDE 13-3/46/4/24 (Plate 18) were the best plants with minimum genetic distance from the recurrent parent Prathyasa (Plate. 20) with euclidean distance of 1.25, 2.35, 3.06, 4.01, 5.60, 6.92 and 7.83 respectively. Along with the minimum euclidean distance, all these progenies had red kernels of Prathyasa parent. However, the progenies ICDE 13-3/46/4/7 and

Plate 12. Phenotypic characteristics of ICDE 13-3/46/4/31 progeny; A: plant, B: panicle, C: seeds and kernels

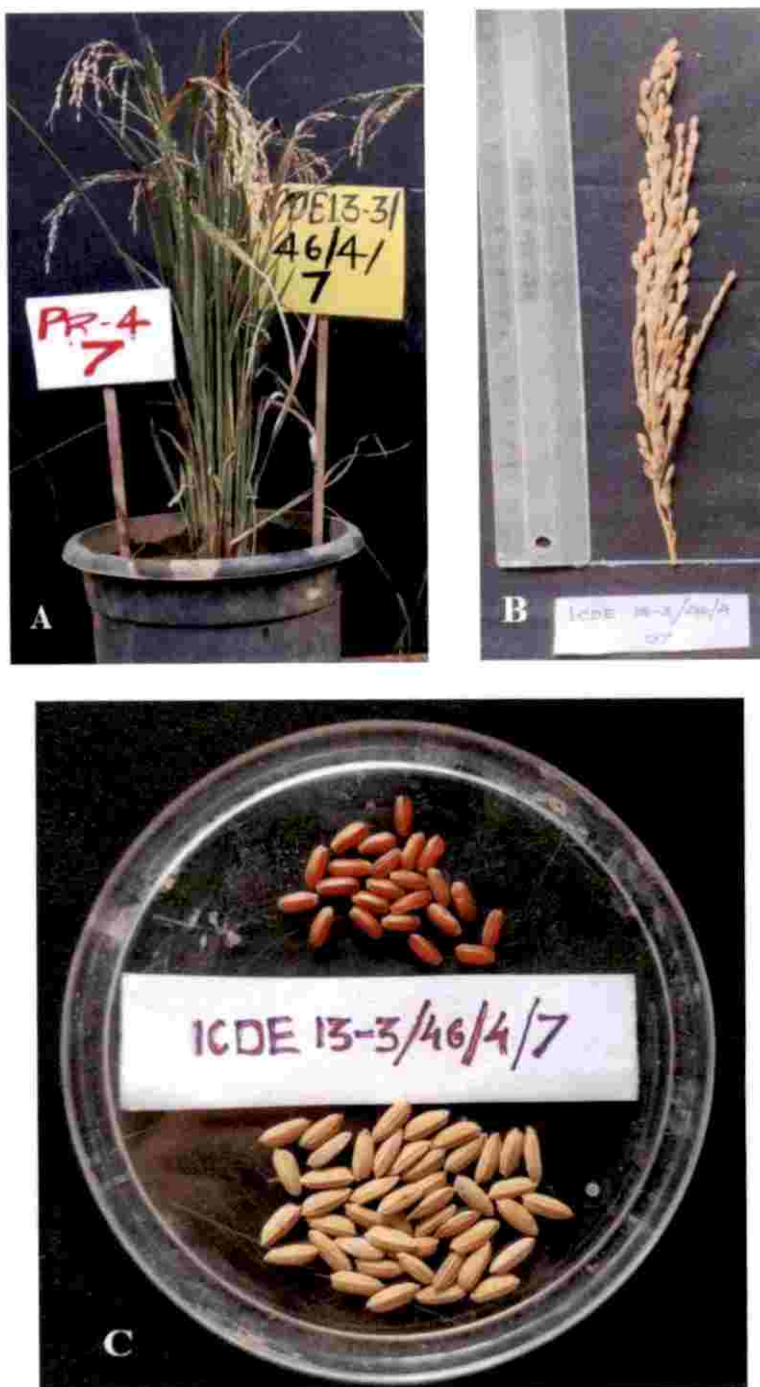

Plate 13. Phenotypic characteristics of ICDE 13-3/46/4/7 progeny; A: plant, B: panicle, C: seeds and kernels

