GENOME WIDE MARKER ASSAY FOR THE RECOVERY OF RECURRENT PARENT GENOME IN RICE (*Oryza sativa*)

By

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(2013-09-114)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled "GENOME WIDE MARKER ASSAY FOR THE RECOVERY OF RECURRENT PARENT GENOME IN RICE (*Oryza sativa*)" 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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Certified that this thesis entitled "**GENOME WIDE MARKER ASSAY FOR THE RECOVERY OF RECURRENT PARENT GENOME IN RICE (*Oryza sativa*)**" is a record of research work done independently by Ms. Bhagyalekshmi R. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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BHAGYALEKSHMI R.

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LIST OF ABBREVIATIONS

%	-	per cent
µl	-	Micro litre
µM	-	Micro molar
^{0} C	-	Degree Celsius
BC_2F_1	-	Second filial back cross generation
bp	-	base pairs
BSA	-	Bulk Segregant Analysis
CAPS	-	Cleaved Amplified Polymorphic Sequences
cm	-	Centimeter
dNTP	-	Deoxyribonucleoside triphosphate
EDTA	-	Ethylene diamine tetra acetic acid
et al.	-	and co-workers/co-authors
F_1	-	First filial generation
Fig.	-	Figure
g	-	Gram
i.e.	-	that is
ISSR	-	Inter-simple sequence repeats
kg	-	Kilogram
m	-	Meter
M	-	Molar
mg	-	Milligram
min	-	Minutes
mM	-	Milli molar
Nacl	-	Sodium chloride
ng	-	Nanogram
PCR	-	Polymerase Chain Reaction
QTL	-	Quantitative Trait Loci

RFLP	-	Restriction Fragment Length Polymorphism
RAPD	-	Random amplified polymorphic marker
RNA	-	Ribonucleic acid
RNase	-	Ribonuclease
RPG	-	Recurrent parent genome
rpm	-	revolutions per minute
S.E(d)	-	Standard Error deviation
SE	-	Standard Error
spp.	-	Species
SSR	-	Simple Sequence Repeat
STS	-	Sequence Tagged Site
TAE	-	Tris –Acetate- EDTA
Taq	-	*Thermus aquaticus*
Tm	-	Annealing Temperature
v/v	-	Volume/ volume
Viz.,	-	Namely
w/v	-	weight/volume

INTRODUCTION

1. INTRODUCTION

Rice (*Oryza sativa*) is the principle food crop of the world and rice production has increased simultaneously with the growing population, with the use of high-input demanding, high yielding, and semi-dwarf varieties (Gnanamanickam, 2009). Asia accounts for 92% of world's production and in India, rice occupies an area of about 44 million hectares with production of 100.0 million tonnes (FAO, 2012). It is the second largest rice producer in the world after China, with 21% of the global production share.

The projected increase in the world's population, reduction in the land for cultivation of rice, emerging different types of diseases, pests and changes in climate are the major issues that must be addressed. Sustainable crop varieties which are resistant to biotic and abiotic stresses can tackle these issues. Bacterial leaf Blight (BB) caused by *Xanthomonas oryzae pv. oryzae (Xoo),* is a potential destructive disease that limits rice production up to 81% in irrigated and rain fed lowland ecosystems of India (Kumar *et al.*, 2012). In India its increased occurrence has been reported as major constraint in both northwestern (Goel *et al.*, 1989). In Kerala, the two major rice growing regions viz. Palakkad and Kuttanad are highly liable to BB disease causing damages upto 35.3% during kharif season. In Kuttanad the severity is marked more in both punja (Nov-Dec to Feb-Mar) and additional crop (Jun-Jul to Sep-Oct) (Mary, 1996).

Since there is no valid and effective chemical and biological control measures for the management of BB disease, improving resistance of the host plant is considered to be most potential alternative to protect the plants against the Bacterial blight (Khush *et al.*, 1989) and it is considered as the most efficient, economical and environmentally safe method. Since the cultivars having a single gene for resistance proved to be susceptible to pathogen mutation, therefore pyramiding two or more major resistance gene has been proven to deliver durable resistance against Xoo (Rajpurohit *et al.*, 2010).

The problems associated with conventional breeding can be tackled by Marker Assisted Selection (MAS) strategy utilizes molecular markers which are tightly-linked to a target loci for the phenotypic screening. By determining the allele of a DNA marker, we can find the plants that possess desired genes or quantitative trait loci (QTLs) based on their genotype rather than their phenotype. With the use of adequate and reliable information about molecular markers, MAS helps to discriminate between "Resistant" and "Susceptible" plants. Several major resistance genes (R) for BB have been identified and fine mapped for the improvement of bacterial blight resistance through MAS (Chen *et al.*, 2008).

Marker assisted backcrossing (MABC) is an efficient breeding method used in MAS where a specific trait(s) are introgressed into the genomic background of recurrent parent (RP) from a donor parent (DP) by using molecular markers (Hospital, 2005). With the knowledge of different cultivars having economically important traits, MABC can be used as a potential strategy to develop rice varieties of desired quality by incorporating a useful trait of interest into an elite variety which is popular and widely accepted by the farmers. Since this method employs molecular markers in research, there is no need of genetic transformation and therefore, there is no chance for raising ethical issues as in case of transgenic crops. Unlike in conventional breeding method, the varieties having desired traits like high yield, tolerant to biotic and abiotic stresses, quality and fragrance can be developed through MABC within a short period of time.

The cv. Aiswarya (PTB 52), released by Regional Agricultural Research Station, Pattambi, is a semi dwarf elite variety of Kerala with productivity 5-5.5t/ha. It is mostly preferred by farmers of Palakkad and other districts of Kerala. Since it is susceptible to Bacterial leaf Blight (BB) disease, it would result in greater yield loss.

As part of the ongoing research project funded by Department of Biotechnology (DBT) in the Dept. of Plant Breeding and Genetics, College of Agriculture, Vellayani, the BC_2F_1 plants of Aiswarya pyramided with two/ three genes for resistance to Bacterial Blight xa13, Xa 21, and Xa 33 has been developed. Samba mahsuri, pyramided with three genes for resistance xa13, Xa21 and Xa33 developed by Indian Institute of Rice Research (IIRR) Hyderabad, was taken as the donor parent.

The present study was undertaken as a continuation programme with an objective to estimate the reconstitution of genome of Aiswarya (RP) rice variety in the BC_2F_1 plants pyramided with genes for resistance to Bacterial Leaf Blight through molecular markers covering the entire genome of Aiswarya, so that the plants which shows high genetic similarity with Aiswarya can be multiplied to release as Essentially Derived Variety.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Rice (*Oryza sativa*) (2n=24), belonging to Poaceae family, is one of the oldest domesticated crop. It was cultivated 10,000 years ago in South and Southeast Asia and China and even now it is the principle food crop for more than half of the world's population. The rice genome is the smallest among cereal crops in which the whole genome sequencing was done for the first time and the genome is estimated to have a size between 400 to 430 Mb. Rice shares a large synteny with other cereal crops, making an excellent model cereal (Gale and Devos, 1998).

2.1 BACTERIAL LEAF BLIGHT OF RICE

Bacterial blight of rice, caused by *Xanthomonas oryzae pv.oryzae*, is a serious disease of rice especially in tropical lowland rice environments (Gnanamanickam *et al.,* 2009). Farmers in the Fukuoka area of Japan have first reported BB disease during 1884–1910.

Xanthomonas oryzae pv. oryzae (Xoo) is a non spore forming, rod shaped, gram-negative bacterium with 0.55 to 0.75 x 1.35 to 2.17 μm size (Ishiyama, 1992). The bacterium causes systemic infection in the xylem of the host rice plant. The bacterium may exist in two forms: i) the Dry form, Xoo is found in the vascular vessel and xylem parenchyma of dried plants. ii) the Growth form, bacterial cells are present in stubble and in the root system of perennial wild plants, especially Leesia spp. The pathogens can survive in the inactive stage also. The dry form can be activated and turn into the growth form after receiving moisture under favourable conditions.

Infection cycle starts when the pathogen enters its hosts through hydathodes and wounds. The bacterium initially multiplies in the intercellular spaces of the epithelial cells and it then spreads through the xylem. And after a few days, bacterial cells and EPS (extracellular polysaccharide) fill the xylem vessels and ooze out from hydathodes, forming beads or strands of exudate on the

leaf surface, which is a characteristic sign of the disease and a source of secondary inoculum (Nino-Liu *et al.*, 2006). Usually symptoms are observed at the tillering stage, but the incidence of disease increases with plant growth, peaking at the flowering stage (reproductive stage). Another major destructive manifestation of the disease is called Kresek, where the entire leaves of the plant turn pale yellow and wilt and this can be observed from the seedling to the early tillering stage, which results in partial or total crop failure. Temperatures between 28 and 34°C favour kresek development.

Irrespective of such infections, some of the rice cultivars/varieties has been found to exhibit genetic resistance against *X. oryzae pv. oryzae*. Thus, host plant resistance can be used as a tool for disease management (Nino-Liu *et al.*, 2005).

2.2 IDENTIFICATION AND MOLECULAR MAPPING OF BACTERIAL BLIGHT RESISTANCE GENES

The most preferred strategy for disease management is the development of varietal resistance against different races of the pathogen (Naveed *et al.*, 2010). This can be efficiently achieved only by identification and mapping of major resistant genes that confers broad spectrum resistance to the host plant.

The success of plant breeding programs lies in the effort for the identification and characterization of major genes for resistance. To date, 38 BB resistance genes have been identified from cultivated rice and wild rice (Zhang, 2005; Chun et al., 2012). Twenty eight BB resistant genes are dominant (Xa1, Xa2, Xa3, Xa4, Xa7, Xa10, Xa11, X12, Xa14, Xa16, Xa17, Xa18, Xa21, Xa22 (t), Xa23, Xa25 (t), Xa26, Xa27, Xa29, Xa30, Xa30 (t), Xa31 (t), Xa32 (t), Xa34, Xa35 (t), Xa36 (t), Xa38, Xa 39) and eleven are recessive genes (xa5, -xa5(t), xa8, xa13, xa15, xa19, xa20, xa24, xa28, xa31 and xa32, Nino-Lui *et al.*, 2006; Singh *et al.*, 2001; Natrajkumar *et al.*, 2012).

Many BB resistance genes, *i.e.* Xa3, Xa4, Xa6, xa9, Xa10, Xa21, Xa22, Xa23, Xa26 and Xa30, have been reported to be located on chromosome 11. It confers resistance to a broad range of Xoo strains. This resistance gene was first tagged with RAPD marker (Ronald *et al.*, 1992). Later, the RFLP marker RG103 is linked tightly to Xa21 gene at a distance of 1.2 cM. Then, pTA248, a PCR-base STS marker was isolated by map-based cloning and it is located on long arm of chromosome 11. Also, Xa21 was the first BB resistance gene successfully cloned. It was fine mapping of Xa10 revealed that the gene for resistance was flanked between markers M491 and M419 on Nipponbare genome which consisted of six candidate genes (Gu *et al.*, 2008).

2.2.1 Molecular genetics of xa13

The xa13 gene was first identified in cultivar BJ1 by Ogawa et al. (1988) and it is a completely recessive gene found in chromosome 8, which specifically offers resistance to Philippine Xoo race 6. Zhang *et al.* (1996) reported that xa13 is closely linked to a RAPD marker AC5-900 and three RFLP markers RG136, RZ28 and CD0116 on the telomeric region of rice chromosome 8. Chu *et al.* (2006) cloned and fine-mapped xa13, to a DNA fragment of 14.8 kb by using the map-based cloning strategy. Sequence analysis of this fragment indicated that This fragment possesses two apparently intact candidate genes (an extensin-like gene and a homologue of nodulin MtN3) and the 5' end of a predicted hypothetical gene. The study reported that E6a, SR6, ST9 and SR11 were the four PCR-based markers, that were found to be tightly linked to the xa13 locus. These markers will be useful tools for the marker-assisted selection of xa13 in breeding programs.

2.2.2 Molecular genetics of Xa 21

At International Rice Research Institute, a new dominant BB resistance gene was identified in the wild sps. *Oryza longismaninata* and it is found to provide resistance to all the six known races of BB in the Philippines (Khush *et al.*,1989).

Ikeda *et al.* (1990) reported that the translational product of the gene Xa21 carries a leucine-rich repeat motif and a serine-threonine kinase-like domain, which is very essential for cell-surface recognition of a pathogen ligand and subsequent activation of an intracellular defense response (Song *et al.*, 1995). This receptor directly or indirectly 'recognizes' a signal generated via a corresponding avirulence (avr) gene product encoded by the pathogen, in this case the AvrXa21 peptide of Xoo. The signalling cascade culminating in defense responses that halt the pathogen's growth were initiated by the formation of receptor–ligand complex (Song *et al.*, 1995) This dominant gene Xa21 located in chromosome 11 confers broad spectrum resistance to most isolates of X. oryzae pv. oryzae (Khush *et al.*, 1989). Thus, Xa21 has been one of the most preferred genes for improving resistance in rice against Bacterial Blight.

2.2.3 Molecular genetics of Xa33

The BB resistance gene, Xa33 present in the *O. nivara* located on the seventh chromosome and offers wider spectrum of resistance to BB. Eight genes were identified in the region which encodes serine-threonine kinase appears to be a candidate for the Xa33 gene. Kumar et al. (2012) mapped the resistance gene or genes present in IRGC 105710, an accession of *Oryza nivara*. International Rice Germplasm Collection (IRGC) accession number 105710 exhibiting high level and broad-spectrum resistance to Xanthomonas oryzae pv. Oryzae was crossed with the bacterial blight (BB)-susceptible varieties 'TN1' and 'Samba Mahsuri' (SM) and then backcrossed to generate backcross mapping populations. A total of 49 SSR markers located between the genomic regions spanned by RM5711 and RM6728, 2,011 individuals of BC_2F_2 population derived from the cross IRGC 105710/TN1//TN1 were screened and, the gene was fine mapped between two SSR markers (RMWR7.1 and RMWR7.6) with a genetic distance of 0.9 and 1.2 cM, respectively, from the gene.

2.3 DNA MARKERS AND MARKER-ASSISTED SELECTION (MAS)

DNA marker or molecular marker is a sequence of DNA with a known location in the chromosome and that can be used for identifying and mapping several agronomically important traits. Molecular markers are 'landmarks' linked to the trait of interest which is co-inherited along with the trait. They increase the efficiency of backcrossing by allowing selection of genotype with maximum percentage of recurrent parent genome (Hospital, 2005). RAPD, RFLP, AFLP and SSR markers are most commonly used and implemented in rice genomics. The dominant nature, lack of reproducibility and use of radiochemicals limits the use of RAPD and RAPD markers, while SSR marker are co-dominant, simple, accurate, efficient, cost-effective, represent single-loci and can detect high levels of polymorphism (Babu *et al.*, 2004).

Simple sequence repeats (SSRs) are repeat sequences of two to six nucleotides which are abundant and dispersed throughout the genome (Tautz, 1989). The unique sequences flanking the SSR (also known as microsatellite) are utilized to design forward and reverse primers to amplify the corresponding SSR loci, which are also referred to as sequence tagged microsatellite sites (STMS) markers (Beckmann and Soller, 1990). It constitutes excellent genetic markers with locus identity and can be multiplexed to achieve higher throughput (Mitchell *et al.*, 1997).

Cleaved Amplified Polymorphic Sequence (CAPS) are new generation co-dominant molecular markers uses amplified DNA fragments followed by restriction endonuclease digestion to display RFLP in order to identify SNPs in target region (Dubcovsky, 2004). One of the major applications of molecular markers to rice breeding is using marker assisted selection as it improves the efficiency of plant breeding through precise transfer of trait of interest and thereby enabling fastest recovery of the recurrent parent genome. It is based on the principle that if a gene is tightly linked to an easily identifiable genetic marker, it

may be more efficient to select in a breeding programme for the marker than for the trait itself. Therefore, MAS strategy can be used to incorporate valuable traits into elite lines that are suitable for cultivar release (Dubcovsky, 2004; Collard and Mackill, 2008). McCouch *et al.* (2001) experimentally validated a total of 2414 new di-, tri- and tetra-nucleotide non-redundant SSR primer pairs for rice (Oryza sativa), representing 2240 unique marker loci and this release makes available a total of 2740 experimentally confirmed SSR markers for rice. Marker assisted selection (MAS) can be successfully employed in pyramiding more than two genes from a wild cultivar into an elite variety and rapid recovery of recurrent parent genome has found to be efficient in developing BB resistant variety (Singh *et al.*, 2001; Sundaram *et al.*, 2008; Shanti *et al.*, 2010).

2.4 MARKER ASISTED BACKCROSS BREEDING FOR BB RESISTANCE

Progressive developments in biotechnology makes a plant breeder to develop more efficient selection systems to replace traditional selection systems. Marker assisted backcrossing involves pyramiding of two or more genes controlling a trait of interest while retaining the essential characteristics of the recurrent parent (Collard *et al.*, 2008). Gene pyramiding has proved to be an effective strategy for conferring durable resistance in host plants since it involves stacking of two or more genes for a specific trait, which leads to the consecutive expression without any breakdown of resistance (Singh *et al.*, 2001). In MABC we can integrate a gene linked to a specific trait from an elite source (the donor parent) to an exclusive breeding line (the RP). After three or four backcrosses, the resultant product will be an improved line comprised only the targeted gene from the donor parent, having the genetic background of the recipient parent line. The successive backcrossing helps to recover the RP genome there by removing the genetic background of DP.

MABC can also be used to develop near isogenic lines (NILs) by minimizing carried-over donor segments flanking the target locus, providing precise introgression of individual genes for detailed characterization of the QTLs

and background selection can speed up the recovery of recipient genome (Frisch *et al.* 2005)

Since the conventional plant breeding mainly depends on phenotypic selection, which is time consuming process and it is less efficient in multiple gene pyramiding because of dominance and epistasis effect in multiple gene transfer (Collard and Mackill, 2008) and linkage drag (Young and Tanksley, 1989), all these can be avoided by molecular marker assisted breeding method.

Huang *et al.* (1997) successfully transferred four bacterial blight resistance genes, Xa-4, xa-5, xa-13 and Xa-21 using RFLP and PCR-based markers. The higher levels of resistance shown by pyramided lines than lines having single gene and this study had resulted in the development of PCR primers for resistance genes xa-5 and xa-13.

Sanchez *et al.* (2000a) pyramided three genes for bacterial blight resistance (xa5, xa13, and Xa21), to improve the resistance of the three NPT lines, IR65598-112 and the two sister lines IR65600-42 and IR65600-96 to Xoo, through MAS based on STS markers. In this study the BC_3F_3 NILs with more than one BB resistance gene showed a wider resistance spectrum of resistance to the Xoo races, than those with single resistance gene for BB.

At Punjab Agricultural University (PAU), Singh *et al.* (2001) pyramided three genes for bacterial blight resistance (xa5, xa13 and Xa2l) into cv. PR106, using marker-assisted selection. 17 isolates of the pathogen from the Punjab and six races of Xoo from the Philippines were inoculated to Pyramided lines of PR106 with genes for resistance showed that the lines with combination of genes provided broad spectrum resistance to the pathogen population. Also, the dominant gene for resistance Xa21 was found to be more effective than xa5 and xa13 (recessive).

