PYRAMIDING BACTERIAL LEAF BLIGHT RESISTANCE GENES INTO POPULAR RICE VARIETIES OF KERALA THROUGH MARKER ASSISTED SELECTION

by

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(2013 - 21 - 111)

THESIS

Submitted in partial fulfilment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING AND GENETICS

COLLEGE OF AGRICULTURE

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KERALA, INDIA

2017

DECLARATION

I, hereby declare that this thesis entitled **"PYRAMIDING BACTERIAL LEAF BLIGHT RESISTANCE GENES INTO POPULAR RICE VARIETIES OF KERALA THROUGH MARKER ASSISTED SELECTION"** 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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ACKNOWLEDGEMENT

The work presented in this thesis would not have been possible without my close association with many people. I take this opportunity to extend my sincere gratitude and appreciation to all those who made this Ph.D thesis possible.

First and foremost, I would like to extend my sincere gratitude to my research guide Dr. Jayalekshmy V G Professor, Department of Plant Breeding and Genetics, College of Agriculture, Vellayani for introducing me to this exciting field of Plant science and for her dedicated help, advice, inspiration, encouragement and continuous support, throughout my Ph.D. Her enthusiasm, integral view on research and her mission for providing high-quality work, has made a deep impression on me. She has taught me another aspect of life, that, "goodness can never be defied and good human beings can never be denied". Her constant guidance, cooperation, motivation and support have always kept me going ahead.

I feel highly obliged and have immense pleasure in expressing my sincere thanks to the members of my Advisory Committee Dr. Arya K Professor and Head, (Plant Breeding and Genetics), Dr. Leenakuamry S, Profeesor and Head RRS, Moncompu and registrar KAU Thrissur, Dr. Kamala Nayar Professor, (Plant Pathology), College of Agriculture, Vellayani, Dr. Surendran M, Assistant Professor, RRS, Moncompu for their intellectual enlightment and persistent suggestions throughout the pursuit of this study.

I cordially offer my profound gratitude to my teachers in Department of Plant Breeding and Genetics Dr. P. Manju, Dr. D.S. Radha Devi, Dr. Maya

Devi, P., Dr. Sunny K. Oommen, Dr. K. M. Abdul Khader, Dr. Wilson D, Lekha Rani, Dr. Mareen Abraham and Dr. Beena Thomas for their excellent teaching and guidance which enabled me complete my course work.

I owe my profound and special thanks to Dr. George Thomas, Scientist EII Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, for his cooperation, timely help and judicious support.

My heartfelt thanks to my lab mates, Sumina, Anila, Remya, Neetu, Anchu, Anju, Sheeja, Reshma, Prinita, Tintu and Naveen for always standing by my side and sharing a great relationship as compassionate friends. I will always cherish the warmth shown by them.

I am extremely grateful to skilled labours Beena S, Joli and all workers of College of Agriculture, Vellayani and RRS, Moncompu for their help and co-operation during my research.

A special mention of thanks to my friends Rajiv, Kranti, Srinivas, Rajanand,Vidya, Shajma and Gangadhar for their constant support and cooperation during my stay. Their timely help and friendship shall always be remembered.

I find myself in short of words to express my warmest thanks to my beloved junior friends Brunda ,Ravi Boli, Lishma, Rajashekar, Pavan, Siddesh, Hari, Vinay, Bibishan, Loki, Srinivas, Murali , Sathishkumar, Nikhitha, Asha, Athul, Reshma , Jeena, Sainath, Pritin, Srikant, Vijit, Jincy, Girish and Jeslam in whose cheerful company I have never felt my work as burden.

I pay my devotion to my loving parents who have relentlessly thrived for shaping my life and future and to whom I owe all my success, I have achieved so far. I would like to thank my brother (Nagu) and sister (Rajashree) for their support in all its forms and critiques.

I owe immense gratitude to Department of Biotechnology for providing Senior Research Scholarship.

I finish with a final silence of gratitude to the almighty.

Ramaling Hundekar

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

17	Foreground selection in BC_1F_1 plants of the cross Aiswarya x ISM using *Xa33* linked marker RMWR 7.1	63-64
18	Foreground selection in BC_1F_1 plants with *Xa38* gene of the cross Aiswarya x PR-114 using *Xa38* linked functional marker Oso4g53050-1	64-65
19	Foreground selection in BC_1F_1 plants of the cross Prathyasa x ISM using *xa*13 linked marker xa13 pro and *Xa*21 linked pTA248.	64-65
20.	Foreground selection in BC_1F_1 plants of the cross Prathyasa Samba Mahsuri using *Xa33* linked marker RMWR 7.1.	64-65
21	Foreground selection in BC_1F_1 plants with *Xa38* gene of the cross Prathyasa x PR-114 using *Xa38* linked functional marker Oso4g53050-1	64-65
22	*Xoo* strain isolated from RRS Moncompu Kuttanad region.	92-93
23	Subcultured petri dish of *Xoo* strain	92-93
24	Disease severity in donors and recipients	92-93
25	Field screening of donors and recipients for *Xoo*	93-94
26	Disease infection rate in donors	93-94
27	Foreground selection in Aiswarya derived pyramided lines using *xa*13 linked marker xa13 pro, *Xa*21 linked pTA248 and *Xa*33 linked with RMWR 7.1	97-98
28	Foreground selection in Prathyasa derived pyramided lines using *xa*13 linked marker xa13 pro, *Xa*21 linked pTA248 and *Xa*33 linked with RMWR 7.1	99-100

LIST OF ABBREVIATIONS

%	-	per cent
μ	-	Mean
μl	-	Micro litre
μM	-	Micro molar
^{0}C	-	Degree Celsius
$\chi 2$	-	Chi-Square
ANOVA	-	Analysis of Variance
BC_1F_1	-	First filial back cross generation
bp	-	base pairs
BSA	-	Bulk Segregant Analysis
CAPS	-	Cleaved Amplified Polymorphic Sequences
CD	-	Critical difference
cm	-	centimeter
d.f	-	degrees of freedom
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
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* L.) is agronomically and nutritionally, most important food crop of the world serving as the staple food for nearly 3.2 billion people. More than 90 percent of the world's rice is grown and consumed in Asia (Khush 2005). It is one of the staple food crops of India and is grown all over the country in diverse ecosystems. Kerala encompasses a total of 38.90 lakh hectares of gross cropped area. At present, rice is grown here, in a gross area of 162.10 thousand hectares producing 697.30 thousand tonnes with productivity of 2565 kg/ha (Anon., 2015).

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a devastating disease of rice in Asia and Africa resulting in an estimated loss of US$ 250 million annually (Zhang *et al.*, 1998). The yield losses due to bacterial blight can be as high as 50% when plants are infected at the maximum tillering stage (Wei *et al.*, 2007). This disease is not only widespread throughout Asia but also occurs in Australia, United States and in several rice growing countries of Latin America (Chu *et al.*, 2006). Major rice growing areas of Kerala are highly prone to BB disease with damage of 35.3% during additional crop (*Kharif*) season. In Kerala even though the disease occurs both during Punja (October-November to February-March) and additional crop (June-July to September -October) it becomes severe during the additional crop season (Mary, 1996).

Control measures for BB include cultural practices, chemical control, biological control, disease forecasting and most importantly, host genetic resistance. As the chemical control for BB is not effective, the utilization of resistant varieties carrying resistance genes have been considered to be the most suitable way for controlling the disease (Nino-Liu *et al.*, 2006). Several donors carrying diverse genes for BB resistance in rice have been used to develop BB resistant varieties (Khush *et al.*, 1989).

Long-term cultivation of rice varieties carrying single resistance gene has resulted in a significant shift in pathogen-race frequency and consequent

breakdown of resistance (Mew *et al.,* 1992). Pyramiding of multiple resistance genes in the background of modern high yielding varieties is a tangible solution to resistance breakdown. The probability of simultaneous pathogen mutations for virulence to defeat two or more effective genes is much lower than with a single gene (Mundt, 1990). Conventional plant breeding mainly depends on phenotypic selection which is time consuming process and less efficient because of dominance and epistatic effect in multiple gene transfer (Collard and Mackill, 2008) and linkage drag (Young and Tanksley, 1989). Conventional breeding tools are inefficient for gene pyramiding, particularly in the case of recessively inherited resistance genes.

Marker-assisted selection (MAS) addresses the limitations of conventional breeding enabling the evaluation of the expression status of resistance genes and allows for pyramiding of multiple resistance genes in a desirable genetic background. Marker-assisted backcross breeding (MABB),which involves two steps - (1) MAS for the gene of interest, known as foreground selection and (2) MAS for recovery of the recurrent parent genome, known as background selection (Hospital *et al.,* 1992) is the most effective way of transferring specific gene(s) to agronomically superior variety/ parental lines. Molecular markers associated with the genes responsible for the trait are integrated with backcross breeding program to choose precise segment and desired genotype containing only required genes. Tightly linked DNA markers have been developed for several BB resistance genes. The BB resistance genes, *viz., Xa1 , xa5, xal3, Xa2l, Xa26, Xa27, Xa33, Xa38* and *Xa40* have been cloned and used for breeding programs (Bhasin *et al.,* 2012; Kumar *et al.,* 2012; Kim *et al.,* 2015). With the exception of *xa5* and *xa13,* the BB resistance genes are dominant in nature and the markers developed from the sequencing information of these genes are widely used in MAS (Gu *et al.,* 2005; Chu *et al.,* 2006). For carrying out foreground selection for bacterial blight resistance in rice, several sequence tagged site (STS) markers RG556, RG136, pTA248, M5 and Npb181 are available for *xa5, xa13, Xa2l, Xa7* and *Xa4* respectively (Huang *et al.,* 1997; Singh *et al.,* 2001; Sundaram *et al.,* 2008; Shanti *et al.,* 2010; Xu *et al.,* 2012) The pTA248 marker is 0.2 cM from *Xa21*

(Ronald *et al.,* 1992). The gene *Xa33* was fine mapped between two SSR markers (RMWR7.1 and RMWR7.6) located at a genetic distance of 0.9 and 1.2 cM, respectively from the gene and flanking it (Kumar *et al.,* 2012). The *Xa38* locus delimited to a 38.4-kb region on the long arm of chromosome 4 and linked to one of the primer pair, designated Os04g53050-1 (Bhasin *et al.,* 2012).These markers have been employed to identify germplasm containing these genes (Blair and McCouch, 1997) and to develop rice cultivars with single and multiple resistance genes which are now widely cultivated in many countries (Singh *et al.*, 2001; Sundaram *et al.,* 2008; Fu *et al.,* 2012).

Background selection for recovery of recurrent parent genome is usually carried out using a set of simple sequence repeat (SSR) markers as these microsatellite markers are, highly sequence specific, co-dominant, multiallelic ,highly polymorphic ,uniformly dispersed and efficiently analyzed by PCR (McCouch *et al.*, 2002). Another effective PCR marker is Cleaved Amplified Polymorphic sequence (CAPS) (Konieczny and Ausubel, 1993; Dubcovsky, 2004; Li *et al.*, 2005). They are co-dominant genetic markers, but CAPS requires restriction endonuclease digestion to detect polymorphism (SNP) in the region of interest. Numerous researchers have done pyramiding of *Xa2l, xa5* and *xa13* important genes for resistance to BB along with rapid background recovery of the recurrent parent genome by marker assisted selection (Singh *et al.*, 2001; Kim *et al.*, 2009; Suh *et al.*, 2011; Huang *et al.*, 2012).

Prathyasa is a semi dwarf, short duration, medium tillering variety with medium bold, red kernelled grains. However, this variety which was released specifically to suit the additional crop season, due to its short duration, is highly susceptible to BB. A potentially high yielding variety alone cannot lead to increase in productivity as compared to a high yielder with moderate resistance to major pests and diseases. Aiswarya is another popular variety that is preferred by farmers of Palakkad and other districts of Kerala and is even suitable for upland cultivation. It is also reported as a restorer for wild abortive (WA) cytoplasm and

therefore a prospective parent in hybrid rice breeding programme. But this variety is also susceptible to BB disease in Kerala.

Under this circumstance the present investigation is planned to introgress effective BB resistance genes *xa13, Xa21, Xa*33 and *Xa38* into the popular varieties, Prathyasa and Aiswarya varieties through marker assisted selection (MAS) with the following major objectives.

1. Pyramiding the genes for resistance to bacterial blight (*xa*13, *Xa*21, *Xa*33 and *Xa38*) into the popular varieties, Prathyasa and Aiswarya based on host plant resistance.
2. Evaluation of donor parents for resistance to bacterial blight under artificial inoculation.
3. Foreground selection for the genes using molecular markers, to obtain lines with the two/three resistance genes combination in the background of recipient cultivars.

Review of Literature

2. REVIEW OF LITERATURE

Rice is one of the oldest domesticated crops which constitute a major source of calories for urban and rural inhabitants as well as model monocot plant for genetic and genomic studies. Besides its economic importance, rice has a small genome (430 Mb) size as compared to sorghum (1000 Mb), maize (3000 Mb), barley (5000 Mb) and wheat(16000 Mb) (Arumugnathan and Earle, 1991) and it is three times larger than *Arabidopsis*. The database gramene houses more than 8,000 entries for QTLs detected in rice. Information from gramene is being used in MAS breeding for rice improvement to meet a number of challenges. The review of literature is presented under following heads.

2.1. Bacterial leaf blight of rice
2.2. Causal organism and symptoms of BB disease
2.3. The infection of *Xoo* in rice plants
2.4. Identification and molecular mapping of bacterial blight resistance genes
2.5. DNA markers and marker-assisted selection (MAS)
2.6. Screening for BB resistance in rice
2.7. Gene pyramiding for bacterial blight resistance

2.1. BACTERIAL BLIGHT OF RICE

Bacterial blight (BB) is one of the most destructive bacterial diseases of rice caused by *Xanthomonas oryzae* pv.*oryzae* (*Xoo*) in irrigated and rain fed lowland ecosystems throughout Asia. In India, bacterial blight was first reported by Srinivasan *et al.* (1959) from Maharashtra where it was reported to be widespread and destructive since 1951. Bhapkar *et al.* (1960) first described the typical symptoms of bacterial blight. Studies showed the disease to be present in most of the rice growing states of India (Mizukami, 1964; Srivastava, 1967). Since the introduction and cultivation of new high yielding but susceptible rice cultivars over a large acreage, the disease has become one of the most serious problems in rice in India. It has also been reported from Sri Lanka, China, Taiwan,

Korea, Thailand, Malaysia, Indonesia, Australia, Vietnam and the Philippines and is now considered to be one of the most destructive diseases of rice in Asia. It has neither been known to occur in North America nor in Europe, with the possible exception of the USSR (Vzoroff, 1938). In Kerala it was first observed in 1976 in Palghat district. Later Mary (1980) reported that the disease was endemic in the two major rice growing regions, Palghat and Kuttanad. However in Kuttanad, it was found to recur in epiphytotic proportion almost every year during the additional crop season from June-July to September –October (Nair *et al.*, 1990: Sreekumar, 1991).

2.2. CAUSAL ORGANISM AND SYMPTOMS OF BB DISEASE

Bacterial blight is caused by the short rod-shaped bacterium with round ends 1-2×0.8-1 um, monotrichous flagellum 6-8 μm, gram-negative, non-spore-forming and aerobic bacteria (Ishiyama, 1992). Colonies of *Xoo* are circular, convex, whitish yellow to straw yellow, with smooth surface, entire margin, and opaque against transmitted light (Plate1). *Xoo* survive on rice stubbles, straw and weed hosts. The over wintering of bacterium occurs in two forms: i) the dry form, *Xoo* is found in the vascular vessel and xylem parenchyma of dried plants. If they are moistened by rain in winter, these dry form bacteria gradually die. ii) The growth form, bacterial cells are found in stubble and in the root system of perennial wild plants, especially *Leesia* spp. The pathogens survive in an inactive stage. The dry form can be activated and turn into the growth form after receiving moisture under favourable conditions.

The bacterial blight is a vascular disease spreads through the xylem vessels. Lesions usually begin at the margin, a few centimetres (cm) from the tip, as water-soaked stripes. It can occur at all stages of the rice plants. At the seedling stage, the symptom first appears as tiny water-soaked spots at the margin of the rice leaf

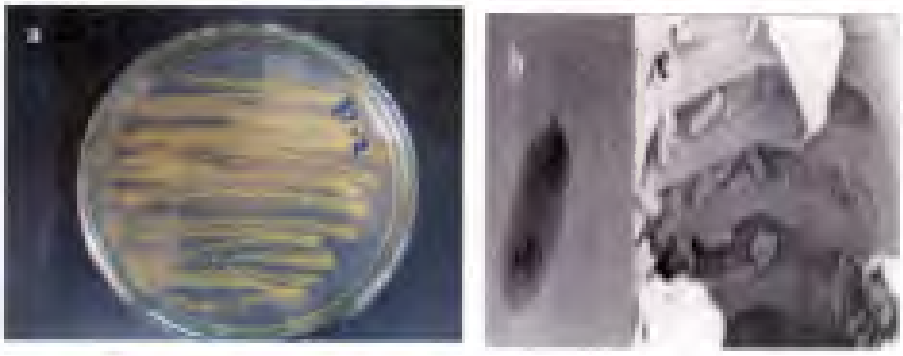


Plate 1.Characteristics of *Xanthomonas oryzae* pv. *oryzae* (a) colonies (b) rod shape of pathogen (c) Xoo in rice xylem vessel with scanning electron micrograph (Khush *et al.*, 1989)

Plate 2.: The BB disease symptom (a) leaf blight symptom occur on adult plant (b) Kresek symptom occur on the seedling plant, (c) and (d) characteristics of BB symptom

blade. Then, it enlarges and the rice leaves turn yellow and wither. The symptom of the disease at the seedling stage is known as kresek (Ou, 1985). The lesions may start at anywhere on the leaf blade at the site of an injury. The lesions can occur on leaf sheath of susceptible cultivars. The affected leaves will turn yellow, roll up and wilt rapidly (Plate 2).

At the tillering and reproductive stages, the symptom is known as leaf blight, a systemic infection that produces tannish-grey to white lesions along the vein. If plant produces panicles, the sterility percentage and number of immature grains will increase. Grains from diseased plants were easily broken during milling. Milky or opaque dewdrops of bacterial exudates on the surface of young lesions can be observed in the early morning. They dry up to form small, yellowish, spherical beads. They are shaken off by wind and drop into the field water. Droplets can be detected by drawing the leaves through the fingers and feeling the stickiness (Khush *et al.*, 1989).

BB can cause damage at vegetative and reproductive stages of rice plants. It can decrease photosynthetic area and reduce photosynthesis system of plant leaves. High fertilizer input condition can induce the disease development (IRRI, 2010). Irrigation water is considered to contribute the spread of BB disease, as it carries the bacterial ooze that drop in the rice field water. The pathogen can survives around 15 days in the field water (Nino-Liu *et al.*, 2006).

2.3. THE INFECTION OF *BB* IN RICE PLANTS

Primarily, *Xoo* enters its hosts through hydathodes and wounds. Bacterial cells on the leaf surface may become suspended in guttation fluid as it exudates at night and then enter the plant by swimming, or passively as the fluid is withdrawn into the leaf in the morning. Bacteria multiply in intercellular spaces of the underlying epithelial cells and then enter and spread into the plant through the xylem. Within a few days, bacterial cells and EPS(extracellular polysacchride) fill the xylem vessels and ooze out from hydathodes, forming beads or strands of

exudates on the leaf surface, a characteristic sign of the disease and a source of secondary inoculums (Nino-Liu *et al.*, 2006). As the bacterium develops in the plant it can also spread into mesophyll (Nino-Liu *et al.*, 2005). The bacteria multiply outside the hydathodes of susceptible rice cultivars and gain entrance through them within 24 hours after spray inoculation. In contrary, they are trapped and embedded in a thin layer of exudates secreted from the water pores of resistant cultivars. The water pore has been related to resistance to hadathodal invasion by *Xoo*. On the other hand, the hydathode apertures in susceptible rice plants are much larger due to the reduced growth of the outer ledges, allowing *Xoo* to pass through freely. In the resistance varieties, bacterial cells were irregular in shape and enveloped by abundant fibrillar material (FM) and apparently dead (Huang and Cleene, 1989).

2.4 IDENTIFICATION AND MOLECULAR MAPPING OF BACTERIAL LEAF BLIGHT RESISTANCE GENES

Through rigorous screening and selection, a number of resistant cultivars have been produced and utilized, but resistance was overcome by the development of mutant strains of pathogen and by the dynamic change in *Xoo* populations. To date 40 BB resistance genes have been identified from cultivated rice and wild rice (Zhang, 2005 and Chun *et al.*, 2012) as shown in Table 1. All of them have been considered to follow the gene-for-gene theory.Twenty nine BB genes are dominant *(Xa1, Xa2, Xa3, Xa4, Xa7, Xa10, Xa11, XI2, 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, Xa39 and Xa40*) and eleven are recessive genes *(xa5, xa5(t), xa8, xa13, xa15, xa19, xa20, xa24, xa28, xa31* and *xa32)* (Nino-Liu *et al.*, 2006 and Singh *et al.*, 2007). Three resistance genes *Xa15, xa19* and *xa20* were induced by mutagenesis (Lee *et al.*, 2003). Six resistance genes *Xa21, Xa1, xa5, xa13, Xa26* and *Xa27* have already been cloned and seven resistance genes *(Xa2, Xa4, Xa7, Xa30, Xa33, Xa38* and *Xa40)* have been physically mapped (Chu *et al.*, 2006; Bhasin *et al.*, 2012 and Kim *et al.*, 2015).

There are two major classes of resistance (R) genes related to disease in rice. *Xa21* was first R gene of the RLK (Receptor –Like Kinase) class to be cloned within the broad spectrum of resistance and is therefore the most abundantly used gene in rice breeding programs. The second class (NBS)-LRR (Leucine rich -repeats) is the largest R gene class conferring resistance against bacteria, fungi and viruses (Hulbert *et al.*, 2001). *Xa21* represents this major class of R genes encoding a nucleotide-binding –LRR protein (Yoshimura *et al.* 1998). *Xa27* is unique in a sense that it is only expressed upon inoculation with *Xoo* strains harboring TAL effectors genes avr *Xa27* (Gu *et al.*, 2005). *xa5* and *xa13* are the two recessive genes that have been characterized in rice to encode a small subunit of the transcription factor IIA (TFIIA) and a novel plasma membrane respectively (White and Yang, 2009 and Verdier *et al.*, 2012).

A recessive resistance gene *xa5* was first reported by Murty and Khush (1972). It was naturally found only within the *Aus* subpopulation of rice (Garris *et al.*, 2003). This recessive R gene was mapped to the telomeric region on short arm of chromosome 5 (Blair and McCouch, 1997). RFLP markers RG556, RG207, RZ390 and SSR markers RM122 and RM390 were closely linked to *xa5* gene (Yoshimura *et al.*, 1995; Blair and McCouch, 1997). The *xa5* is particularly strong and has broad resistance to all BB isolates in Korea (Jeung *et al.*, 2006). Moreover, Iyer-Pascuzzi and McCouch (2007) presented a set of CAPS markers for easy, quick and direct identification of cultivars or progeny carrying *xa5*-mediated resistance and provide evidence that these markers are 100% predictive of the presence of the *xa5* allele.