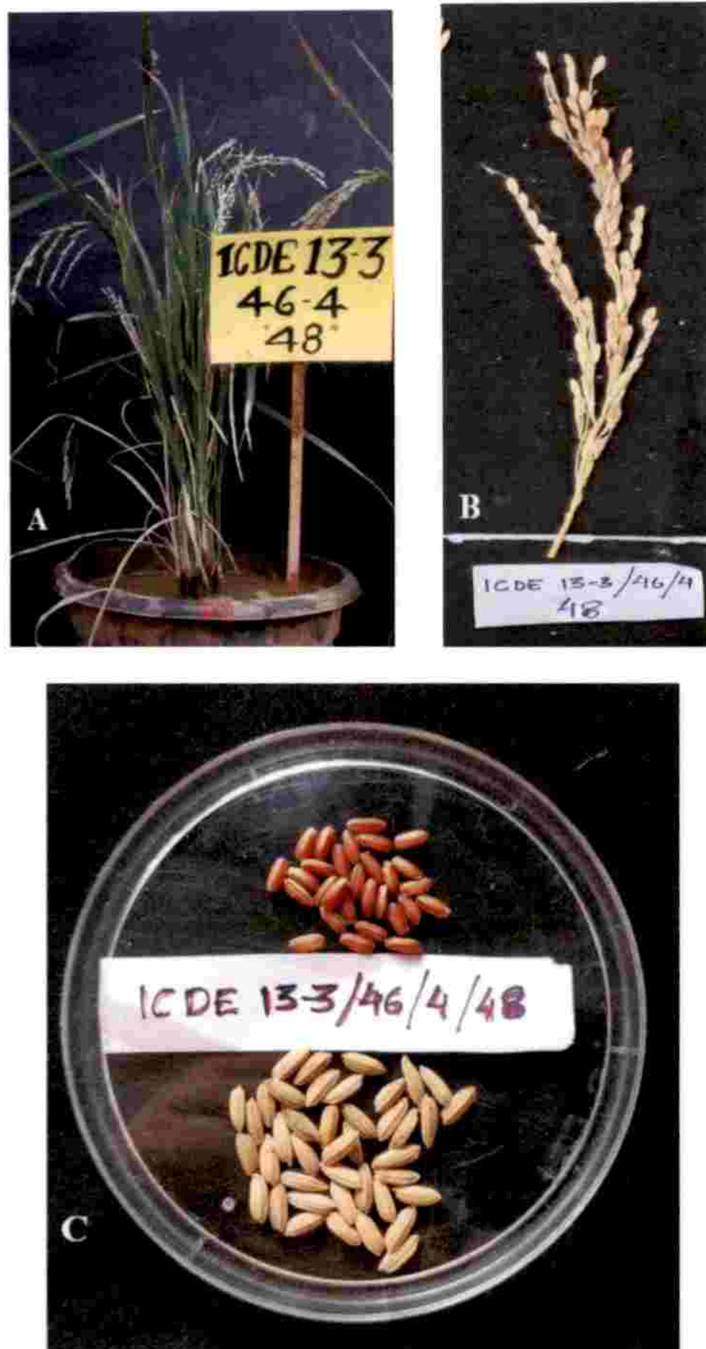

Plate 14. Phenotypic characteristics of ICDE 13-3/46/4/48 progeny; A: plant, B: panicle, C: seeds and kernels

Plate 15. Phenotypic characteristics of ICDE 13-3/46/4/50 progeny; A: plant, B: panicle, C: seeds and kernels