Haung *et al.* (2003) introgressed bacterial blight resistance genes Xa21 and Xa4 from IRBB60 into a hybrid rice line, Shunhui527 using Marker Assisted Selection. The improved lines expressed high level of resistance to the Xoo strain CI-C VIII.

Joseph *et al.* (2004) introgressed four bacterial blight resistance genes Xa4, xa8, xa13and Xa 21, were found to be effective against Xoo infection. The IRBB55 lines carrying xa13 and Xa21 was found equally effective as three/four gene pyramid lines. This two genes pyramided lines of IRBB55 were combined with the basmati quality traits of Pusa Basmati-1 (recurrent parent). Background analysis using 252 polymorphic AFLP markers showed 80.4 to 86.7% recurrent parent alleles in BC_1F_3 selections.

Two bacterial blight resistance genes Xa21 and Xa7 were pyramided into Minghui 63 background and the improved lines with homozygous genotypes showed high level of resistance than heterozygous genotypes having a combination of both Xa21 and Xa7 (Zhang *et al.*, 2006).

Perez *et al.* (2008) introgressed three Bacterial blight resistance genes Xa4, Xa7 and Xa21 into a Temparature sensitive genetic male sterile plant (TGMS) line using MAS. The F2 plants having all the three genes (Xa4+Xa7+Xa21) showed more resistance to all Xoo races used in this study.

Sundaram *et al.* (2008) introgressed three major BB resistance genes (xa5, xa13 and Xa21) into an elite variety, Samba Mahsuri from a donor line (SS111 3) having all the three genes in a homozygous condition. The three gene pyramids in the BC_4F_1 generation showed a recovery of 97% of recurrent parent (Samba mahsuri) when screened with polymorphic microsatellite markers.

Zhou *et al.* (2009) developed a high yielding elite variety, Lu-You-Zhan highly resistant to both BB and BLS by pyramiding Xa23, derived from wild rice and a non host maize resistance gene, Rxo1, using both marker assisted selection

(MAS) and genetic engineering. The study demonstrated that MAS combined with transgenic technologies are effective in achieving high level of resistance against multiple plant diseases.

Shanti *et al.* (2010) used marker-assisted backcross-breeding program for the introgression of four bacterial blight resistance genes namely Xa4, xa5, xa13 and Xa21 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 showed very high level of disease resistance to 10 highly virulent isolates of Xanthomonas oryzae pv. oryzae.

Bharani *et al.* (2010) combined three BB resistance genes (Xa21, xa5 and xa13) FOM isoline IRBB6060 by molecular marker assisted selection into high yielding susceptible rice cultivars popular in South India viz., ADT43 and ADT47. The pyramided genotypes with two or three gene combination were found to be more resistant.

Basavaraj *et al.* (2010) used 'Improved Pusa Basmati 1' (having xa13 and Xa21 genes) as the recurrent parent and cultivar 'Tetep' (carrying the blast resistance gene Pi54 and ShB resistance quality trait loci (QTL), qSBR11-1) as the donor. Foreground selection combined with phenotypic selection in BC_2F_5 lines using flanking markers identified 7 families homozygous for xa13, Xa21 and Pi54 and 89.5% recovery was found in the background analysis.

Rajpurohit *et al.* (2010) carried out introgression of two genes for BB resistance, Xa2l and xa13 along with sd-1, a semi dwarfing gene in the traditional Indian basmati rice Type 3 Basmati from a rice cultivar PR106-P2 through MAS using SSR and ISSR markers. And they reported that the BC_2F_3 progenies possessing both Xa21 and xa13 were highly BB resistant.

Pandey *et al.* (2012) aimed to improve two traditional, BB susceptible Basmati varieties (Taraori basmati and Basmati 386) by introgressing two BB resistance genes Xa21 and xa13 from Improved Samba Mahsuri through the process of MAS for a single generation (BC1) coupled with phenotype-based selection for short plant stature and high yield. The two gene (Xa21 and xa13) pyramid Basmati lines of BC_1F_5 generation exhibited significant levels of resistance.

Huang *et al.* (2012) used marker assisted selection for introgressing four bacterial blight resistance genes Xa7, Xa21, Xa22 and Xa23 into elite hybrid restorer line Huahui 1035. They obtained ten promising lines of Huahui 1035 restorer background showing BB resistance and their respective F_1 hybrids with a cytoplasmic male sterile line i.e. Jinke1A.

Salgotra *et al.* (2012) reported combining three genes, two for BB resistance (Xa21 and xa13) and one aroma gene (fgr) for the first time using functional marker-assisted selection in rice variety IRS 5441-2 (Oryza sativa L.) from a BB resistant variety IRBB59 having all the three BB resistance genes xa5, xa13 and Xa21. The BC_1F_3 generation expressed enhanced resistance to BLB along with Basmati quality and they were found to be effective against the most virulent BLB isolate, as the donor line IRBB59.

Suh *et al.* (2013) introgressed Xa4, xa5 & Xa21 from an indica donor IRBB57, into Mangeumbyeo, which is high yielding but BB-susceptible elite japonica rice cultivar with good grain quality. The study led to the development of three elite advanced backcross breeding lines (ABL). The multiple gene pyramided lines exhibited higher resistance to Xoo than the lines having individual resistance genes. The background aanalysis done with SSR markers revealed 92.1% genome recovery of the ABL.

Win *et al.* (2013) successfully pyramided three genes for BB resistance including xa5, Xa21 & xa33 to MK-75, a susceptible variety. The results of BB resistance evaluation against both Thai and Myanmar Xoo strains indicated that multiple gene pyramided lines (xa5Xa21xa33 or xa5Xa21 or xa5xa33 or Xa21xa33) had increased level of resistance and wider resistance spectrum than normal MK-75.

Magar *et al.* (2014) introgressed BB resistance genes viz., xa13 & Xa21 from B95-1 to a high yielding rice variety, MTU1010 (Cottondora Sannalu). The foreground selection was done using gene linked primers viz., xa13 promotor and pTA 248 for xa13 & Xa 21 respectively and the genetic analysis of F_2 populations revealed that the genes (xa13 & Xa21) followed Mendelian inheritance.

Pradhan *et al.* (2015) employed backcross breeding to transferred three major BB resistance genes (Xa21, xa13 and xa5) from the pyramided lines with two gene combinations Xa21+xa13 and Xa21+xa5 into Jalmagna variety from The BC_3F_2 plants of BB pyramided lines showed the maximum recipient parent genome recovery of 95%.

Abhilash *et al.* (2016a) transferred BB resistance gene Xa21 & Pi54, gene for blast resistance into RPHR-1005 and analyzed the backcross derived plants for their resistance against BB and blast. RPBio Patho-2 was used as a donor for both the resistance genes.

Abhilash *et al.* (2016 b) transferred Xa21 and Xa33 genes for BB resistance and Pi2andPi54 for Blast resistance to RPHR-1005 using RPBio Patho-1(possessing Xa21+Pi2), RPBioPatho-2 (possessing Xa21+Pi54) and FBR1 15EM (possessing Xa33) as the donors. In the BC_2F_2 generation, the plants having the gene combination Xa21+Pi2, Xa21+Pi54 and Xa33 in homozygous condition possessed >92% recovery of the recurrent parent genome (RPG).

Ellur *et al.* (2016a) introgressed the blast resistance genes, Pi2 and Pi54 and bacterial blight (BB) resistance genes xa13 & Xa21 into the genetic background of Pusa Basmati 1121 (PB1121) and Pusa Basmati 6. The background analysis showed 95.8% recovery of recurrent parent genome (RPG) in one of the near-isogenic lines (NILs) namely, Pusa 1728-23-33-31-56. In phenotypic analysis also, it showed high degree of resemblance to PB6.

Ellur *et al.* (2016b) introduced Xa38 gene in PB1121, a variety susceptible to bacterial blight (BB) disease from PR114-Xa38 using a modified marker-assisted backcross breeding (MABB) scheme. Both the NILs of PB1121, carrying gene for resistance Xa38 alone and another line carrying xa13 + Xa21 showed resistance against the Xoo races 1, 2, 3 and 6. The background selection was done for evaluating the recovery of RPG and it was found to be.9% in the developed NILs. Additionally, Xa38 also resisted Xoo race 5 to which xa13 + Xa21 was susceptible. This strategy was very effective in reducing the linkage drag to <0.5 mb upstream and <1.9 mb downstream of Xa38.

Srikanth *et al.* (2016) introgressed three major resistant genes into Vallabh Basmati 22 through marker-assisted breeding (MAB) using Improved Samba Mahsuri (having Xa21 + xa13) and Tetep (having Pi 54) as the donor parents through two sets of crosses. At each generation, foreground selection was done to identify plants possessing Xa21 + xa13 + Pi54 in homozygous condition. In the ICF_4, four promising three-gene pyramid lines of Vallabh Basmati2 showing high level of resistance against both BB and blast along with high yield and grain type similar to the recurrent parent have been identified.

Karma Mahsuri, a popular high yielding rice variety susceptible to BLB was pyramided with xa5, xa13 and Xa21 from IRBB59. The presence of these genes were confirmed by using gene specific primers viz., xa5R, xa5S, xa13 promoter and Xa21F/R respectively. By genetic analysis, the presence of all the three resistant genes for BB (xa5, xa13 & Xa21) were confirmed in 22 lines of BC_2F_3 population (Deshmukh *et al.*, 2017).

Kabir *et al.* (2017) transferred two genes Xa21 and SUB1 QTL for BB resistance and Submergence tolerance respectively to BRRI dhan52, a unique submergence tolerant rice variety. Here the genes for both BB as well as submergence were detected by using Sequence tagged site (STS) and simple sequence repeat (SSR) markers. The percentage of recipient genome recovery in the best plant 1, 2 and 3 selected from ten double heterozygous (Xa21 and SUB1 QTL) BC_1F_1 plants were 78.7%, 75.83% and 75.4%, respectively.

Recently, Baliyan *et al.* (2018) reported that MAS along with stringent phenotypic selection without compromising the Basmati traits were successfully carried out in CSR-30 is a salt-tolerant Basmati variety. The BB-resistant donor variety IRBB-60 having Xa21, xa13 & xa5 from were introgressed into the CSR-30 through marker-assisted selection (MAS). In this study 131 polymorphic SSR markers were used for the background analysis and the results revealed that recurrent parent genome (RPG) recovery ranged up to 97.1% in the BC_3F_1 three-gene-pyramided genotypes.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 PLANT MATERIALS

The seeds from BC_1F_1 plants developed in Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, as part of the project Entitled "Development Of Rice Varieties For Kerala With Pyramided Genes For Resistance To Bacterial Leaf Blight By Marker Assisted Selection", having more than 50% homozygousity yielding two/three gene combination was taken for the present study. Seeds of donor parent, Improved Samba Mahsuri (pyramided with xa13 and Xa21), Samba Mahsuri (pyramided with Xa33) were collected from IIRR (Indian Institute of Rice Research) Hyderbad.

Table 1. Donor varieties used in the study

Genes	Donors	Resistance Source	Chr. No.	Reference
xa13	Improved Samba Mahsuri (BPT5204)	SS1113	8	Sundaram et al.2011
Xa21			11	Ronald et al.1992
Xa33	Samba Mahsuri(Fbr-1-1)	IRGC105710	7	Kumar et al. 2012

Table 2. Susceptible variety used in the study

Recipient	Source	Characters
Aiswarya(PTB52)	RARS, Kerala Agricultural University (KAU), Pattambi	Semidwarf, Short duration, medium bold red kernels

3.2 DNA ISOLATION

HiPurA Plant Genomic DNA Miniprep Purification Kit (MB507) was used to carry out genomic DNA isolation. 100 mg of finely cut leaf material (after removing the mid rib, since it is a source of carbohydrate contamination) were ground with liquid nitrogen to make a fine powder using sterilized mortar and pestle. The finely ground leaf tissue was mixed with 400 µl of lysis buffer, and transferred to a 2.0 ml clean collection tube. After giving a vigorous vortexing, 20µl of RNase (20 mg/ml) was added and incubated at room temperature (15-25^{0}C) for 10 minutes. The mixture was then incubated at 65^{0}C for 10 minutes with intermittent mixing. Then 130µl of precipitation buffer was added to the lysate and incubated for 5 minutes on ice. After that, the entire sample was added to the Hishredder placed in a 2.0 ml collection tube and centrifuged for 5minutes at 14000 rpm. The resultant flow-through was transferred to a 2.0ml collection tube without disturbing the cell debris. To the lysate, added 1.5volumes of dil. Binding buffer (The binding buffer was prepared by diluting the 14ml Binding Buffer Concentrate with 7ml of 100% ethanol) and from this solution 650µl of this lysate was added to HiElute Miniprep Spin Column, and carried out centrifugation at 8000 rpm for 1minute. The same step was repeated once again and flow-through was discarded. To the same collection tube after discarding the flow-through, 500µl diluted wash solution (15ml wash solution concentrate added to 35 ml 100% ethanol) and centrifuged at 8000 rpm for 1 minute. Discarded the flow-through and reused the collection tube. Again added 500 µl of diluted wash buffer to the column and centrifuged for 2 minutes at 14000 rpm. The tube with column was again centrifuged for 2 minutes at 14000 rpm after discarding the flow-through inorder to dry the membrane. To a new 2.0 ml collection tube added 100µl of elution buffer, incubated at room temperature (RT) for 5 minutes. For eluting DNA, centrifugation was carried at 10000 rpm for 1 minute. This step was repeated again with another 100µl of elution buffer for getting high yield of DNA. Finally the elute was transferred to a fresh capped 2.0 ml collection tube and was stored at -20^{0}C.

3.2.1 AGAROSE GEL ELECTROPHORESIS

Reagents (Stock solutions)

a) 50X TAE Buffer (1000 ml)

Tris base	240g
Acetic acid	57.1ml
0.5M EDTA (pH-8.0)	186.12g
Distilled H_2O	942.9ml

b) 6X loading dye (10 ml)

Sucrose	4.0g
Bromophenol blue	0.025g
Distilled H_2O	10ml

After DNA isolation, agarose gel electrophoresis was carried out to resolve the genomic DNA in a horizontal gel electrophoresis Unit of BIO-SYS. For that, 0.8% (w/v) agarose gel was prepared using 1x TAE buffer by boiling it in a microwave oven. After melting, the solution was cooled to 42-45°C and mixed with 0.3 μg/ml of ethidium bromide. The solution was then poured on to a preset, gel casting tray and allowed the gel to solidify for 15-20 min. After removing the comb the casting tray was submerged in electrophoresis chamber containing 1X TAE buffer in such a way that the wells are placed closest to the negative terminal of the chamber. The DNA samples were prepared by mixing with loading dye in the ratio 5:1 respectively and loaded into the corresponding wells of gel. The negative terminal (cathode) and positive terminal (anode) of the electrophoresis unit were attached to the power supply and set a voltage of 60V to run the gel. The power was turned off when the loading dye reaches about $3/4^{th}$ of the gel. The gel was visualized under U.V light using SYNGENE gel documentation system inorder to check the intactness, shearing of DNA and RNA

contamination. Single intact band of high molecular weight showed that DNA was pure.

3.2.2 QUANTITATIVE AND QUALITATIVE ANALYSIS OF DNA

Quantification of the genomic DNA was estimated by using UV-Visible spectrophotometer by measuring the absorbance (A) at 260 nm. Here, 5µl of DNA was mixed with 0.1X TAE which is then added to 3ml of distilled water. By using distilled water as blank, the absorbance was taken at 260 nm and 280 nm. Since the absorbance, 1.0 O.D. at 260 nm is equivalent to 50mg of DNA per ml; the concentration of DNA was estimated from the following formula:

Concentration of DNA (mg/ml) = A260 x 50 x dilution factor.

The quality of DNA was checked by calculating A260/A280 ratio. The ratio of A260/A280 in the range 1.8±0.05 was taken as pure DNA.

3.3 MOLECULAR MARKERS

Simple Sequence Repeat markers which are specific and closely linked to the BB resistance genes viz., xa13 prom primers for xa13, pTA248 for Xa21 and RMWR7.1 for Xa33 were used in the foreground selection to identify the plants having specific genes for resistance to BB. Since functional nucleotide polymorphism specific for the recessive gene xa 13 have been clearly identified, the functional marker which target the indels in the promoter of Os8N is used. pTA248, marker is located at a genetic distance of 0.2 cM from the gene Xa21 (Ronald *et al.*, 1992), while the marker RMWR7.1 flanks the gene for resistance Xa33 at a genetic distance of 0.9 cM (Kumar *et al.*, 2011). The SSR primers were obtained from Sigma Aldrich Chemicals Pvt. Ltd.

Table 3. SSR markers used for evaluation of BB resistance genes to Xanthomonas oryzae pv. oryzae

R Gene	Marker	Sequence	Annealing temperature (Tm)
xa13	xa 13 proF	5' GGCCATGGCTCAGTGTTTAT 3'	55.3°C
	xa 13proR	5' GAGCTCCAGCTCTCCAAATG 3'	
Xa21	pTA 248 F	5'AGACGCGGAAGGGTGGTTCCC GGA3'	55.3°C
	pTA248R	5'AGACCGGTAATCGAAAGATGA AA 3'	
Xa33	RMWR7.1F	5'TTTTATCCCCTTCTTCCTTC3'	56°C
	RMWR7.1 R	5'CGTGTTTTGTGTGTCTTTTG3'	

3.4 POLYMERASE CHAIN REACTION (PCR)

The DNA isolated from BC_2F_1 plants were used for PCR amplification using the three specific SSR primers viz., xa13 prom primers, pTA248 and RMW7.1 respectively for xa13, Xa21 and Xa33. The PCR reaction was carried out in 25 µl reaction mixture containing 50ng genomic DNA, 1 unit of Taq DNA polymerase, 10X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$ and 0.01 mg/ml gelatin), 10mM dNTPs and 10 pM of both forward and reverse primer. The reactions were carried out in Eppendorf master cycler nexus gradient PCR. Following PCR conditions were used:

DNA template was initially denatured at 94°C for 5 min followed by 35 cycles of PCR amplification under the following parameters: a 30sec to 1 min denaturation at 94°C, a 30sec to 1 min annealing at 55.3°C for xa13 and Xa 21, and 56°C for Xa33, 72°C for 1 min of primer extension and final extension 5-7 min at 72°C (5 min for pTA248, and xa13pro, 7min for RMWR 7.1). Amplified products were stored at -20°C till next use. The amplified product was

electrophoretically resolved in 2% agarose gel containing 0.3 µg/ml of ethidium bromide in 1x TAE buffer and visualized under SYNGENE G-Box F3 gel documentation unit.

As a standard reference to score the polymorphisms in the DNA profile, a 100 bp DNA ladder was used for comparing the size of the genotypes showing resistant and susceptible bands

3.5 FOREGROUND SELECTION FOR RESISTANCE GENES IN BC_2F_1 PLANTS

The BC_2F_1 plants were raised from the seeds of backcrossed progenies BC_1F_1 to identify the plants having two/three gene combination, by undertaking foreground selection as follows.