Khan *et al.* (2000) observed that BB incidence is increasing in Pakistan especially in "Kaller" belt which is famous for rice cultivation. Although conventional approach for the identification of different resistance genes in rice germplasm is also being used (Kihupi *et al.*, 2001; Lee *et al.*, 2003), it is time consuming and need artificial inoculation of all the lines with different types of the pathogen. Cruz *et al.* (1996) observed in their study that different races of the BB pathogen exist in the same rice field on the same cultivar. *Xoo* populations

collected from different districts of Indian Punjab found high level of diversity in pathogen population and BB resistance gene *xa*8 and *Xa21* are effective against the prevalent isolates in Indian Punjab followed by *xa5* and *Xa7* (Sodhi *et al.*, 2003).

xa5(t) was identified from rice cultivar Ajaya (IET8585). This variety is highly resistant to all pathotypes present in India. The F_2 population was screened with RAPD and SSR markers. Two SSR markers RM39 and RM31 on long arm of chromosome 5 were tightly linked to the resistance gene (Rao, 2003). In the same way, *xa31* (t) was identified in wild rice *O. glaberrima* using SSR analysis. This recessive gene was also located on chromosome 5. The flanking markers were RM548 and RM593 with the distance of 1.7 and 1.1 cM, respectively (Singh *et al.*, 2007).

Xa27 was identified in wild rice *O. minuta* Acc.101141 (Amante-Bordeos *et al.*,1992). It was reportedly located between RFLP markers RG424 and RG162 (70.4- 104.6 cM) on the long arm of chromosome 6, which is about 22.1 cM away from *Xa7*. The fine genetic mapping of this resistance gene was carried out. *Xa27* was tightly linked with markers M964 and M1197 and co-segregated with markers M631, M1230 and M449. The resistance reaction of *Xa27* showed semi-dominant or a dosage effect in the cv.CO39 genetic background (Gu *et al.*, 2004). *xa32*(t) was identified in wild rice *O. barthii*. It was mapped with SSR markers using bulk segregation analysis (BSA). BSA indicated presence of gene in *O. Barthii* on the terminal region of chromosome 6 at a distance of 9.3 cM proximal to RM588 (Singh *et al.*, 2007).

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 had found to be tightly linked to *Xa21* gene at a distance of 1.2 cM. Then, a PCR-base STS marker pTA248 was developed and can be used in MAS and adopted in a map-based cloning strategy. *Xa21* was the first BB resistance gene successfully

cloned. It was isolated using map-based cloning and found to be a member of a complex locus located on long arm of chromosome 11. Fine mapping of *Xa10* revealed that the resistance gene was flanked between markers M491 and M419 on Nipponbare genome which consisted of six candidate genes (Gu *et al.*, 2008).

Arif *et al.* (2008) conducted a molecular survey for the detection of *Xa*4 gene in Pakistan rice germplasm (*Xa4* is the dominant resistant gene showing resistance against many bacterial strains). They screened more than 100 genotypes along with basmati lines for the presence and absence of *Xa4* gene in Pakistani rice germplasm.

The BB resistance gene present in the *O. nivara* accession was identified to be novel based on its unique map location on chromosome 7 and wider spectrum of BB resistance; this gene has been named *Xa33*. In total, eight genes were identified in the region and a putative gene encoding serine threonine kinase appears to be a candidate for the *Xa33* gene (Kumar *et al.*, 2012).

BB resistance gene *Xa*38 identified from *Oryza nivara* accession IRGC 81825 was mapped on chromosome 4L. Based on gene annotation, three nucleotide binding site–leucine-rich repeat genes present in the target region were cloned from *O. nivara* and sequenced. One of the loci, LOC_Os04g53050, had a 48-base-pair deletion in *O. nivara* accession IRGC 81825 compared to the cultivated rice (Bhasin *et al.*, 2012).

Kim *et al.* (2015) identified *Xa* 40 gene in the japonica advanced backcross breeding lines derived from the indica line IR65482-7-216-1-2 in the background of cultivar Junam and are resistant to all Korean BB races, including K3a. Genetic analysis using graphical mapping indicated that resistance (R) was controlled by a new resistance gene linked with the flanking markers RM27320 and ID55. WA18-5 located within an approximately 80-kb region between 28.14 and 28.22 Mbp on chromosome 11.

2.5. DNA MARKERS AND MARKER-ASSISTED SELECTION (MAS)

DNA markers or molecular markers are typically derived from a small region of DNA that show sequence polymorphism. DNA markers are tools for simultaneously advancing our understanding of plant genome and increasing the efficiency of plant breeding. They can be used to dissect the genetic basis of complex phenotypes in to Mendelian components in order to obtain information about gene dosage, epitasis, pleiotropy and genotype x environment interaction (Toojinda 1999). The use of DNA markers, which permit the genetic dissection of the progeny at each generation, increases the speed of selection process (Babu *et al.*, 2004). The closely linked DNA markers to the target genomic regions can be used to accelerate fixation of favourable alleles in the next selection step. Molecular markers could also increase the efficiency of backcrossing by allowing selection of genotype with the maximum percentage of recurrent parent genome (Hospital, 2005).Commonly four types of markers *i.e.* RAPD, RFLP, AFLP and SSR are used and implemented in rice genomics. However, RAPD and RFLP markers have limitations. RFLP using southern analysis is laborious, time-consuming, costly and involves the use of radiochemical, while SSR marker is simple, accurate, efficient, cost-effective, represent single-loci and can detect high levels of polymorphism (Babu *et al.*, 2004).

Simple sequence repeats (SSRs) are tandem arrays of two to six nucleotide base pair repeats that occur ubiquitously throughout the genome (Tautz 1989). The unique sequences flanking the SSR (also known as microsatellite) are generally conserved within a species. The flanking sequences 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).

Markers are used for selecting qualitative as well as quantitative traits. MAS can aid selecting for all target alleles that are difficult to assay phenotypically

especially in early generations, where breeders usually restrict their selection activities to highly heritable traits because a visual selection for complex traits like yield is not possible with only few plants per plot being available, MAS is said to be effective, cost- and time-saving (Eathington *et al.,* 1997). An important prerequisite for successful early-generation selection with MAS are large populations and low heritability of the selected traits, as under individual selection, the relative efficiency of MAS is greatest for characters with low heritability (Lande and Thompson, 1990).

MAS in rice breeding have mainly been utilized for the pyramiding of disease resistances, namely bacterial blight (Singh *et al.*, 2001) and blast (Narayanan *et al.*, 2004). In 2006, two rice lines showing strong submergence tolerance were developed by introgressing a locus conferring submergence tolerance from cultivar 'FR13A' into the variety 'Swarna' (Xu *et al.,* 2006).

MAS have been used to transfer favourable alleles of genes/QTL for biotic and abiotic stress resistance tolerance into the desired rice genetic background (Babu *et al.*, 2004 and Toojinda *et al.,* 2005). Gandhi (2007) crossed a deep rooted upland japonica rice variety from the Philippines with a high yielding Indica variety using MAS. The new variety MAS 946-1 consumes up to 60% less water than traditional varieties and became the first drought tolerant aerobic nee variety released in India.

Guangzhan63S (GZ63S) is an elite rice photoperiodic and thermo-sensitive male sterile (P/TGMS) line from which many widely cultivated two-line super-hybrid varieties in the Yangzi River basin have been derived. However, GZ63S is susceptible to rice blast as well as bacterial blight. The blast resistance gene *Pi*9 and the bacterial blight resistance gene *Xa23* in GZ63S and its derived hybrid (Liangyou6326) were pyramided by marker-assisted selection (MAS). The improved sterile line and its derived hybrid showed high levels of resistance to all eight tested *Xanthomonas oryzae* pv. *oryzae* strains (Ni *et al.,*2015) .

Naik *et al.* (2015) introgressed two resistance genes *Xa21* and *Gm4* into rice variety Akshaydhan by marker aided backcross with foreground selection scheme to speed up the process. Donor for gene *Xa*21 and *Gm*4 were used from B95-1/Abhaya(breeding line of Samba Mahsuri with *Xa*21 and *Gm*4) .

Hajira *et al.* (2016) developed simple PCR-based functional markers for BB resistance *xa13* and *xa5* genes. For *xa13*, they designed a functional PCR-based marker, xa13-prom targeting the InDel polymorphism in the promoter of candidate gene *Os8N3* located on chromosome 8 of rice. With respect to *xa5*, they also developed a multiplex-PCR based functional marker system, named xa5FM, consisting of two sets of primer pairs targeting the 2-bp functional nucleotide polymorphism in the exon II of the gene *TFIIA□5* (candidate for *xa5*), has been developed. Both xa13-prom and xa5FM can differentiate the resistant and susceptible alleles for *xa13* and *xa5*, respectively.

Soumya and Sindhumole (2016) collected forty traditional rice genotypes from the germplasm of Division of Plant Breeding and Genetics, Regional Agricultural Research Station, Pattambi, and initially screened in field for BB resistance. Out of them, only twenty genotypes which had either no or less symptoms of BB disease, were utilized for marker assisted selection. Marker assisted selection in rice germplasm accessions for *Xa5* gene, using RM122; a closely linked microsatellite marker was used. Five genotypes *viz.*, Karuthakuruka, Kuruva, Kokkankoli, Kochuvithu (Thamarakulam) and Punjaparathu had no symptoms of BB, indicating that BB resistance in these genotypes is due to the gene *Xa5*.

2.6. SCREENING FOR BB RESISTANCE IN RICE

Naveed *et al.* (2010) conducted a research on screening of BB resistance in rice varieties. They continued DNA analysis of 88 rice germplasm which included basmati breeding lines and different basmati varieties. The banding pattern of all the individuals were either identical with that of the IRBB-5 (having *xa5* gene) or

with that of the IR-24 (without *xa5*) gene. The size of the band corresponding to IRBB-5 was 240 bp whereas the band corresponding to IR-24 was 230 bp in size. During this polymorphic survey, out of 88 rice lines, 45 rice lines along with IRBB-5 amplified 240 bp size fragments indicating the presence of *xa5* gene.

Ramalingam *et al.* (2001) performed similar type of molecular survey for the presence of bacterial blight resistance genes *xa5, xa13* and *Xa21* in Chinese rice germplasm. They surveyed 56 germplasm, 23 genotypes were reported to carry allele 3, 30 genotypes had allele 2 and no allele was reported in one genotype.

Swamy *et al.* (2006) tested six distinct Indian isolates of *Xanthomonas oryzae* on rice genetic backgrounds carrying single or multiple BB resistance genes. Three BB resistance genes were introduced into a susceptible line, MH2R, by marker assisted selection, which resulted in a gain of resistance. In addition, Pusa Basmati 1 (PB1) transgenic lines carrying the BB resistance gene, *Xa21*, and IR72- *Xa21* transgenic lines were evaluated after inoculation with the six *Xoo* isolates. It was found that PB1 lines expressing the *Xa21* gene were susceptible to the six *Xoo* isolates.

Huang *et al.* (1997) used gene specific PCR markers for the identification of pyramided line in rice carrying different combinations of BB resistant genes. They obtained lines with different combination of genes, 2 lines for *Xa4*/*xa5*/*xa13*, 3 lines for *Xa4*/*xa5*/*xa21*, 3 lines for *Xa4*/*xa13*/*Xa21*, 3 lines for *xa5*/*xa13*/*Xa21* and 2 lines with all four genes (*Xa4*/*xa5*/*xa13*/*Xa21*). They also observed that *xa5* in any resistance genes combination showed resistant against six BB Philippine races.

Ijaz *et al.* (2008) revealed that twenty six cultivars of rice have two recessive genes for resistance to BB. One of these governs resistance to race 1 (PXO 61) while the other gene confers resistance to race 6 (PXO 99). One cultivar Pankiraj has three recessive genes for resistance, among which two convey resistance to race 1 and while third gene conveys resistance to race 6. Another cultivar Kalonchi has one dominant and one recessive gene for resistance to race 1 and

another recessive gene for resistance to Race 6. Allelism test with *Xa4* and *xa5* showed that the dominant and recessive genes of Kalonchi that confer resistance to race 1 are allelic to *Xa4* and *xa5* respectively. Similarly one of the recessive gene of Pankiraj and single recessive genes conferring resistance to race 1 in 22 cultivars are allelic to *xa5*.

Salgotra *et al.* (2012) screened 29 recombinants for BB resistant genes along with aroma (*fgr*) gene using a suite of different markers. Twenty genotypes were found in homozygous condition for *Xa*21 and *xa*13 genes. Restriction digestion analysis with *BsrI*, gave nine resistant recombinants, of which seven were in homozygous and two were in heterozygous conditions. Recombinants viz., IRS 5441-2-21, IRS 5441-2-79, IRS 5441-2-81, IRS 5441-2-85 and IRS 5441-2-91 possessed all the three BB resistance genes and *fgr* in the homozygous condition. Recombinants with enhanced resistance to BB, basmati quality and desirable agronomic traits were identified.

Hittalmani *et al.* (2013) evaluated landraces of rice for blast and bacterial blight (BB) resistance, via tightly linked SSR markers and by phenotyping for flowering time, maturity and grain yield. Marker RG64 on Chromosome 6 linked to *Pi*-2, a major dominant blast resistance gene and marker pTA248 on Chromosome 11 linked to *Xa*21, a resistant gene to bacterial leaf blight were used to detect the presence of resistant alleles. Three different types of bands of 1 kb carrying *Xa*21 resistant allele and two susceptible alleles of 700 and 750 bp were amplified using *pTA*248. Fourteen rice genotypes were resistant for BB, 46 genotypes showed susceptible banding pattern, and 87 genotypes were in heterozygous condition for resistance. Twenty eight genotypes carried resistant alleles for both blast and bacterial leaf among Gowri Sanna, Ponni, Antharsali and Doddabyranellu were popularly preferred by the farmers. These can serve as donor lines for transferring of both resistances simultaneously.

2.6.1 RACES AND GENES

Lore *et al.* (2011) classified *Xoo* population from the Punjab state into seven distinct pathotypes (PbXo- 1 to PbXo-7) by inducing differential reactions on a set of near-isoganic lines in the background of IR24 and some international, national and regional cultivars of rice. Known BB resistance genes (*Xa*1, *Xa*3, *Xa*10, *Xa*11, *Xa*14, *Xa*18) were ineffective, whereas *xa*13, *Xa*4 + *xa*13, *xa*5 + *xa*13, *xa*13 + *Xa*21, *Xa*4 + *xa*5 + *xa*13, *Xa*4 + *xa*5 + *Xa*21, *Xa*4 + *xa*13 + *Xa*21, *xa*5 + *xa*13 + *Xa*21 and *Xa*4 + *xa*5 + *xa*13 + *Xa*21 and rice line IET8585 / Ajaya were effective against all the seven pathotypes analysed. *Xa*21 was effective against all the pathotypes except PbXo-3 and PbXo-4. PbXo-7, the most dominant pathotype, was found to be virulent and induced susceptible/moderately susceptible reaction on 22 of the 40 test genotypes followed by PbXo-1, PbXo-5 and PbXo-6. The least virulent pathotype was PbXo-2.

Banito *et al.* (2012) inoculated 13 *X. oryzae* pv. *oryzae* strains on 21 rice varieties and near isogenic lines in screen house. The results revealed differential reactions of these genotypes in disease expression. AMMI analysis identified three groups of genotypes: resistant group made up of 12 lines IRBB1, IRBB2, IRBB3, IRBB4, IRBB7, IRBB8, IRBB10, IRBB11, IRBB13, IRBB14, IRBB21 and IR24, medium resistant group made up of three cultivars grown in Togo, NERICA4, NERICA8 and NERICA14, and one cultivar from Africa rice, Giganté, and susceptible group including five genotypes TGR203 and IR841 from ITRA, NERICA19 and TOG5681 from Africa rice, and the line IRBB5.

Debnath *et al.* (2013) collected six isolates of *Xoo* from different parts of West Bengal and screened against near isogenic lines of rice to identify variability in virulence. All the NILs were found to possess varying degree of susceptibility to resistance against all the isolates with significant differences in disease progress. The pyramided lines showed broad spectra of resistance against all the Xoo isolates and most durable resistant monogenes were *xa*5, *xa*13 and *Xa*21.

Yadav *et al.* (2013) screened 386 Indian rice germplasm lines including commercially cultivated rice varieties for the presence of four bacterial blight resistance genes *i.e. Xa4, xa5, xa13 and Xa21.* Two genotypes, Pusa 1460 (*xa13* and *Xa21*) and Pusa 1463-02-1-1 (*xa13* and *Xa21*) were shown to carry more than one BB resistance genes.

Purushothaman and Niza (2013) studied pathotype of 14 isolates of Kerala on six near isogenic lines and three rice differentials. The isolates *viz.*, Kodallur (XKOR-3) and Moncompu (XMOU-1) showed resistant reaction on all six isogenic lines *viz.*, IRBB-4 (*Xa4*), IRBB-5 (*xa5*), IRBB-13 (*xa13*), IRBB-21 (*Xa21*) IRBB-57 (*Xa4/xa5/Xa21*) and (*Xa4/xa5/xa13/Xa21*) the three rice differentials *viz.*, Ajaya, IR-8 and IR-24.

Mondal *et al.* (2014) collected 780 *Xoo* isolates from 13 different states of north-west and eastern India and was grouped into six races based on disease reaction with 12 near isogenic lines (NILs). Race 4 and 6 were predominant in north-western parts of India; while races 2 and 3 were present in the north-eastern hills and plains. The detailed analysis of disease reaction, revealed that race 1 and race 2 are less virulent compared to the other races. It is interesting to note the co-existence of both less virulent (like race 1) and more virulent races (like race 5) in the *Xoo* population in a particular location. Among the R-genes the most vulnerable are *Xa*1 and *Xa*2 showing susceptible reaction to all the races while *Xa*4, *Xa*11, *xa*13, and *Xa*21 were found to be stable showing resistant to moderately resistant type BB reaction.

Bharathkumar *et al.* (2014) analysed the virulence of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) in rice using 200 isolates on near isogenic lines (NILs). These isolates were assembled from major rice growing areas of Punjab, Haryana, Uttar Pradesh, Bihar, West Bengal, Odisha, Andhra Pradesh, Tamilnadu and Kerala state. From these isolates, 20 pathotypes (races) on NILs and 12 pathotypes on rice differentials were identified based on disease reaction. In this analysis, more number of pathotypes were identified in Bihar state (10

pathotypes) followed by Uttar Pradesh (7 pathotypes), Haryana, West Bengal, Andhra Pradesh, Kerala (6 pathotypes), Odisha (4 pathotypes), Punjab (3 pathotypes) and Tamilnadu (2 pathotypes). Among these, pathotype I was virulent to all rice cultivar harbouring single resistant gene (Xa4, xa5, Xa7, Xa10 and xa13) and it was found to be prevalent throughout India. Significantly, isolates of pathotype III prevailing in Haryana state were noted that they breakdown the resistance of IRBB21 (*Xa21*) and IRBB52 (*Xa4+Xa21*). The results revealed that almost all the isolates were able to overcome NILs which harbored a single BB resistance gene (*i.e.* IRRB4) or two BB resistance genes (*i.e.* IRBB52/DV85), and not those NILs (*i.e.* IRBB57-IRBB60), which harbored either a 3-gene/4-gene combinations.

Khoshkdaman *et al.* (2014) evaluated against BB on 12 near-isogenic lines and 14 pyramiding lines from International Network for Genetic Evaluation of rice (INGER) and 8 local and improved Iranian varieties under natural photoperiod condition in the field. Infection levels of pyramiding lines containing two to five resistance genes, IRBB53 and IRBB61 with resistance gene combination, *Xa5 + Xa13 and Xa4 + Xa5 + Xa7,* respectively were not so clear. Among near-isogenic lines IRBB1, IRBB2, IRBB4 and IRBB10 carrying resistance gene *Xa1, Xa2, Xa4* and *Xa10* were susceptible; IRBB8, IRBB11, IRBB3, IRBB5 and IRBB13 were moderately susceptible; (having resistance gene *Xa8, Xa11, Xa3, Xa5* and *Xa13*) IRBB14, IRBB21 and IRBB7 with respectively resistance gene *Xa14, Xa21* and *Xa7* were moderately resistance to bacterial blight.

Amgai *et al.* (2015) screened ninety six Nepalese rice accessions using eight Simple Sequence Repeats (SSR) markers and one Sequence Tagged Sites (STS) marker for presence and absence of BB resistance gene. They detected BB resistance gene *Xa-10* on five accessions, *Xa-13* on six accessions, *Xa-7* on 23 accessions, *Xa-3* and *Xa-4* on 52 accessions, *Xa-5* on 25 accessions, *Xa-8* on 30 rice accessions. None of the rice accessions tested has *Xa-21.* Presence of *Xa-13*

on susceptible check variety CNTRL-85033 confirmed that this resistant gene is not working in Nepalese rice field.

Singh *et al.* (2015) conducted an experiment during the 2013 monsoon season to screen 35 wild rice accessions against the *BX043* strain of *Xoo* and identify the presence of bacterial blight resistance genes *Xa21*, *xa13*, *xa5*, *Xa4*, and *Xa2*. On the basis of disease severity 11 accessions showed moderate resistance, 21 were moderately susceptible, and 3 accessions showed susceptible response to the *BX043* strain of *Xoo*. While none of the accessions were found to be resistant. The genetic frequency of the 5 resistance genes varied from 00.00% to 45.71%. The accession NKSWR-25 harboured 3 resistance genes, *xa5*, *Xa4*, and *Xa2*, while accessions NKSWR-16, NKSWR-32, NKSWR-36, NKSWR-41, NKSWR-42, NKSWR-53, NKSWR-64, NKSWR-97 and NKSWR-99 each possessed 2 resistance genes of *xa5*, *Xa4*, and *Xa2*.

2.7. GENE PYRAMIDING FOR BACTERIAL BLIGHT RESISTANCE

Pyramiding of multiple resistance is an effective solution to resistance breakdown .The probability of simultaneous pathogen mutations for virulence to defeat two or more effective genes is much lower than with a single gene (Mundt, 1990).When two or more genes are introgressed, phenotypical evaluation is unable to distinguish the effect of individual gene precisely since each gene confers resistance to and combats multiple races of the pathogen. Moreover, in the presence of a dominant and a recessive allele, the effect of the recessive gene is masked. The success of BB gene pyramiding using MAS has been reported from several breeding programs.

Huang et *al.* (1997) used marker-assisted selection to pyramid four bacterial blight resistance genes *Xa-4, xa-5, xa-13* and *Xa-21.* They developed PCR markers for the two recessive genes *xa-5* and *xa-13* to speed up the gene pyramiding process and to facilitate future marker aided selection and used these to survey a

range of rice germplasm. The pyramided lines showed a wider spectrum and a higher level of resistance than lines with only a single gene.

Sanchez *et al.* (2000) used STS marker-assisted selection to improve the resistance of the new plant type (NPT) rice lines IR65598-112, IR65600- 42 and IR65600-96 with high yield potential to bacterial blight. Sequence tagged site (STS) markers for the two resistance genes were developed based on DNA sequence linked with restriction fragment length polymorphism (RFLP) markers (RG556 and RG207 for *xa5* and RG136 for *xa13).* Marker polymorphism for *xa5* was detected after digestion of RG556 PCR products with *Mae II* enzyme and digestion of RG136 PCR product with *Hinf 1* enzyme for *xa13.*

Singh *et al.* (2001) carried out pyramiding of three bacterial blight resistance genes (*xa5, Xa13 and Xa2l*) using marker assisted selection into indica rice cultivar PR106 widely grown in Punjab, India. Lines of PR106 with pyramided genes were evaluated after inoculation with 17isolates of the pathogen from the Punjab and six races of *Xoo* from the Philippines. Genes in combinations were found to provide high level of resistance to the predominant, *Xoo* isolates from the Punjab and six races from the Philippines.

Joseph *et al.* (2004) combined the important basmati quality traits with resistance to bacterial blight by a combination of phenotypic and molecular marker assisted selection. The two- gene pyramid line IRBB55 carrying *xa13* and *Xa21* was found equally effective as three/four gene pyramid lines. The two BB resistance genes present in IRBB55 were combined with the basmati quality traits of Pusa Basmati-1(PB-1) used as recurrent parent.