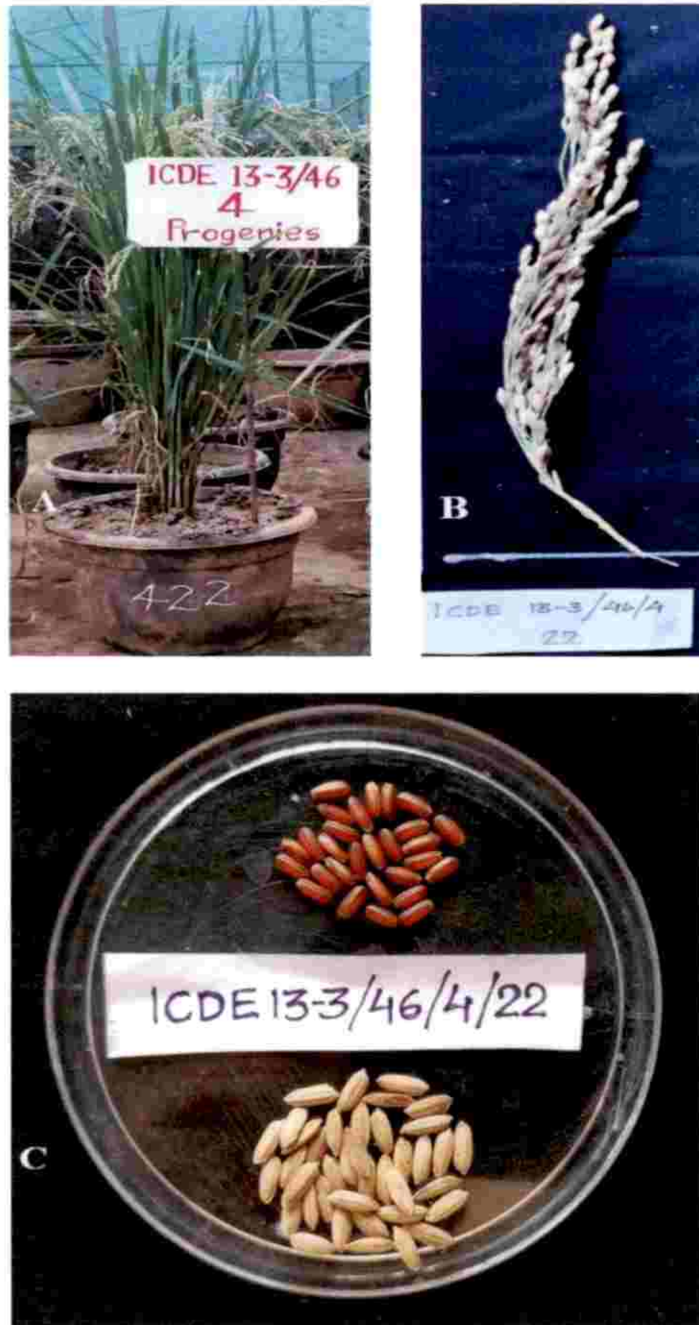

Plate 16. Phenotypic characteristics of ICDE 13-3/46/4/22 progeny; A: plant, B: panicle, C: seeds and kernels

Plate 17. Phenotypic characteristics of ICDE 13-3/46/4/53 progeny; A: plant, B: panicle, C: seeds and kernels

Plate 18. Phenotypic characteristics of ICDE 13-3/46/4/24 progeny; A: plant, B: panicle, C: seeds and kernels

Plate 19. Phenotypic characteristics of ICDE 13-3/46/4/46 progeny; A: plant, B: panicle, C: seeds and kernels