3.5.1 Plant Materials

After background selection, the seeds of BC_1F_1 plants having two/three gene combination were taken and sown in the nursery. After twenty one days i.e., at the tillering stage, leaf samples from these plants were collected for DNA isolation and then it is used for foreground selection with the gene specific markers linked to gens for resistance xa13, Xa 21 and Xa33.

3.5.2 Foreground selection of BC_2F_1 generation

Genomic DNA from these BC_2F_1 plants was isolated by the procedure of HiPurA Plant Genomic DNA Miniprep Purification Kit. Here the DNA markers closely linked to the BB resistance genes such as pTA248 (Xa21 gene), xa 13 pro (xa13 gene), RMWR7.1 (Xa33) were used to identify the presence of the above mentioned resistance genes by the PCR analysis described earlier.

3.5.3 Background selection of BC_2F_1 generation

The BC_2F_1 plants having the respective BB resistance genes identified by foreground selection were screened with Aiswarya specific markers to calculate the recovery of Recurrent Parent Genome.

3.5.4 Back ground Selection for Molecular Markers Specific to the Recurrent Parents

Identification of parental polymorphism in both recurrent and donor followed by background selection in BC_2F_1 were performed at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala by detailed procedure described below.

3.5.4.1. Plant Materials

A total of 23 progenies obtained by the cross between (Samba mahsuri X Aiswarya) X (Samba mahsuri X Aiswarya) were genotyped with 44 microsatellite loci that are polymorphic between Samba mahsuri and Aiswarya.

3.5.4.2. PCR for Assaying Microsatellite Loci

For assaying the microsatellite loci, carried out polymerase chain reaction in a 10 µl reaction volume containing 2 ng template DNA, 0.4 µM of M13 tailed sequence specific forward primer, 1.2 µM of FAM labeled M13 primer and 1.6 µM of the sequence specific reverse primer, 2µM each of dNTPs, 1X PCR buffer containing 2.5 mM $MgCl_2$, 0.5 unit Taq DNA polymerase (AmpliTaq Gold, Applied Biosystems, Foster City, CA, USA), The PCR was performed in GeneAmp® PCR System 9700 (Applied Biosystems) under the following conditions:

Initial denaturation at 94 °C for 5 min followed by 30 cycles of 30 sec at 94 °C; 45 sec at 56 °C, 45 sec at 72 °C, followed by 8 cycles of 30 sec at 94 °C, 45

sec at 53 °C, 45 sec at 72 °C and a final extension at 72 °C for 10 min. For checking nonspecific amplification non template control was taken.

Then capillary electrophoresis was carried out for the PCR products on an ABI Prism 3730 genetic analyzer (Applied Biosystems), where the mixtures were denatured at 94° C for 5 minutes and snap cooled on ice and then it is subjected to capillary electrophoresis. For capillary electrophoresis, 2 µl of PCR product was mixed with 0.1 µl of size standard (GENESCAN® 400 HD [ROX] size standard, Applied Biosystems) and 10 µl of Hi-Di formamide (Applied Biosystems). s

3.5.4.3 Microsatellite Allele Calling and Allele Binning

Allele sizing was done after capillary electrophoresis, by using the software Gene mapper 4 (Applied Biosystems) which calculates allele sizes (in base pairs) by comparing the fragment peaks with the internal size standard. The software provides Electropherograms that can be viewed visually in the software Gene mapper 4 inorder to ensure that clear peaks were found for the expected marker sizes and that stutter peaks were not called. In cases where there were more than one peak, because of the presence of stutter bands, the last stable peak on the chromatogram was considered as the size of an allele. Raw allele size calls were binned manually to assign whole integer allele values.

3.5.4.4 Assessment of Recovery of Recurrent Parent Genome

The microsatellite markers which were found to be polymorphic to the respective parents were used to genotype foreground selected plants at each backcross generation. For estimating the amount of recurrent parent genome contribution 'G' in the BC_2F_1 progenies was calculated by the following formula (Sundaram *et al.*, 2008)

$G = [(X + 1/2Y) \times 100]/N$

Where,

N = total number of parental polymorphic markers screened

X = number of markers found homozygous to recurrent parent allele

Y = number of markers found heterozygous for recurrent parent allele

RESULTS

4. RESULTS

Bacterial blight is a major devastating disease caused by Xanthomonas oryzae pv. oryzae results in high yield loss in the major rice growing areas in the world. In order to tackle the problem, advance in biotechnology is employed to improve the efficiency in the breeding programmes. Till date, about 40 genes conferring resistance to BLB were identified. Pyramiding two/ more resistance genes gives durable resistance instead of a single gene, also it avoids the resistance break down due to pathogen mutation. Marker assisted selection uses molecular markers linked to the genes for identifying the lines pyramided with resistance genes. The present study uses marker assisted backcross strategy to calculate the recovery of recurrent parent genome in the plants pyramided with three genes for resistance viz., xa13, Xa21 and Xa33. The results obtained are mentioned under following headings.

4.1. Validation of BB genes for resistance in the parental genotypes.

4.2. Screening of Aiswarya and Samba mahsuri with SSR markers to locate Aiswarya specific Markers.

4.3. Foreground selection in BC_2F_1 plants to find plants with 2/3 gene combination.

4.4. Background screening for the assessment of the recovery of recurrent parent.

4.1 VALIDATION OF BB RESISTANCE GENES IN THE PARENTAL GENOTYPES

4.1.1 Isolation of Genomic DNA

The recurrent parent, Aiswarya was collected from Regional Agricultural Research Station, Kerala Agricultural University (KAU), Pattambi. The two donors used in this study included viz., Improved Samba Mahsuri (pyramided

with xa13 and Xa21), Improved Samba Mahsuri (pyramided with Xa33) collected from IIRR (Indian Institute of Rice Research) Hyderbad. These genotypes were grown in nursery of Department of Plant Breeding and Genetics, College of Agriculture, Vellayani. Genomic DNA was isolated from the young leaves of 21 days old seedlings of the donors and recipient genotypes by using HiPurA Plant Genomic DNA Miniprep Purification Kit (MB507). The quality and quantity of extracted genomic DNA was checked both by agarose gel electrophoresis and UV spectrophotometer respectively.

4.1.2 Qualitative and Quantitative Analysis of DNA

The quality of genomic DNA of all the parental genotypes was resolved in 0.8% (w/v) agarose gel. A single discrete band of high molecular weight DNA was obtained without any smear, and this indicates that the DNA extracted from the parental genotypes was of good quality without any enzymatic degradation or mechanical disruption.

The quantity of genomic DNA was calculated by ratio of absorbance at 260 and 280 nm respectively. UV absorbance at 260 nm along with distilled water as blank. The amount of protein and RNA in the genomic DNA was also recorded at 280 nm using the same UV spectrophotometer. The A_{260}/A_{280} range from 1.8-2.00 which indicates that the DNA was free from contaminants like RNA and proteins. The ratio of absorbance at 260 nm and 280 nm of the parental genotypes, Improved Samba Mahsuri (xa13 and Xa21), Samba mahsuri (Xa33), and cv. Aiswarya calculated were 1.80, 2.00 and 2.0 respectively.

4.1.3 Polymerase Chain Reaction (PCR)

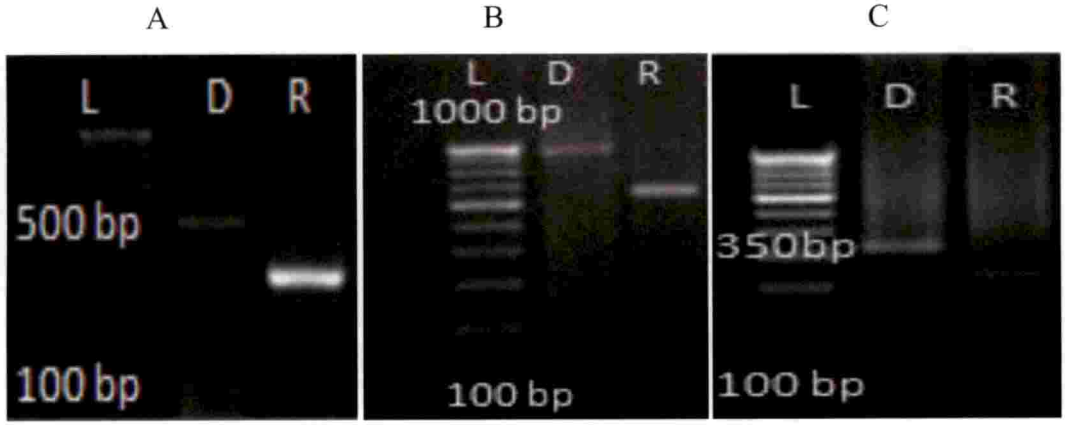
DNA isolated from the parental genotypes were subjected to PCR amplification using gene specific markers. The marker polymorphism was analysed for all the three genes viz., xa13, Xa21, Xa33 in the donor parents with specific markers xa13 pro, pTA 248 and RMW 7.1 respectively.

The amplification product of the marker specific to xa13 showed that the Improved Samba Mahsuri (donor) amplification was observed at 500 bp while the recipient Aiswarya gave rise to the product at 250 bp.

The amplification product of pTA248 specific to the gene Xa21 (Plate 1) showed that the allele size of the donor parent, Improved Samba mahsuri, is at 1000bp which was absent in the susceptible parent Aiswarya that gave rise to a product at 650bp.

The marker profile for Xa33 gene by the gene specific marker RMW7.1 showed that the allele size of the donor was produced ~350bp and of size ~250bp in recipient parents (Plate 1).

Plate1. Validation of *xa*13 and *Xa*21 genes and Xa33 in donor parents with xa13 pro, pTA248 and RMW.1 markers



A. Amplification profile for xa13 B. Amplification profile for Xa21
C. Amplification profile for Xa33 where D=Improved Samba mahsuri (Donor) for xa13 and Xa 21, and D= Samba mahsuri for xa33, R= Aiswarya (Recipient)

4.2. SCREENING OF AISWARYA AND SAMBA MAHSURI WITH SSR MARKERS TO LOCATE AISWARYA SPECIFIC MARKERS.

4.2.1. Selection of microsatellite loci and custom synthesis of primers

A total of 320 primers were selected from the public domain (http://www.gramene.org). The loci included dinucleotide, trinucleotide, tetranucleotide and complex repeats. The loci with high polymorphism information content (PIC) were selected in such a way that it covers whole genome of rice i.e., 12 chromosomes. The chromosome wise distribution of loci chosen is as follows: 29 loci in chromosome 1, 25 loci in chromosome 2, 25 loci in chromosome 3, 25 loci in chromosome 4, 28 loci in chromosome 5, 41 loci in chromosome 6, 33 loci in chromosome 7, 21 loci in chromosome 8, 27 loci in chromosome 9, 17 loci in chromosome 10, 23 loci in chromosome 10, 20 loci chromosome 12. Out of 320 microsatellites used 44 were found to be specific to the recurrent parent Aiswarya (Table 4) and the allele size of both donor and recurrent parent were estimated for each of the polymorphic primer (Table 5).

4.3. FOREGROUND SELECTION IN BC_2F_1 PLANTS FOR RESISTANCE GENES.

The harvested BC_1F_1 seeds of the crosses having 2/3 gene combination with more than 50% homozygousity to the recurrent parent were sown in nursery and 30 days old plants used for foreground selection. The genomic DNA of the BC_2F_1 plants was isolated using same protocol of Hipure mini prep plant genomic DNA isolation kit (MB 507). Quantitative and qualitative analysis were done by u-visible spectrophotometer and 0.8% gel electrophoresis. The results showed that the A260/A280 ratio ranged 1.8-2.00 were of high purity likewise the intact single band visualized in the Gel Documentation system also reveals the DNA. The number of accessions used in the study is shown in Table 6. A total of 149 plants were screened in the foreground selection. Details of the foreground selection in backcrossed population showing the lines pyramided with one gene and two gene combinations is given in Table 7.

Table 4. Details of 44 loci specific to the recurrent parent Aiswarya

Sl. No.	Loci	Chr. no.	Forward primer(5'-3')	Reverse primer(3'-5')	Reference
1	RM212	1	ccactttcagctactaccag	cacccatttgtctctcattatg	Chen et.al.,1997
2	RM493	1	tagctccaacaggatcgacc	gtacgtaaacgcggaaggtg	Temnykh t.al.,2001(a)
3	RM 312	1	gtatgcatatttgataagag	aagtcaccgagtttaccttc	Temnykh et.al.,2000
4	RM6	2	gtcccctccacccaattc	tcgtctactgttggctgcac	Panaud et.al.,1996
5	RM207	2	ccattcgtgagaagatctga	cacctcatcctcgtaacgcc	Chen et.al.,1997
6	RM110	2	tcgaagccatccaccaacgaag	tccgtacgccgacgaggtcgag	Temnykh et.al.,2000
7	RM300	2	ggttaggcatcgtcacgg	tcacctcaccacacgacacg	Temnykh et.al.,2000
8	RM450	2	aaaccacagtagtacgccgg	tcctagggtgaagaaagggg	Temnykh et.al.,2001
9	RM517	3	ggcttactggcttcgatttg	cgtctcctttggttagtgcc	Temnykh et.al.,2001
10	RM520	3	aggagcaagaaaagttcccc	gccaatgtgtacgcaatag	Temnykh et.al.,2001
11	RM261	4	ctacttctccccttgtgtcg	tgtaccatcgccaaatctcc	Chen et.al.,1997
12	RM267	5	tgcagacatagagaaggaagtg	agcaacagcacaacttgatg	Temnykh et.al.,2001
13	RM509	5	tagtgagggagtggaaacgg	atcgtccccacaatctcatc	Temnykh et.al.,2001
14	RM548	5	tcggtgagaaactgagagtacg	aaggaggccatctcaatgtg	Temnykh et.al.,2001
15	RM204	5	gtgactgacttggtcataggg	gctagccatgctctcgtacc	Chen et.al.,1997

Table 4. Contd.

16	RM225	6	tgcccatatggtctggatg	gaaagtggatcaggaaggc	Chen et.al.,1997
17	RM454	6	ctcaagcttagctgctgctg	*gtgatcagtgcaccatagcg*	Temnykh et.al.,2001
18	RM469	6	agctgaacaagccctgaaag	gacttgggcagtgtgacatg	Temnykh et.al.,2001
19	RM584	6	agaaagtggatcaggaaggc	gatcctgcaggtaaccacac	Temnykh et.al.,2001
20	RM190	6	ctttgtctatctcaagacac	ttgcagatgttcttcctgatg	Akagi et al.,1996
21	RM214	7	ctgatgatagaaacctcttctc	aagaacactgacttcacaa	Chen et al.,1997
22	RM420	7	ggacagaatgtgaagacagtcg	actaatccaccaacgcatcc	Temnykh et al.,2001
23	RM445	7	cgtaacatgcatatcacgcc	atatgccgatatgcgtagcc	Temnykh et al.,2001
24	RM172	7	tgcagctgcgccacagccatag	caaccacgacaccgccgtgttg	Temnykh et.al.,2000
25	RM478	7	cagctggggaagagagagag	tcagaaactaaacgcacccc	Temnykh et.al.,2001(a)
26	RM25	8	ggaaagaatgatcttttcatgg	ctaccatcaaaaccaatgttc	Chen et.al.,1997
27	RM223	8	gagtgagcttgggctgaaac	gaaggcaagtcttggcactg	Akagi et al.,1996
28	RM447	8	cccttgtgctgtctcctctc	acgggcttcttctccttctc	Temnykh et al.,2001
29	RM502	8	gcgatcgatggctacgac	acaacccaacaagaaggacg	Temnykh et al.,2001
30	RM285	8	ctgtgggcccaatatgtcac	ggcggtgacatggagaaag	Temnykh et.al.,2000
31	RM105	9	gtcgtcgacccatcggagccac	tggtcgaggtggggatcgggtc	Temnykh et.al.,2000
32	RM566	9	acccaactacgatcagctcg	ctccaggaacacgctctttc	Temnykh t.al.,2001(a)
33	RM 553	9	aactccacatgattccaccc	gagaaggtggttgcagaagc	Temnykh et.al.,2000

Table 4. Contd.

34	RM278	9	gtagtgagcctaacaataatc	tcaactcagcatctctgtcc	Temnykh et.al.,2000
35	RM257	9	cagttccgagcaagagtactc	ggatcggacgtggcatatg	Chen et al.,1997
36	RM271	10	tcagatctacaattccatcc	tcggtgagacctagagagcc	Temnykh et.al.,2000
37	RM 228	10	ctggccattagtccttgg	gcttgcggctctgcttac	Temnykh et.al.,2000
38	RM 216	10	gcatggccgatggtaaag	tgtataaaaccacacggcca	Temnykh et.al.,2000
39	RM484	10	tctccctcctcaccattgtc	tgctgccctctctctctc	Temnykh et al.,2001
40	RM4B	11	ttgacgaggtcagcactgac	agggtgtatccgactcatcg	Panaud et al.,1996
41	RM206	11	cccatgcgtttaactattct	cgttccatcgatccgtatgg	Chen et.al.,1997
42	RM277	12	cggtcaaatcatcacctgac	caaggcttgcaagggaag	Temnykh et.al.,2000
43	RM313	12	tgctacaagtgttcttcaggac	gctcacctttgtgttccac	Temnykh et.al.,2000
44	RM235	12	agaagctagggctaacgaac	tcacctggtcagcctctttc	Chen et.al.,1997

Table 5. Allele size of Samba mahsuri and Aiswarya for the 44 polymorphic SSR primers.

Sl. No.	Loci	Chr. no.	Allele size of Samba mahsuri (bp)	Allele size of Aiswarya (bp)	Distance between alleles (bp)
1	RM212	1	111	131	20
2	RM493	1	92	96	4
3	RM 312	1	204	220	4
4	RM6	2	123	126	3
5	RM207	2	136	132	4
6	RM110	2	136	142	6
7	RM300	2	123	127	4
8	RM450	2	117	137	20
9	RM517	3	259	281	22
10	RM520	3	268	264	4
11	RM261	4	129	122	7
12	RM267	5	153	155	2
13	RM509	5	140	142	2
14	RM548	5	107	105	2
15	RM204	5	268	270	2
16	RM225	6	110	104	6
17	RM454	6	121	139	18
18	RM469	6	270	273	3
19	RM584	6	91	98	9
20	RM190	6	157	176	19
21	RM214	7	158	164	6
22	RM420	7	112	131	19
23	RM445	7	181	185	4
24	RM172	7	257	253	4
25	RM478	7	205	211	6
26	RM25	8	154	156	2
27	RM223	8	103	116	13

Table 5. contd.