Bacterial blight resistance genes *Xa21* and *Xa4* from IRBB60 were introgressed into a hybrid rice line, Shunhui527 using MAS. The improved lines expressed high level of resistance to the *Xoo* strain CI-C VIII (Haung *et al.,* 2003). In addition, *Xa21* and *Xa7* were pyramided into Minghui 63 background and the improved lines showed high level of resistance to BB (Zhang *et al.,* 2006).

Moreover, TGMS rice was introgressed with *Xa4*, *Xa7* and *Xa21* for the development two-line hybrids. The plants carrying gene combinations exhibited highly resistance phenotype (Perez *et al.*, 2008).

Sundaram *et al.* (2008) used PCR based molecular marker in a backcross breeding program to introgress three major BB resistance genes (*Xa21, xa13* and *xa5*) into Samba Mahsuri from a donor line (SS111 3) in which all the three genes were present in a homozygous condition. At each backcross generation, markers closely linked to the three genes were used to select plants possessing these resistance genes (foreground selection) and microsatellite markers polymorphic between donor and recurrent parent were used to select plants that have maximum contribution from the recurrent parent genome (background selection).

Zhou *et al.* (2009) reported the development of a high yielding elite line, Lu-You-Zhan highly resistant to both bacterial blight (BB) and bacterial leaf streak (BLS). *Xa*23 with a wide-spectrum resistance to BB derived from wild rice and a non host maize resistance gene, Rxo1 was pyramided using both marker assisted selection (MAS) and genetic engineering.

Basavaraj *et al.* (2010) carried out the introgression of BB resistance genes *xa13* and *Xa21* into Pusa6B and PRR78 (the maintainer parent and the restorer parent of the hybrid Pusa RH10) using a marker assisted backcross breeding program. They were able *to* recover the recurrent parent genome ranging from 85.14 to 97.30% and 87.04 to 92.81% in the 10 best BC_2F_5 families of Pusa 6B and PRR78, respectively.

Kottapalli *et al.* (2010) introgressed three bacterial blight resistance genes, *xa*5, *xa*13 and *Xa*2l into a fine grain rice variety, Samba Mahsuri, using sequence tagged site (STS) markers. They adopted four different pyramiding schemes to minimize loss of recessive resistance genes in advanced backcross generations.

Bharani *et al.* (2010) pyramided BB resistance genes (*Xa*21, *xa*l3 and *xa*5) through molecular marker assisted selection into high yielding susceptible rice

cultivars ADT43 and ADT47. The genotypes with different resistance gene combinations were challenged with two *Xoo* isolates under field conditions. Plants with both dominant and recessive gene in homozygous condition either *Xa*21/*xa*13 or *Xa*2I/*xa*5 combinations were found to be resistant.

Rajpurohit *et al.* (2010) carried out the pyramiding of bacterial blight resistance genes *Xa*2l and *xa*13 along with the semi dwarfing gene sd-1 in the traditional Indian basmati rice cultivar Type 3 Basmati and marker assisted background profiling of selected BC_2F_3 progenies using rice SSR and ISSR markers. They found that the pyramided lines were BB resistant and had excellent cooking quality and strong aroma.

Huang *et al.* (2012) introgressed four bacterial blight ressitance genes *Xa7, Xa21, Xa22 and Xa23* into elite hybrid restorer line Huahui 1035 by marker assisted selection. Ten promising BB resistant lines were identified in Huahui 1035 restorer background and their respective F_1 hybrids with a cytoplasmic male sterile line *i.e.* Jinke1A.

Tabanao *et al.* (2013) determined the presence of introgressed resistance genes in improved parent lines and assess their genetic similarity to original Mestizo 1 and Mestiso 3 parents, and evaluate disease resistance and agronomic performance of the improved hybrids. With target gene selection, 21 backcross lines were confirmed to have 2-3 *Xa* genes, exhibiting high levels of resistance. Most of these improved maintainer and restorer lines had recovered at least 80% of the recurrent parent genome based on genome-wide marker assay using simple sequence repeats.

Guvvala *et al.* (2013) pyramided four resistance genes (*Xa4, xa5, xa13* and *Xa21*) into popular cv. Mahsuri and two hybrid rice parental lines, PRR78 and KMR3 using marker assisted back cross breeding. Nine pyramid families of each background were evaluated under both artificial and natural disease conditions. Pyramids were inoculated with sixteen *Xanthomonas oryzae* pv.*oryzae* isolates

collected from different parts of India. BF16 was highly virulent on recurrent parents with a yield loss of 23% in Mahsuri, 28% in PRR78 and 24% in KMR3. BF7 (10% loss in Mahsuri and 13% in KMR3) and BF10 (22% in PRR78) were least virulent.

Dokku *et al.* (2013) transferred three resistance genes i.e. *xa*5, *xa*13 and *Xa*21 from IRBB 60 through marker-assisted backcross breeding to supplement the *Xa*4 gene present in rice variety Tapaswini. The precise transfer was aided through effective foreground selection using STS markers RG 556, RG 136 and pTA248 linked to the three target genes. Background selection based on morphological and grain quality traits and SSR markers led to the recovery of 85–96 % of the recurrent parent genome in the gene pyramids. In the screening assays, the gene pyramids having four genes exhibited higher level of resistance against the disease and resulted in identification and release of CRMAS-2622-7-6(IT-6) with pyramided genes as improved Tapaswini.

Suh *et al.* (2013) developed three elite advanced backcross breeding lines (ABL) of a BB-susceptible elite japonica rice cultivar, Mangeumbyeo, with three resistance genes by foreground and phenotypic selection from an indica donor (IRBB57) using a marker-assisted backcrossing (MAB) breeding strategy. The background genome recovery of the ABL expressed more than 92.1% using genome wide SSR marker analysis.

Rekha (2013) introgressed the BB resistance genes into rice variety CSR-30 from donors Pusa Basamati-1460 (having genes *Xa 21* and *xa13* and IRBB-60 having genes *Xa 21, xa13* and *xa* 5) by using marker-assisted selection. Foreground selection was carried out in F_1 plants of both the crosses using specific STS markers pTA 248, RG136 and RG-556 linked *Xa21, xa13* and *xa*5 genes respectively. In foreground selection 10/250 BC_1F_2 plants were found to have all the three resistance genes. The background genome recovery ranged from 44.2-78.9%. The pyramided lines exhibited very high level of resistance when inoculated *Xanthomonas oryzae* strain.

Hari *et al.* (2013) introgressed a major dominant gene conferring resistance against BB (*i.e. Xa*21) and blast (*i.e.Pi*54) into IR 58025B. An introgression line of Samba Mahsuri (*i.e.* SM2154) possessing *Xa*21 and *Pi*54 genes in homozygous condition and fine-grain type were used as donor parent and backcross breeding strategy was adopted for targeted introgression of the resistance genes. At BC_2F_5, four backcross-derived lines possessing resistance against BB and blast, devoid of aroma, high yield, short plant stature, long-slender grain type and with recurrent parent genome recovery ranging from 88.8% to 98.6% were selected and advanced for further evaluation. The improved versions of IR 58025B, *viz.*, SB54-11-143-9-44-5, SB54-11-143-9-44-98, SB54-11-143-9-44-111 and SB54-11-143-9-44-171 behaved as perfect maintainers when test crossed with WA-CMS lines.

Win *et al.* (2013) used high yielding Manawthukha rice line 'Yn 3248-2-128-76-4-3-75' (MK-75) with fragrance and intermediate amylose content as a recipient parent to improve its resistance to BB. RGDU-07097-1-8M-9 (RG-9), an improved line carrying xa5, *Xa21* and *xa33* were used as the donor parent. Resistant alleles of *xa5, Xa21* and *xa33* from RG-9 were successfully transferred into MK-75 by using marker-assisted backcrossing (MAB) method while maintaining the cooking quality of MK-75. Twenty eight selected BC_3F_2 introgression lines (MK-75 ILs) carrying different combinations of these three loci were tested for BB resistance at seedling and maximum tillering stages against ten Thai and five Myanmar Xoo strains. The triple and double resistance gene-introgressed lines (*xa5, Xa21, xa33 or xa5, Xa21 or xa5, xa33 or Xa21, xa33*) had higher level of resistance and wider resistance spectrum than MK-75 against both Thai and Myanmar *Xoo* strains.

Magar *et al.* (2014) introgressed BB resistance genes viz., *xa13* and *Xa21* from B95-1 to popular high yielding and fine grain rice variety, MTU1010 (Cottondora Sannalu).They verified for the presence of target genes by using gene linked primers viz., *xa13* promotor and pTA 248. The F_1 plants confirmed as true hybrids for both the genes were advanced to F_2 generation and foreground

selection was done using gene linked markers. Genetic analysis in F_2 populations confirmed that the genes (*xa13* & *Xa21*) governing BB resistance followed Mendelian inheritance.

Pradhan *et al.* (2015) employed to transfer three major BB resistance genes (*Xa21, xa13* and *xa5*) into Jalmagna variety. During backcross generations, markers closely linked to the three genes were used to select plants possessing these resistance genes and markers polymorphic between donor and recurrent parent were used to select plants that have maximum contribution from the recurrent parent genome. A selected BC_3F_1 plant was selfed to generate homozygous BC_3F_2 plants with different combinations of BB resistance genes.

Park *et al.* (2015) used Unkwang carrying *Xa3* as a recurrent parent and SR30075 carrying *Xa4+xa5+Xa21+Stvb-i* as a donor parent. RL-1(Resistant Line, BC_1F_7), RL2, RL3, RL4, and RL5 (BC_2F_6) were bred through bio-assay of K3a race inoculation and phenotypic selection of agronomic traits. The presence of introduced genes was confirmed by testing the resistance levels against bacterial blight and rice stripe virus and then double-checked by using DNA marker. RL1 has all target genes, *Xa3+xa5+Xa21+Stvb-i.* RL2, RL3, and RL5 have *Xa3+Xa21+Stvb-i* whereas RL4 has only *Xa21.* The combinations of bacterial blight resistant genes (*Xa3+xa5+Xa21 and Xa3+Xa21*) were found to be promising, as the rice lines carrying these genes enhanced a strong resistant reaction against 16 bacterial blight isolates.

Abhilash *et al.* (2016a) introgressed two major genes, viz., *Xa*21 and Pi54 conferring resistance against BB and blast, respectively into RPHR-1005, the male parent of DRRH3 through marker assisted backcross breeding (MABB) 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 four major resistance genes (i.e., *Xa21* and *Xa33* for BB resistance and *Pi2*and*Pi54* 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. At BC_2F_2, plants possessing the gene combination *Xa21+Pi2, Xa21+Pi54* and *Xa33* in homozygous condition and with >92% recovery of the recurrent parent genome (RPG) were identified and intercrossed to combine all the four resistance genes. Twenty two homozygous pyramid lines of RPHR-1005 comprising of three single-gene containing lines, six 2-gene containing lines ,eight 3-gene containing lines, and five 4-gene containing lines were identified among the double intercross lines at F_3generation ($DICF_3$). They were then evaluated for their resistance against BB and blast, fertility restoration ability and for key agro-morphological traits. While single gene containing lines were resistant to either BB or blast, the 2-gene, 3-gene, and4-gene pyramid lines showed good level of resistance against both and /or either of the two diseases. Most of the 2-gene, 3-gene, and 4-gene containing pyramid lines showed yield levels and other key agro morphological and grain quality traits comparable to the original recurrent parent and showed complete fertility restoration ability, with a few showing higher yield as compared to RPHR-1005.

Ellur *et al.* (2016a) was employed to incorporate the blast resistance, Pi2 and Pi54 and bacterial blight (BB) resistance genes *xa*13 and *Xa*21 into the genetic background of Pusa Basmati 1121 (PB1121) and Pusa Basmati 6. Foreground selection for target gene(s) was followed by arduous phenotypic and background selection which fast-tracked the recovery of recurrent parent genome (RPG) to an extent of 95.8% in one of the near-isogenic lines (NILs) namely, Pusa 1728-23-33-31-56, which also showed high degree of resemblance to recurrent parent, PB6 in phenotype. PB1121-NILs and PB6-NILs carrying BB resistance genes *xa*13+*Xa* 21 were resistant against *Xanthomonas oryzae* pv. *oryzae* races of north-western, southern and eastern parts of the country.

Ellur *et al.* (2016b) reported that PB1121 is highly susceptible to bacterial blight (BB) disease. A novel BB resistance gene *Xa38* was incorporated in PB1121 from donor parent PR114-*Xa38* using a modified marker-assisted

backcross breeding (MABB) scheme. The NILs of PB1121 carrying *Xa38* were compared with PB1121 NILs carrying *xa13* + *Xa21* for their resistance to BB. Both NILs showed resistance against the Xoo races 1, 2, 3 and 6. Additionally, *Xa38* also resisted *Xoo* race 5 to which *xa13* + *Xa21* was susceptible. The PB1121 NILs carrying *Xa38* gene will provide effective control of BB in the Basmati growing region.

Srikanth *et al.* (2016) introgressed three major resistant genes (i.e., *Xa21* and *xa13* for BB resistance and *Pi54* for blast resistance) into Vallab Basmati 22 through the strategy of marker-assisted breeding (MAB) using Improved Samba Mahsuri (possessing *Xa21* + *xa13*) and Tetep (possessing *Pi 54*) as the donor parents through two sets of crosses. At each generation, plants possessing *Xa21* and *xa13* or *Pi54* in heterozygous condition were identified from the two crosses with help of gene-specific markers through foreground selection. At each generation, plants possessing *Xa21* + *xa13* + *Pi54* in homozygous condition were identified with the help of gene specific markers and advanced further through selfing. At ICF_4, four promising three-gene pyramid lines of Vallabh Basmati22 possessing high level of resistance against both BB and blast along with high yield and grain type similar to the recurrent parent have been identified.

Table 1 List of genes identified for bacterial blight resistance

Gene	Chr.	Resistance source	Cultivar/Variety	Isolate/race	Reference
Xa1	4	-	Kogyoku Koganemaru Pi No.1	Japanese race 1 and II	Sakaguchi, 1967
Xa2	4	Temperate japonica	Tetep	Japanese race I and II	He *et al.* 2006
Xa3	11	Indica	Wase Aikoku Chukogu-45 IR20. IR22	Japanese race and III	Ezuka *et al.* 1975
Xa4	11	Japonica	1R1529-680-3 TKM6	Philippine race I	Petpisit *et al.*1977
xa5	5	Indica	IRI545-248, BJ-1, IR29I-7, DV85	Japanese races	Petpisit *et al.* 1977
xa5(t)	5	Aus	Ajaya	Indian races	Rao, 2003
Xa6	ND	-	Malagetsunsong IR994102.IRI698-24, Zenith	Philippine race I	Sidhu *et al.* 1978
Xa7	6	Aus	DV85, DV87	Philippine race I	Sidhu *et al.* 1978
xa8	7	-	PI23I129	Philippine isolates	Sidhu *et al.* 1978
xa9	11	-	Sateng	Philippine isolates	Singh *et al.* 1983
Xal0	11	-	CAS209	Japanese isolates	Yoshimura *et al.* 1983
Xa11	3	Indica	IR8, RP9-3	Japanese isolates	Ogawa and Yamamoto, 1986

Table 1. (Continued....)

Gene	Chr.	Resistance source	Cultivar/Variety	Isolate/race	Reference
Xa12	4	Japonica	Java 14	Japanese and Indonesian isolates	Ogawa *et al.* 1978
xa13	8	-	BJ1,AC191-1, AUS274-1	Philippine race VI	Zhang *et al.* 1996
Xa14	4	Japonica	TN1	Japanese isolates	Kurata and Yamazaki 2006
xa15	ND	-	M41	Japanese isolates	Nakai *et al.* 1988
Xa16	ND	Indica	Tetep and IR24	Japanese isolates	Kurata and Yamazaki, 2006
Xa17	ND	Japonica	Asominori	Japanese race II	Kurata and Yamazaki, 2006
Xa18	ND	Indica, Japonica	Toyonishiki	Burmese isolates	Ogawa and Yamamoto, 1986
xa19	ND	-	XM5	Japanese isolates	Kurata and Yamazaki 2006
xa20	ND	-	XM6	Japanese isolates	Kurata and Yamazaki 2006
Xa21	11	Wild spp of *Oryza*	*0. longtstaminiita*	Philippine and Japanese isolates	Khush *et al.* 1989
Xa22(t)	11	-	Zhachanglong	Chinese isolates	Wang *et al.* 2003
Xa23	11	Wild spp of *Oryza*	*0. nivaraO. rufipogon*	Indian isolates PX099	Zhang *et al.* 2001
xa24	2	-	DV85. DV86 Aus29	Philippine races 4, 6,10	Khush and Angeles, 1999
Xa25	12	Indica	Minghui63	Philippine race	Chen *et al.* 2002
Xa26	11	Indica	Nep Bha Bong To Minghui63	Philippine race 1,2,3,4 and 5	Sun *et al.* 2004

Table 1. (Continued....)

Gene	Chr.	Resistance source	Cultivar/Variety	Isolate/race	Reference
Xa27	6	Wild spp of *Oryza*	O. minuta, Arai Raj	Philippine race 2	Gu *et al.* 2004
Xa28	ND	Indica	Lota Sail	Philippine race 2	Lee *et al.* 2003
Xa29	1	Wild spp of *Oryza*	*O. officinalis*	-	Tan *et al.* 2004
Xa30(t)	11	Wild spp of *Oryza*	CBB30	Phillipine race 6	Jin *et al.* 2007
Xa31(t)	4	-	Zhachanglong	PXO61 and OS105	Wang *et al.* 2009
xa32(t)	6	Wild spp *Oryza*	*O. barthii*	-	Zheng *et al.* 2009
Xa33	7	Wild spp of *Oryza*	*O. nivara*	Indian isolates	Kumar *et al.* 2012
xa33(t)	6	Indica	Ba7	Thailand	Korinsak *et al* . 2009
xa34(t)	1	*AUS*	*BG 1222*	Chinese races	Chen *et al.* 2011
Xa35(t)	11	Wild spp *Oryza*	*O .minuta*	Philippines	Sibin *et al.* 2010
Xa36(t)	11	-	C4059	China	Lili *et al.* 2010
Xa38	4	Wild spp *Oryza*	*O. nivara* IRGC81825,	Indian isolates	Bhasin *et al.* 2012
Xa39(t)	11	Indica	FF329	Philippines PXO349	Zhang *et al.* 2014
Xa40	11	Indica	IR65482-7-216-1-2	Korean isolates	Kim *et al.* 2015

ND- Not Determined

Materials and Methods

3. MATERIALS AND METHODS

The research pertaining to "Pyramiding Bacterial Leaf Blight resistance genes into popular rice varieties of Kerala through marker assisted selection" was undertaken at the College of Agriculture, Vellayani, Thiruvananthapuram and Rice Research Station Moncompu, Alappuzha, Kerala.

The study involved utilization of both classical genetics as well as modern molecular tools. The materials and methods used for these studies are described below.

3.1 PLANT MATERIAL

Breeder seeds of two traditional high yielding popular rice varieties Aiswarya and Prathyasa were collected from Regional Agricultural Research Station, Pattambi, Kerala Agricultural University (KAU), Palakkad, Kerala and Rice Research Station, Moncompu, KAU, Alappuzha, Kerala respectively (Table 2 and Plate 3A). Seeds of donor parents such as Improved Samba Mahsuri (pyramided with *xa13* and *Xa21*), Improved Samba Mahsuri (pyramided with *Xa33*) were collected from IIRR (Indian Institute of Rice Research) Hyderbad and PR-114(pyramided with *Xa*38) from Dr. Kuldheep Singh, PAU, Ludhiana (Table 3 and Plate3B).

3.2 MOLECULAR MARKERS

Reported specific DNA markers closely linked to the BB resistance genes viz., *Xa21, xa13, Xa33* and *Xa38* were used. The sequence and annealing temperature of each marker is given in Table 4. The pTA248 marker at a genetic distance of 0.2 cM from *Xa21* (Ronald *et al.,* 1992), The marker RMWR7.1 was located at a genetic distance of 0.9 cM on one side and the marker RMWR7.6 at a distance of 1.2 cM on the other side flanking the BB resistance gene, *Xa33.* (Kumar *et al.,* 2012). The marker Os04g53050-1 at a genetic distance of 38.4-kb located region on the chromosome 4L and linked to *Xa*38 (Bhasin *et al.,* 2012).

Table 2. Popular varieties used as recipients in the study

Sl.No	Recipients	Source	Characters
1.	Aiswarya(PTB52)	Regional Agricultural Research Station, Kerala Agricultural University (KAU), Pattambi	Semidwarf, Short duration,Medium tillering with medium bold red kernels
2.	Prathyasa(MO21)	Rice Research station KAU, Moncompu, Alappuzha, Kerala	Medium duration, Medium tillering with Red long bold kernels

Table 3: Donors of genes for resistance to BB

Sl.No	Donors	Resistance Source	Genes	Chr.	Reference
1	Improved Samba Mahsuri (BPT5204)	SS1113	*xa13*	8	Sundaram *et al.*2011
			Xa21	11	Ronald *et al.*1992
2	Improved Samba Mahsuri(Fbr-1-1)	IRGC105710	*Xa33*	7	Kumar *et al.* 2012
3	PR-114	IRGC 81825	*Xa38*	4	Bhasin *et al.*2012

Plate 3. Recipients and Donors used for the study

A.Recipients

B.Donors

Table 4. Details of marker used for evaluation of BB resistance and linkage to bacterial blight resistance genes.

Gene	Marker	Sequence	Annealing temperature(Tm)
Xa21	pTA 248 F	5'AGACGCGGAAGGGTGGTTCCCGGA3'	55°C
	pTA248R	5'AGACCGGTAATCGAAAGATGAAA 3'	
xa13	xa 13 proF	5' GGCCATGGCTCAGTGTTTAT 3'	55°C
	xa 13proR	5' GAGCTCCAGCTCTCCAAATG 3'	
Xa33	RMWR7.1F	5'TTTTATCCCCTTCTTCCTTC3'	56°C
	RMWR7.1 R	5'CGTGTTTTGTGTGTCTTTTG3'	
	RMWR7.6F	5'CAACAAACACCTCCATGGTC3'	
	RMWR7.6R	5'GGGAATGAGCAAAAATTGG3'	
Xa38	Oso4g53050-1 F	5'TCTTCTATTGCTAACATTGGTG3'	56°C
	Oso4g53050-1R	5'TCGCATTCATTTTCAGAG3'	

The markers used in this study were obtained from the Sigma-Aldrich Chemicals Bangalore.

3.3 ISOLATION OF GENOMIC DNA

Genomic DNA from these accessions were isolated using the procedure of QIAGEN DNeasy plant mini kit. Samples were disrupted (≤100mg wet weight or ≤ ≥20mg lyophilized tissue) using the mortar and pestle with liquid nitrogen. 400µl of buffer AP_1 and 4µl of RNAse A were added, vortexed and incubated for 10min at 65°C. The tube was inverted 2-3 times during incubation. 130µl buffer P3 was added and mixed and incubated for 5 min on ice. The lysate was centrifuged for 5 min at 20,000 x g (14000rpm), the lysate was pipetted into a QIA shredder spin column placed in a 2ml collection tube and centrifuged for 2 min at 20,000 x g. The flow–through was transferred into a new tube without disturbing the pellet if present. 1.5 volumes of buffer AW1 was added by pipette and mixed well. Then 650 µl of the mixture was transferred into a DNeasy mini spin column placed in a 2 ml collection tube and centrifuged for 1 min at ≥ 6000 x g (≥8000rpm).The flow –through was discarded and this step with the remaining sample were repeated. The spin column was placed into a new 2 ml collection tube, 500 µl Buffer AW2 were added and centrifuged for 1 min at ≥6000 x g. The flow through was discarded, another 500 µl Buffer AW_2 were added and centrifuged for 2 min at ≥20000 x g. The spin column was transferred to a new 1.5ml or 2ml micro centrifuge tube and 100 µl Buffer AE was added for elution. Then Incubated for 5 min at room temperature (15-25° C) and Centrifuged for 1 min at ≥6000 x g. These DNA samples were stored at -20°C.