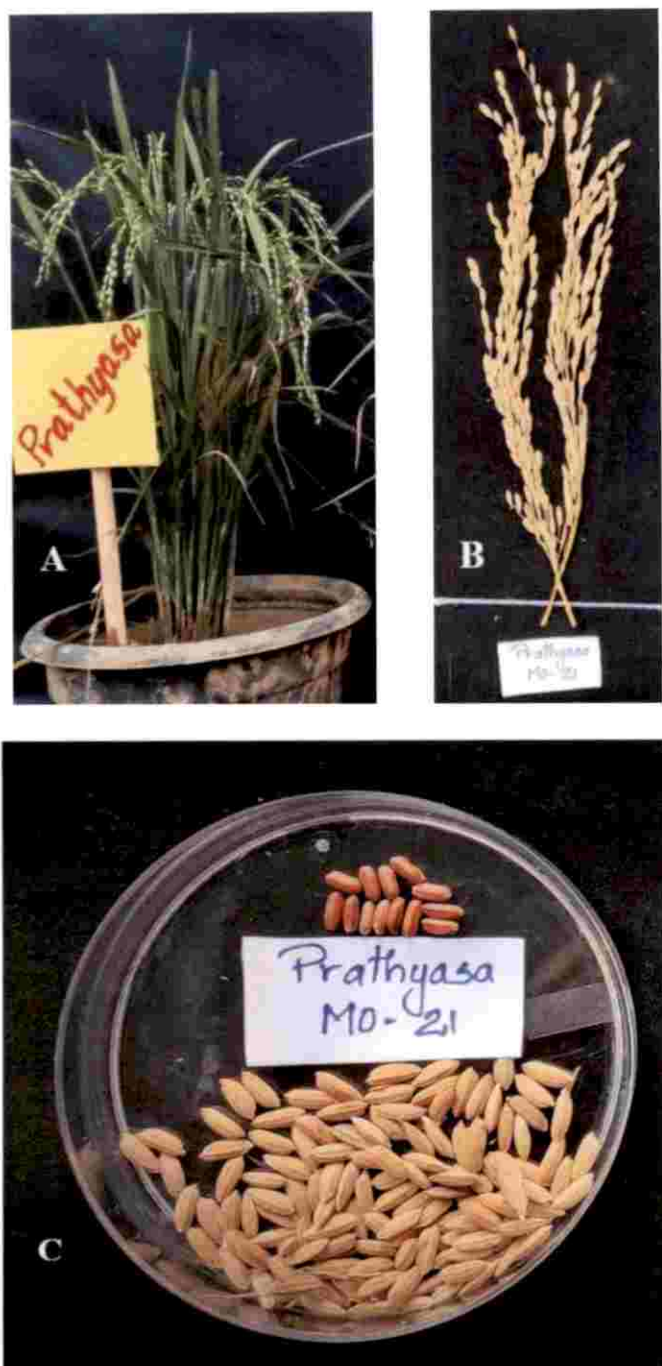

Plate 20. Phenotypic characteristics of recurrent parent Prathyasa (MO 21); A: plant, B: panicle, C: seeds and kernels

ICDE 13-3/46/4/48 only had homozygous *xa13* and *Xa21* R-genes. The remaining progenies, ICDE 13-3/46/4/31 had a heterozygous combination of R-genes *xa13* and *Xa21*, whereas ICDE 13-3/46/4/50, ICDE 13-3/46/4/22, ICDE 13-3/46/4/53 and ICDE 13-3/46/4/24 had heterozygous *xa13* and homozygous *Xa21*.

The dendrogram generated using the proximity dissimilarity matrix based on euclidean distance method grouped the R-gene pyramids ICDE 13-3/46/4/31, ICDE 13-3/46/4/7 and ICDE 13-3/46/4/48 along with the recurrent parent Prathyasa (Figure 3). So considering the three facts i.e. the combination of genes, the euclidean distance and the kernel colour, progenies ICDE 13-3/46/4/7 and ICDE 13-3/46/4/48 are the best ones in a breeder point of view. These progenies have a homozygous combination of R-genes (*xa13* and *Xa21*), maximum proximity with recurrent parent Prathyasa and inherited the red kernels of Prathyasa. These progenies can be advanced further for either yield trials and phenotypic evaluation of disease resistance or backcrossing with the recurrent parent for improving recurrent parent genome. According to Joseph *et al.* (2004) the low recovery of recurrent parent genome in some of the pyramided lines can be improved by additional round of backcrossing. However they also developed an improved version of Pusa Basmati 1 from BC_1F_3 lines itself by introgressing two BLB resistance gene *xa13* and *Xa21*. Sundaram *et al.* (2008) developed the EDV of Samba Mahsuri introgressed with three BLB R-genes namely Improved Samba Mahsuri from BC_4F_2 lines after several rounds of selection for agromorphological evaluation and phenotypic evaluation of resistance genes. Pradhan *et al.*, (2015) developed the EDV of Jalmagna with three BLB resistance genes from BC_3F_3 populations of MAB.

Besides these progenies, ICDE 13-3/46/4/41 (ED-8.45) with a combination of heterozygous *xa13* and homozygous *Xa21* genes and ICDE 13-3/46/4/46 (ED-11.46, Plate. 19) with a combination of homozygous *xa13* and *xa5* genes can be advanced to further breeding steps either backcrossing with the recurrent parent or forwarding to BC_2F_3 population. This breeding line can be utilized as a NIL to introgress a third gene *xa5* into Prathyasa by intermating.