28	RM447	8	259	261	2
29	RM502	8	134	142	8
30	RM285	8	133	139	6
31	RM105	9	143	141	2
32	RM566	9	142	140	2
33	RM 553	9	158	160	2
34	RM278	9	117	108	9
35	RM257	9	258	264	6
36	RM271	10	98	100	2
37	RM 228	10	292	294	2
38	RM 216	10	148	104	44
39	RM484	10	128	140	12
40	RM4B	11	145	143	2
			154	152	2
41	RM206	11	107	138	21
42	RM277	12	118	120	2
43	RM313	12	110	114	4
44	RM235	12	128	98	30

Table 6. Details of total no. of accessions used in foreground selection

Sl. No.	Parental line	Accession no.
1.	ICAB133-17	1-29
2.	ICAB133-3	30-93
3.	ICAB-22-1/119	94-139
4.	ICAB133-21	140-189
5.	ICAB133-27	190-210
6.	ICAB160-5/115	211-219
7.	ICAB159-2	220-229
8.	ICAB159-2/152	230-259
9.	ICAB22-10/128	260-279
10.	ICAB133-16	280-302
11.	ICAB133-16/153	303-332
12.	ICAB101	501
13.	ICAB135-50	502-519
14.	ICAB168	520-564

4.3.1 Foreground selection for xa13 gene

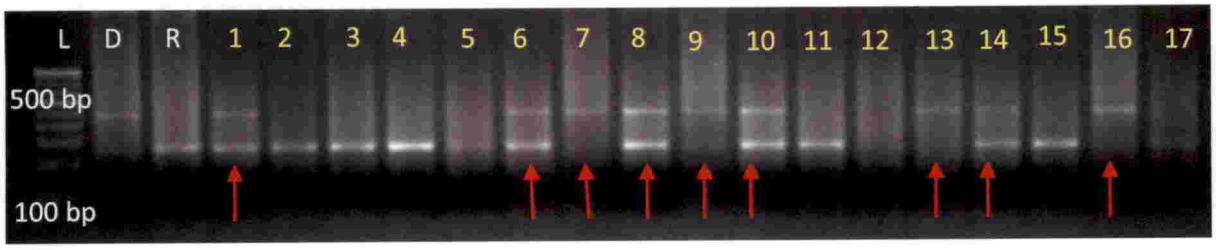
A total of 149 plants obtained were subjected to foreground selection with xa13 prom primer, gene specific primer for xa13 gene. Out of 149 plants 79 plants were found to possess xa13 gene. The allele for resistance has a size of 500 bp whereas the susceptible allele possesses a size of 250 bp. (Plate 2).

4.3.2 Foreground selection for Xa21 gene

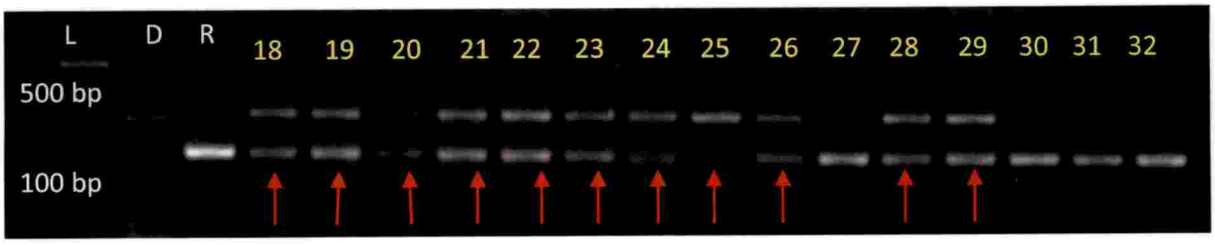
For the foreground selction of Xa21 gene, the popular gene specific primer used for detecting its presence pTA 248 was used. The resistant allele of the donor is found to be 1000 bp whereas the recipient parent possesses the susceptible allele at 650 bp. Out of 149 plants screened a total of 38 plants were

Plate 2: Foreground selection for xa13

Accession no: 1-17

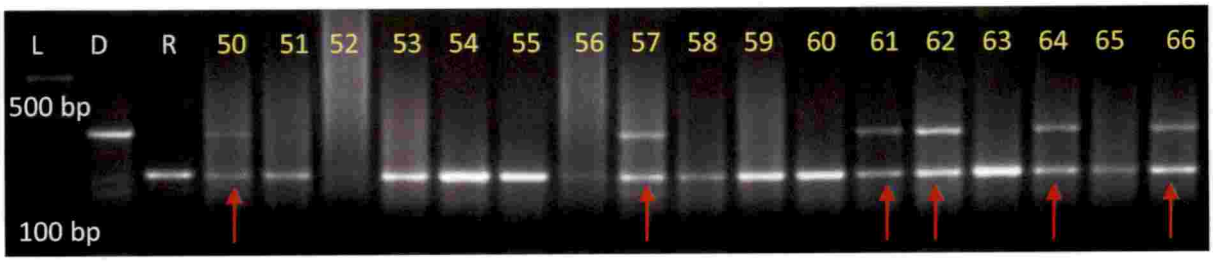


Accession no: 18-32



Accession no: 33-49

Accession no: 50-66

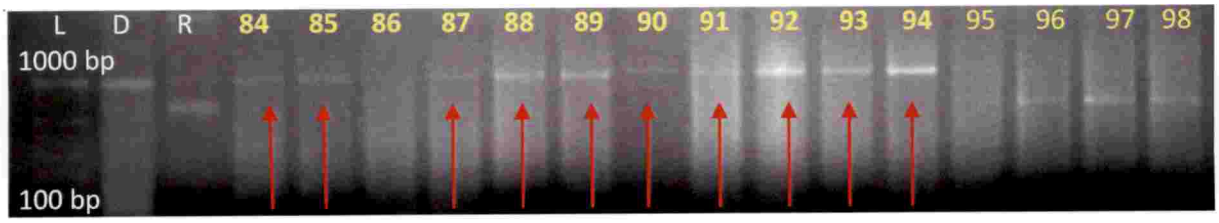


*Red arrow indicates the samples having xa13 gene

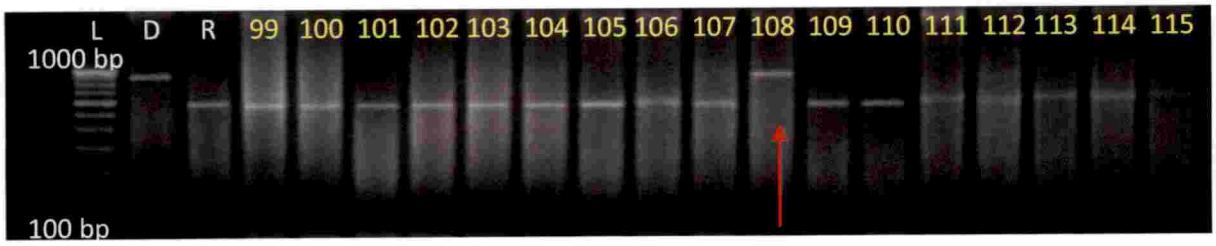
Plate 3. Contd.

Accession no: 67-83

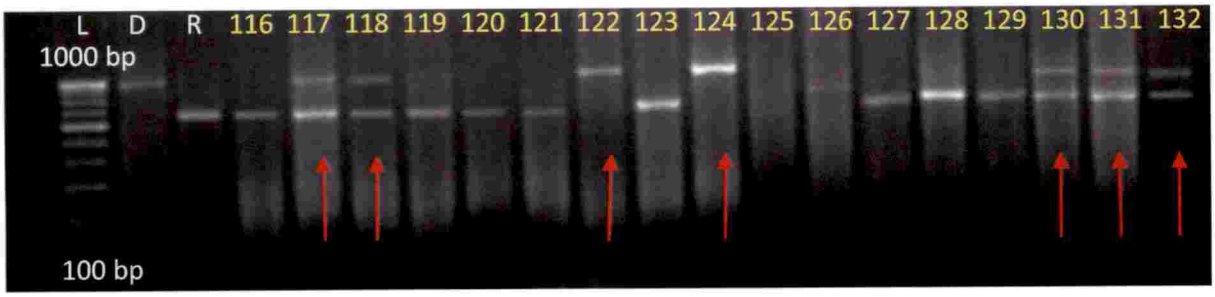
Accession no: 84-98



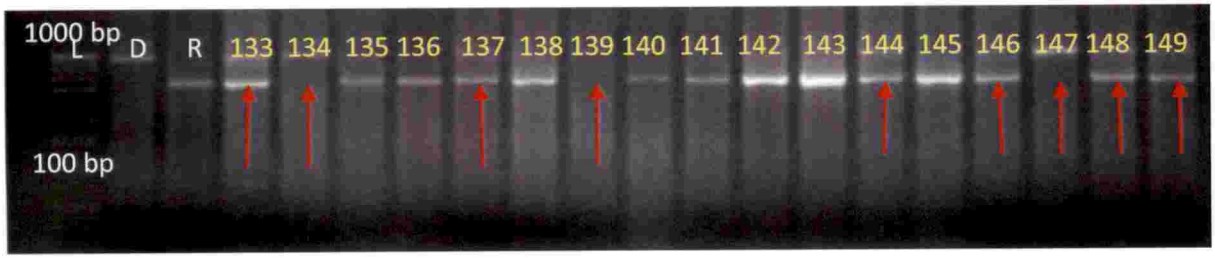
Accession no: 99-115



Accession no: 116-132



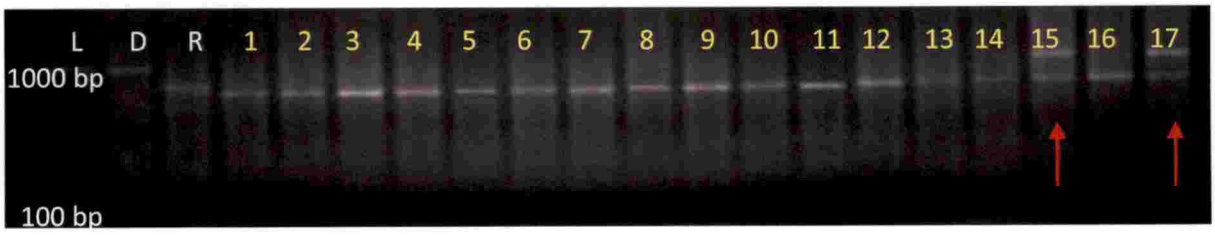
Accession no: 133-149



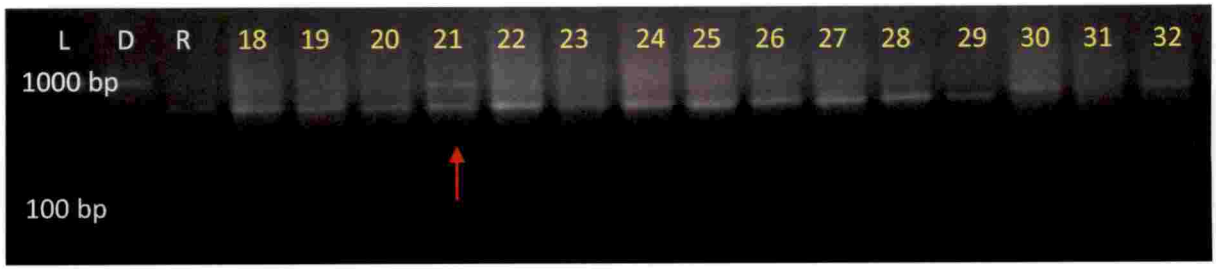
*Red arrow indicates the samples with Xa21 gene.

Plate 3: Foreground selection for Xa21 gene

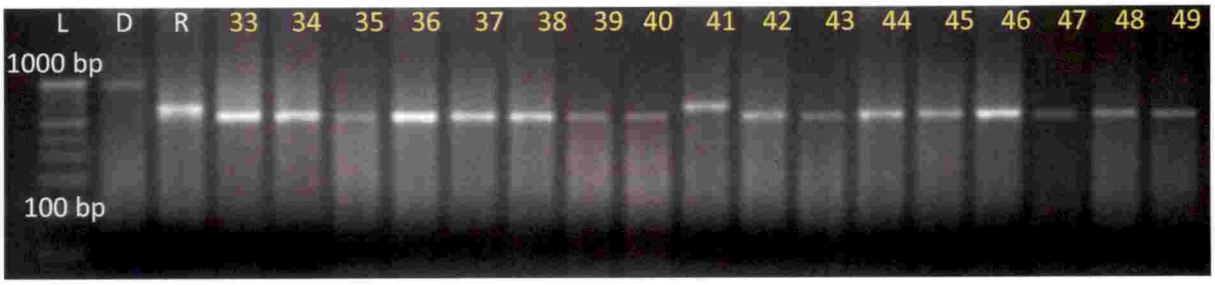
Accession no: 1-17



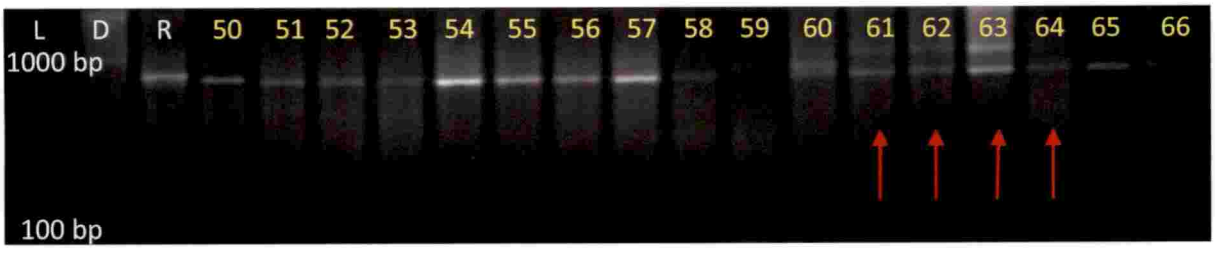
Accession no: 18-32



Accession no: 33-49



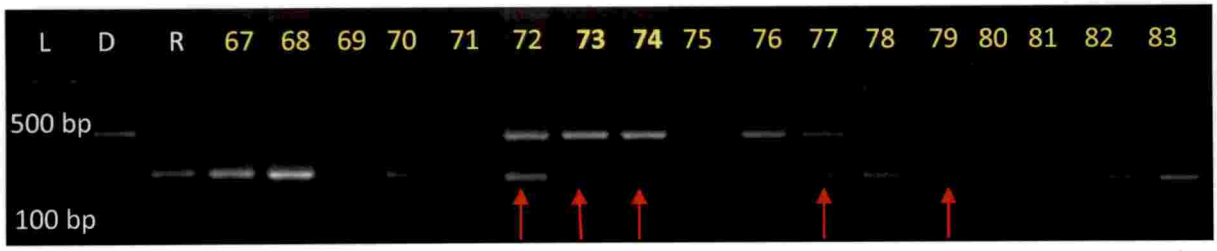
Accession no: 50-66



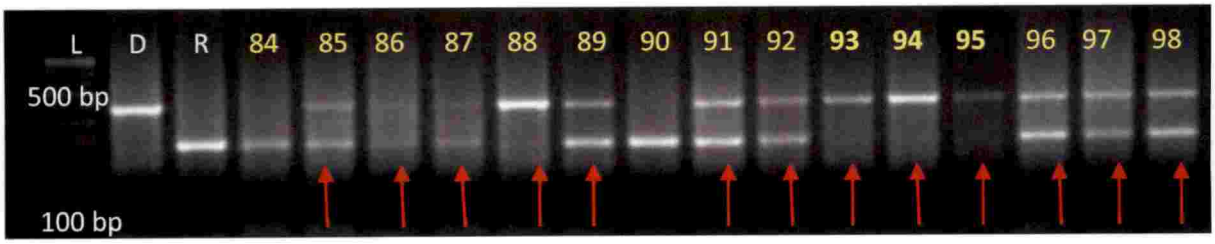
***Red arrow indicates the samples with Xa21 gene.**

Plate 2. Contd.