3.4 AGAROSE GEL ELECTROPHORESIS

Stock solutions

50X TAE Buffer

Tris base	240g
Acetic acid	57.1ml
0.5M EDTA (pH-8.0)	186.12g
Final volume (Distilled H_2O)	1000ml

6X loading dye

Sucrose	4.0g
Bromophenol blue	0.025g
Volume (Distilled H_2O)	10ml

(Loading dye solution was stored at 4°C)

Agarose gel electrophoresis was carried out in a BIO-SYS, horizontal gel electrophoresis Unit. Agarose (0.8g) was weighed and melted in 1x TAE buffer. After cooling the solution to 42-45°C, ethidium bromide was added at the rate of 3µl for 100ml. The solution was then poured on to a preset, sealed gel casting tray with a comb fixed in position, to a height of 3mm-5mm. The gel was allowed to solidify for 15-20 min. The comb and sealing tapes were then removed and tray was submerged in electrophoresis tank filled with 1x TAE buffer ensuring that the buffer covered the gel to height of 1mm. Required volume of DNA sample and loading dye [glycerol 30% + bromophenol blue] were mixed in the ratio 5:1 and loaded into the slots of gel using a micropipette near the negative terminal. The cathode and anode of the electrophoresis unit were attached to the power supply and a constant voltage of 60V was used for the run. The power was turned off when the loading dye moved about $3/4^{th}$ of the gel. The gel was documented using SYNGENE gel documentation system.

3.5 QUANTIFICATION OF DNA

After ensuring the presence of DNA in samples by electrophoresis the quality and quantity of DNA was measured as follows

5µlof DNA dissolved in 0.1X TAE was added to 3ml of distilled water and read against distilled water used as blank at an absorbance of 260 nm and 280 nm, in an UV spectrophotometer. The concentration of DNA in sample was calculated using the formula;

$$\text{Amount of DNA (ng/ml)} = A260 \times \frac{\text{Volume of dist.water (µl)} \times 0.05 \times 1000}{\text{Amount of DNA (µl)}}$$

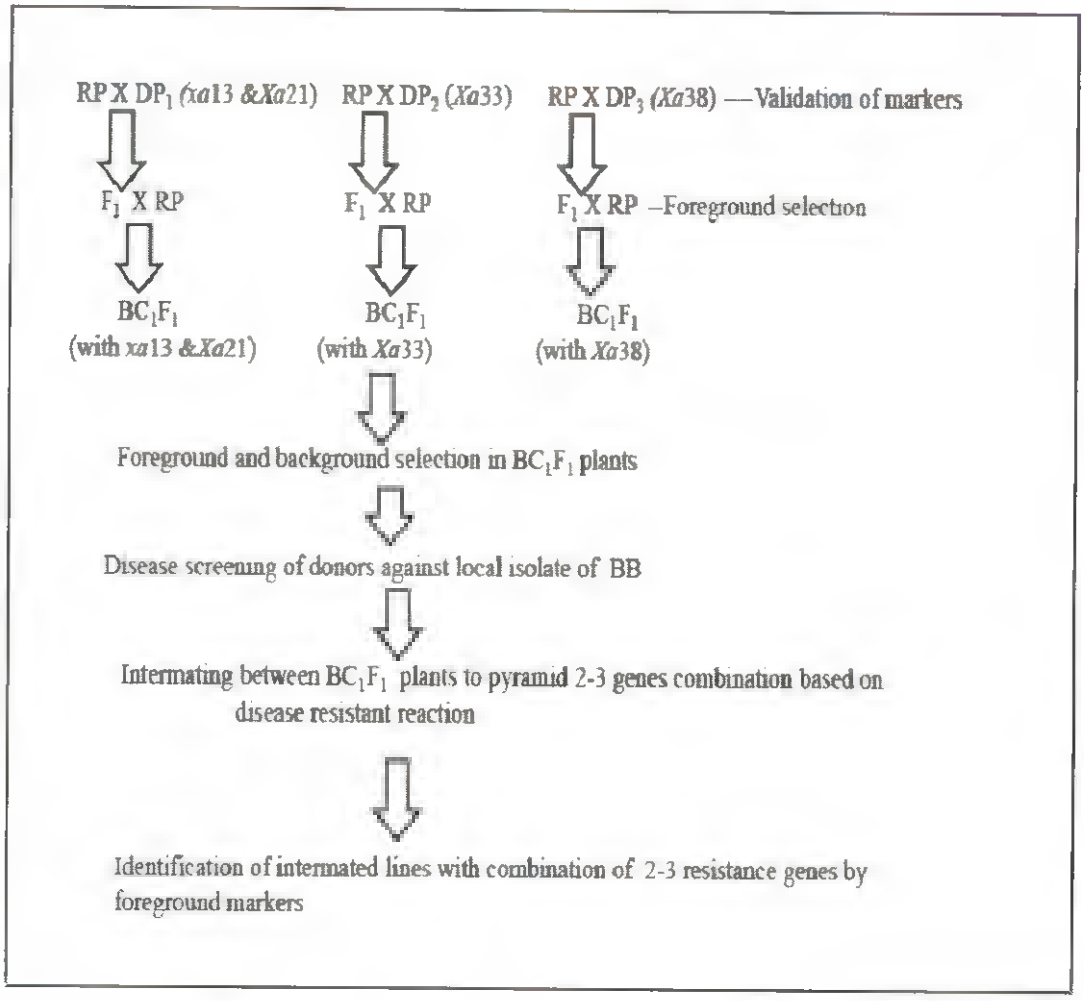
Where, A260 = Absorbance at 260nm.

The quality of DNA was judged from ratio of absorbance values at 260 nm and 280 nm. A ratio of 1.8-2.0 indicated best quality of DNA.

3.6 VALIDATIONS OF THE MICROSATELLITE MARKERS FOR BB RESISTANCE GENES

The annealing temperature of each primer was standardized by using gradient PCR as given in Table 4.

The PCR mixture contained 50 ng template DNA, 10 pmoles of each primer, 10 mM dNTPs, 10X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$ and 0.01 mg/ml gelatin) and 1 U of Taq DNA polymerase in a reaction volume of 25 µl. Amplifications were carried out in an Eppendorf master cycler nexus gradient PCR. Template DNA was initially denatured at 94°C for 5 min followed by 35 cycles of PCR amplification with the following parameters: a 30sec to 1 min denaturation at 94°C, a 30sec to 1 min annealing at 55°C , 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, RMWR 7.6 and Oso4g53050-1). After completion of amplification, PCR products were stored at –20°C. The amplified product was



RP=Recurrent parent DP=Donor parent

Fig.1. Schematic diagram of crossing and selection for BB resistance genes

electrophoretically resolved on a 2% agarose gel containing 0.5 µg/ml of ethidium bromide in 1x TBE buffer and visualized under SYNGENE G-Box F3gel documentation unit.

3.7 INITIAL HYBRIDIZATION BETWEEN TWO RECURRENT PARENTS AND THREE DONOR FOR THE TRANSFER OF RESISTANT GENES

Initial hybridization using the two recurrent parents and three donors (Table 2 and 3) was undertaken at Department of Plant breeding and Genetics, College of Agriculture, Vellayani as described below.

3.7.1 Raising of Parents

Recipient/Recurrent (Female) parents *viz*., Aiswarya and Prathaysa used in the hybridization programme were medium to short duration varieties and the donors used, *viz*., Improved Samba Mahsuri (*xa13* and *Xa21*), ISM (*Xa33*) and PR-114(*Xa38*), were long duration varieties. These varieties were raised in the nursery by staggered sowing for obtaining synchronization of flowering. Seedlings of these varieties were transplanted to main field, when they attended the maturity of 21 days.

3.7.2 Hybridization Programme

The recipient female plants were uprooted in series from the main field at boot leaf initiation stage and planted in pots filled with wetland soil. After the plants had established in the pots the crossing was conducted as follows:

Emasculation was done in the recipient parents by the clipping method. On the previous day evening, top 1/3 portion in each spikelet was clipped-off in a slanting position using scissors. Then six anthers in each spikelet were removed with the help of the needle. The entire process of emasculation was done carefully without causing any damage to the gynoecium. After emasculation, the panicles

were moistened to maintain receptivity of stigma until the period of pollination, which is conducted on the next day morning. The emasculated spikelets were then covered with butter paper bag in order to prevent contamination from any foreign pollen.

Crossing between the emasculated recipient and the donor parent was done on the next day morning as follows:

At 9.00AM, the bloomed panicle from the male parent was collected and dipped in a glass containing water, for 1-2 minutes and was exposed to 200 watt bulbs inside the pollination chamber. After half an hour, spikelets of these panicles started exerting their anthers. The pollen from exerted anthers were dusted on the emasculated spikelets of the recipient female parents. After conducting the pollination, the appropriate information pertaining to pollination were labelled on butter paper cover (Plate 4 and 5). After three to four weeks the matured crossed seeds obtained from female parents were harvested and stored in refrigerator.

3.8 FOREGROUND SELECTION FOR RESISTANCE GENES IN F_1 PLANTS

Resistance genes of the F_1 plants raised from seeds obtained from crossing programme were identified, by undertaking foreground selection as follows.

3.8.1 Plant Materials

Seeds from first hybridization programme (3.7.2) were sown in the nursery on different staggered dates. Twenty one days old seedlings from this nursery were then transplanted to the main field and individual plants of the crosses were labelled. At tillering stage, leaf samples from these F_1 plants were collected for DNA isolation. The DNA samples obtained were then used for foreground selection with the gene specific marker.

Plate 4. Panicle with set seeds

Plate 5. Back crossed panicles

3.8.2 Isolation of Genomic DNA and PCR Analysis

Genomic DNA from these F_1 plants was isolated by the procedure of QIAGEN DNeasy plant mini kit (3.3). In F_1 generation, DNA markers closely linked to the BB resistance genes such as pTA248 (*Xa21* gene), xa 13 pro (*xa13* gene), RMWR7.1 (*Xa33*) and Oso4g53050-1(*Xa*38) were used to confirm the presence of specified resistance genes.

3.8.3 Backcross Breeding Programme

The F_1 plants having the respective BB resistance genes identified by foreground selection were back crossed with the respective recurrent parents Aiswarya and Prathaysa. The BC_1F_1 seeds obtained from these backcrosses were harvested.

3.8.4 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_1F_1 were performed at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala by detailed procedure described below.

3.8.4.1 Selection of Microsatellite Loci

A total of 270 microsatellite loci were chosen from the public domain (http://www.gramene.org) and presented in Appendix 1. The loci included dinucleotide, trinucleotide, tetranucleotide and complex repeats. The loci with high recorded polymorphism information content (PIC) were selected in such a way as to cover the rice genome as much as possible. The chromosome-wise distribution of loci chosen is as follows: chromosome 1- 26 loci; chromosome 2 - 20 loci: chromosome 3 - 23 loci; chromosome 4 - 24 loci; chromosome 5 - 25 loci; chromosome 6 - 35 loci; chromosome 7 – 28 loci; chromosome 8 – 17 loci :

chromosome 9 – 21 loci; chromosome 10 – 13 loci; chromosome 11- 21 loci; chromosome 12- 17 loci.

3.8.4.2 *Custom Synthesis of Primers*

The microsatellite alleles were detected by conducting the capillary electrophoresis in an automated DNA sequencing machine. In this method three primers were used in each PCR assay: a sequence specific forward primer with an M13 sequence (TGTAAAACGACGGCCAGT) attached to the 5'end, a sequence specific reverse primer and the universal fluorescent labelled M13 primer. The amount of the M13 tailed forward primer used was less than that of both the M13 labelled primer and the reverse primer, which allowed the forward primer and reverses primer to initiate the reaction and enabled the fluorescent labelled M13 primer to take the place of the forward primer in the remaining PCR cycles when the limited forward primer was depleted. The thermocycling conditions were set in such a way that during the first few cycles, the forward primer with its M13 sequence was incorporated into the accumulating PCR products. Later, when the forward primer was used up, the annealing temperature was lowered to facilitate annealing of the universal fluorescent labeled M13 primer and fluorescent labeled alleles amenable for fragment separation in automated DNA sequencing machine was generated. Also in the calculation of the final allele size, 18 bp of the M13 sequence was subtracted from the generated allele to obtain the correct allele size. In this work, the forward primers of all the 270 primer loci were tailed with M13 sequence (TGTAAAACGACGGCCAGT), the reverse primers were retained as such, and the universal M13 primer (TGTAAAACGACGGCCAGT) was fluorescently labeled with 6-carboxy-fluorescine (FAM).

The primers of the selected loci including the 6-FAM modified M13 primer were custom synthesized with Sigma Genosys (Bengaluru, India).

3.8.4.3 Plant Materials

The plant materials used in this study included two resistant gene donors Improved Samba Mahsuri and PR 114 and two recurring parents Aiswarya and Prathyasa. Seeds of the four varieties were germinated in the plastic cups. Sufficient quantity of healthy tender leaves were collected from a single individual from each variety and stored in -80°C in polythene bags until DNA extraction.

The quality and quantity of the DNA was assessed against 100 ng of undigested λ DNA. The genomic DNA was diluted to a concentration of 2 ng/µl and stored at 4 °C as working solution while the stock DNA (undiluted) was stored at -20 °C in aliquot.

3.8.4.4. PCR for Assaying Microsatellite Loci

For assaying the microsatellite loci, polymerase chain reaction (PCR) was carried out in a 10 µl reaction volume containing 2 ng template DNA, 0.5 unit *Taq* DNA polymerase (AmpliTaq Gold, Applied Biosystems, Foster City, CA, USA), 1X PCR buffer containing 2.5 mM $MgCl_2$, 2µM each of dNTPs, 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. Cycling was performed in a PCR machine 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. Non template control was provided in each run to check for nonspecific amplification.

PCR products were subjected to capillary electrophoresis on an ABI Prism 3730 genetic analyzer (Applied Biosystems). For capillary electrophoresis, the samples were prepared by mixing 2 µl of PCR product with 10 µl of Hi-Di

formamide (Applied Biosystems) and 0.1 µl of size standard (GENESCAN® 400 HD [ROX] size standard, Applied Biosystems). The mixtures were denatured at 94° C for 5 minutes and snap cooled on ice before subjecting to capillary electrophoresis.

3.8.4.5 Microsatellite Allele Calling and Allele Binning

Following capillary electrophoresis, allele sizing was performed using the software Gene mapper 4 (Applied Biosystems) which computes allele sizes (in base pairs) by comparing the fragment peaks with the internal size standard. Electropherograms were reviewed visually in the software Gene mapper to ensure that clear peaks were found for the expected marker sizes and that stutter peaks were not called. To synchronize the allele detection process across individuals and loci, the last stable peak on the chromatogram (in cases where there were more than one peak, because of the presence of stutter bands), was considered as an allele. Raw allele size calls were binned manually to assign whole integer allele values.

3.8.4.4 Assessment of Recovery of Recurrent Parent Genome

The parental polymorphic microsatellite markers were used to genotype foreground selected plants at each backcross generation to estimate the amount of recurrent parent genome contribution 'G' which was calculated as per 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 showing homozygosity for recurrent parent allele
Y = number of markers showing heterozygosity for parental alleles

3.9 ARTIFICIAL SCREENING OF DONORS AND RECIPIENTS FOR RESISTANCE AGAINST BB

The donor and recipient parents used in this study were screened by artificial inoculation using the virulent culture of the BB pathogen *Xanthomonas oryzae* pv. *oryzae.*

3.9.1 Isolation of Bacteria (*Xanthomonas oryzae* pv. *oryzae)*

Infected rice leaves showing bacterial blight symptoms were collected from the BB infected fields of RRS, Moncompu. These leaves were surface-sterilized with 2% sodium hypochlorite for 1 min and washed twice with sterile distilled water. The leaves were then cut into 0.5 cm pieces in few drops of distilled water placed on a glass slides. Bacterial cells were allowed to ooze from leaves into sterile water and ooze was streaked for single-colony isolation on Peptone Sucrose Agar (PSA) (Appendix 2) plated in petridishes .The BB pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) was sub cultured on PSA slants and preserved in glycerol at −20°C for conducting further resistance screening programme.

3.9.2 Pot Evaluation Study

Experimental material including three donors and two recipients were screened by artificial inoculation of *Xoo* isolate. The experiment was conducted in RRS, Moncompu.

Varieties=5 (Donors *viz.,* ISM (*xa*13 and *Xa*21), ISM (*Xa*33) and PR-114(*Xa*38) and recipients Aiswarya and Prathyasa)

Replications =4

Design= Completely Randomised Design (CRD)

The experiment was laid out in Completely Randomised Design (CRD) with four replications at RRS, Moncompu. Artificial inoculation of 3 donors and 2

recipients with a virulent strain of (*Xanthomonas oryzae* pv. *oryzae)* was done by clip inoculation technique. In this technique a plant was clipped at maximum tillering stage with a pair of scissors dipped in the bacterial suspension. It was also applied on the entire leaf surface using a cotton swab. The plants were covered with polythene bags and a high level humidity was maintained by sprinkling water twice a day so that favourable microclimate was created to initiate infection.

The typical symptoms of bacterial blight first appeared as water soaked lesions which later turned to yellow .These lesions spread gradually downwards from the tip of leaves. Plant reaction to the inoculation was recorded 14 days after inoculation. The lesion length in each plant was measured on five individual leaves and average lesion length was obtained (Waheed *et al.*, 2009). Individual plant of each variety was scored by using standard evaluation scale (IRRI 2013) (Table 5). The data recorded in the evaluation experiment was converted to angular transformation. These observations were analysed statistically by using data analysis tool pack in Microsoft excel programme.

$$\textbf{Disease severity}\ (\%) = \frac{\text{Lesion leaf length}}{\text{Total leaf length}} \times 100$$

3.9.3 Field Screening of Bacterial Blight Disease

The recipient parents (Aiswarya and Prathyasa) and donors (ISM (*xa13* and *Xa21*), ISM (*Xa33*) PR-114(*Xa38*)) were sown in nursery during rabi season. 21 days old seedlings were transplanted in main field and each variety planted in two rows 3meters length. After 60 days *i.e.* at maximum tillering stage, leaves were clipped inoculated with *Xoo* isolate. Initial symptoms were recorded 15 days after inoculation. Percentage of disease incidence recorded in five plants of each parental variety by following formula and calculated mean value.

Table 5.SES scale for evaluation of cultures to BB of rice (IRRI 2013).

Infection %	Score	Host Behaviour
0 - 3%	1	Highly Resistant
4- 6%	2	Resistant
7 - 12%	3	Resistant
13 - 25%	4	Moderately Resistant
26 - 50%	5	Moderately Susceptible
51 - 75%	6	Susceptible
76 - 87%	7	Susceptible
88 - 94%	8	Highly Susceptible
95 - 100%	9	Highly Susceptible

$$\text{Percentage of Disease Incidence/Plant} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves}} \times 100$$

3.10 INTERMATING BETWEEN BC_1F_1 FOR PYRAMIDING THE GENES

Based on the results of artificial screening of donors against *Xanthomonas oryzae* pv. *oryzae,* intermating was done to pyramid the effective resistance genes. BC_1F_1 plants having resistant genes and highest background percent of genome were selected based on foreground and background markers. BC_1F_1 seeds were sown in different staggering dates to synchronise the flowering (Plate 6). The DNA was isolated from 45 days old plant for marker analysis. Intermating was performed between BC_1F_1 to pyramid two to three genes combinations from which the seeds were harvested.

3.11 SCREENING OF INTERMATED BC_1F_1 PLANTS BY FOREGROUND MARKERS

Seeds from intermated plants were sown in nursery. DNA isolated from these plants was used for foreground markers selection and also for recording morphological traits.

3.12 PHYSIO-MORPHOLOGICAL TRAITS OF PARENTAL, BC_1F_1 AND INTERMATED BACK CROSS LINES.

For every plant, observations of the following characters were recorded.

3.12.1. Plant Height (cm)

It was measured from base of main tiller to the tip of the panicle (excluding awn) at the time of harvest and expressed in centimetre.

Plate 6. BC_1F_1 lines in the field for intermating

Plate7: Molecular screening of pyramided lines

3.12.2. Effective Number of Tillers Plant^{-1}

Effective number of tillers plant^{-1} was recorded at physiological maturity.

3.12.3. Days to Maturity

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

3.12.4. Length/Breadth (L/B) Ratio of Grain

The length and breadth of grain was measured by using dial calliper. The ratio of length/breadth was determined by dividing the length of a grain by its corresponding breadth.

3.12.5. Kernel Colour

Rice seeds were manually dehusked and visual observation was taken based on following chart:

Table 6. Kernel colour characterization

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

3.13 STATISTICAL ANALYSIS

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

3.13.1 Completely Randomized Design (CRD)

CRD analysis was performed to analyze disease severity and concluded the experimental results by using following formulae.

ANOVA for CRD analysis

Sources	df	Sum of Squares(SS)	Mean sum of Squares (MSS)	Observed F
Between Treatments	t-1	SST	MST	MST/MSE
Within treatments(Error)	t(r-1)	SSE	MSE	
Total	tr-1	TSS		

Standard Error difference (SE(d)) $= \sqrt{\frac{2MSE}{ri}}$

C.D. = t X SE (d)

and t is the critical t value for error degrees of freedom at 5% level.

3.13.2 Descriptive Statistical Analysis

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

3.13.2.1 Range

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

3.13.2.2 Arithmetic Mean

It is calculated by the following formula:

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

Where,

ΣX =sum of all the observations,

N = total number of observations.

3.13.2.3 Standard Deviation

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

Where,

X = Individual reading

$\bar{X}$ = mean

N = sample size

3.13.2.4 Standard Error

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

Where,

S.D =Standard Deviation

N = total number of observations.

3.13.3 Chi-Square Test

Goodness of fit for all loci to an expected 1:1 segregation ratio was tested by means of chi-square analysis in BC_1F_1 population controlling by single resistance gene.

Following expression was used to calculate the chi-square values:

$\chi 2$ (n-1) d.f = Σ (O-E) ^{2}/E

Where,

n = number of phenotypic classes

d.f = degrees of freedom

O = Observed number of plants in each phenotypic class

E = Expected number of plants in each phenotypic class

3.13.4 Euclidean Distance

Proximity dissimilarity matrix was analyzed using Euclidean distance method for four morphological characters by estimating Euclidean distance as formula suggested by Shifriss and Sacks, (1980).

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

Where,

Xik =Performance of the i^{th} parent for k^{th} character

Xjk= Performance of the j^{th} parent for k^{th} character

Sk= Standard deviation of the K^{th} character

Genetic divergence (genetic distance) among pyramided lines was measured by Euclidian distance method (Cruz and Regazzi, 1994) using Statistical Package for the Social Sciences (SPSS version 22.0).

Results

4. RESULTS

Bacterial Blight (BB) caused by the bacteria *Xanthomonas oryzae* pv. *oryzae* is a major disease of rice causing significant economic losses to farmers. As chemical control of the disease is ineffective, development of host plant resistance is considered as one of the best ecofriendly and sustainable strategy to tackle the disease. As many as 40 major genes conferring resistance to various races of the pathogen *Xanthomonas oryzae* pv. *oryzae* have been identified and utilized in rice breeding programs. However, large-scale and long-term cultivation of varieties carrying a single gene for resistance results in a significant shift in pathogen race frequency which results in consequent breakdown of resistance in these cultivars. To combat the problem of resistance breakdown, pyramiding of resistance genes into different cultivars is being carried out. Pyramiding of resistance genes is now possible with molecular markers that are developed for individual genes. The present investigation was carried out to pyramid multiple resistance genes into popular rice varieties of Kerala *viz.,* Aiswarya and Prathyasa, through marker aided selection. The results obtained are presented under the following sections.