Use of functional markers linked to the resistance genes integrated with phenotype-based selection resulted in development of two best 2 R-gene pyramided BC_2F_2 lines of Prathyasa (progenies ICDE 13-3/46/4/7 and ICDE 13-3/46/4/48) with the genes *xa13* and *Xa21* from a population of 289 plants. The combined effort of molecular markers and phenotypic selection reduced the number of progenies to be backcrossed with the recurrent parent or to be advanced to the next generation for further breeding and improving the lines. According to Collard and Mackill (2008), the combined effort can reduce the burden over conventional breeding method to develop elite varieties.

According to Singh *et al.* (2001), advanced lines with resistance gene combinations have practical breeding value by providing a wider spectrum of resistance against most of the existing isolates of BLB in the region and will have a high impact on yield stability and sustainability of rice crop. In the light of this advancing the line with *xa13* and *xa5* genes to BC_3F_1 generation could help improving the recurrent parent to triple gene pyramided variety.

The plants ICDE 13-3/46/4/7 and ICDE 13-3/46/4/48 with two bacterial blight resistance genes (*xa13* and *Xa21*) in the homozygous state were the best BC_2F_2 plants identified with a maximum phenotypic resemblance to Prathyasa. These plants can be either subjected to selection by forwarding to BC_2F_3 generation or backcrossing with the recurrent parent for maximizing recurrent parent genome in the progeny. Even though the plants with the combination of *xa13* and *xa5* were more divergent from Prathyasa parent, the presence of red kernels in them signifies its selection for either backcrossing or generating BC_2F_3 population for fixation of resistance genes. These plants can be utilized to generate triple gene pyramided essentially derived variety of Prathyasa to provide durable resistance to bacterial leaf blight.

Summary

6. SUMMARY

Bacterial leaf blight (BLB) disease of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most important threat to sustainable rice production throughout the world. The tropical hot humid climate required for the rice cultivation is quite conducive for the spread and development of the disease, causing yield losses upto 80% depending on the stage of the crop, cultivar susceptibility and the environmental conditions. Besides this, it adversely affects the quality of the grain. Enhancement of host plant resistance is the most effective, economical and environmentally safe method available for management of BLB as the chemical control of this disease is ineffective and causing health hazards (Nino-Liu *et al.*, 2005). The resistance gene pyramided lines showed broad-spectrum durable complete resistance than single resistance genes (Singh *et al.*, 2001). The present study was a part of gene pyramiding carried out to introgress BLB resistance genes *xa13*, *Xa21* and *xa5* in the backcross progenies of Prathyasa variety of rice through marker assisted selection.

Foreground selection of BLB resistance genes in the BC_2F_2 plants was undertaken using PCR based markers. Total genomic DNA was isolated by the CTAB method from young leaf bits collected from each individual and the parents. The quantity of DNA was estimated by means of UV spectrophotometer. The quality was estimated by the ratio of absorbance at 260 nm to 280 nm, which ranged from 1.79 to 1.89 for all samples.

PCR amplification was carried out using the gene specific functional markers xa3pro, pTA248, and xa5FM linked to BLB resistance genes *xa13*, *Xa21* and *xa5* respectively for parents. A clear distinct polymorphism was observed between the donors and recipients for all molecular markers. Hence the PCR amplification was carried out for all BC_2F_2 individuals.

Among the 289 plants subjected to molecular analysis, 155 plants were found to have resistance genes in combination. Besides this, thirty-two plants with homozygous *xa13*, thirty-one plants with heterozygous *xa13*, three plants with

homozygous *Xa21* and two plants with heterozygous *Xa21* were also identified. Sixty six plants did not show the presence of resistance genes.

Among the 155 plants with gene combination, 136 BC_2F_2 plants showed the presence of homozygous *xa13* and *Xa21* genes in combination and a single plant showed presence of homozygous *xa13* and *xa5* genes. Four plants with homozygous *xa13* and heterozygous *Xa21*, seven plants with heterozygous *xa13* and homozygous *Xa21*, six plants with heterozygous *xa13* and heterozygous *Xa21* and a plant with heterozygous *xa13* and homozygous *xa5* were also identified.