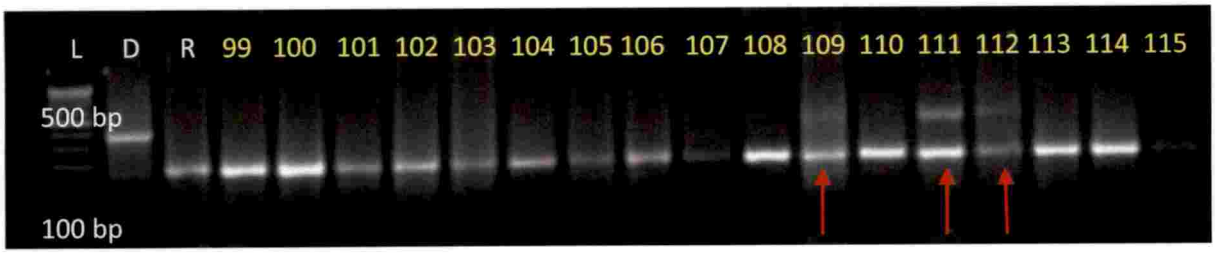
Accession no: 67-83



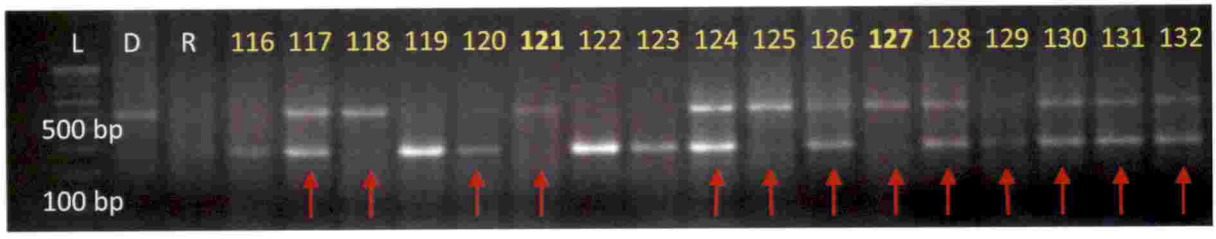
Accession no: 84-98



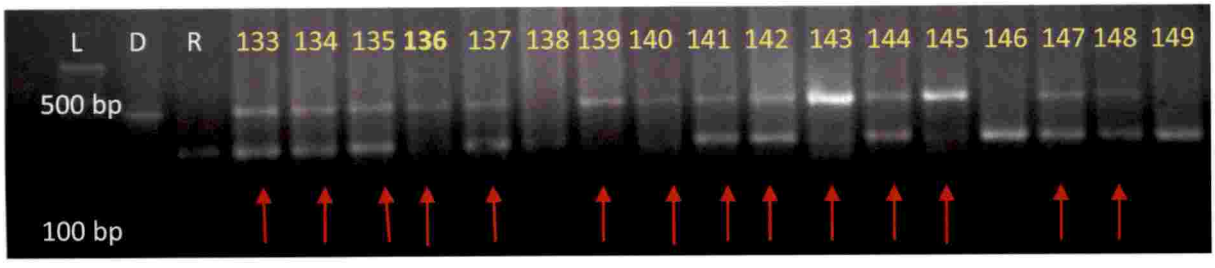
Accession no: 99-115



Accession no: 116-132



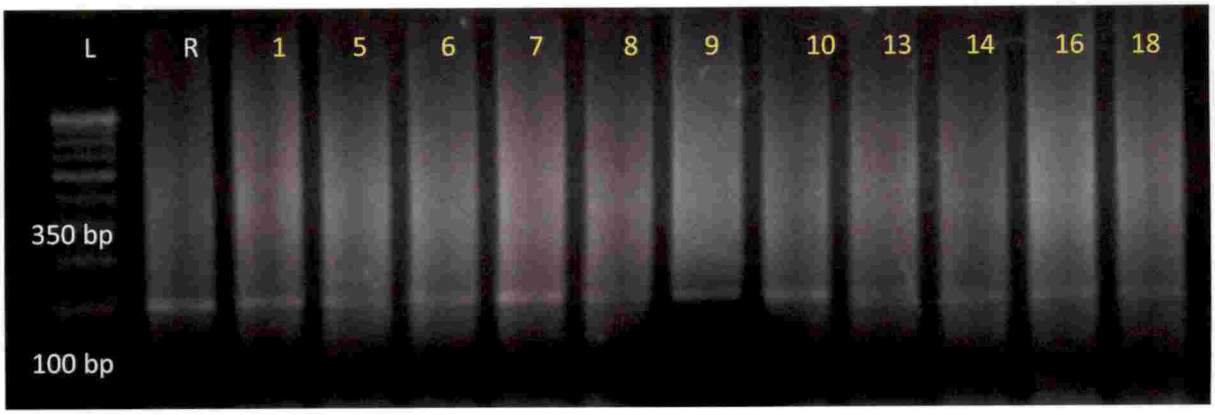
Accession no: 133-149



***Red arrow indicates the samples having xa13 gene**

Plate 4: Foreground selection for Xa33

Accessions with either xa13 or Xa21



Accessions with either xa13 or Xa21

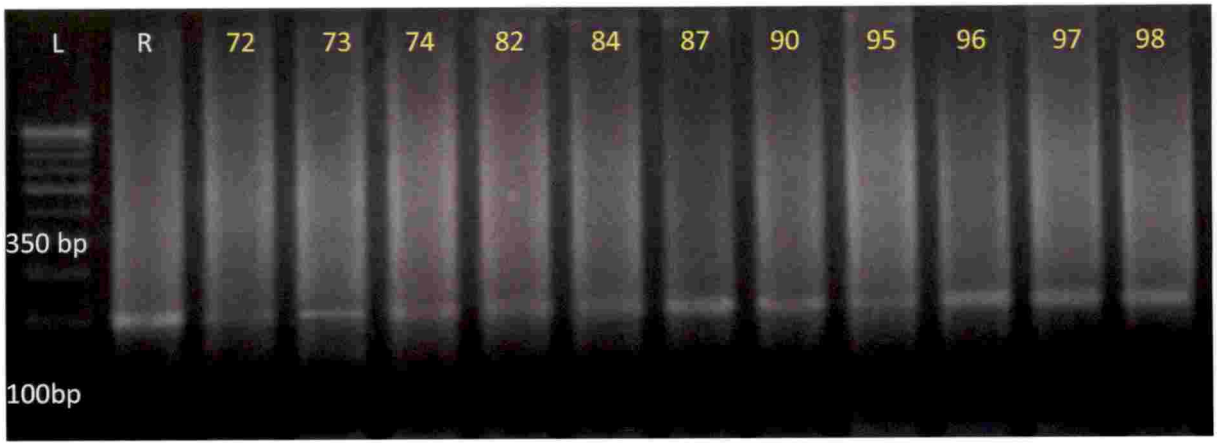
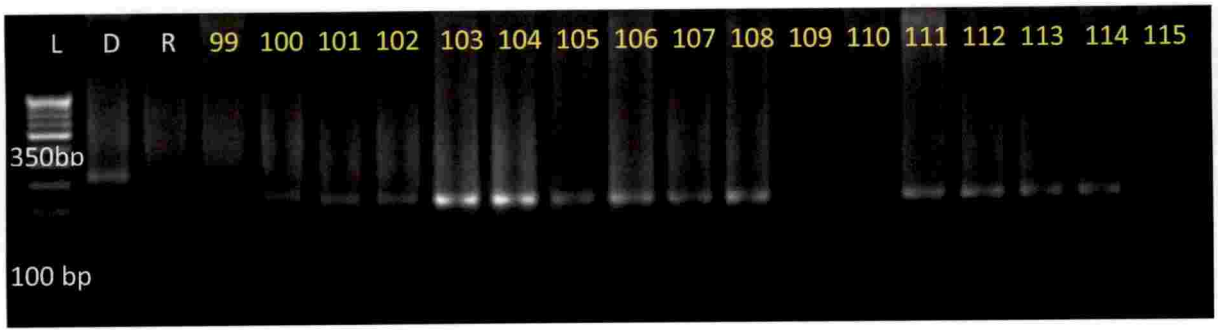
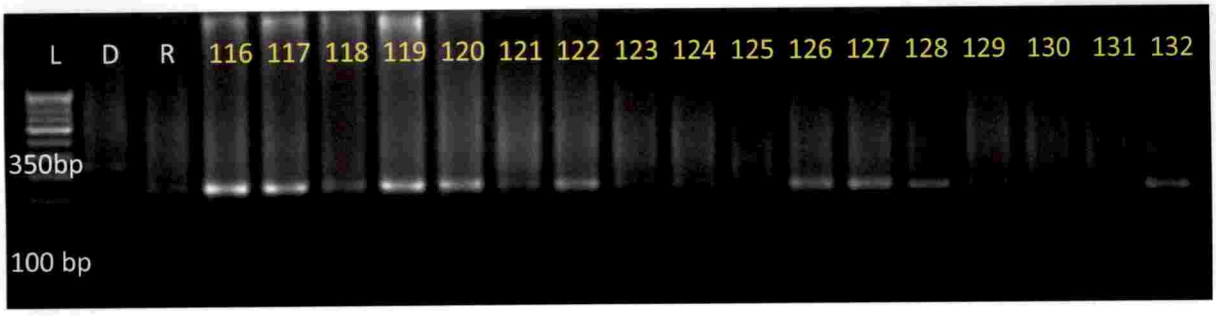


Plate 4. Contd.

Accession no: 99-115



Accession no: 116-132



Accession no: 133-149

found to possess one of the dominant resistance gene for BB used in the study (Plate 3).

4.3.3 Foreground selection for Xa33 gene

Foreground selection for Xa33 gene was carried out in all the 149 plants derived from the backcrossed progenies of Aiswarya. The gene specific marker for identifying this particular was RMW7.1. The PCR profile shows the allele size of the resistance gene is nearly 350 bp whereas the susceptible allele found in the recipient is nearly 250 bp. Foreground selection of Xa33 gene were carried out only in plants having either xa13 or Xa21 gene identified in the foreground selection. Further the seeds from parents possessing three gene combination (xa13, Xa21 and Xa33), 62 plants were raised and all these plants were screened for Xa33 (Plate 4).

Table 7. Details of Foreground selection in BC_2F_1 plants of Aiswarya showing the presence of genes for resistance

R gene	Total no. of plants having the gene for BB resistance
xa13	79
Xa21	38
xa13 and Xa21	23

4.4. BACKGROUND SCREENING FOR THE ASSESSMENT OF THE RECOVERY OF RECURRENT PARENT.

4.4.1. Screening of BC_2F_1 plants pyramided with 2/3 gene combination using 44 specific markers and scoring homozygous and heterozygous loci.

In foreground selection, 149 BC_2F_1 plants were screened for all the three genes for resistance to BB. And out of these, 23 plants were found to possess two gene combination of xa13 and Xa21 (Table 8). These plants were subjected to background selection.

In background selection method, for the assay of microsatellite loci, PCR was carried out with the respective polymorphic SSR primers. The resultant PCR product was then diluted using Hidi-Formamaide and ROX400 and then subjected to capillary electrophoresis. The allele size was computed by using the software GeneMapper. It compares the fragment peaks with internal size standard for calculates the allele size in base pairs. The GeneMapper provides Electropherograms to identify the clear peaks indicates the expected marker sizes (shutter peaks were ignored). The genotypic data of background selection gives information about heterozygous and homozygous loci present in all the progenies. The genotypic plot of homozygous and heterozygous loci can be visualized as in Fig.1 and 2 respectively. Details of genotyping of all the 23 backcrossed progenies pyramided with xa13 and Xa21 using 44 polymorphic SSR primers were given in Table 9. The 44 polymorphic markers were distributed in the entire genome (12 chromosomes).

In chromosome number 1, three polymorphic loci were screened viz., RM212, RM312, RM493 respectively and these markers are found to be homozygous in almost all samples. Chromosome no.2 was screened with five polymorphic microsatellites and out of these RM300 and RM450 are more homozygous in nature whereas RM 207 is highly heterozygous in nature. Chromosome number 3 was screened with two polymorphic microsatellites RM517 and RM520. Out of these two markers, RM 517 is heterozygous whereas RM520 is homozygous in all samples. RM261 was the only microsatellite used

Table 8. Details of BC_2F_1 lines having two-gene combination (xa13 and Xa21)

Sl. No.	Progeny no.	Sample no.	Accession no.
1	ICAB 22-1/119/17	110	21
2	ICAB 159-2/152/1	220	61
3	ICAB 159-2/152/2	221	62
4	ICAB 159-2/152/6	225	64
5	ICAB 159-2/152/8	227	66
6	ICAB 159-3/153/23	325	88
7	ICAB 159-3/153/26	328	90
8	ICAB 159-3/153/27	329	91
9	ICAB 159-3/153/28	330	92
10	ICAB 159-3/153/30	332	94
11	ICAB/168/10	529	116
12	ICAB/168/11	530	117
13	ICAB/168/12	531	118
14	ICAB/168/24	532	130
15	ICAB/168/25	543	131
16	ICAB/168/26	545	132
17	ICAB/168/27	546	133
18	ICAB/168/28	547	134
19	ICAB/168/31	550	137
20	ICAB/168/33	552	139
21	ICAB/168/38	557	144
22	ICAB/168/41	560	148
23	ICAB/168/42	571	149

Table 9: Genotyping of 23 backcrossed progenies pyramided with xa13 and Xa21 using 44 polymorphic SSR primers

Sl. No.	Loci*	Parents - Allele size		Allele size of BC2F1 Samples of Aiswarya corresponding to the alleles of parents (S* and A*)						
		S*	A*	529**	530**	531**	543**	544**	545**	546**
1	RM212 (1)	111	131	**131**	**131**	**131**	**131**	**131**	**131**	**131**
2	RM312 (1)	92	96	**96**	**96**	**96**	**96**	**96**	**96**	**96**
3	RM493(1)	204	220	**220**	**220**	**220**	**220**	**220**	**220**	**220**
4	RM300 (2)	123	126	**126**	**126**	**126**	**126**	**126**	**126**	**126**
5	RM450 (2)	136	132	**132**	**132**	**132**	**132**	**132**	**132**	**132**
6	RM 6(2)	136	142	**142**	**142**	**142**	**142**	**142**	**138 142**	**142**
7	RM207(2)	123	127	123 127	123	123	123 127	123	123	123
8	RM110(2)	117	137	117 137	**137**	117 137	117 137	117 137	117 137	137
9	RM517 (3)	259	281	259 281	259	259	259 281	259	258	259
10	RM520 (3)	268	264	**264**	**264**	**264**	**264**	264	264	264
11	RM261(4)	129	122	**122**	122 129	122 129	129	122	122	122
12	RM267 (5)	153	155	153 155	153	153 155	155	153	153	153
13	RM509 (5)	140	142	**142**	**142**	**142**	142	142	140 142	142
14	RM190(6)	107	105	**105**	107	107 105	107 105	107	107	107
15	RM548 (5)	268	270	**270**	**270**	**270**	270	270	268 270	270
16	RM204 (6)	110	104	**104**	**104**	**104**	104	104	104	104
17	RM225 (6)	121	139	**139**	**139**	**139**	139	139	139	139
18	RM454(6)	270	273	**273**	270	**273**	273	270 273	270 273	270 273
19	RM469 (6)	91	98	**98**	92 98	**98**	98	98	98	98
20	RM584 (6)	157	176	**176**	**176**	**176**	176	176	176	176

Table 9: contd.

21	RM172 (7)	158	164	158	164	158		158	164	158	164	158	164	158	164	164					
22	RM214 (7)	112	131	112	131	112		112		112	131	112		112		112	131				
23	RM420(7)	181	185	181	185	181	185	181	185	181	185	181	185	181	185	185					
24	RM445 (7)	257	253	**253**		**253**		**253**		**253**		**253**		**253**		**253**					
25	RM478(7)	205	211	**211**		**211**		**211**		**211**		**211**		**211**		**211**					
26	RM223 (8)	154	156	154		154		154		**156**		154		154		154					
27	RM447 (8)	103	116	103	116	103		103		103	116	103	116	103	116	103	116				
28	RM502 (8)	259	261	259	261	261		259		259	261	259	261	259	261	259	261				
29	RM25(8)	134	142	134	142	134		134	142	134	142	134		**142**		134					
30	RM285 (9)	133	139	**139**		133		133	139	**139**		133		**139**		133	139				
31	RM257(9)	143	141	141	143	143		**143**		**143**		141	143	**141**		**141**					
32	RM278(9)	142	140	**140**		**140**		**140**		**140**		**140**		**140**		**140**					
33	RM553 (9)	158	160	**160**		**160**		**160**		**160**		**160**		**160**		**160**					
34	RM105(9)	117	108	**108**		**108**		**108**		**108**		**108**		**108**		**108**					
35	RM566(9)	258	264	**264**		**264**		**264**		**264**		**264**		**264**		**264**					
36	RM271 (10)	98	100	98	100	100		98		98	100	98		98	100	98					
37	RM484 (10)	292	294	**294**		292		292		**294**		292		**294**		294					
38	RM228(10)	148	104	**104**		148		148	104	**104**		148	105	148	104	148	104				
39	RM216(10)	128	140	**140**		**140**		**140**		**140**		140		**140**		140					
40	RM4 B (11)	145	143	**143**		X		X		143		X		143		143					
		154	152	**152**		154	152	154	152	154	152	154	152	154	152	154					
41	RM206(11)	107	138	**138**		**138**		X		**138**		**138**		**138**		**138**					
42	RM277 (12)	118	120	118	120	118		118		118		118		120		118					
43	RM313 (12)	110	114	110	114	110		110		110	114	110		110	114	110					
44	RM235(12)	128	98	**98**		**98**		**98**		**98**		**98**		**98**		**98**					

S*=Samba mahsuri, A*=Aiswarya

** represents samples of BC_2F_1 plants of Aiswarya

Bolded digits shows the loci homozygous to Aiswarya

'X' – Missing values

Table 9. contd.

Sl. No.	Loci*	Parents - Allele size		Allele size of BC2F1 Samples of Aiswarya corresponding to the alleles of parents(S* and A*)						
		S*	A*	547**	550**	552**	557**	560**	561**	110**
1	RM212 (1)	111	131	**131**	**131**	**131**	**131**	**131**	**131**	**131**
2	RM312 (1)	92	96	**96**	**96**	**96**	**96**	**96**	92	92
3	RM493(1)	204	220	**220**	**220**	**220**	**220**	**220**	**220**	**204** **220**
4	RM300 (2)	122	126	**126**	**126**	122 126	**126**	**126**	126	122 220
5	RM450 (2)	136	132	**132**	**132**	136	**132**	**132**	132	136
6	RM 6(2)	138	142	**142**	**142**	138	**142**	138 142	138 142	138 142
7	RM207(2)	123	127	123	123 127	123 127	123	123	123	123 127
8	RM110(2)	117	137	**137**	**137**	117	117	117	117 137	117 137
9	RM517 (3)	259	281	259	259 281	259	259	258	258	281
10	RM520 (3)	268	264	**264**	**264**	**264**	**264**	**264**	**264**	**264**
11	RM261(4)	129	122	**122**	**122**	**122**	**129**	**122**	**122**	**122**
12	RM267 (5)	153	155	153	153 155	153 155	153	153	153	153 155
13	RM509 (5)	140	142	**142**	**142**	**140** **142**	**142**	**142**	**140** **142**	**142**
14	RM190(6)	107	105	107	107 105	107	107	107 105	107	105
15	RM548 (5)	268	270	268 270	268 270	268	268 270	268 270	268 270	268 270
16	RM204 (6)	110	104	**104**	**104**	**104**	**104**	**104**	**104**	**104**
17	RM225 (6)	121	139	**139**	**139**	**139**	**139**	**139**	**139**	**139**
18	RM454(6)	270	273	270 273	270 273	**273**	270	270 273	270 273	270 273
19	RM469 (6)	91	98	**98**	**98**	**98**	**98**	**98**	**98**	**98**
20	RM584 (6)	157	176	157 176	**176**	**176**	**176**	**176**	**176**	**176**
21	RM172 (7)	158	164	158	158 164	158 164	158 164	158 164	158 164	**164**
22	RM214 (7)	112	131	112	112	112 131	112 131	112	112	112
23	RM420(7)	181	185	181	181 185	**185**	185	181 185	**181** **185**	**185**
24	RM445 (7)	257	253	**253**	**253**	**253**	**253**	**253**	**253**	**253**

Table 9. contd.

25	RM478(7)	205	211	**211**		**211**		**211**		**211**		**211**		**211**		**205**	**211**		
26	RM223 (8)	154	156	154		154		**156**		**154**		154		**154**		**156**			
27	RM447 (8)	103	116	103	116	103	116	103	106	103	116	103	116	103	116	103	116		
28	RM502 (8)	259	261	259	261	259	261	259		259	261	259	261	259	261	261			
29	RM25(8)	134	142	134		**142**		134		134		134	142	142		134	142		
30	RM257(9)	143	141	143	141	143	141	143	141	**143**	**141**	143	141	143	141	141			
31	RM278(9)	142	140	**140**		**140**		142	140	**140**		**140**		**140**		**140**			
32	RM285 (9)	133	139	133	139	139		X		133		133	139	133		139			
33	RM553 (9)	158	160	**160**		**160**		160	158	**160**		**160**		**160**		**160**			
34	RM105(9)	117	108	**108**		**108**		117	**108**	**108**		**108**		**108**		**108**			
35	RM566(9)	258	264	**264**		**264**		258	**264**	**264**		**264**		**264**		**264**			
36	RM271 (10)	98	100	**100**		98	100	98	100	98	100	98		98	100	**100**			
37	RM484(10)	292	294	292		292		292		294		**294**		292		**294**			
38	RM228(10)	148	104	**104**		**104**		**104**		**104**		**104**		**104**		**104**			
39	RM216(10)	128	140	140		**140**		**140**		**140**		X		**140**		**140**			
40	RM4 B (11)	145	143	143		143		143		X		X		X		143			
		154	152	152		152		152	154	152	154	152	154	152	154	152	154		
41	RM206(11)	107	138	**138**		**138**		**138**		**138**		**138**		**138**		**138**			
42	RM277 (12)	118	120	118		118	120	118		118		118		118		**120**			
43	RM313 (12)	110	114	110		110	114	110		110		110		110		**114**			
44	RM235(12)	128	98	**98**		**98**		**128**	**98**	**98**		**98**		**98**		**128**	**98**		

S*=Samba mahsuri, A*=Aiswarya

** represents samples of BC_2F_1 plants of Aiswarya

Bolded digits shows the loci homozygous to Aiswarya

'X' – Missing values

Table 9.Contd.