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

4.2. Hybridization between donors and recipients

4.3. Foreground selection in F_1 plants for resistance genes

4.4. Backcross breeding for recovery of recurrent parent genome

4.5. Foreground selection in backcross generation BC_1F_1

4.6. Phenotypic analysis of BC_1F_1 plants with resistance genes

4.7. Background marker analysis in BC_1F_1 generation

4.8. Screening of donors against local isolate

4.9. Intermating between BC_1F_1 and Identification of two or three resistance genes combination in BC_1F_1 intermated lines by foreground selection

4.1 VALIDATION OF BB RESISTANCE GENES IN THE PARENTAL GENOTYPES

4.1.1 Isolation of Genomic DNA

The plant materials used in this study included three donors *viz.*, Improved Samba Mahsuri (pyramided with *xa13* and *Xa21*), Improved Samba Mahsuri (pyramided with *Xa33*) collected from IIRR (Indian Institute of Rice Research) Hyderbad and PR-114(pyramided with *Xa*38) from Dr. Kuldheep Singh, PAU, Ludhiana. Two recurrent parents, Aiswarya and Prathyasa, collected from Regional Agricultural Research Station, Kerala Agricultural University (KAU), Pattambi, and Rice Research Station KAU, Moncompu, respectively. These genotypes were grown in nursery of Department of Plant Breeding and Genetics, College of Agriculture, Vellayani. Genomic DNA was isolated from the young leaf of 3-4 week old seedlings of the donors and recipient genotypes by using Qiagen Dneasy Mini kit (Germany). The quantity and quality of extracted genomic DNA was checked both by agarose gel electrophoresis and UV spectrophotometer. DNA isolation was fast and accurate with the help of Dneasy Mini kit and no RNA and protein contamination were found in isolated samples.

4.1.2 Qualitative and Quantitative Estimation of DNA

The quantity of genomic DNA estimated at UV absorbance at 260 nm along with blank samples. The amount of protein and RNA in the genomic DNA was also recorded at 280 nm using the same UV spectrophotometer. The ratio of absorbance at 260 nm and 280 nm ranged from 1.8-2.00 which indicated that the DNA was free from contaminants like RNA and proteins etc. The ratio of absorbance at 260 nm and 280 nm of the parental genotypes, Improved Samba Mahsuri (*xa13* and *Xa21),* ISM *(Xa33),* PR-114(*Xa38),* Aiswarya and Prathyasa calculated were1.80, 2.00, 1.80, 2.00 and 1.83 respectively (Table 7). The quantity of DNA of the parental genotypes ranged from 400 (ISM *(Xa33))* to 550 ng/µl in (Prathyasa). This showed that Dneasy mini kit protocol yielded high amount of

Table: 7 Quantity and quality of genomic DNA isolated from parental genotypes of rice

Sl.No	Samples	O.D of DNA at A260	O.D of DNA at A280	Ratio of A260/A280	Quantity of DNA(ng/µl)
1	ISM(*xa13* and *Xa21)*	0.009	0.005	1.80	450
2	ISM*(Xa33)*	0.008	0.004	2.00	400
3	PR-114(*Xa38)*	0.009	0.005	1.80	450
4	Aiswarya	0.010	0.005	2.00	500
5	Prathyasa	0.011	0.006	1.83	550

genomic DNA. The quality of genomic DNA of all the parental genotypes was also checked on agarose gel for its base pair size and RNA contamination. Resolution of genomic DNA on 0.8 per cent (w/v) submerged agarose gel showed a discrete single band of high molecular weight DNA. This showed that the genomic DNA of the parental genotypes was free from any mechanical or enzymatic degradation and was intact and of good quality.

4.1.3 Polymerase Chain Reaction (PCR)

DNA extracted from the parental genotypes was used for PCR amplification using the specific microsatellite markers. The marker polymorphism was analysed in the resistant parent Improved Samba Mahsuri for the resistance genes *viz., xa13* and *Xa21* with linked markers. The amplification profile produced by the marker pTA248 specific to the gene *Xa*21 (Plate 8) showed that the donor parent, Improved Samba Mahsuri, produced a product at 1000bp which was absent in both the susceptible parents Aiswarya and Prathyasa that gave rise to a product at 650bp.

The amplification profile produced by the marker *xa*13 pro specific to the gene *xa*13 showed that the donor parent Improved Samba Mahsuri produced a product at 500bp which was absent in both the susceptible parents Aiswarya and Prathyasa that gave rise to a product at 250bp.

The markers RMWR7.1 and RMWR7.6 are reported to be flanking the novel BB resistance gene *Xa*33.Both these markers were used to evaluate the amplification profile of donors and recipients. Marker RMWR7.1 specific to the gene *Xa*33 produced an allele of size ~350bp in donor parent and of size ~250bp in recipient parents (Plate 9). Similarly amplification with primer RMWR7.6 showed alleles of size 190 bp and ~210 bp in donors and recurrent parents respectively. The marker Oso4g53050-1 specific to gene *Xa*38 amplified an allele of size ~ 250bp in donor parent (PR-114) but not in its recipient parents (Aiswarya and Prathyasa). Oso4g53050-1 produced an allele of size 300-320bp in

Plate 8. Validation of *xa*13 and *Xa*21 genes in donor parent (ISM) with xa13 promoter and pTA248 markers

ISM=Improved Samba Mahsuri (*xa*13 and *Xa*21) A=Aiswarya P=Prathyasa

Plate 9. Validation of *Xa33* gene with flanking markers RMWR 7.1, RMWR 7.6 and *Xa38* in PR-114 with Oso4g53050-1 marker

S= ISM (*Xa33*) PR-114 A=Aiswarya P=Prathyasa

susceptible parents. This validation confirmed the absence of resistance genes in the recurrent parent chosen for study.

4.2 HYBRIDIZATION BETWEEN DONORS AND RECIPIENTS

4.2.1 Raising of Parents for Hybridization Programme

Donor parents were raised in the nursery by staggered sowing with eight days interval to synchronize flowering. Donor parents (Male parents) were long duration varieties (140 days) and were sown one month before the recipient parents. Twenty one day old seedlings were transplanted to main field. At boot leaf initiation stage plants lifted from the field and transferred to pots. Clipping method of emasculation was followed in crossing programme. Three donors were crossed with two recipients to transfer genes to the recurrent parents. The F_1 seeds harvested from resultant six crosses. The number of crossed seeds ranged from 100 to 150 (Table 8). The maximum number of crossed seeds (150) were obtained from cross Aiswarya x PR-114(*Xa38*). The lowest number of crossed seeds (100) from cross Prathyasa x Improved Samba Mahsuri (*Xa33*).

4.3 FOREGROUND SELECTION IN F_1 PLANTS FOR RESISTANCE GENES

The harvested F_1 seeds of the respective crosses were sown in nursery and 45 days old plants used for foreground selection. The number of positive heterozygous plants for respective markers is shown in Table 9. The total plants screened ranged from 25 to 43. The lowest numbers of plants screened in the crosses between Prathyasa x ISM (*xa*13 and *Xa*21) and Prathyasa x PR-114 (*Xa*38). The highest number of plants were screened in Aiswarya x PR-114 (*Xa*38).

4.3.1 Cross between Aiswarya and ISM (*xa*13 and *Xa*21)

One hundred and twenty five F_1 seeds of the cross Aiswarya x ISM were obtained. Out of the 50 healthy and fully matured seeds were kept for germination

Table 8 .Details of hybridization between donors and recipients.

Sl. No	Crosses	No. of panicles pollinated	No. of set seeds
1	Aiswarya x PR-114(*Xa*38)	35	150
2	Prathyasa x PR-114(*Xa*38)	28	120
3	Aiswarya x Improved Samba Mahsuri (*Xa*33)	30	134
4	Prathyasa x Improved Samba Mahsuri (*Xa*33)	24	100
5.	Aiswarya x Improved Samba Mahsuri (*xa*13 and *Xa*21)	35	125
6	Prathyasa x Improved Samba Mahsuri (*xa*13 and *Xa*21)	40	150

Table 9. Details of heterozygous plants in different crosses.

Sl. No	crosses	Total plants screened	Plants with heterozygous loci	Plants with Homozygous loci
1	Aiswarya x ISM (*xa*13and *Xa*21)	26	24	2
2	Aiswarya x ISM (*Xa*33)	42	33	9
3	Aiswarya x PR-114 (Xa^{38})	43	33	10
4	Prathyasa x ISM(*Xa*33)	32	29	3
5	Prathyasa x ISM (*xa*13 and *Xa*21)	25	21	4
6	Prathyasa x PR-114 (*Xa*38)	25	23	2
	Total	193	163	30

twenty six seeds germinated. DNA isolation was carried out from 45 day old seedlings from these twenty six F_1 plants following Qiagen DNeasy mini kit to carry out the foreground selection. The quality of genomic DNA checked in 0.8% agarose gel and quantity of DNA was calculated based on spectrophotometer value.

The presence of the resistance genes *xa*13 and *Xa*21 were checked in all the F_1 plants using the specific microsatellite markers *xa*13 pro and pTA248, respectively (Plate 10). Out of twenty six F_1 plants, twenty four plants were found heterozygous with respect to both the BB resistance genes *xa13* and *Xa*21.

4.3.2 Cross between Aiswarya and ISM (*Xa*33)

One hundred and thirty four F_1 seeds were harvested from this cross. Out of which only forty two seeds germinated due to high percentage of shrivelled seeds. DNA isolation was made from these forty two plants which were subjected to foreground selection.

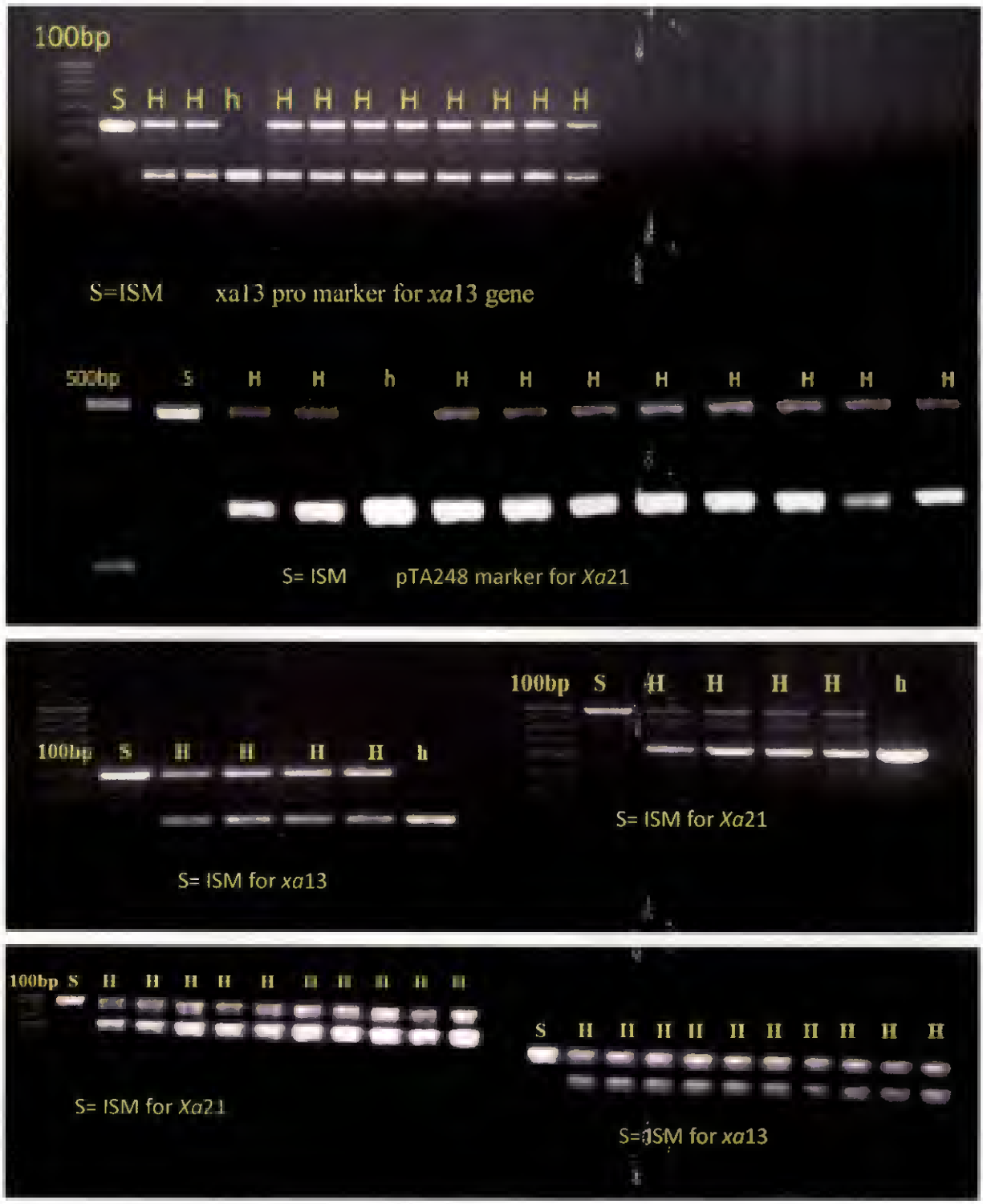
The BB dominant resistance gene *Xa*33 was found in heterozygous condition in thirty three plants by PCR analysis using markers RMWR 7.1 linked to the gene (Plate11).

4.3.3 Cross between Aiswarya and PR-114

One hundred and fifty seeds were obtained from this cross and from this forty three seeds germinated in petri dishes and were planted in nursery after eight days. Twenty one days old plants were planted in main field to obtain more number of tillers. DNA was isolated from these seedlings for foreground selection.

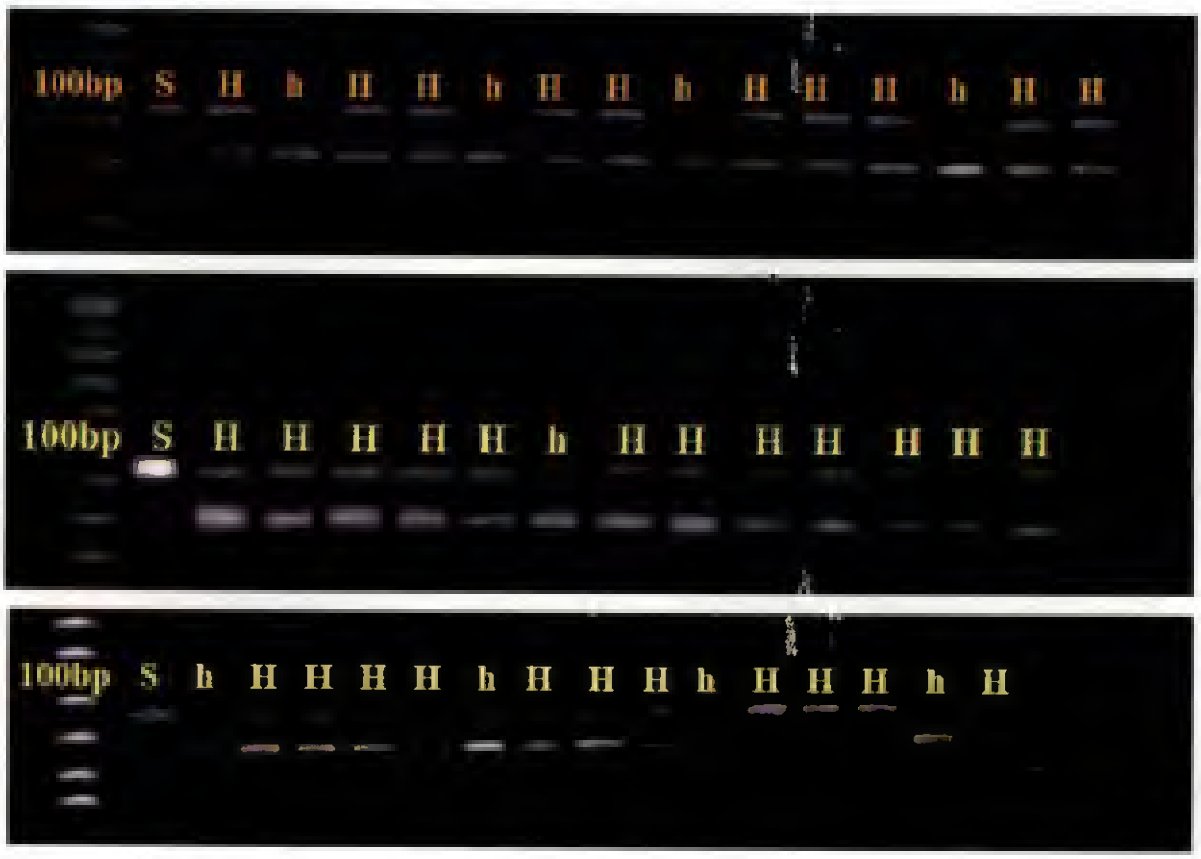
Allele specific to *Xa*38 gene was found in heterozygous condition in thirty three plants when analysed with the functional marker Oso4g53050-1 (Plate12).

Plate 10: Foreground selection in cross Aiswarya x ISM F_1 plants using *xa*13 linked marker xa13 pro and *Xa*21 linked pTA248.



H= Heterozygous resistant plants h=homozygous susceptible plants

Plate11. Foreground selection in cross Aiswarya x ISM F_1 plants using *Xa33* linked marker RMWR 7.1.



S=Improved Samba Mahsuri (*Xa33*) H= Heterozygous resistant plants h=homozygous susceptible plants

Plate 12.Foreground selection in cross Aiswarya x PR-114 F_1 plants using *Xa38* linked functional marker Oso4g53050-1

S= PR-114 H= Heterozygous resistant plants h=homozygous susceptible plants

4.3.4 Cross between Prathyasa and ISM (*Xa21* and *xa13*)

The seedlings from twenty five germinated seeds were transferred to nursery after eight days. Twenty one days old seedlings were transplanted in main field. At the stage of maximum tillering leaf samples were collected for DNA isolation.

The foreground selection was carried out in these F_1 plants using the specific STS markers pTA248 and functional marker *xa*13 pro to detect the presence of resistance genes *Xa21* and *xa13* respectively. A total of twenty one out of twenty five F_1 plants, were found to have heterozygous loci for the BB resistance genes *Xa*21 and *xa*13 (Plate 13). Two plants showed homozygous susceptible allele.

4.3.5 Cross between Prathyasa and ISM (*Xa*33)

Thirty two seeds germinated in petridish were planted in nursery. After twenty one days these seedlings were transplanted in main field. Twenty nine plants showed heterozygous loci (Plate 14).

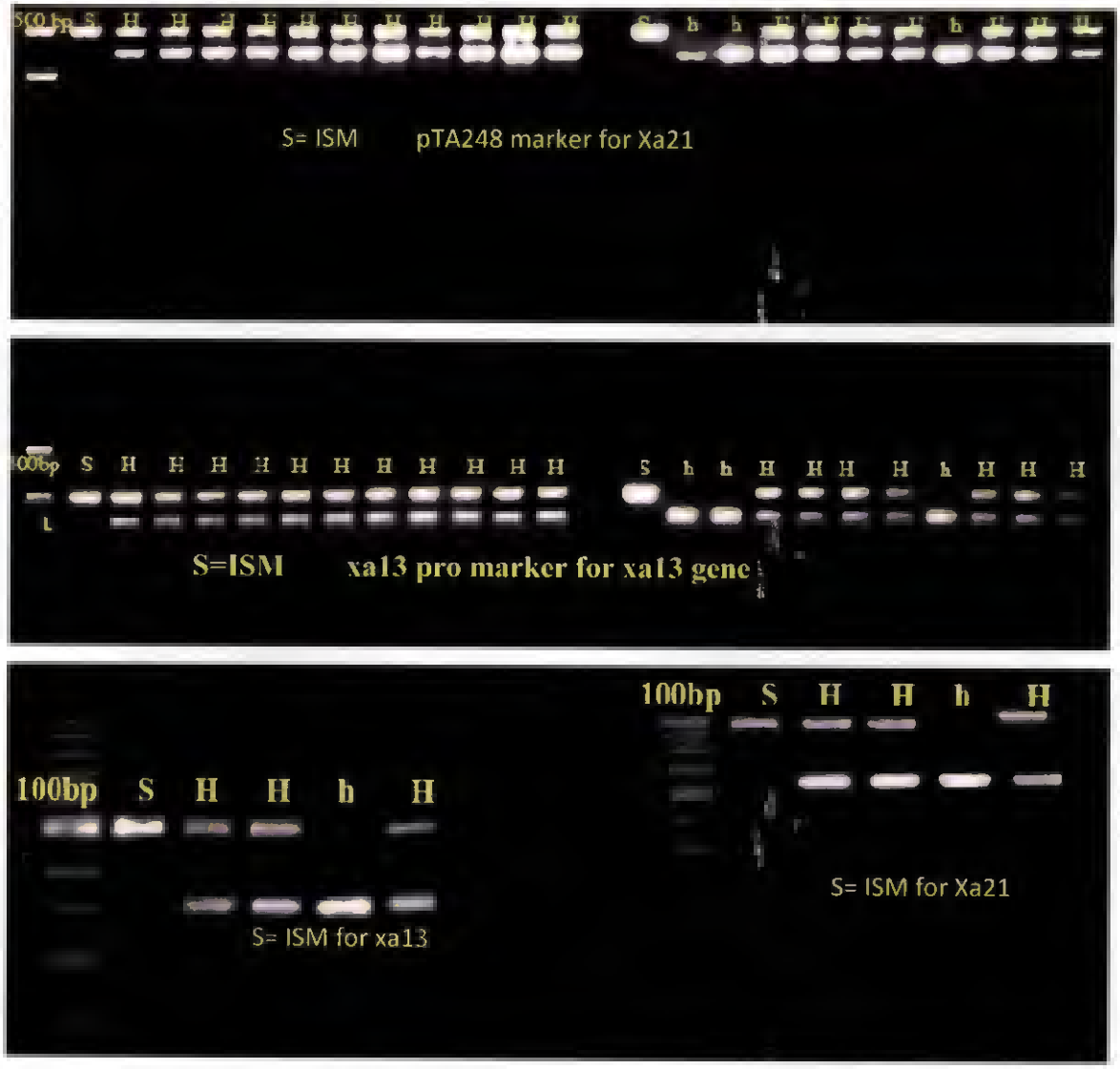
4.3.6 Cross between Prathyasa and PR-114

Twenty eight panicles were emasculated and pollinated and one hundred twenty F_1 seeds were harvested. These seeds were sown and twenty five seeds germinated in petridish used for PCR analysis. DNA was isolated and screened with functional marker Oso4g53050-1. Twenty three plants had showed the presence of heterozygous loci. (Plate 15).