In order to identify the best BC_2F_2 lines with resistance genes in combination and having maximum phenotypic similarity with Prathyasa, observations on seven quantitative characters such as plant height, days to maturity, number of productive tillers plant^{-1}, length of panicle, number of grains panicle^{-1}, 1000 grain weight and length/breadth (L/B) ratio of grain of each individual were recorded along with the qualitative character kernel colour, in the BC_2F_2 segregants and parents.

Euclidean distance based on these seven quantitative characters was calculated from recurrent parent Prathyasa. In the pyramided progenies the distance varied from 1.25 to 57.20. The analysis revealed that the progenies ICDE 13-3/46/4/31, ICDE 13-3/46/4/7, ICDE 13-3/46/4/48, ICDE 13-3/46/4/50, ICDE 13-3/46/4/22, ICDE 13-3/46/4/53, and ICDE 13-3/46/4/24 were having the minimum Euclidean distance of 1.25, 2.35, 3.06, 4.01, 5.60, 6.92 and 7.83 respectively from recurrent parent Prathyasa and had inherited the exact red kernels of recurrent parent. Among these plants, ICDE 13-3/46/4/7 and ICDE 13-3/46/4/48 had homozygous combination *xa13* and *Xa21* (*xa13xa13Xa21Xa21*), ICDE 13-3/46/4/31 had heterozygous combination of *xa13* and *Xa21* (*Xa13xa13Xa21xa21*) and remaining progenies ICDE 13-3/46/4/50, ICDE 13-3/46/4/22, ICDE 13-3/46/4/53, and ICDE 13-3/46/4/24 had heterozygous *xa13* and homozygous *Xa21* genes (*Xa13xa13Xa21Xa21*).

The plants with a combination of *xa13* and *xa5*, ICDE 13-3/46/4/46 (*xa13xa13xa5xa5*) and ICDE 13-3/46/4/41 (*Xa13xa13xa5xa5*) had a genetic distance of 11.46 and 8.45 respectively from the recurrent parent. Both these plants

showed red kernels. These plants had maximum similarity with Prathyasa for number of productive tillers and length/breadth ratio of the grain.

The twenty two pyramided BC_2F_2 lines having euclidean distance from Prathyasa below 15 units when clustered based on the proximity dissimilarity matrix, the progenies ICDE 13-3/46/4/31, ICDE 13-3/46/4/7 and ICDE 13-3/46/4/48 were grouped along with Prathyasa. It revealed the extent similarity of these progenies with the recurrent parent. These progenies had maximum similarity with Prathyasa in case of plant height, days to maturity, number of productive tillers, length of panicle and length/breadth ratio of grain.

These advanced breeding lines derived through marker assisted selection and phenotypic selection can be of practical value in providing durable resistance in the breeding programme for evolving BLB resistant varieties suited to Kerala. Thus, this study had identified two BLB resistance gene pyramided BC_2F_2 lines of Prathyasa, ICDE 13-3/46/4/7 and ICDE 13-3/46/4/48 with homozygous genes *xa13* and *Xa21*. These lines can be either subjected to selection and phenotypic evaluation by forwarding to BC_2F_3 generation or backcrossing with the recurrent parent for maximizing recurrent parent genome in the progeny. The lines are best to develop essentially derived variety of Prathyasa with BLB resistance

The line ICDE 13-3/46/4/46 with homozygous *xa13* and *xa5* can be improved as a breeding line (Near isogenic line) of the variety to introduce a third gene *xa5*, to the pyramided essentially derived variety of Prathyasa.

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MARKER ASSISTED SELECTION FOR BACTERIAL LEAF BLIGHT RESISTANCE GENES IN THE BACKCROSS PROGENIES OF PRATHYASA VARIETY OF RICE (*Oryza sativa* L.)

by

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ABSTRACT

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ABSTRACT

Bacterial leaf blight disease of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most devastating disease of rice in the world. Major rice growing countries are affected by this disease leading to losses upto 80% in susceptible cultivars. Exploitation of host plant resistance is the most suitable practical strategy for the disease management in an eco-friendly manner. Till date, about 42 BLB resistance genes, conferring resistance against various strains of *Xoo*, have been identified from the rice germplasm collections worldwide. So, the present study entitled "Marker assisted selection for bacterial leaf blight resistance genes in the backcross progenies of Prathyasa variety of rice (*Oryza sativa* L.)" was undertaken at College of Agriculture, Vellayani during 2017-2019 to identify lines in the BC_2F_2 progeny of Prathyasa pyramided with two/three genes (*xa13*, *Xa21,* and *xa5*) through marker assisted selection for resistance to bacterial leaf blight and to evaluate these lines morphologically to assess Prathyasa phenome recovery.