Sl. No.	Loci*	Parents - Allele size		Allele size of BC2F1 Samples of Aiswarya corresponding to the alleles of parents(S* and A*)				
		S*	A*	220**	221**	225**	227**	325**
1	RM212 (1)	111	131	**131**	**131**	**131**	**131**	**131**
2	RM312 (1)	92	96	**96**	**96**	**96**	**96**	**96**
3	RM493(1)	204	220	**220**	**220**	**220**	**220**	**220**
4	RM300 (2)	123	126	**126**	**126**	**126**	**126**	**126**
5	RM450 (2)	136	132	136	**132**	136	136	136
6	RM 6(2)	136	142	**142**	**142**	**142**	138	**142**
7	RM207(2)	123	127	123 127	**127**	**127**	**127**	**127**
8	RM110(2)	117	137	137	**137**	**137**	117 137	----
9	RM517 (3)	258	281	281	258 281	258 281	258 281	**281**
10	RM520 (3)	268	264	264	**264**	**264**	**264**	**264**
11	RM261(4)	129	122	122 129	**122**	**122**	**122**	**122**
12	RM267 (5)	153	155	**155**	**155**	**155**	**155**	**155**
13	RM509 (5)	140	142	**142**	**142**	**142**	**142**	**142**
14	RM190(6)	107	105	105	107 105	107 105	107 105	107
15	RM548 (5)	268	270	**270**	**270**	**270**	**270**	**270**
16	RM204 (6)	110	104	**104**	**104**	**104**	**104**	110
17	RM225 (6)	121	139	**139**	**139**	**139**	**139**	121
18	RM454(6)	270	273	273	270 273	270 273	**273**	**273**
19	RM469 (6)	91	98	**98**	**98**	**98**	**98**	**98**
20	RM584 (6)	157	176	**176**	**176**	**176**	**176**	157
21	RM172 (7)	158	164	**164**	**164**	**164**	**164**	**164**
22	RM214 (7)	112	131	131	112 131	112 131	112 131	112 131
23	RM420(7)	181	185	**185**	**185**	**185**	**185**	**185**
24	RM445 (7)	257	253	**253**	**253**	**253**	**253**	**253**
25	RM478(7)	205	211	**211**	**211**	**211**	**211**	**211**

Table 9. contd.

26	RM223 (8)	154	156	**156**			**156**			**156**			**156**			154	156
27	RM447 (8)	103	116	103	116		103	116		103	116		103	116		103	
28	RM502 (8)	259	261	259	261		259	261		259	261		259	261		259	
29	RM25(8)	134	142	**142**			**142**			**142**			**142**			**142**	
30	RM257(9)	143	141	143	141		143	141		143	141		143	141		143	141
31	RM278(9)	142	140	142	140		142	140		142	140		142	140		142	140
32	RM285(9)	133	139	133	139		133	139		133	139		133	139		133	
33	RM553 (9)	158	160	160	158		160	158		160	158		**158**			160	158
34	RM105(9)	117	108	**108**			**108**			117	108		**108**			**108**	
35	RM566(9)	258	264	264			258	264		258	264		**264**			**264**	
36	RM271 (10)	98	100	**100**			**100**			**100**			**100**			98	100
37	RM484 (10)	292	294	**294**			**294**			**294**			292			292	
38	RM228(10)	148	104	**104**			**104**			**104**			**104**			**104**	
39	RM216(10)	128	140	**140**			**140**			**140**			**140**			**140**	
40	RM4 B (11)	145	143	143			143			143			143			143	
		154	152	292	294		292	294		292	294		292	294		292	294
41	RM206(11)	107	138	**138**			**138**			**138**			**138**			**X**	
42	RM277 (12)	118	120	118	120		118	120		118	120		118	120		118	
43	RM313 (12)	110	114	110	114		110	114		110	114		110	114		110	
44	RM235(12)	128	98	**98**			**98**			**98**			**98**			**98**	

S*=Samba mahsuri, A*=Aiswarya

** represents samples of BC_2F_1 plants of Aiswarya

Bolded digits shows the loci homozygous to Aiswarya

'X' – Missing values

Table 9. Contd.

Sl. No.	Loci*	Parents - Allele size		Allele size of BC2F1 Samples of Aiswarya corresponding to the alleles of parents(S* and A*)			
		S*	A*	328**	329**	330**	332**
1	RM212 (1)	111	131	**131**	**131**	**131**	**131**
2	RM312 (1)	92	96	**96**	**96**	**96**	**96**
3	RM493(1)	204	220	**220**	**220**	**220**	**220**
4	RM300 (2)	123	126	**126**	**126**	**126**	**126**
5	RM450 (2)	136	132	**132**	136	**132**	**132**
6	RM 6(2)	136	142	138 142	**142**	**142**	**142**
7	RM207(2)	123	127	**127**	123	123	123
8	RM110(2)	117	137	**137**	**137**	**137**	**137**
9	RM517 (3)	258	281	**281**	**281**	**281**	**281**
10	RM520 (3)	268	264	**264**	**264**	**264**	**264**
11	RM261(4)	129	122	**122**	**122**	**122**	**122**
12	RM267 (5)	153	155	**155**	X	155	153 155
13	RM509 (5)	140	142	**142**	**142**	**142**	**142**
14	RM190(6)	107	105	X	107 105	X	107 105
15	RM548 (5)	268	270	**270**	**270**	268	268
16	RM204 (6)	110	104	110 104	110	110	110 104
17	RM225 (6)	121	139	121 139	121	121 139	121
18	RM454(6)	270	273	**273**	**273**	**273**	**273**
19	RM469 (6)	91	98	**98**	**98**	**98**	**98**
20	RM584 (6)	157	176	157 176	157 176	157 176	157
21	RM172 (7)	158	164	**164**	**164**	**164**	**164**
22	RM214 (7)	112	131	**131**	**131**	**131**	131
23	RM420(7)	181	185	X	**185**	X	**185**
24	RM445 (7)	257	253	X	**253**	X	**253**

Table 9. Contd.

25	RM478(7)	205	211	**211**		**211**		**211**		**211**			
26	RM223 (8)	154	156	154	156	154	156	154	156	156			
27		103	116	103		103	116	103	116	103	116		
28	RM502 (8)	259	261	259		259	261	259	261	X			
29	RM25(8)	134	142	**142**		**142**		X		**142**			
30	RM257(9)	143	141	X		**141**		X		143	141		
31	RM278(9)	142	**140**	**140**		142	140	X		142	140		
32	RM285(9)	133	139	133		133		133		133			
33	RM553 (9)	158	160	160		160		160		160	158		
34	RM105(9)	117	108	**108**		**108**		**108**		**108**			
35	RM566(9)	258	264	258	264	**264**		**264**		**264**			
36	RM271 (10)	98	100	98	100	98	100	**100**		**100**			
37	RM484 (10)	292	294	292		292		292		X			
38	RM228(10)	148	104	**104**		**104**		**104**		**104**			
39	RM216(10)	128	140	**140**		**140**		**140**		**140**			
40	RM4 B (11)	145	143	145		143		X		X			
		154	152	292	294	292	294	292	294	X			
41	RM206(11)	107	138	X		X		X		X			
42	RM277 (12)	118	120	X		118		118		118			
43	RM313 (12)	110	114	110		110		110		110			
44	RM235(12)	128	98	**98**		**98**		**98**		**98**			

S*=Samba mahsuri, A*=Aiswarya

** represents samples of BC_2F_1 plants of Aiswarya

Bolded digits shows the loci homozygous to Aiswarya

'X' – Missing values

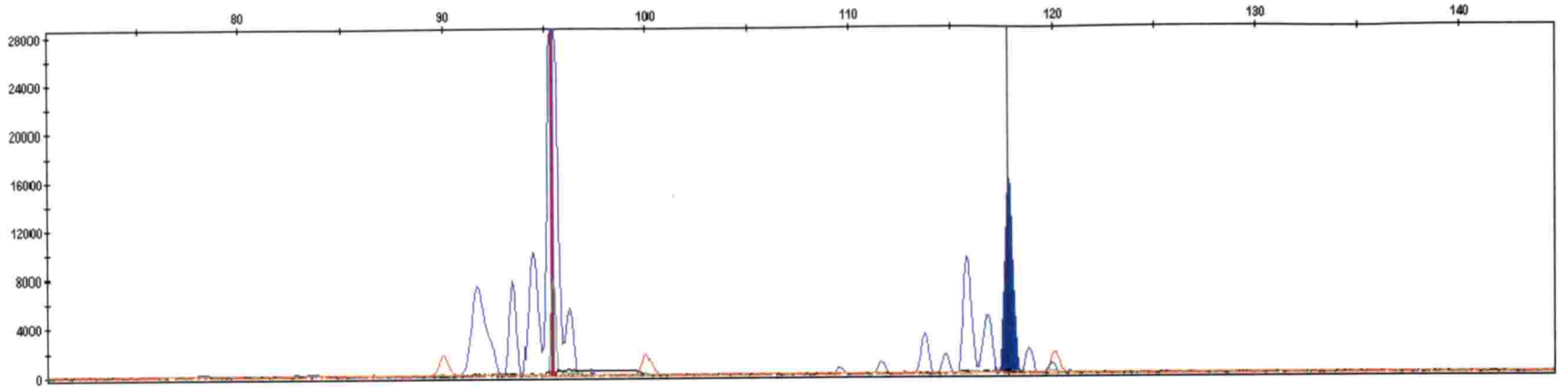


Fig. 1. Genotypic plot obtained in GeneMapper software for Heterozygous loci.
Pink peak represents allele size of donor parent and blue peak-denotes allele size of the recurrent parent

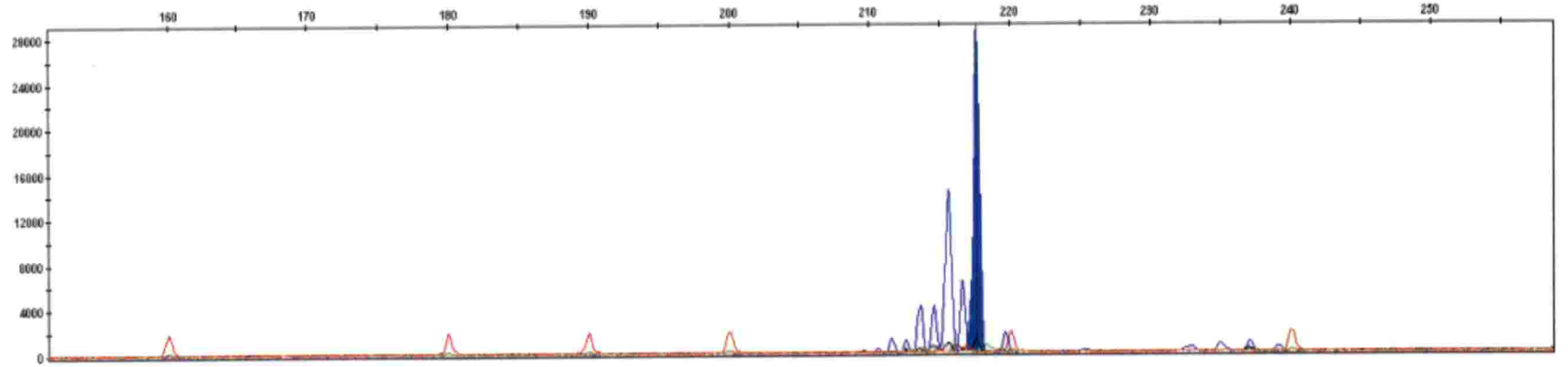


Fig. 2. Genotypic plot obtained in GeneMapper software for Homozygous loci.
Clear Blue peak-denotes allele size of the recurrent parent

for screening in chromosome number 4 and it is found to be heterozygous in four samples viz., 530,531,543 and 220. In chromosome number 5, three polymorphic loci were screened, out of these RM548 was found to be highly homozygous when compared with other loci. Chromosome number 6 was screened with six polymorphic markers and out of these RM454 shows heterozygous nature in samples 531, 544, 546, 547, 550, 560, 561 and 110, whereas RM 190 is heterozygous in sample no.s 550, 560, 543, 545, 221, 225, 227. Chromosome number 7 was screened with 5 microsatellite markers and out of these RM445 and RM478 was found to be homozygous in all the 23 samples screened. In chromosome no.8, the polymorphic microsatellite markers RM447, RM502 and RM25 were showing heterozygousity with the recurrent parent in more than twenty samples. Chromosome no.9, out of six loci screened RM278, RM553, RM105, RM566 are showing homozygousity with recurrent parent in more than 10 samples. While in chromosome number 10, four polymorphic primers were used and the results shows that RM 216 is showing homozygousity with the recurrent parent in all the samples screened while other three primers were found to be homozygous and heterozygous in different samples screened. In case of chromosome number 11, only two polymorphic loci were screened, in which RM206 is found to be homozygous in all samples while RM4B was showing both homozygous and heterozygous nature in all the screened samples. Finally in chromosome number 12, three polymorphic primers were used to screen all the 23 samples viz., RM 277, RM313 and RM235. Out of these only RM235 was showing homozygousity with the recurrent parent while the other two were heterozygous in nature in the screened samples. The graphical representation of total number of homozygous and heterozygous loci for all the 23 samples taken for background study were given in Fig 3&4.

4.4.2. Estimation of percentage recovery of Aiswarya (Recurrent parent)

Twenty three plants having two gene combinations (xa13+Xa21) were selected for assessing the recovery of recurrent parent genome. The 44 microsatellite markers which are found to be specific to the recurrent parent were

Table 10. Number of microsatellite loci tested and the percentage recovery of recurrent parent in the Backcross program

Sl. No	Progeny no.	Sample no.	Total no. of loci	No. of loci Homozygous with Recurrent parent	No. of loci Heterozygous with Recurrent parent	% Recovery of recurrent parent
1	ICAB 22-1/119/17	221	44	30	14	84.09
2	ICAB 159-2/152/1	529	44	29	14	81.81
3	ICAB 159-2/152/2	545	44	29	14	81. 81
4	ICAB 159-2/152/6	220	44	30	11	80.68
5	ICAB 159-2/152/8	543	444	28	15	80.68
6	ICAB 159-3/153/23	227	44	29	12	79.54
7	ICAB 159-3/153/26	225	44	27	16	79.54
8	ICAB 159-3/153/27	550	44	26	14	75
9	ICAB 159-3/153/28	110	44	26	13	73.86
10	ICAB 159-3/153/30	328	38	24	8	73.68
11	ICAB/168/10	546	44	29	6	72.70
12	ICAB/168/11	329	42	25	8	69.04
13	ICAB/168/12	530	44	23	15	69.3
14	ICAB/168/24	332	40	23	8	67.5
15	ICAB/168/25	547	44	26	6	65.9

Table 10. Contd.

16	ICAB/168/26	325	44	23	7	60.22
17	ICAB/168/27	330	37	20	7	63.5
18	ICAB/168/28	544	44	23	9	62.5
19	ICAB/168/31	557	44	23	8	61.3
20	ICAB/168/33	561	44	21	12	61.36
21	ICAB/168/38	531	43	21	10	60.4
22	ICAB/168/41	560	43	21	10	60.4
23	ICAB/168/42	552	43	17	16	58.13

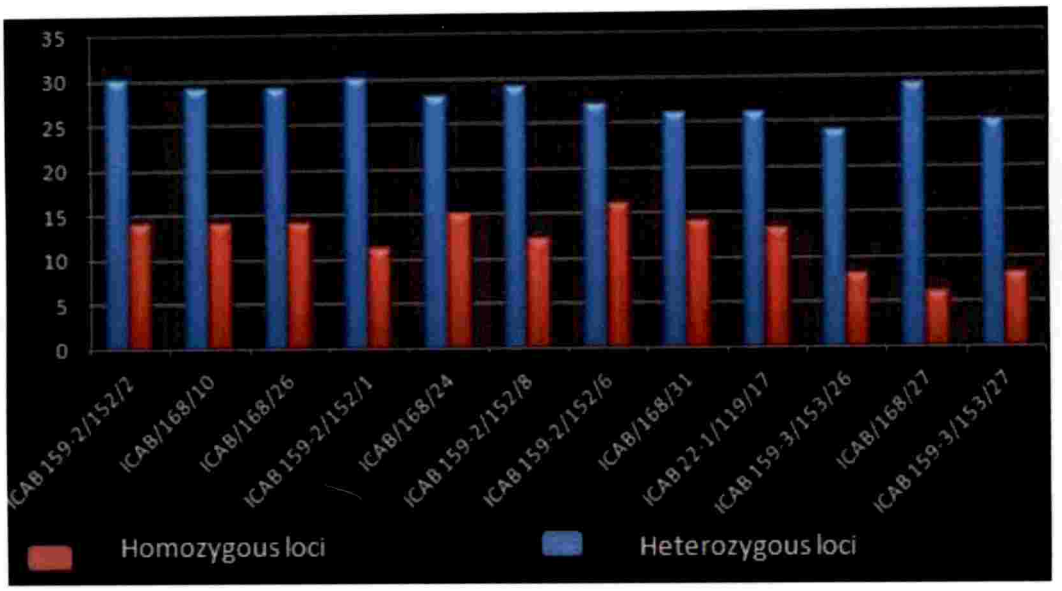


Fig.3.Graphical representation of homozygous and heterozygous loci in plants having two gene combination (ICAB 159-2/152/2 to ICAB 159-3/153/27)

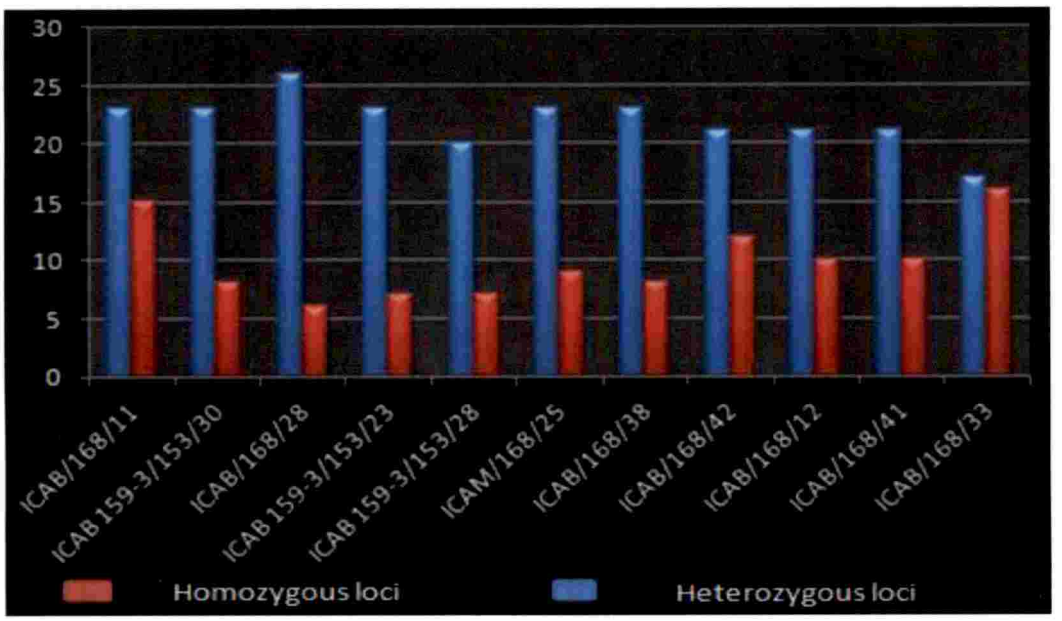


Fig.4. Graphical representation of homozygous and heterozygous loci in plants having two gene combination (ICAB 168/11 to ICAB 168/33).

used for screening the plants with two-gene combinations. The results of background selection showed the number of markers which shows homozygousity and heterozygousity for the recurrent parent allele. The genomic contribution of the parents in the BC_2F_1 genotypes based on SSR marker data analysis were evaluated by using the formula.