4.4. BACKCROSS BREEDING FOR RECOVERY OF RECURRENT PARENT GENOME

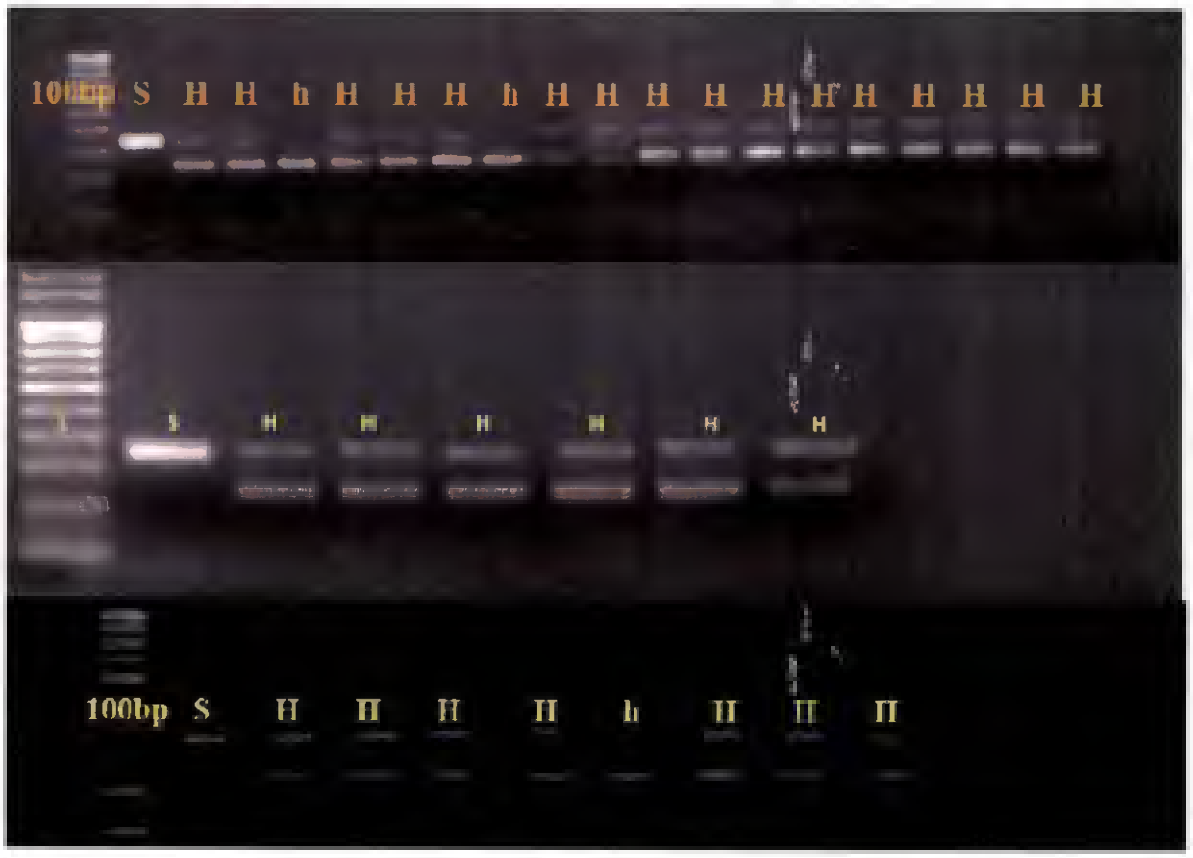
The F_1 plants with heterozygous loci linked to the respective resistance genes (*xa13*and *Xa21, Xa33,* and *Xa38*) used as the female parents were sown in

Plate 13: Foreground selection in cross Prathyasa x ISM F_1 plants using *xa*13 linked marker xa13 pro and *Xa*21 linked pTA248.



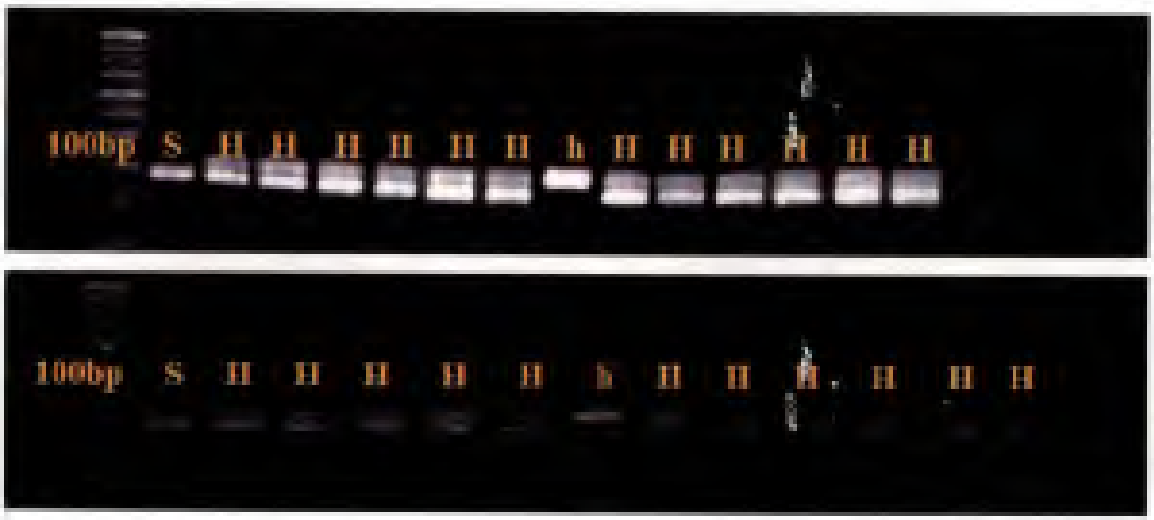
H= Heterozygous resistant plants h=homozygous susceptible plants

Plate14. Foreground selection in cross Prathyasa x ISM F_1 plants using *Xa*33 linked marker RMWR 7.1



S= Improved Samba Mahsuri H=Heterozygous resistant plants h=homozygous susceptible plants

Plate15. Foreground selection in cross Prathyasa x PR-114 F_1 plants using *Xa38* linked functional marker Oso4g53050-1



S= PR-114 H= Heterozygous resistant plants h=homozygous susceptible plants

the field and transferred to pots in tillering stage. The recurrent parents Aiswarya and Prathyasa that represented the male parents were sown on three different staggered dates. Backcrossing was then performed between F_1 plants (female) and plants of recurrent parents (male) with synchronized flowering following which the BC_1F_1 seeds were harvested at maturity (Table 10). The number of BC_1F_1 seeds ranged from 120 in the cross Prathyasa x ISM (*Xa*33) to 545 in the cross Prathyasa x ISM (*xa*13 and *Xa*21).

4.5. FOREGROUND SELECTION IN BACKCROSS POPULATION

Foreground selection was performed with respective resistance gene in back cross population of six cross combination by using foreground markers. Based on genotypic data chi square was calculated for analysis of Mendelian segregation ratio for single gene model (Table11).

4.5.1. Aiswarya x ISM (*xa13* and *Xa21*) Backcross Generation Plants

From the 400 back cross seeds sown, one hundred seventy two germinated. DNA was isolated from 45 days old seedlings for foreground selection. Out of 172 plants screened eighty three plants showed presence of gene *xa13* and seventy seven plants had *Xa21* gene (Table12). Only forty two plants had both genes in heterozygous condition (Plate 16(a) to 16(m)). These plants were used as female parent in intermating programme.

4.5.2. Aiswarya x ISM (*Xa33*) Backcross Generation Plants

Out of the 320 backcrossed seeds, 122 seeds germinated. DNA isolated from these plants was used for foreground selection. Forty six plants showed the presence of heterozygous loci of the *Xa*33 gene (Plate17 (a) to 17 (h).). Chi square test for goodness of fit was analysed in BC_1F_1. Calculated $\chi 2$ value was 7.38, (table value significant at 5%, 1 df was 3.84; significant at 1% 1df was 6.63). It showed significant deviation from 1:1 segregation ratio.

Table 10. Details of back cross breeding programme

Sl. No	Crosses	No. of panicles emasculated and pollinated	No. of set seeds
1	(Aiswarya x PR-114(*Xa*38))x Aiswarya	60	400
2	(Aiswarya x Improved Samba Mahsuri (*Xa*33)) x Aiswarya	58	320
3	(Aiswarya x Improved Samba Mahsuri (*Xa*13 and *Xa*21)) x Aiswarya	68	400
4	(Prathyasa x Improved Samba Mahsuri (*Xa*33)) x Prathyasa	50	120
5.	(Prathyasa x Improved Samba Mahsuri (*Xa*13 and *Xa*21)) x Prathyasa	70	545
6	(Prathyasa x PR-114(*Xa*38)) x Prathyasa	45	180

Table11. Chi-square analysis for single resistance genes in the BC_1F_1 population

BC_1F_1 generation	Total	Segregation ratio	Observed ratio		Calculate $\chi 2$ value	P value
			Resistant (Rr)	Susceptible (rr)		
Aiswarya x ISM(*Xa*33)	122	1:1	46	76	7.38**	0.006
Prathyasa x ISM(*Xa*33)	72	1:1	32	40	0.89	0.34
Aiswarya x PR-114(*Xa*38)	178	1:1	80	98	1.82	0.17
Prathyasa x PR-114(*Xa*38)	78	1:1	37	41	0.21	0.64

Chi square table value * Significant at 5%, 1 df (3.84); ** significant at 1% 1df (6.63).

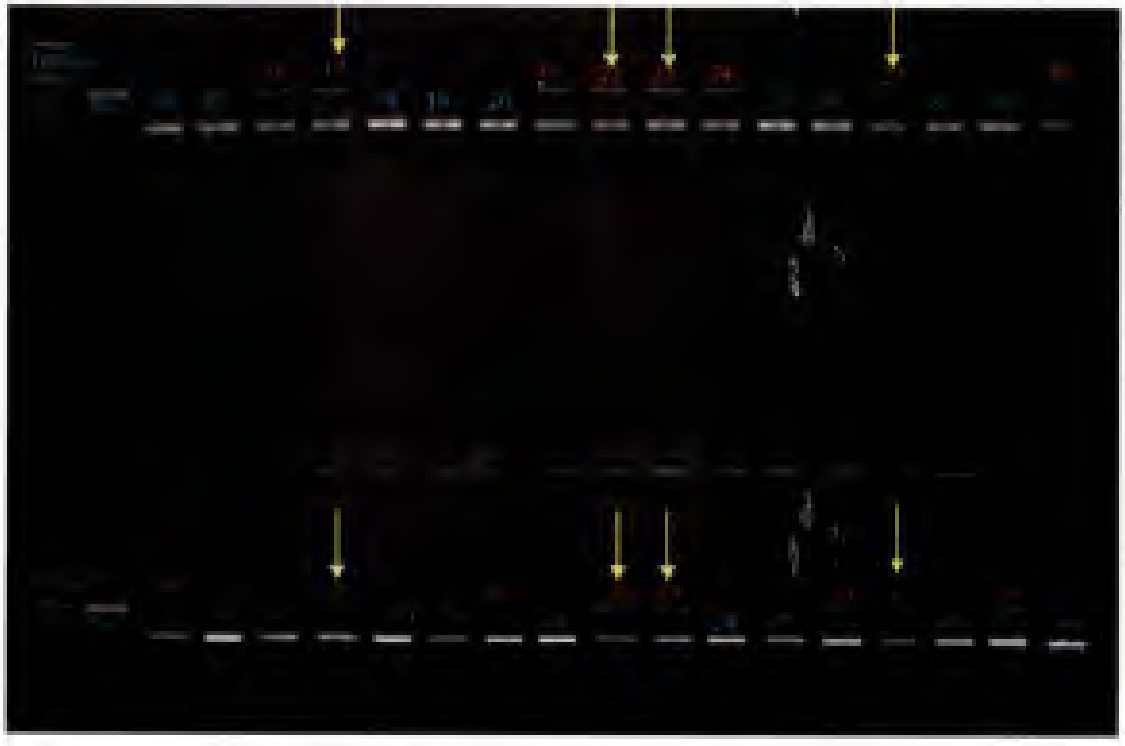
Table 12. Details of foreground selection in BC_1F_1 generation.

Sl. No	Backcross	Total No. of plants	Heterozygous plants		Foreground selected plants
1	Aiswarya x Improved Samba Mahsuri (*Xa13 and Xa21*)	172	*xa13*	*Xa21*	*xa13* and *Xa21*
			83	77	42
2	Aiswarya x Improved Samba Mahsuri(*Xa33)*	122	46		46
3	Aiswarya x PR-114 *(Xa38)*	178	80		80
4	Prathyasa x Improved Samba Mahsuri *(xa13 and Xa21*)	154	*xa13*	*Xa21*	*xa13* and *Xa21*
			74	68	42
5.	Prathyasa x Improved Samba Mahsuri (*Xa33*)	72	32		32
6	Prathyasa x PR-114(*Xa38)*	78	37		37
Total		776	-		279

Plate16 Foreground selection in BC_1F_1 plants of the cross Aiswarya x ISM using *xa*13 linked marker xa13 pro and *Xa*21 linked pTA248

Plate 16 (a)

Plate 16 (b)



Arrow mark = Samples with both *xa* 13and *Xa21*genes

Plate16(c)

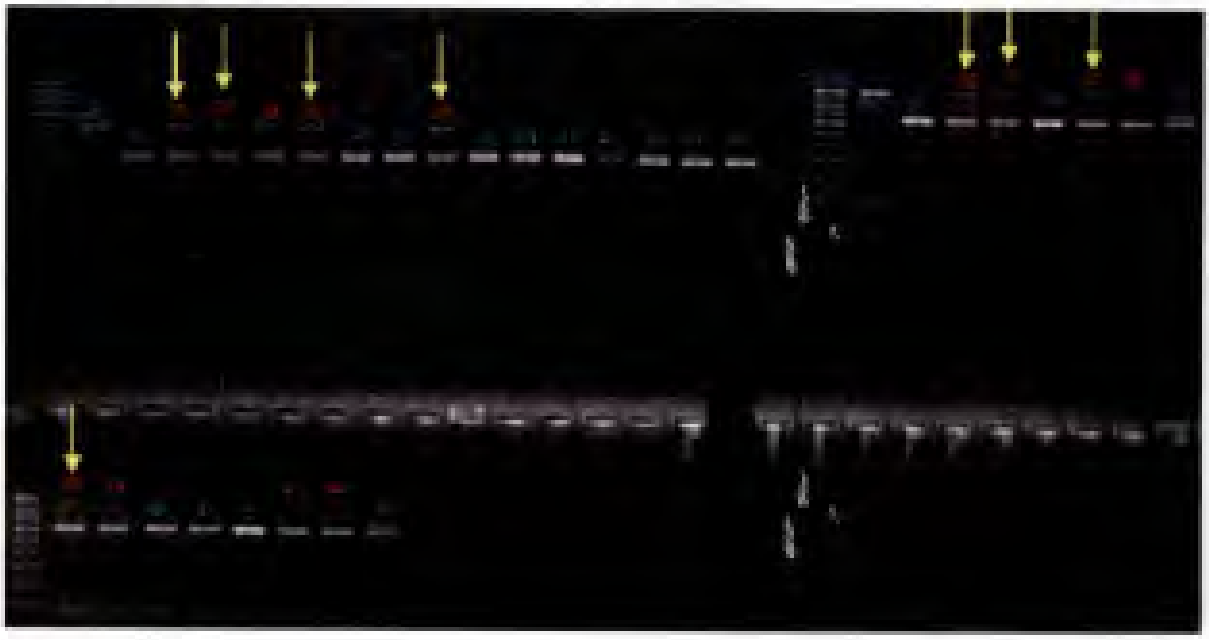
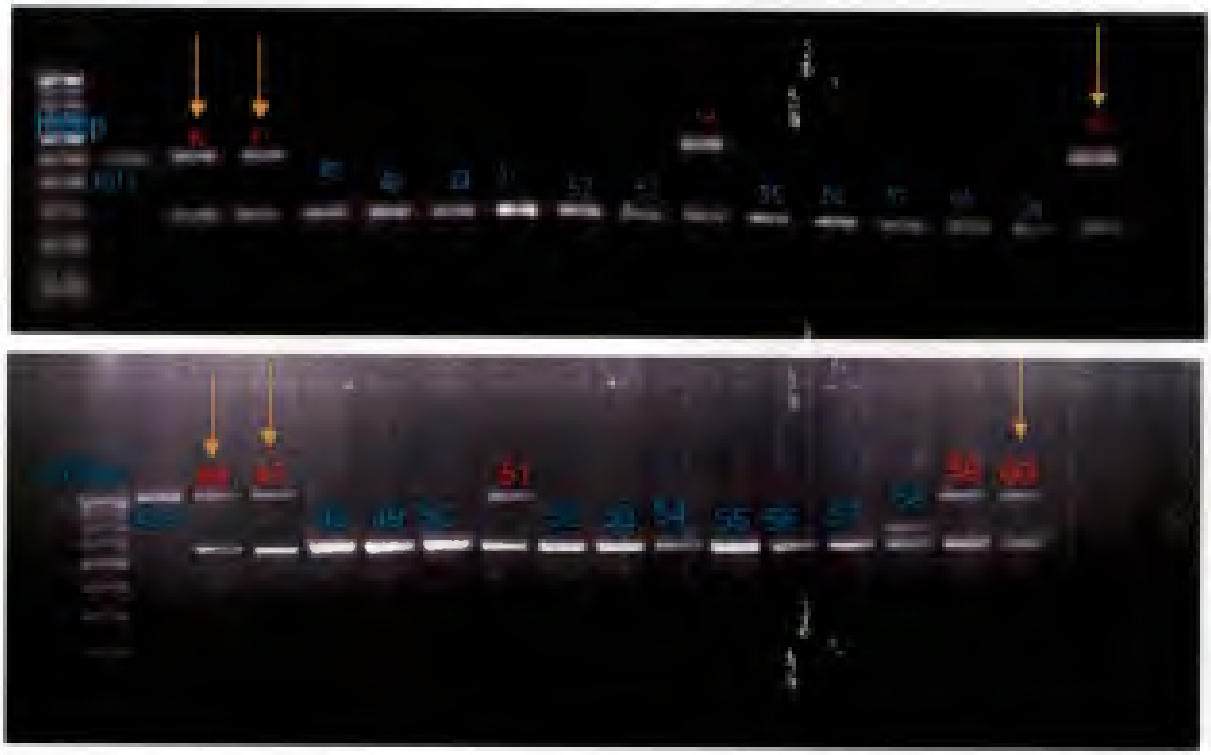


Plate16(d)



Arrow mark = Samples with both *xa* 13and *Xa21*genes

Plate 16(e)

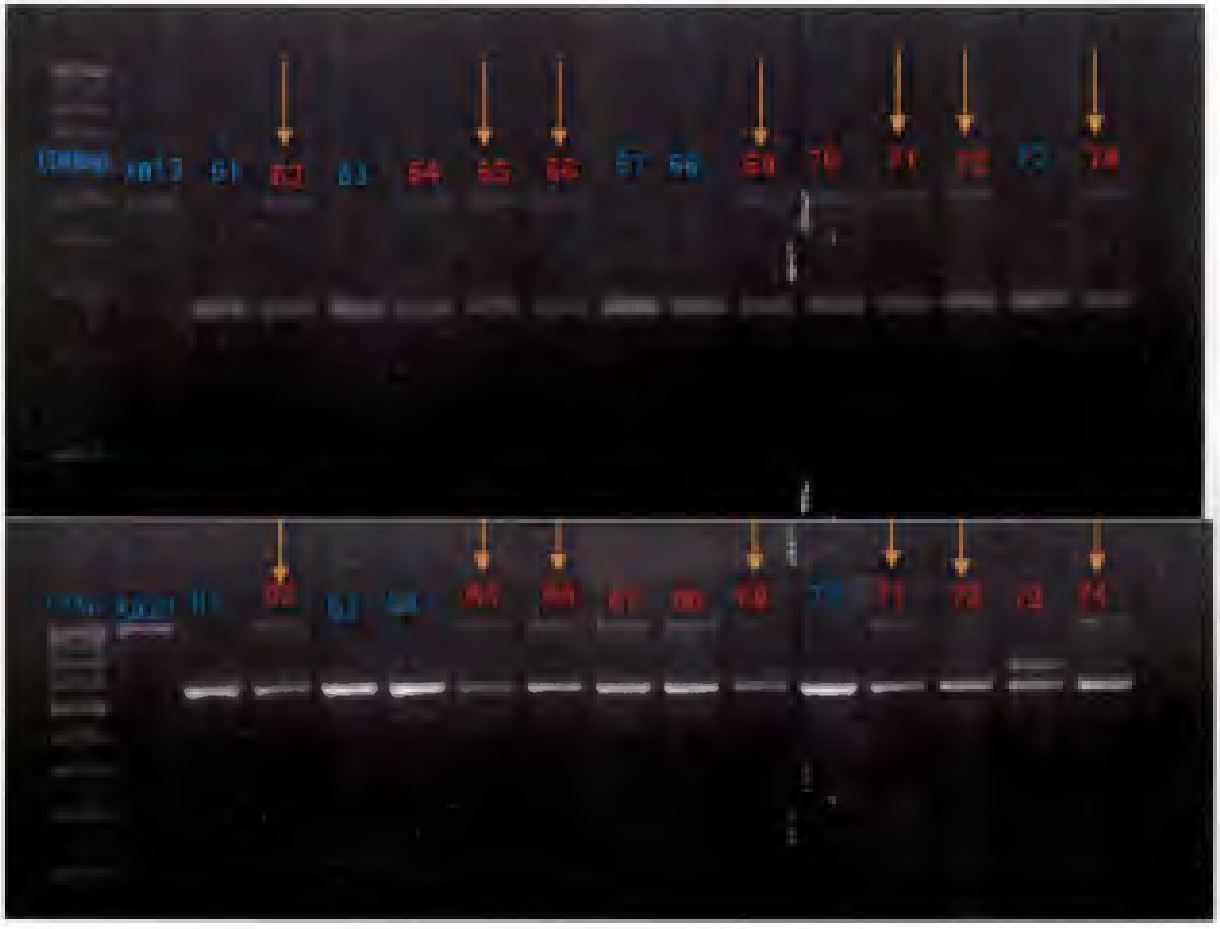
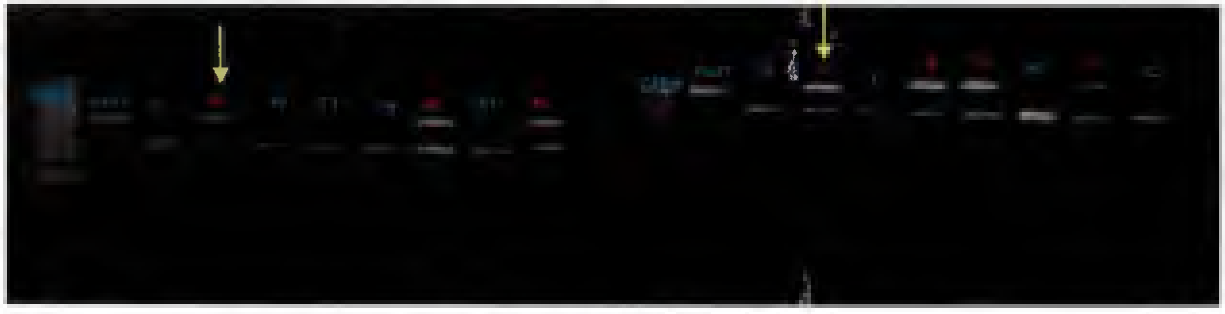


Plate16 (f)



Arrow mark = Samples with both *xa* 13and *Xa21*genes

Plate16(g)