In this study, the recurrent parent was Prathyasa (MO 21) susceptible to bacterial leaf blight and donor parent was Improved Samba Mahsuri (RPBio-226) resistant to bacterial leaf blight with *xa13, Xa21* and *xa5* resistance genes. BC_2F_2 seeds from ten BC_2F_1 plants selected on the basis of recurrent parent genome recovery was used for the study. The isolated DNA samples from about 289 BC_2F_2 plants and the parents were subjected to quality and quantity analysis. The diluted DNA was utilized for PCR amplification using gene-specific co-dominant functional markers xa13pro, pTA248 and xa5FM for the genes *xa13*, *Xa21* and *xa5* respectively, to identify the presence of genes.

Among the 289 plants subjected to molecular analysis, 155 plants were found to have resistance genes in combination. Besides this, thirty-two plants with homozygous *xa13*, thirty-one plants with heterozygous *xa13*, three plants with homozygous *Xa21* and two plants with heterozygous *Xa21* were also identified.

Among the 155 plants with gene combination, 136 BC_2F_2 plants with the presence of homozygous *xa13* and *Xa21* genes in combination and a single plant with the presence of homozygous *xa13* and *xa5* genes in combination were identified. Four plants with homozygous *xa13* and heterozygous *Xa21*, seven plants with heterozygous *xa13* and homozygous *Xa21*, six plants with heterozygous *xa13*

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and heterozygous *Xa21* and a plant with heterozygous *xa13* and homozygous *xa5* were also identified.

Euclidean distance analysis of the segregants with two gene combination using the quantitative traits such as plant height, days to maturity, number of productive tillers plant^{-1}, length of panicle, number of grains panicle^{-1}, 1000 grain weight and length/breadth ratio of grain revealed the divergence of each individual from the recurrent parent Prathyasa. Plants ICDE 13-3/46/4/31, ICDE 13-3/46/4/7, ICDE 13-3/46/4/48, and ICDE 13-3/46/4/50 were having the minimum euclidean distance of 1.25, 2.35, 3.06, and 4.01 respectively from recurrent parent. These lines had the red kernels of Prathyasa. Among them, ICDE 13-3/46/4/7 and ICDE 13-3/46/4/48 had homozygous combination *xa13* and *Xa21*, ICDE 13-3/46/4/31 had heterozygous combination of *xa13* and *Xa21* and ICDE 13-3/46/4/50 had heterozygous *xa13* and homozygous *Xa21* genes. The plants ICDE 13-3/46/4/46 with a combination of homozygous *xa13* and *xa5* and ICDE 13-3/46/4/41 with a combination of heterozygous *xa13* and homozygous *xa5* had a genetic distance of 11.46 and 8.45 respectively from the recurrent parent. Both these plants showed red kernels.

The plants up to a maximum euclidean distance of 15 units from Prathyasa were selected and upon clustering using the proximity dissimilarity matrix, the plants ICDE 13-3/46/4/31, ICDE 13-3/46/4/7 and ICDE 13-3/46/4/48 were in the same cluster along with the recurrent parent Prathyasa.

The plants ICDE 13-3/46/4/7 and ICDE 13-3/46/4/48 with two bacterial blight resistance genes (*xa13* and *Xa21*) in the homozygous state were the best BC_2F_2 plants identified with a maximum phenotypic resemblance to Prathyasa. These plants can be either subjected to selection by forwarding to BC_2F_3 generation or backcrossing with the recurrent parent for maximizing recurrent parent genome in the progeny. Even though the plants with the combination of *xa13* and *xa5* were more divergent from Prathyasa parent, the presence of red kernels in them signifies its selection for either backcrossing or generating BC_2F_3 population for fixation of resistance genes. These plants can be utilized to generate triple gene pyramided essentially derived variety (EDV) of Prathyasa to provide durable resistance to bacterial leaf blight.