$G = [(X + 1/2Y) \times 100]/N$ (Sundaram *et al.*, 2012).

Where,

N = total number of parental polymorphic markers screened

X = number of markers found homozygous to recurrent parent allele

Y = number of markers found heterozygous for recurrent parent allele

The percentage recovery of recurrent parent genome (Aiswarya) assessed in 23 plants selected in foreground selection from BC_2F_1 lines ranged from 58.13% in ICAB/168/42 to 84.09% in ICAB 22-1/119/17. Five lines ICAB 159-2/152/2, ICAB/168/10, ICAB/168/26, ICAB 159-2/152/1, and ICAB/168/24 were found to have recovery of RPG 84.9%, 81.81%, 81.81%, 80.68%, 80.68% respectively. (Table10). Graphical representation of % recovery of recurrent parent genome in the sdecreasing order is shown in Fig.5.

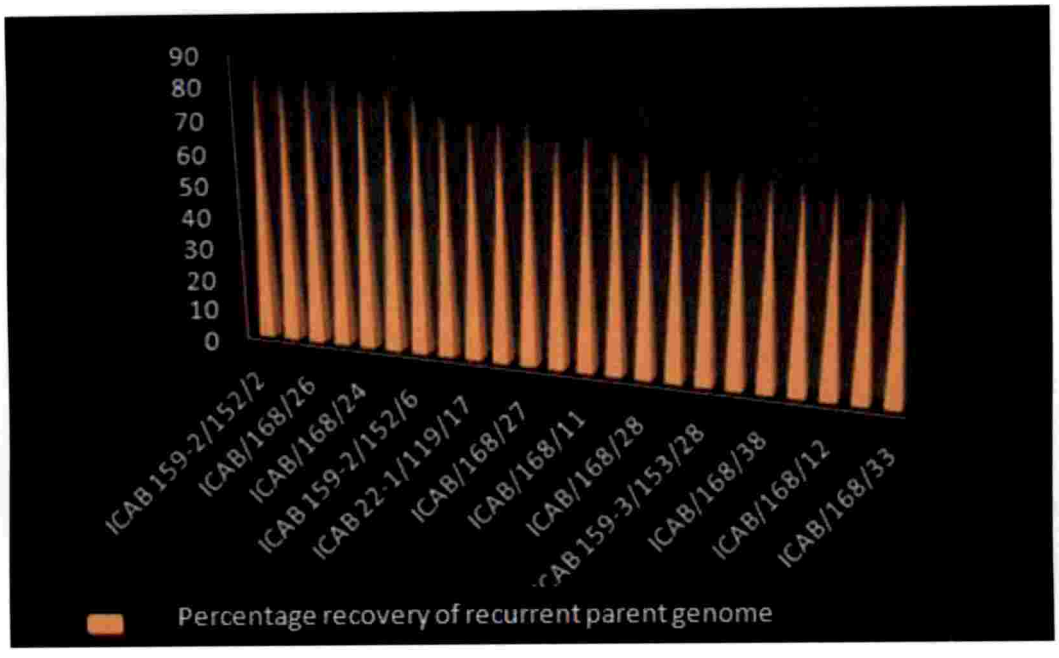


Fig.5. Graph showing % recovery of recurrent Parent genome in decreasing order.

DISCUSSION

5. DISCUSSION

Bacterial leaf blight disease caused by the bacterium *Xanthomonas oryzae pv. Oryzae (Xoo)* is a major constraint amongst rice diseases all over the world. Breeding for developing host plant resistance is the most efficient strategy for its management (Sanchez *et al.*, 2000), as there is no effective chemical control measures are available (but that may often leads to health hazards and environmental pollution). Conventional plant breeding methods are not efficient for pyramiding multiple genes for resistance as it is time consuming and is mainly based on phenotypic selection, which also shows dominance and epistasis effect (Collard and Mackill, 2008) and linkage drag (Young and Tanksley, 1989). With the advancement in biotechnology plant breeding can be performed efficiently and precisely by using molecular markers, which were proved to be novel tools for the selection of resistant varieties even in the absence of pathogens and to reduce the chance of pathogens to overcome resistance due to mutation (Huang *et al.*, 2003). Nearly 40 genes imparting resistance to BB have been identified till now (Basavaraj *et al.*, 2010). When compared to one, two and three gene combinations, multiple resistance genes provide broad spectrum and durable resistance by their synergistic and complementary gene action to a wide range of races (Ogawa and Khush, 1988).

Marker assisted backcrossing (MABC) is one of the most promising approaches that employs molecular markers to identify and select the target genes. Therefore, MABC can be used to develop resistant or high-yielding or quality rice varieties by incorporating a specific gene of interest into an elite variety which is popular among the farmers. On comparing with conventional breeding strategies, MABC is an efficient tool in which even in a large population for the backcross F_1 generations, by using molecular markers we will be able to recover the recurrent parent genotype within two or three backcrosses.

The cv. Aiswarya is a semi dwarf, red kernelled short duration variety of Kerala with productivity 5-5.5t/ha. It is mostly preferred by farmers of Palakkad and other districts of Kerala, but due to its susceptiblity to Bacterial leaf Blight (BB) disease there occurs a greater yield loss. Hence there is a requisite to develop cultivars pyramided with two/ more genes conferring resistance to BB in the background of Aiswarya.

Main aim of the present study entitled "Genome wide marker assay for the recovery of recurrent parent genome in rice (Oryza sativa)" is to assess the percentage introgression of BC_2F_1 plants pyramided with two/ three gene combination. The results of the study presented in chapter 4 are discussed below.

5.1. VALIDATION OF PARENTAL LINES WITH GENE SPECIFIC MARKERS

The parental lines used in the study were Improved Samba mahsuri for xa13 and Xa 21 gene and Samba mahsuri Xa33 gene, these pyramided lines were developed at Indian Institute of Rice Research, Hyderabad. Aiswarya (PTB 52), an elite variety released by RARS pattambi, KAU was taken as the recurrent parent.

Validation for the BB resistance genes xa13, Xa21 and Xa33 were done by using gene specific primers xa13 pro, pTA248 and RMW 7.1 respectively. The xa13 gene was validated by using xa13 prom, a functional SSR primer. The donor parent Samba mahsuri (BPT5204) showed the presence of the resistant allele at 500 bp in the amplification profile with xa13 pro primer. The results showed that an allele of size 1000 bp was amplified by pTA248 primer. Also RMW7.1 primer amplified an allele of about 350 bp in the donor, Samba mahsuri (Fbr-1-1). In the amplification profile resistant alleles were absent in the recipient variety Aiswarya confers the absence of these genes in the cv. Aiswarya. Therefore these three markers were confirmed to use in the study.

5.2. SCREENING OF AISWARYA AND SAMBA MAHSURI WITH SSR MARKERS TO LOCATE AISWARYA SPECIFIC MARKERS

A total of 320 SSR markers were used to identify markers showing polymorphism to Aiswarya. Out of these 320 SSR markers used, 44 markers were found to be specific to Aiswarya were distributed among all the 12 chromosomes covering the entire genome. These markers were selected from www.gramene.org in order to screen the BC_2F_1 plants pyramided with two/three gene combination. The chromosome wise distribution of loci chosen is as follows: 29 loci in chromosome 1, 25 loci in chromosome 2, 25 loci in chromosome 3, 25 loci in chromosome 4, 28 loci in chromosome 5, 41 loci in chromosome 6, 33 loci in chromosome 7, 21 loci in chromosome 8, 27 loci in chromosome 9, 17 loci in chromosome 10, 23 loci in chromosome 10, 20 loci chromosome 12.

. Chromosome wise distribution of polymorphic loci specific to Aiswarya are as follows: 3 loci in chromosome 1, 5 loci in chromosome 2, 2 loci in chromosome 3, 1 loci in chromosome 4, 4 loci in chromosome 5, 5 loci in chromosome 6, 5 loci in chromosome 7, 5 loci in chromosome 8, 5 loci in chromosome 9, 4 loci in chromosome 10, 2 loci in chromosome 11, 3 loci in chromosome 12.

Arunakumari *et al.* (2016) used 617 SSR markers to carry out parental polymorphism survey in the parental lines. Improved Samba Mahsuri (possessing Xa21 and xa13) and NLR145 (possessing Pi54) were donor parents and MTU1010 (recurrent parent). Here, out of 617 markers, 82 markers showed polymorphism between MTU1010 (recurrent parent) and ISM, while 83 markers showed polymorphism between MTU1010 and NLR145.

In a similar study conducted by Yugander *et al.* (2018) introgressed xa38 from Breeder line PR 114 to Improved Samba Mahsuri (ISM) an fine grain type

BB-resistant rice variety with three BB-resistant genes (Xa21, xa13 & xa5) has used 82 polymorphic SSR markers to identify maximum recovery of recurrent parent in BCF_1s.

5.3. FOREGROUND SELECTION IN BC_2F_1 PLANTS TO FIND PLANTS WITH 2/3 GENE COMBINATION

Foreground selection is an essential method in MABC in order to find the presence of respective genes for resistance in the pyramided lines using gene specific markers. In the previous work, done the department of Plant Breeding and Genetics as a part of the same project of DBT, cv. Aiswarya was pyramided with three genes for resistance to Bacterial Blight xa13, Xa21, and Xa33 has been developed. Foreground selection was carried out in all the 149 BC_2F_1 plants raised from BC_1F_1 seeds showing more than 50% homozygousity. Gene specific markers xa13 pro, pTA248 and RMW 7.1 were used identify the three genes for resistance used in the pyramiding programme *viz.,* xa13, Xa21 and Xa33 respectively. The results showed that xa13 gene was present in 79 plants and Xa21 was present in 38 plants. Also 23 plants were found to have two gene combinations of xa13 and Xa21.

Magar *et al.* (2014) transferred BB resistant genes viz., xa13 & Xa21 from B95-1 to MTU1010 susceptible to Bacterial blight disease. Here the foreground selection was done using gene specific markers viz., xa13 pro and pTA 248 and the genetic analysis of F2 populations revealed that the genes (xa13 & Xa21)followed Mendelian inheritance.

Another dominant gene for BB resistance used in the study, Xa33 was absent in all the screened plants. Joshi et al. (2008) reported that there is a chance for losing pyramided genes, where the donors of the resistance genes were two different plants. In the present study we have used three genes for resistance to BB i.e., donors used were Improved Samba mahsuri for xa13 and 21 and Samba

mahsuri for Xa33. This might be a reason for the absence of Xa33 gene in the screened plants.

5.4. BACKGROUND SCREENING FOR THE ASSESSMENT OF THE RECOVERY OF RECURRENT PARENT.

Backcrossing is paramount in all the marker assisted backcross breeding programmes, since it involves the introgression of a target gene from donor parent to recipient. The main objective of backcrossing is to reduce the donor parent genome content into the progenies and essentially to recover the recurrent parent genome within two/three backcrosses where as conventional backcrossing requires six backcrosses for obtaining maximum recovery of RPG (Hospital *et al.*, 2005).

By six backcrosses, the expected recurrent parent (RP) genome recovery would be 99.2% which is most similar to the improved variety. The proportion of the RP genome is recovered at a rate of $1- (1/2)^{t+1}$ for each of the generations of backcrossing (Babu *et al.*, 2004). But in some specific backcross progeny (BC_3 or BC_2) this proportion may change during crossing over which is not possible to detect phenotypically.

The results of foreground selection showed that in the twenty three lines of BC_2F_1 possessed xa13 and Xa21. And recovery of genomic background of Aiswarya was assessed in these twenty three lines. The 44 microsatellite markers which are found to be polymorphic in the initial screening were used for the background study. Electropherograms after capillary electrophoresis shows clear peaks represents the allele size of respective samples when compared with the allele size of the parents for each polymorphic marker used. The results indicated that the maximum recovery of RPG ranged from 84.09% in ICAB 159-2/152/2 and 53.13% in ICAB/168/33. Since we have taken plants from the second backcrossed progeny it is expected to show RPG of more than 80%. Here five lines were found to have recovery of RPG more than 80% i.e., ICAB 159-2/152/2 - 81.81%, ICAB/168/10 - 84.9%, ICAB/168/26 - 81.81%, ICAB 159-2/152/1- 80.68%, and ICAB/168/24 - 80.68%.

Theoretically, the expected recovery of recurrent parent genome in second backcrossed population should be 87.50%. In a similar study conducted by Sundaram *et al.*, (2008) outlined that less than expected genomic contribution of the recurrent parent successive backcrossed populations might be due an unknown "pull" mechanisms that contributes to the inheritance of additional unlinked loci from the donor genome.

Also it is pertinent to note that the present study, background selection has performed with less number of microsatellite markers (44 no.s), probably that couldn't yield much recovery of percentage reconstitution of recurrent parent genome.

Gopalakrishnan *et al.* (2008) reported that Pusa 1460-01-32-6-7-67 (possessing xa13 & Xa21), the recurrent parent genome recovery was assessed in the BC_1F_5 generation by using 69 polymorphic SSR markers and found to possess 86.9% recovery of Pusa Basmati-1 genome. In another study conducted by Sundaram et al. (2016) found more than 90% recovery of MTU1010 genome (RP) in the second backcross generation, by deploying a maximum 109 parental polymorphic SSR markers.

The BC_2F_1 plants identified with xa13 and Xa21 with genome recovery of more than 80% can be proceeded to BC_2F_2 generation for further screening to develop Essentially Derived Variety.

SUMMARY

6. SUMMARY

Rice, being principle food crop all over the world, but its production is limited majorly by various biotic factors such as bacterial, fungal and viral diseases. Bacterial blight (BB) of rice, is one among major destructive disease – caused by *Xanthomonas oryzae pv. oryzae (Xoo)*. BB is a vascular disease resulting in a systemic infection (Mew *et al.*, 1987) that results in the production of tannish-grey to white lesions along the veins. Studies have revealed that breeding with single major genes for resistance, may be ineffective due to on pathogen variation which results in resistance breakdown. Thus, pyramiding of more than one gene for resistance to BB appears to be effective for managing the disease (Gnamanickam *et al.*, 1999).

Marker assisted backcross breeding program can be effectively employed for pyramiding two/more genes coding for BB resistance to susceptible varieties to develop durable resistance. Also it is most widely accepted strategy among plant breeders as it takes lees time frame to develop and release a variety by omitting field level screening procedures. In the present investigation, BC_2F_1 plants pyramided with two gene combinations were identified and estimated the percentage recovery of the recurrent parent.

Validation for the respective genes for resistance xa13, Xa21, Xa33 in the parental lines viz., Improved Samba Mahsuri (xa13 and Xa21) were done using gene specific markers xa13 promoter, pTA248 and RMW 7.1 respectively. The amplification profile for these markers showed the product size of resistance genes 500 bp, 1,000 bp, and 350 bp in the donor parents where as they were absent in the recurrent parent Aiswarya. Therefore these markers were used for the foreground selection in the BC_2F_1 plants.

Foreground selection was performed for the 149 plants raised from the BC_1F_1 seeds two/ three gene combination. A total of 79 plants in BC_2F_1 showed

the presence of the recessive resistance gene for BB, whereas 38 plants were found to possess Xa21, which is a dominant resistance gene. But Xa33, which is also a dominant gene for BB resistance were found to be absent in the screened plants.

In order to conduct background selection, a total of 23 plants having the two gene combination i.e, xa13 and Xa21 were identified and selected. Out of 320 SSR markers used, the background screening was done with 44 polymorphic markers identified in the initial screening were used which covers the entire chromosomes of Aiswarya and the percentage recovery was calculated for the respective plants.

The percentage reconstitution of the recurrent parent genome was calculated inorder to find the lines showing maximum genetic similarity with Aiswarya. The 23 plants identified with two gene combination were used to assess the percentage recovery. Here five lines were found to have recovery of RPG more than 80% i.e., ICAB 159-2/152/2 - 81.81%, ICAB/168/10 - 84.9%, ICAB/168/26 - 81.81%, ICAB 159-2/152/1- 80.68%, and ICAB/168/24 - 80.68% where the minimum percentage of recovery is shown by 53.13% in ICAB/168/33. Also the lines, ICAB 159-3/153/23, ICAB 159-3/153/26, ICAB 159-3/153/27, ICAB 159-3/153/30 and ICAB/168/10 show more than 70% recovery.

REFERENCES

7. REFERENCES

Abhilash, K. V., Balachiranjeevi, C. H., Bhaskar Naik, S., Rambabu, R., Rekha, G., and Madhavi, K. R. 2016a. Marker-assisted introgression of bacterial blight and blast resistance genes into RPHR 1005, restorer line of the popular rice hybrid, DRRH-3. *J. Plant Biochem. Biotechnol.* 25: 400-409.

Abhilash, K. V., Balachiranjeevi, C. H., Bhaskar Naik, S., Rambabu , R., Rekha, G., and Madhavi K. R. 2016b. Development of gene-pyramid lines of the elite restorer line, RPHR-1005 possessing durable bacterial blight and blast resistance. . *Front. Plant Sci.* 7:1195.

Babu, R., Nair, S.K. Prasanna B. M., and Gupta, H.S. 2004. Integrating marker assisted selection in crop breeding – Prospects and challenges. *Curr. Sci.* 7(5): 607-619.

Basavaraj, S. H., Singh, V. K., Singh, A., Singh, A., Singh, A., Yadav, S., Ellur, R. K., Singh, D., Gopalakrishnan, S., Nagarajan, M., Mohapatra,T., Prabhu, K.V., and Singh A. K. 2010. Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. *Mol. Breed.* 26: 293–305.

Beckmann, J. S. and Soller, M. 1990. Towards a unified approach to genetic mapping of eukaryotes based on sequence-tagged microsatellite sites. *Biotechnol.* 8: 930-932.

Bharani, M., Nagarajan, P., Rabindaran, R., Saraswathi, R., Balasubramanian, P., and Ramalingam, J. 2010. Bacterial leaf blight resistance genes (*Xa*21, *xa*13 and *xa*5) pyramiding through molecular marker assisted selection into rice cultivars. *Phytopathol. and Plant prot.* 43: 1032-1043.

Baliyan, N., Malik, R., Rani, R., Mehta, K., Vashisth, U., Dhillon, S., Boora, K. S. 2018. Integrating marker-assisted background analysis with foreground selection for pyramiding bacterial blight resistance genes into Basmati rice. *C. R. Biologies.* 341:1–8.