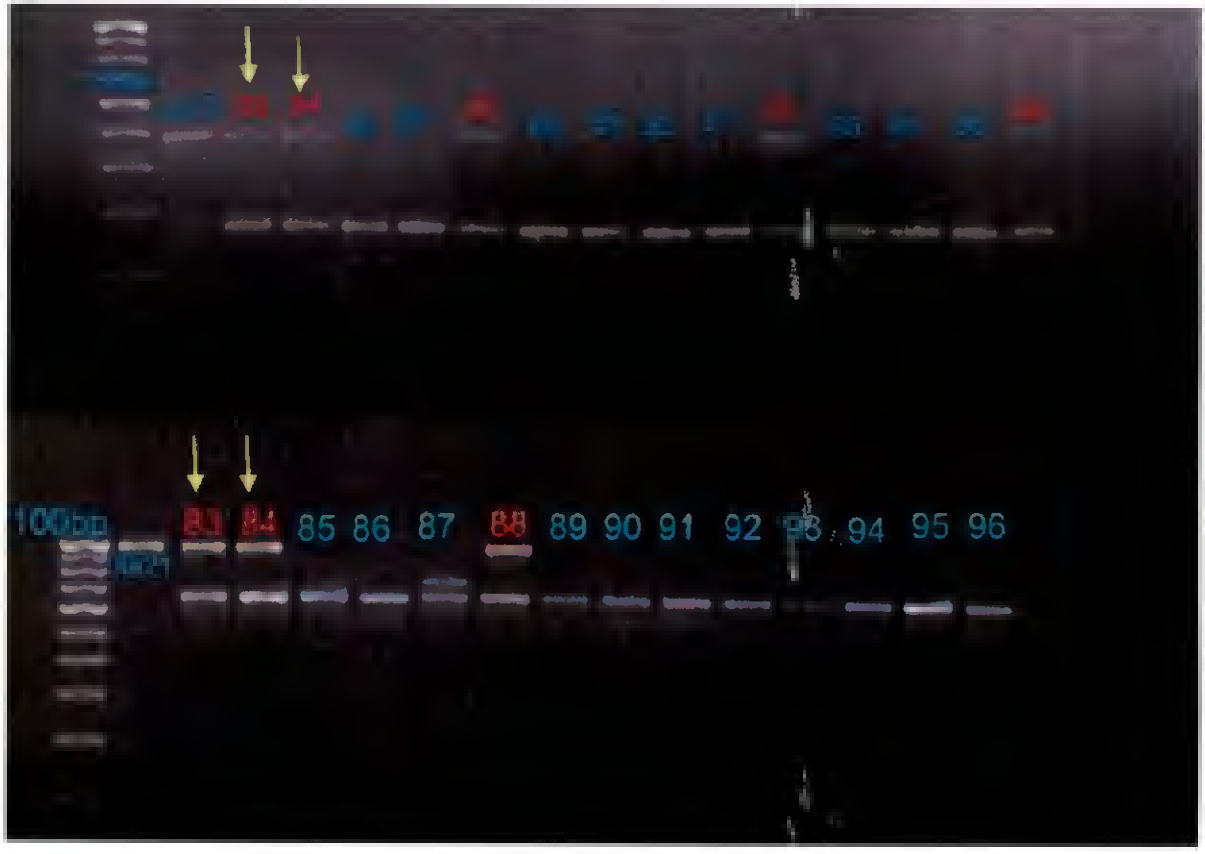
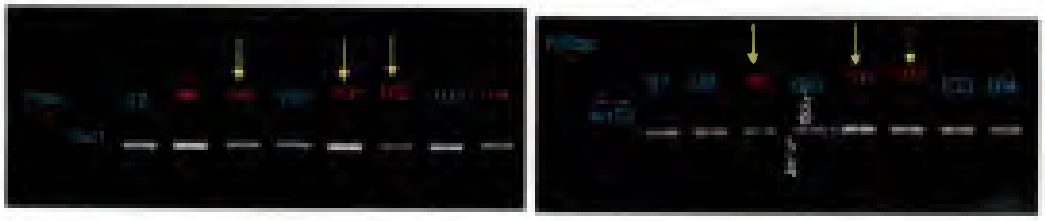


Plate16 (h)



Arrow mark = Samples with both *xa* 13and *Xa21*genes

Plate16 (i)

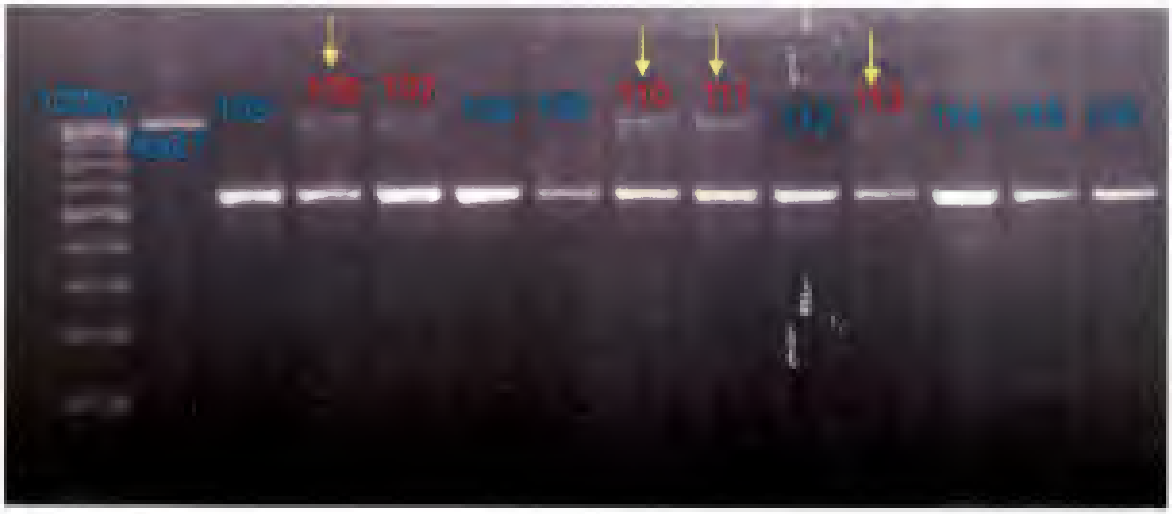


Plate16(j)

Arrow mark = Samples with both *xa* 13and *Xa21*genes

Plate16(k)

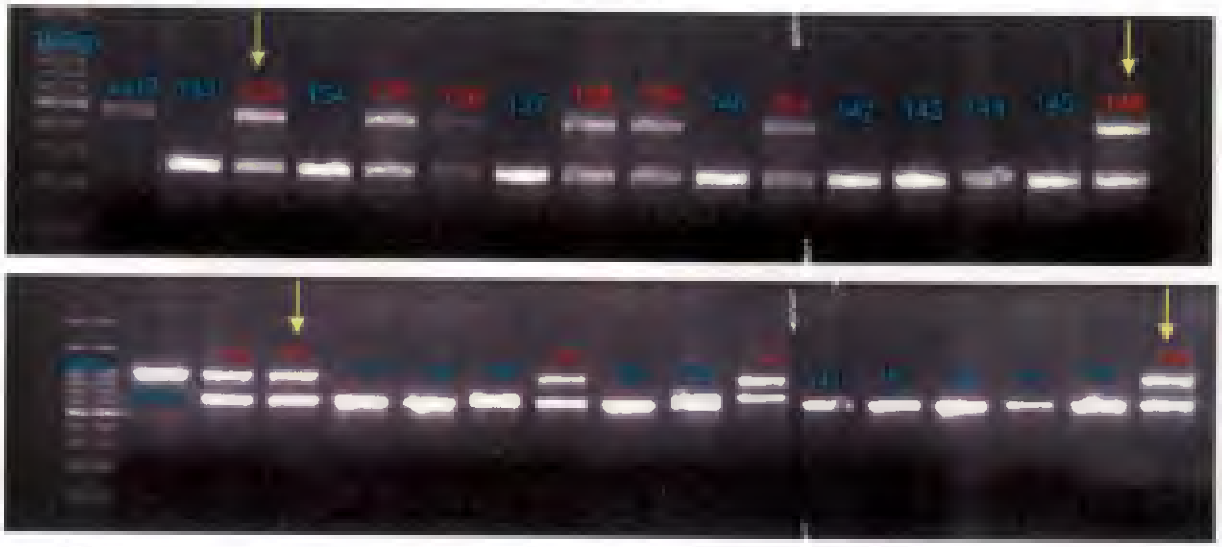


Plate16(l)

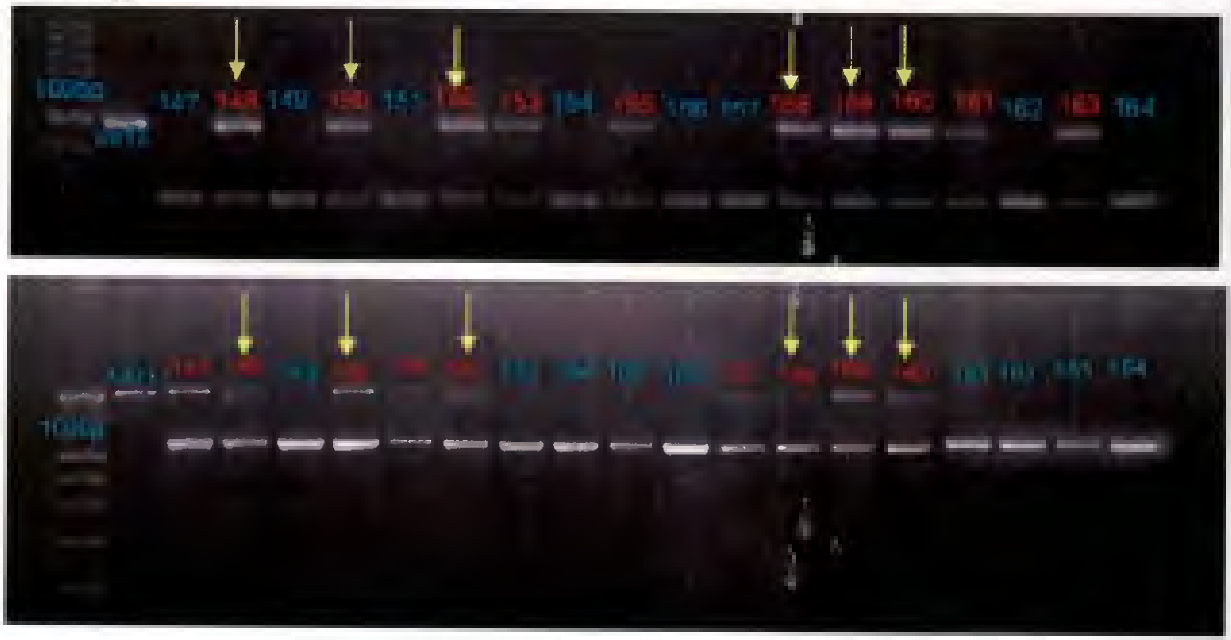


Plate16(m)

Arrow mark = Samples with both *xa* 13and *Xa21* genes

Plate 17. Foreground selection in BC_1F_1 plants of the cross Aiswarya x ISM using *Xa33* linked marker RMWR 7.1

Plate 17(a)

Plate 17 (b)

Plate 17 (c)

Plate 17 (d)

Red color labelled samples with *Xa33* gene

Plate 17(e)

Plate 17 (f)

Plate 17(g)

Plate 17 (h)

Red color labelled samples with *Xa33* gene

4.5.3. Aiswarya x PR-114 (*Xa38*) Backcross Generation Plants

400 BC_1F_1 seeds obtained in this crossing programme were sown and DNA was isolated from the germinated 178 plants and was subjected to foreground selection with the respective primer. Eighty plants had heterozygous loci for marker linked to the gene of interest (Plate18 (a) to 18(i)). Calculated Chi square value was 1.82 and showed non significance deviation and fitted in 1:1 segregation ratio (P value 0.17).

4.5.4. Prathyasa x ISM (*xa13* and *Xa21*) Backcross Generation Plants

Out of 545 seeds obtained by backcrossing twenty one F_1 plants having both *Xa*21 and *xa13* genes, only 178 seed germinated. DNA from these plants was subjected to foreground selection by primer pTA248 and xa13 pro. Seventy four plants showed presence of resistance gene *xa13*. Sixty eight plants had the dominant gene *Xa21* in heterozygous condition. Forty two plants were selected for the presence of both the genes with heterozygous loci for both genes (Plate19 (a) to 19(i)). These plants were female in the intermating crossing programme.

4.5.5 Prathyasa x ISM (*Xa33*) Backcross Generation Plants

120 seeds were obtained by crossing 29 F_1 plants with the recurrent parent. 120 seedlings were transplanted in main field and after 21 days leaf samples were collected for DNA isolation. DNA from these plants was screened with foreground polymorphic marker RMWR7.1 and identified plants with heterozygous loci for the marker linked to *Xa33* gene. Out of seventy two, 32 plants had the marker in heterozygous condition (Plate 20(a) to 20(d)). Calculated chi - square value was 0.89 and showed non significance difference at 1 and 5% level. The results fitted in the expected 1: 1 ratio ($\chi 2$ value = 0.89, P =0.34).

Plate 18 . Foreground selection in BC_1F_1 plants with *Xa38* gene of the cross Aiswarya x PR-114 using *Xa38* linked functional marker Oso4g53050-1

Plate 18 (a)

Plate 18 (b)

Plate 18 (c)

Plate 18 (d)

Red color labelled samples with *Xa38* gene

Plate 18 (e)

Plate 18(f)

Plate 18 (g)

Plate18(h)

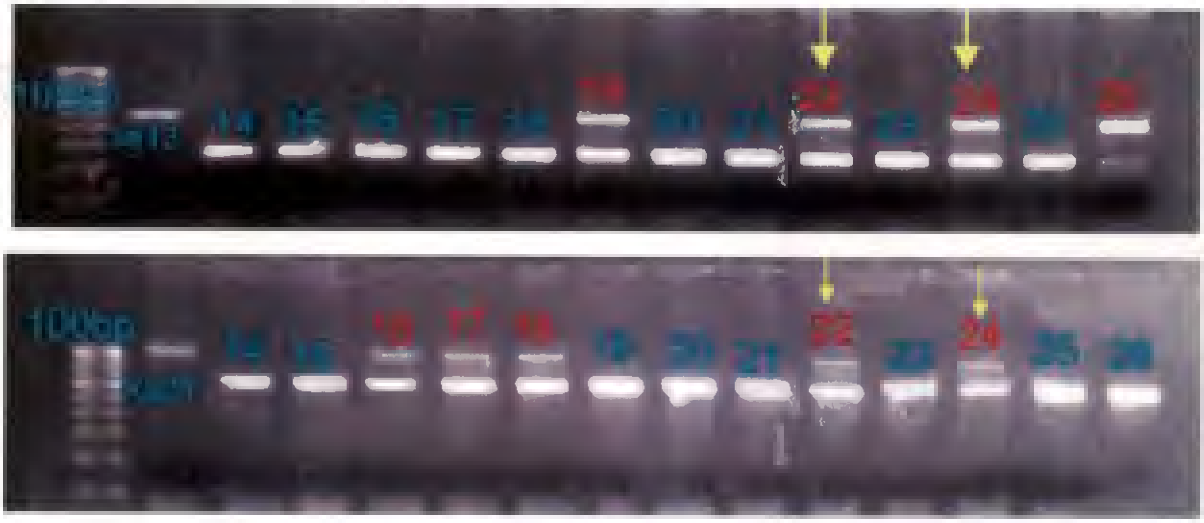
Plate 18(i)

Red color labelled samples with *Xa38* gene

Plate 19. Foreground selection in BC_1F_1 plants of the cross Prathyasa x ISM using *xa*13 linked marker xa13 pro and *Xa*21 linked pTA248.

Plate 19(a)

Plate 19 (b)



Arrow mark = samples with both *xa* 13and *Xa21*genes

Plate 19(c)

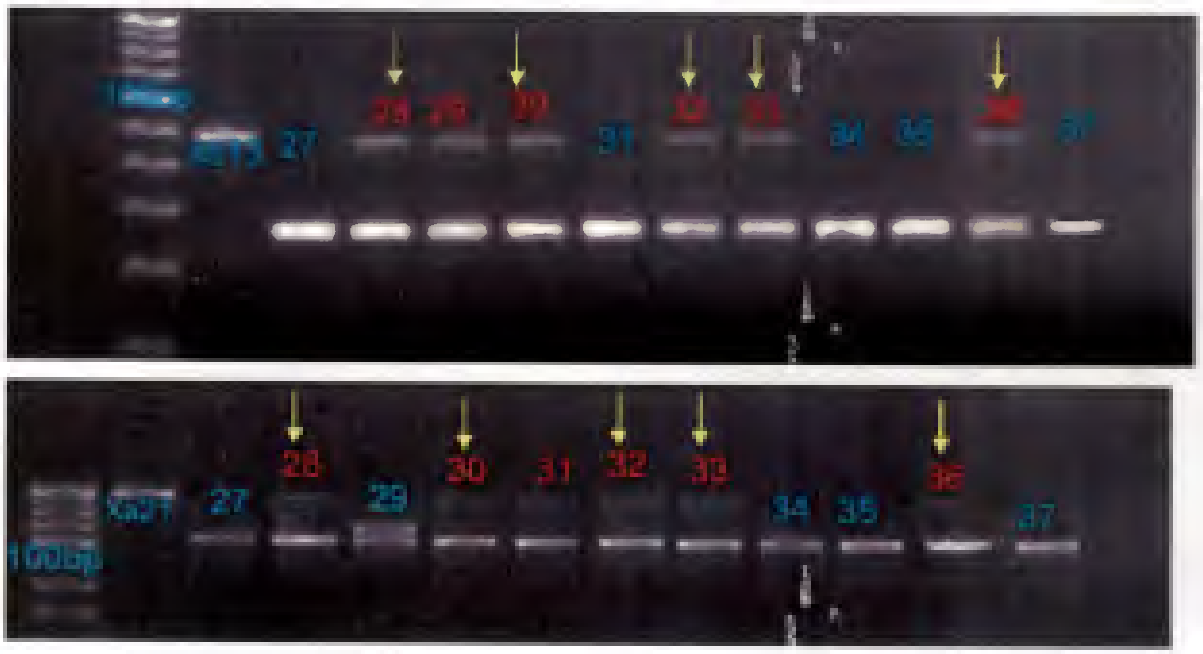
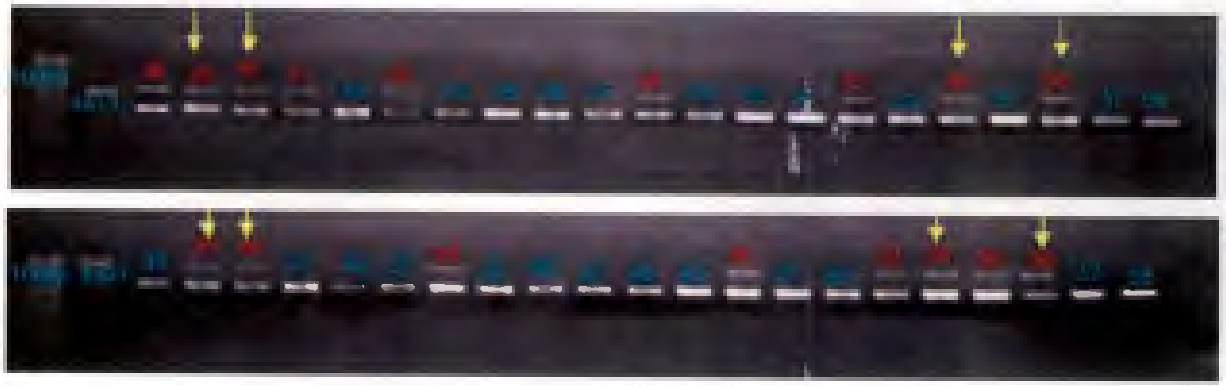


Plate 19(d)



Arrow mark = samples with both *xa* 13and *Xa21*genes

Plate 19(e)

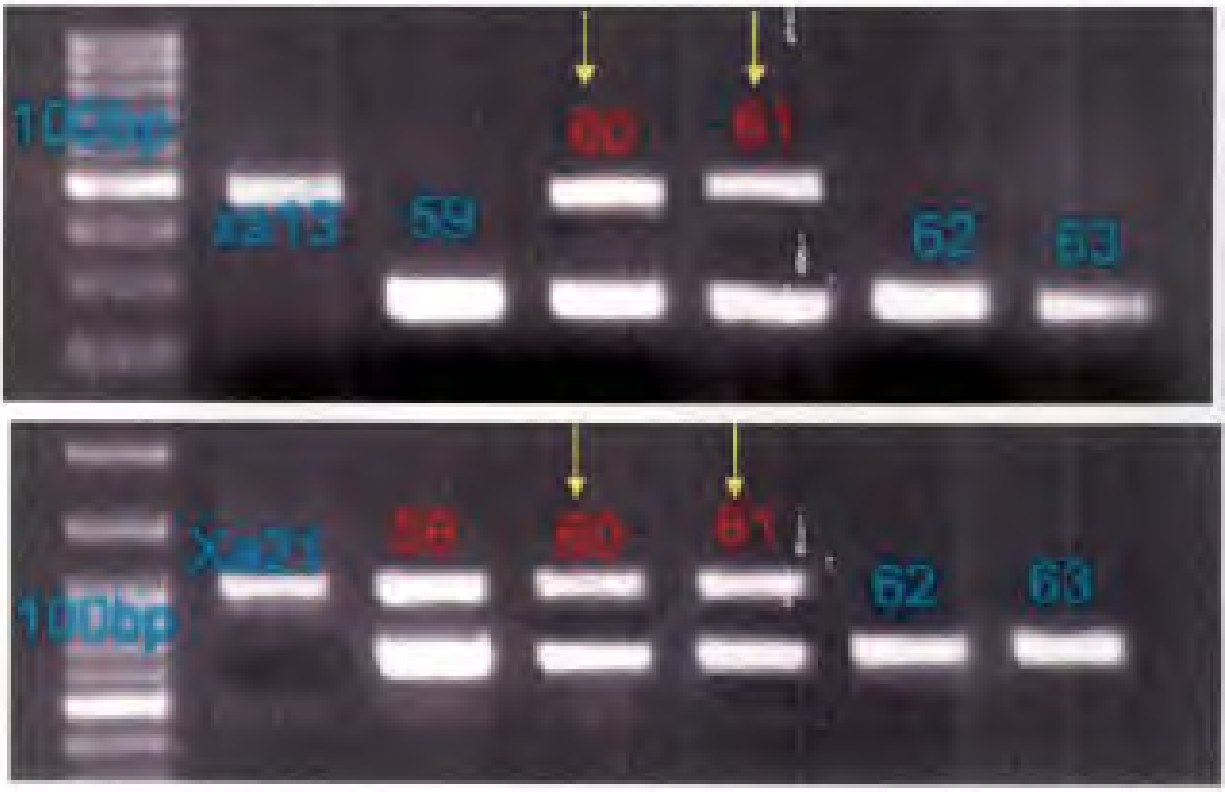
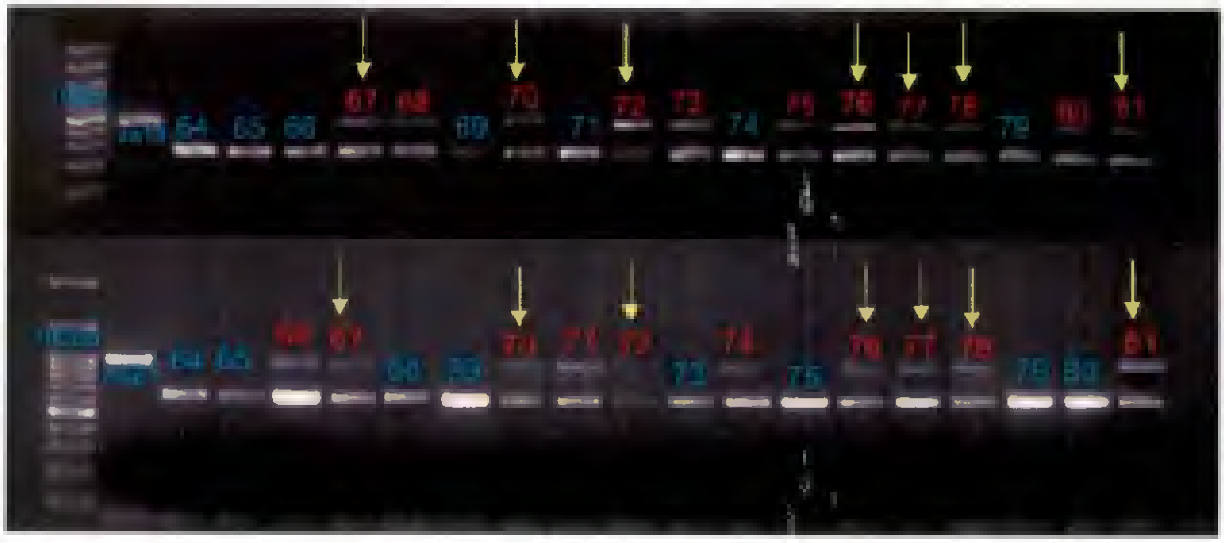