Chen, S., Huang, Z., Zeng, L., Yang, J., Lui, Q and Zhu, X. 2008. High-resolution mapping and gene prediction of Xanthomonas oryza epv. Oryzae resistance gene Xa7. *Molecular Breeding*. 22:433-441.

Chu, Z., Fu, B. H., Yang, C., Xu, Z., Li, A., Sanchez, Y. J., Park, L., Bennetzen, Q., Zhang and Wang, S.2006. Targeting xa13, a recessive gene for bacterial blight resistance in rice. *Theoretical and Applied Genetics*. 112: 455-461.

Chun, X., Hongqi, C. and Xudong, Z. 2012. Identification, Mapping, Isolation of the Genes resisting to Bacterial Blight and Breeding application in Rice. *Molecular Plant Breeding*. 3(12): 121-131.

Collard, B. C. Y. and Mackill, D. J. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society.* 363: 557–572.

Deshmukh,U.C., Verma, R.K., Saxena, R.R., Mohan, P. and Verulkar, S. 2017. Marker Assisted Selection for Bacterial Leaf Blight resistance in Segregating Populations of Karma Mahsuri. *Vegetos- An International Journal of Plant Research.*30:1-4

Devadath, S. 1989. Chemical control of bacterial blight of rice, in: Proceedings of the International Workshop on bacterial blight of rice International Rice Research Institute IRRI, Manila, Philippines. 89–98.

Dokku, P., Das, K. M., and Rao, G. J. N. 2013. Pyramiding of four resistance genes of bacterial blight in Tapaswini, an elite rice cultivar, through marker-assisted selection. *Euphytica*. 2013; 192:87–96.

Dubcovsky, J. 2004. Marker-assisted selection in public breeding programs: the wheat experience. *Crop Sci.* 44: 1895-1896.

Ellur, R. K., Khanna A., Yadav, A., Pathania, S., Rajashekara, H., Singh, V. K., Nagarajan, M., Mondal, K. K., Singh, N. K., Singh, K., Prabhu, K.V., and Singh, A. K. 2016a. Improvement of Basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding. *Plant Sci*. 242, 330–341.

Ellur, R. K., Khanna, A., Yadav, A., Pathania, S., Rajashekara, H., Singh, V. K., Nagarajan, M., Mondal, K. K., Singh, N. K., Singh, K., Prabhu, K.V., and Singh, A. K. 2016b. Marker-aided Incorporation of *Xa38,* a novel bacterial blight resistance gene, in PB1121 and comparison of its resistance spectrum with *xa13* + *Xa21*. Sci. Rep. 6: 29-188.

Frisch, M. and Melchinger A.E. 2005. Selection theory for marker assisted backcrossing. *Genetics* 170: 909-917.

Gale, M.D. and Devos, K. M. 1998. Comparative genetics in the grasses. *Proceedings of the National Academy of Sciences of the USA* 95:1971–1974.

Gnanamanickam, S.S. 2009. Rice and its importance to human life. Prog Biol Con. 8:1-11

Gu, K., Sangha, J. S., Li, Y., and Yin, Z. 2008. High resolution genetic mapping of bacterial blight resistance gene Xa10. *Theor. Appl. Genet.* 116:155-163.

Guillebeau, P. 1998. What to do about the food quality protection act. Or how can we protect the pesticides we need, Proc. S.E. Pecan Growers Assoc. 91:65–69.

Goel, R. K., Kaur, L., Saini, R. G. 1989. Effectiveness of different Xa genes against Xanthomonas oryzae pv. oryzae population causing bacterial blight of rice in Punjab (India). *Rice Genetics Newsletter* 15: 131.

Gopalakrishnan, S., Sharma, R. K., Anand Rajkumar, K., Joseph, M., Singh, V. P., Singh, A. K., Bhat, K.V., Singh, N. K., and Mohapatra, T. 2008. Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. *Plant Breed.* 127:131–139.

Gu, K., Sangha, J. S., Li, Y., and Yin, Z. 2008. High-resolution genetic mapping of bacterial blight resistance gene *Xa10*. *Theor. Appl. Genet.* 116(2): 155-163.

Guvvala, L. D., Koradi, P., Shenoy, V., and Marella, L. S. 2013. Making an Indian traditional rice variety Mahsuri, bacterial blight resistant using marker-assisted selection. *J. Crop Sci. Biotech.* 16:111-21.

Hospital, F. 2005. Selection in backcross programs. *Phil. Trans. R. Soc. B.* 360: 1503-1511.

Huang N, Angels E. R., Domingo J., Mangpantay G., Singh S., Zhang G., Kumar N., Vadivel B. J., Khush G. S. 1997. Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theor. Appl. Genet.* 95:313–20.

Hospital, F. And Charcosset, A. 1997 Marker assisted introgression of quantitative trait loci. *Genetics.* 147:1469-1485

Huang, T.Y., Li, S. G., Wang, Y. P., and Li, H.Y. 2003.Accelerated improvement of bacterial blight resistance of Shuhui527 using molecular marker–assisted selection . *Sheng Wu Gong Cheng Xue Bao* 19(2):153-157.

Huang, B., Xu, J.Y., Hou, M. S., Ali, J., and Mou, T. M. 2012. Introgression of bacterial blight resistance genes *Xa*7, *Xa*21, *Xa*22 and *Xa*23 into hybrid rice restorer lines by molecular marker-assisted selection. *Euphytica* 187:449–459.

Huang, N., Angeles, E. R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kuma, A., Bennett, N. J. and Khush., G. S. 1997. Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theor. Appl. Genet.* 95: 313-320.

Ikeda, R., Khush, G. S. and Tabien, R. E. 1990. A new resistance gene to bacterial blight derived from *O. longistaminata*. Jap. Jour. Breed. 40 (Suppl. 1): 280-281.

Ishiyama, S. 2002. Studies of bacterial leaf blight of rice. *Report Imp. Agril. Stn. Konosu*, 45:233-261.

Invasive Species Compendium. 2017 (lastmodified). Available at https://www.cabi.org/isdatasheet/37964

Joseph, M., Gopalakrishnan, S., Sharma, R. K., Singh, V. P., Singh, A. K., Singh, N. K., and Mohapatra, T. 2004. Combining bacterial blight resistance and basmati quality characteristics by phenotypic and molecular marker assisted selection in rice. *Mol. Breed.* 13:377–387.

Joshi R. K. and Nayak, S. 2010. Gene pyramiding-A broad spectrum technique for developing durable stress resistance in crops. *Biotechnology and Molecular Biology Review.* 5(3). 51-60,

Kabir, M. E., Iftekharuddaula, K. M., Khan, M. A. I., Mian, M. A. K., Ivy, N. A. 2017. Marker assisted introgression of bacterial blight resistance genesinto submergence tolerance rice variety BRRI dhan52. *Bangladesh J. Agril. Res.* 42(3):403-411

Khush, G. S., Mackill, D. J., and Sidhu, G. S. 1989. Breeding rice for resistance to bacterial blight. Bacterial blight of rice, Proceedings of the International Workshop on Bacterial blight of Rice. International Rice Research Institute, Manila, Philippines. pp. 177-207.

Khush, G. S., D. J. Mackill and G. S. Sidhu, 1989. Breeding rice for resistance to bacterial blight. *In* Bacterial blight of rice, p. 207-217. International Rice Research Institute, P. O. Box 933, Manila, Philippines.

Khush, G. S. and Angeles, E. R, 1999. A new gene for resistance to race 6 of bacterial blight in rice, *Oryza sativa. Rice Genet. Newsl.* 16:92–93.

Kottapalli, K. R., Narasu, M. L., and Jena, K. K. 2010. Effective strategy for pyramiding three bacterial blight resistance genes into fine grain rice cultivar, Samba Mahsuri, using sequence tagged site markers. *Biotechnol.* 32:989–996.

Kumar, N. P., Sujatha, K., Laha, G. S., Srinivasarao, K., Mishra, B., Viraktamath, B. C., Hari, Y., Reddy, C. S., Balachandran, S. M., Ram, T., Sheshumadhav, M., Shobharani, N., Neeraja, C. N., Ashokreddy, G., Shaik, H., and Sundaram, R. M. 2012. Identification and fine-mapping of *Xa*33, a novel gene for resistance to *Xanthomonas oryzae* pv. *oryzae*. *Phytopathol.* 102:222–228.

Magar, M. M., Durga Rani, and Anuradha, G. 2014 Marker assisted selection For Bacterial Leaf Blight Resistance In Segregating Populations Of Cottondora Sannalu. *Int. J. Appl. Sci. Biotechnol.* 2(3): 229-237.

Mary, C. A. 1996. Survival of *X. campstris* pv. *oryzae* and its control in Kuttanad. Ph. D Thesis Kerala Agricultural University, Thrissur, p.179.

McCouch, S. R., Teytelman, L., Xu, Y., Lobos, K.B., Clare, K., Walton, M., Fu, B., Maghirang, R., Li, Z., Zing, Y., Zhang, Q., Kono, I., Yano, M., Fjellstrom, R., DeClerck, G., Schneider, D., Cartinhour, S., Ware, D., Stein, L. 2002. Development and mapping of 2240 new SSR markers for rice (Oryza sativa L.). *DNA Res.* 9:199–207

Mitchell, S. E., Kresovich, S., Jester, C. A., and Szewc-McFadden, A. K. 1997. Application of multiplex PCR and fluorescence-based, semi-automated allele sizing technology for genotyping plant genetic resources. *Crop Sci.* 37: 617-624.

Naveed, S. A., Babar, M., Arif, A., Zafar, Y., Sabar, M., Ali, I., Chragh, M. and Arif, M. 2010. Detection of bacterial blight resistant gene *xa5* using linked marker approaches. *Afr. J. Biotechnol.* 9:3549-3554.

Natraj, K. P., Sujatha, K., Laha, G. S., Srinivasa, R. K., Mishra, B., Viraktamath, B. C., Hari, Y., Reddy, C. S., Balachandran, S. M., Ram, T., Sheshu Madhav, M., Shobha Rani, N., Neeraja, C. N., Ashoka Reddy, G., Shaik, H., and Sundaram, R. M. 2012. Identification and fine mapping of Xa33, a novel gene for resistance to x*anthomonas oryzae pv. Oryzae. Phytopathology* 102:222-228.

Nino-Liu, D. O., Ronald, P. C., and Bogdanove, A. J. 2006. Pathogen profile of Xanthomonas oryzae pathovars: model pathogens of a model crop. *Mol. Plant Pathol.* 7(5):303-324.

Nino-Liu, D. O., Ronald, P. C., and Bogdanove, A. J. 2005. A Simple method of mass inoculation of rice effective for both pathovars of *Xanthomonas oryzae,* and the construction of comparable sets of host cDNA libraries spanning early stages of bacterial leaf blight and bacterial leaf streak. *J. Phytopathol.* 153: 500-504.

Ogawa, T., Busto G. A., Tabien, R. E., Khush G. S. 1988. Further study of Xa-4b gene for resistance to bacterial blight of rice. *Rice Genet. Newsl.* 5:104–106

Pandey, M. K., Shobha, R. N., Sundaram, R. M., Laha, G. S., Madhav, M. S., Rao, S.K., Sudharshan, I., Varaprasad, G.S., Subba Rao, Sivaranjani, A. K. P.B.C., and Viraktamath. 2012. Improvement of two traditional Basmati rice varieties for bacterial blight resistance and plant stature through morphological and marker-assisted selection. *Mol. Breed.* 31(1):239-246.

Perez, L. M., Redona, E. D., Mendioro, M. S., Vera Cruz, C. M., and Leung, H. 2008. Introgression of *Xa*4, *Xa*7 and *Xa*21 for resistance to bacterial blight in thermosensitive genetic male sterile rice (*Oryza sativa* L.) for the development of two-line hybrids. *Euphytica* 164(3): 627-636.

Pradhan, S. K., Nayak, D. K., Mohanty, S., Behera, L., Barik, S. R., and Pandit, E. 2015. Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna. *Rice* 8:19

Rajpurohit, D., Kumar, R., Kumar, M., Paul, P., Awasthi, A.A., Basha, P. O., Puri, A., Jhang, T., Singh, K., and Dhaliwal, H. S. 2010. Pyramiding of two bacterial

blight resistance and a semi dwarfing gene in Type 3 Basmati using marker-assisted selection. *Euphytica* 178:111–126.

Ronald, P. C., Albano, B., Tabien, R., Abenes, L., Wu, K., and McCouch, S. R. 1992. Genetic and physical analysis of the rice bacterial blight disease resistance locus *Xa*-21. *Mol. Gen. Genet.*236: 113–120.

Salgotra, R. K., Gupta, R. J., Millwood, M., Balasubramaniam, C. N., and Stewart. 2012. Introgression of bacterial leaf blight resistance and aroma genes using functional marker assisted selection in rice (*Oryza sativa* L). *Euphytica* 187: 313-323.

Sanchez, A. C., Brar, D. S., Huang, N., Khush, G. S. 2000. Sequence tagged site markers-assisted selection for three bacterial blight resistance genes in rice. *Crop Sci.* 40:792–797.

Shanti, M. L., Shenoy, V. V., Devi, G. L., Kumar, V. M., Premalatha, P., Kumar, G. N., Shashidhar, H. E., Zehr, U. B., and Freeman, W. H. 2010. Marker-assisted breeding for resistance to bacterial leaf blight in popular cultivars and parental lines of hybrid rice. *J. Plant Pathol.* 92(2):495–501.

Singh, S., Sindhu, J. S., Huang, N., Vikal, Y., Li, Z., Brar, D. S., Dhaliwal, H. S., and Khush, G. S. 2001. Pyramiding three bacterial blight resistance genes (*xa*5, *xa*13 and *Xa*21) using marker-assisted selection into indica rice cultivar PR106. *Theory of Applied Genetics.* 102:1011–1015.

Song, W.Y., Wang, G.L., Chen, L.L., Kim, H.S., Pi, L.Y. and Holsten, T. 1995. A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. *Science* 270: 1804–1806.

Srikanth. S., Pandey, M. K., Chiranjeevi, C., Hajira, S. K., Vijay Kumar, S., Kousik, M. B., Bhadana, V. P., Madhav. M., Suneetha, K., Subba Rao, L.V., Kumaraswamy, M., Archana, Giri., Laxmi Narasu, B. and Sundaram, R. M. 2016. Introgression of major bacterial blight and blast resistant genes into Vallabh basmati 22, an elite basmati variety. *Int. J. Dev. Res.* 6, (7):8366-8370.

Suh, J. P., Jeung, P., Ung, N. T., and Shin, M. S. 2013. Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. *Rice* 6:5-7.

Sundaram, R. M., Vishnupriya, M. R., Biradar, S. K., Laha, G. S., Reddy, G. A., Rani, N. S., Sharma, N. P., and Sonti, R.V. 2008. Marker-assisted introgression of bacterial blight resistance in Samba Mashuri, an elite indica rice variety. Euphytica 160(3): 411-422.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.*17: 6463-71.

Win, K. M., Korinsak, S., Sirithunya, P., Lanceras-Siangliw, J., Jamboonsri, W., Da, T., Patarapuwadol, S., and Toojind, T. 2013. Marker assisted introgression of multiple genes for bacterial blight resistance into aromatic Myanmar rice MK-75K. *Field Crops Res.* 154: 164-171.

Young, N.D. and Tanskley, S.D. 1989. RFLP Mapping in Plant Breeding: New Tools for an Old Science. *Bio/Technology*7:257–264

Zhang, G., Angeles, E. R., Abenes, M. L. P., Khush, G. S., and Huang, N. 1996. RAPD and RFLP mapping of the bacterial blight resistance gene *xa*13 in rice. *Theor. Appl. Genet.* 93: 65-70.

Zhang, J. Li. X., Jiang, G., Xu, Y., and He, Y. 2006. Pyramiding of *Xa7* and *Xa21* for the improvement of disease resistance to bacterial blight in hybrid rice. *Plant Breed.* 125(6): 600–605.

Zhang, Q. 2005. Highlights in identification and application of resistance genes to bacterial blight. *Chinese J. Rice Sci.* 19:453–459.

Zhou, Y. L., Xu, J. L., Zhou, S. C., Yu, J., Xie, X. W., Xu, M. R., Sun, Y., Zhu, L. H., Fu, B.Y., Gao, Y. M., and Li, Z. K.2009 . Pyramiding *Xa*23 and *Rxo1* for resistance to two bacterial diseases into an elite indica rice variety using molecular approaches. *Mol. Breed.* 23:279-287.

GENOME WIDE MARKER ASSAY FOR THE RECOVERY OF RECURRENT PARENT GENOME IN RICE (*Oryza sativa*)

By

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Abstract of thesis

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8. ABSTRACT

Bacterial leaf blight disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a major constraint in major rice growing areas of the world particularly in Asia. The disease is prelevant in the kharif season and it results in a greater yield loss. Since there is no valid chemical control measure, exploiting host plant resistance is an efficient way to tackle this problem. Approximately 40 genes conferring resistance to BB were identified. Pyramiding of these genes into the background of susceptible parent with good agronomical traits is the best strategy that can be adopted to develop plant varieties durable resistance to BB. So the present study entitled"Genome wide marker assay for the recovery of recurrent parent genome in rice (*Oryza sativa*)" was undertaken in College of Agriculture, Vellayani, Thiruvananthapuram to estimate the reconstitution of genome of Aiswarya (RP) rice variety in the BC_2F_1 plants pyramided with genes for resistance to Bacterial leaf blight through molecular markers covering the entire genome of Aiswarya.

DNA markers closely linked to the BB resistance genes, *viz.*, xa13 pro (xa13 gene), pTA248 (Xa21 gene), RMWR7.1 (Xa33 gene) were used for validation of the marker polymorphism in the donors for the genes. Improved Samba Mahsuri with xa13 and Xa21, Samba Mahsuri with *Xa*33, were taken as donors and Aiswarya was chosen as the recipient parent. The validation of gene specific markers confirmed the absence of the genes in the recurrent parent used in the study. And these markers were further used for foreground selection in BC_2F_1 plants.

Also, the donor and recurrent parents used in the study were screened with 320 SSR primers in order to find the markers specific to the recurrent parent. In this screening out of 320 markers used, 44 were found to be polymorphic and these polymorphic markers were used in the background selection.

Foreground selection was performed initially in all the 149 BC_2F_1 plants to identify the presence of these genes. In the foreground selection, a total of 149 plants were screened and 79 plants were found to have xa13 gene and 38 plants confirmed the presence of Xa21 gene while none of the screened plants showed the presence of Xa33 gene. 23 plants were found to possess two gene combination of xa13+Xa21 and these plants were subjected to background selection to estimate the percentage introgression of the recurrent parent genome.

Background screening of the plants identified with two gene combination using the 44 markers specific to recurrent parent revealed the number of markers showing homozygousity and heterozygousity with the recurrent parent. With this information percentage recovery of RPG was calculated and found that among 23 plants, the maximum recovery found was 84.09% and a total of 5 lines were showing more than 80% recovery of recurrent parent genome.

The present study could identify BC_2F_1 plants identified with xa13 and Xa21 with genome recovery of more than 80% and further screening can be done in the BC_2F_2 generation to develop Essentially Derived Variety.