Plate 19(f)



Arrow mark = samples with both *xa* 13and *Xa21*genes

Plate 19(g)

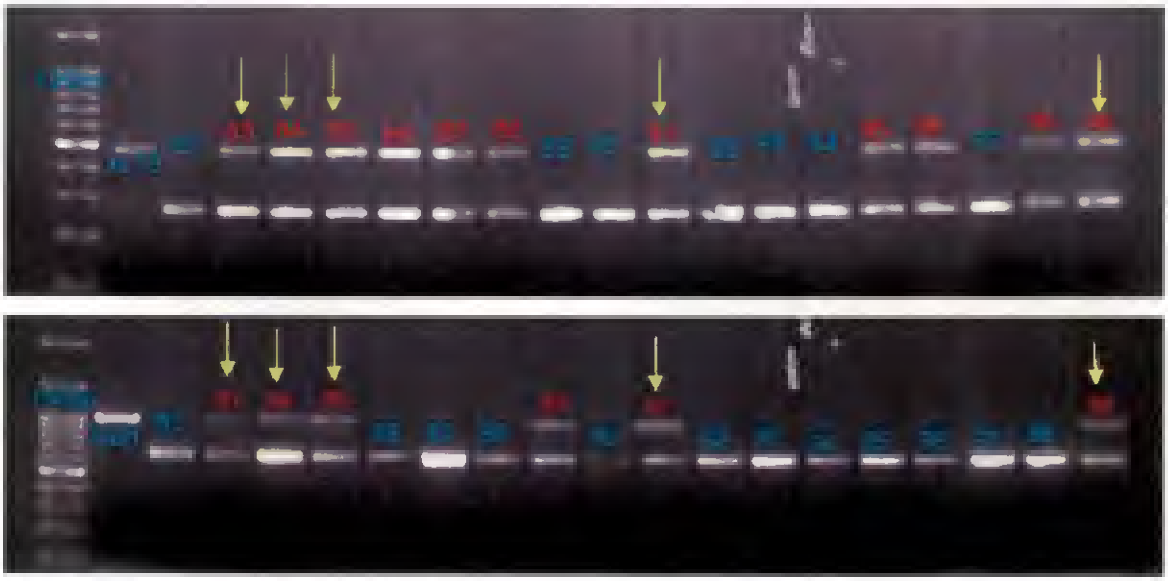
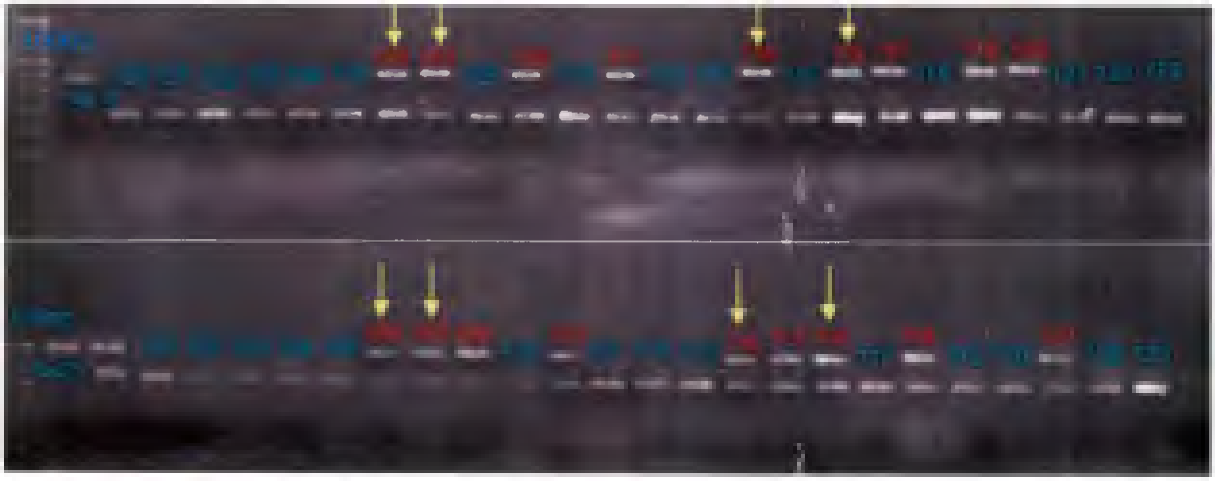


Plate 19(h)



Arrow mark = samples with both *xa* 13and *Xa21*genes

Plate 19(i)

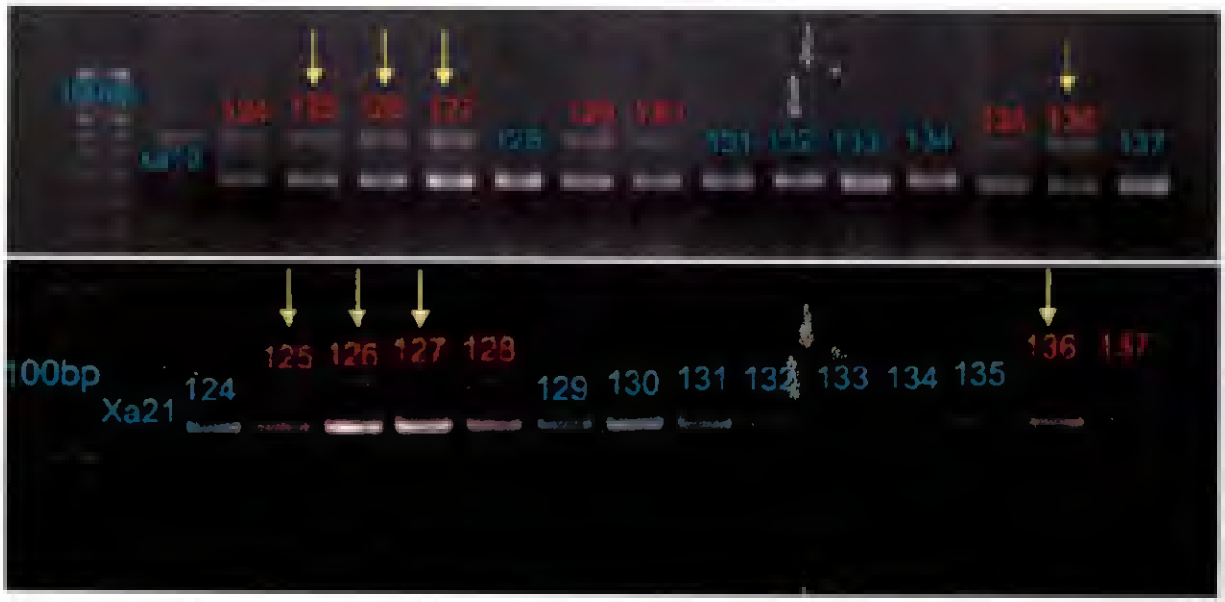


Plate 19(j)

Arrow mark = samples with both *xa* 13and *Xa21*genes

Plate 20. Foreground selection in BC_1F_1 plants of the cross Prathyasa Improved Samba Mahsuri using *Xa33* linked marker RMWR 7.1

Plate20(a)

Plate20(b)

Plate20(c)

Plate20(d)

Red color labelled samples with *Xa33* gene

Plate21.Foreground selection in BC_1F_1 plants with *Xa38* gene of the cross Prathyasa x PR-114 using *Xa38* linked functional marker Oso4g53050-1

Plate21 (a)

Plate 21(b)

Plate21(c)

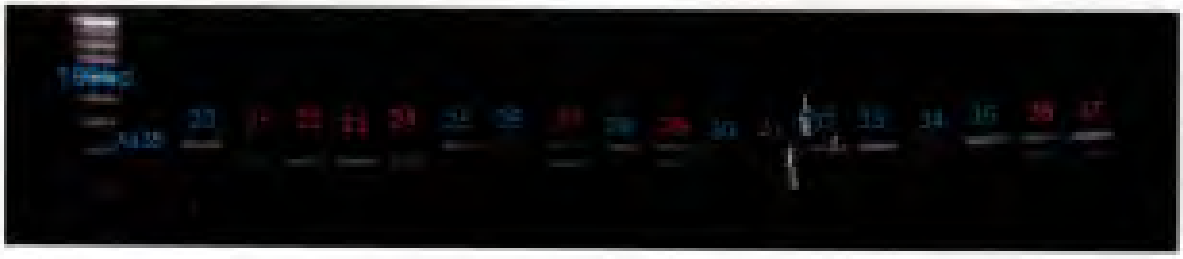
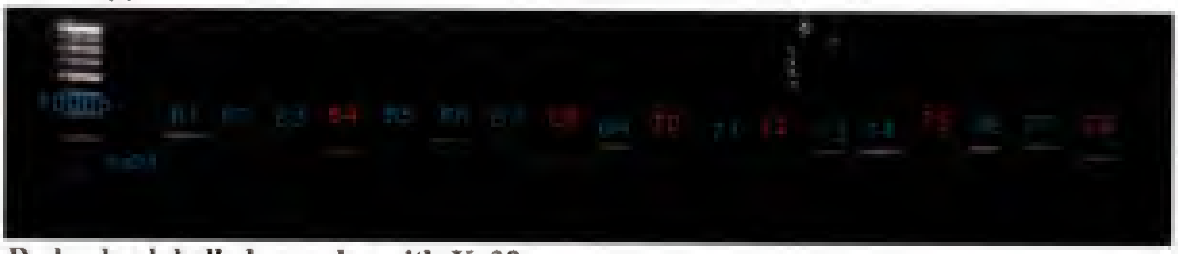


Plate 21(d)

Plate21(e)



Red color labelled samples with *Xa38* gene

4.5.6. Prathyasa x PR-114 (*Xa38*) Backcross Generation Plants

From the 180 back cross seeds seventy eight germinated. DNA from these plants were subjected to foreground selection by using foreground marker Os04g53050-1 .Thirty seven BC_1F_1 plants had resistance gene *Xa*38 (Plate21 (a) to 21(e)). Calculated $\chi 2$ value was 0.21(P value 0.64) and it was fitted in 1:1 Mendelian ratio.

4.6. PHENOTYPIC ANALYSIS OF BC_1F_1 PLANTS WITH RESISTANCE

Phenotypic data in four metric traits and one qualitative trait were recorded in the BC_1F_1 plants and the parental genotypes (Table 13-18). Significant variation was observed for plant height, effective number of tillers per plant, grain length/breadth ratio, kernel colour and days to maturity in the BC_1F_1 plants.

4.6.1. Population Derived from Aiswarya x ISM

4.6.1.1 Plant Height (cm)

Aiswarya x ISM (*xa13* and *Xa21*)

Plant height of the parental genotypes Aiswarya and ISM was 98 cm and 100 cm respectively. Plant height of the BC_1F_1 plants ranged from 65 to 117cm with average value of 89.98± 2.04. Plant A-102 had a maximum height of 117 cm and genotype A-133 had a minimum height of 65 cm. All backcross plants showed height similar to the parental lines except A-33(108cm), A-35(107.50 cm), A-38(110cm),A-102(117cm),A-113(106.25cm) and A-148(105cm) which were taller than the better parent.

Aiswarya x ISM (*Xa33*)

BC_1F_1 plants of this cross also showed a similar height range from 70 cm to 105cm with mean value of 87±1.51. Seven plants (B-8, B-12, B-33, B-43, B-63

Table 13.Phenotypic data of the BC_1F_1 plants of Aiswarya with the genes *xa*13 and *Xa21*

Plant No	Plant Height(cm)	Productive Tillers	L/B ratio	Kernel Colour	Days to Maturity
A-2	92.50	12	2.80	Light red	135
A-8	97.50	13	2.41	Brown	140
A-17	70.00	10	2.85	Brown	125
A-22	101.25	15	2.65	Light red	120
A-23	97.50	15	2.97	Light red	126
A-27	67.50	5	3.51	Variegated brown	135
A-32	102.50	6	3.37	Light red	128
A-33	108.00	6	2.33	Brown	130
A-35	107.50	5	2.31	Light red	135
A-38	110.00	5	3.13	Brown	126
A-46	73.75	4	3.55	Light red	120
A-47	80.00	9	2.22	Brown	128
A-60	88.00	16	3.78	Light red	136
A-62	95.00	11	2.68	Brown	125
A-65	80.00	13	2.29	Light red	130
A-66	70.00	10	2.28	White	130
A-69	87.50	12	2.88	White	135
A-71	91.25	10	2.80	Variegated brown	132
A-72	70.00	8	3.13	Brown	130
A-74	72.50	6	2.28	Light red	130
A-76	90.00	11	2.80	Light red	126
A-83	100.00	10	2.88	Light red	125
A-84	87.50	6	3.05	Light red	122
A-99	102.50	6	2.80	Brown	130
A-101	95.00	11	3.01	Brown	135
A-102	117.00	15	2.41	Brown	128
A-106	88.75	15	2.87	Light brown	125
A-110	100.00	10	2.50	Light red	123
A-111	102.50	8	2.80	Light red	130
A-113	106.25	12	2.64	Variegated brown	135
A-120	93.75	10	2.98	Red	138
A-129	80.00	8	3.14	Light red	126
A-133	65.00	6	3.03	Brown	139
A-146	92.50	11	2.70	Light red	135
A-148	105.00	10	2.26	Variegated brown	132
A-150	97.50	6	2.77	Light red	129
A-152	85.00	6	2.43	Variegated brown	130
A-158	90.00	8	3.39	White	126
A-159	88.75	9	2.32	Brown	125
A-160	67.50	7	2.69	Brown	130
A-168	80.00	10	2.41	Light red	128
A-170	82.50	9	3.05	Light red	130
Aiswarya	98.00	8	2.69	Red	120
ISM	100.00	12	2.86	White	150
Mean	89.98±2.04	9.4±0.50	2.79±0.06		129.06±0.76

B-72, B-79 and B-114) exhibited plant height ±100cm and were on par with parents.

4.6. 1.2. Number of Productive Tillers Plant^{-1}

Aiswarya x ISM (*xa13* and *Xa21*)

Numbers of productive tillers in the parental genotypes Aiswarya and ISM was 8 and 12 respectively. Number of productive tillers in the BC_1F_1 plants ranged between 4 and 16 with average value of 9.4±0.50. Four Plants A- 72, A-111, A-129, and A-158 showed numbers of productive tillers similar to Aiswarya parent. Eight BC_1F_1 plants (A-17, A-66, A-71, A-83, A-110, A-120, A-148 and A-168) had number of productive tillers intermediate between the two parents.

Aiswarya x ISM (*Xa33*)

This backcross derived plants also showed a similar variation in the number of productive tillers between 4 and 15 with average value of 10.02 ±0.41. Six plants (B-16, B-27, B-44, B-51, B-72 and B-90) had 8 numbers of productive tillers and were more similar to Aiswarya.

4.6.1.3. Length/Breadth Ratio of Grain

Aiswarya x ISM (*xa13* and *Xa21*)

Length/breadth ratio of grain in the parental genotypes Aiswarya and ISM was 2.69 and 2.86 respectively. Length/breadth ratio of grain among BC_1F_1 plants ranged from 2.22 to 3.78 and with average L/B ratio 2.79±0.06. Four BC_1F_1 plants (A-22, A-62, A-113 and A-160) showed length/breadth ratio and which were closer to the recurrent parent.

Table 14. Phenotypic data of the BC_1F_1 plants of Aiswarya with the gene *Xa33*

Plant No.	Plant Height (cm)	Productive Tillers	L/B ratio	Kernel Colour	Days to Maturity
B-6	95	10	3.01	Light brown	125
B-7	77.5	14	3.04	Variegated brown	130
B-8	105	12	2.71	Light red	135
B-9	80	14	2.33	Brown	126
B-10	87.5	12	2.48	Light brown	129
B-12	102.5	14	3.12	Variegated brown	128
B-16	75	8	3.37	Light brown	130
B-17	88	12	2.85	Variegated brown	125
B-18	95	11	2.64	Brown	130
B-19	80	13	3.12	White	139
B-23	70	10	2.72	Light brown	135
B-24	87.5	12	2.52	Dark red	140
B-25	91.25	10	2.85	Light red	138
B-27	70	8	3.23	Variegated brown	135
B-30	72.5	6	3.05	Dark brown	133
B-32	90	11	2.33	Light red	136
B-33	100	10	2.48	Light brown	138
B-39	87.5	6	3.12	Brown	125
B-43	102.5	6	3.23	Dark brown	123
B-44	75	8	2.51	Light red	128
B-45	88	4	2.86	Dark brown	125
B-46	95	7	3.37	Dark brown	130
B-47	80	6	2.29	Variegated brown	135
B-49	87.5	7	3.45	Light red	132
B-50	91.25	7	2.73	Light red	136
B-51	70	8	3.67	Light red	131
B-54	72.5	13	2.41	Light red	129
B-61	90	11	2.48	Light red	131
B-63	100	15	3.12	Variegated brown	125
B-64	87.5	15	3.08	Dark brown	128
B-68	70	10	3.05	Light red	132
B-69	72.5	6	3.01	Light red	130
B-71	90	10	3.05	Brown	129
B-72	100	8	2.71	Light red	134
B-73	87.5	12	2.42	Light brown	131
B-79	102.5	11	2.48	Variegated brown	130
B-80	75	12	3.12	Dark brown	135
B-82	88	10	3.32	Light red	133
B-90	95	8	2.68	Light red	136
B-92	87.5	9	2.48	Light red	138
B-97	92.5	6	3.12	Light red	125
B-112	97.5	11	2.72	Dark brown	123
B-114	100	12	2.52	Light red	128
B-116	90	13	2.85	Red	125
B-117	95	12	2.98	Light red	130
B-118	87.5	11	2.85	Red	135
Aiswarya	98.00	8	2.69	red	120
ISM	100.00	12	2.86	White	150
Mean	87±1.51	10.02±0.41	2.86±0.05		130.96±0.67

Aiswarya x ISM (*Xa33*)

This set of BC_1F_1 plants showed a wider range of length breadth ratio (2.29 - 3.67) and mean value of 2.86 ±0.05. BC_1F_1 plants *viz*., B-8, B-18, B-23, B-50, B-72, B-90, and B-112 recorded L/B ratio 2.71, 2.64, 2.72, 2.73, 2.71, 2.68, 2.72 which were closer to recurrent parent value. Seven plants (B-16, B-27, B-39, B-46, B-49, B-51 and B-82) exhibited higher L/B ratio.

4.6. 1.4. Kernel Colour

Aiswarya x ISM (*xa13* and *Xa21*)

Parents Aiswarya and ISM had red and white kernel colours respectively. Most of BC_1F_1 plants (20) showed light red to red kernel colour. Only three plants (A-66, A-69 and A-158) exhibited white kernel colour. Some plants had brown to light brown kernel colour.

Aiswarya x ISM (*Xa33*)

Twenty one BC_1F_1 plants exhibited light red colour of kernel. Twenty four plants had brown kernel colour.

4.6. 1. 5 Days to Maturity

Aiswarya x ISM (*xa13* and *Xa21*)

Parents Aiswarya and ISM matured in 120 and 150 days respectively. The average maturity days among BC_1F_1 plant was 129.06 ±0.76. BC_1F_1 plant A-8 showed highest maturity days (140) which were higher than recurrent parent Aiswarya. None of the plants exhibited higher maturity duration than donor parent ISM. BC_1F_1 plants A-22 and A-46 had taken time to similar to that of the Aiswarya in maturing.

Aiswarya x ISM (*Xa33*)

In this set of BC_1F_1 plants maturity days ranged from 123 days (B-43 and B-112) to 140 days (B-24) with average value of130.96±0.67.All most all the plants matured before the long duration donor parent. None of the BC_1F_1 plant matured before the recurrent parent.

4.6. 2. Aiswarya x PR-114(*Xa*38) Derived Backcross Generation

4.6. 2. 1 Plant Height (cm)

Parents Aiswarya and PR-114 had plant height 98 cm and 108 cm respectively. The BC_1F_1 plant height ranged from 70cm (C-166) to 117.5 cm (C-45) with mean value of 89.19±1.05. Twelve plants exhibited higher plant height than recurrent parent Aiswarya. Plant C-45(117.5cm) showed plant height more than donor PR-114. Most of the plants exhibited lesser plant height than Aiswarya.

4.6. 2 .2 Number of Productive Tillers Plant $^{-1}$

Parents Aiswarya and PR-114 had 8 and 10 number of productive tillers. The number of productive tillers in BC_1F_1 plants varied from 4 to 16 with mean of 8.44±0.34. The maximum number of productive tillers (16) observed in C-3 and C-35 and minimum number of tillers (4) observed in C-32, C-71, C-140 and C-153. Eight productive tillers were observed in twelve plants (C-33, C-41, C-49, C-54, C-70, C-78, C-93, C-101, C-113, C-130, C-137 and C-167) which are similar to that of the recurrent parent.

4.6.2.3. Length/Breadth Ratio of Grain

Grain Length/Breadth ratio of Aiswarya and PR-114 were 2.69 and 3.57 respectively. Length/breadth ratio in the BC_1F_1 plants varied between 2.19 and 3.94 with an average value of 2.95 ±0.05. Two BC1F1 plants (C-63 (3.83) and

Table 15. Phenotypic data of the BC_1F_1 plants of Aiswarya with the gene *Xa* 38

Plant No.	Plant Height(cm)	Productive Tillers	L/B ratio	Kernel Colour	Days to Maturity
C-2	85	6	3.25	Light red	135
C-3	88.75	16	2.71	Brown	130
C- 7	100	10	2.31	White	128
C-8	92.5	7	2.55	Red	132
C-10	88.75	7	2.74	Dark brown	130
C-12	97.5	7	2.89	Light red	125
C-13	93.75	6	3.46	Brown	122
C-15	80	6	2.21	Light red	126
C-18	85	7	3.33	Light brown	130
C-20	92.5	5	3.52	Light red	122
C-23	105	9	2.63	Brown	124
C-29	97.5	5	2.76	Brown	125
C-32	85	4	3.30	White	128
C-33	88.75	8	3.50	Dark brown	132
C-35	90	16	3.51	Light red	135
C-36	102.5	10	3.34	Brown	122
C-39	97.5	7	3.03	Dark brown	128
C-40	85	7	3.53	Brown	130
C-41	102.5	8	2.79	Light red	125
C-43	102.5	13	3.01	Light red	132
C-44	95	11	2.42	Dark red	135
C-45	117.5	15	2.26	Light red	120
C-46	88.75	15	3.34	Dark brown	128
C-47	100	10	3.46	Light red	136
C-49	102.5	8	2.21	Brown	125
C-50	106.25	12	3.33	brown	130
C-52	93.75	10	3.52	Light brown	130
C-54	80	8	2.74	Light red	135
C-60	75	6	2.89	Brown	132
C-62	92.5	11	2.63	Variegated brown	130
C-63	105	10	3.83	Brown	130
C-67	97.5	6	2.19	Light red	126
C-68	85	6	3.01	Light red	125
C-70	88.75	8	2.42	Light red	122
C-71	90	4	2.26	Light red	130
C-72	102.5	7	2.88	Brown	135
C-73	97.5	6	3.12	Variegated brown	132
C-74	80	7	2.79	Light red	136
C-77	87.5	7	3.01	Light red	120
C-78	92.5	8	2.42	brown	134
C-85	85	13	2.26	Light red	115
C-86	85.75	11	3.34	Variegated brown	135

Table 15. (Continued...)

Plant No.	Plant Height(cm)	Productive Tillers	L/B ratio	Kernel Colour	Days to Maturity
C-88	85	15	3.46	Red	130
C-90	80	15	2.21	Brown	132
C-91	77.5	10	3.33	Light red	125
C-93	92.5	8	3.52	Light red	138
C-94	88.75	12	2.74	Brown	135
C-96	97.5	6	3.13	Light red	126
C-101	93.75	8	3.05	Brown	120
C-103	80	13	2.42	light red	128
C-106	85	6	3.34	Light red	136
C-109	92.5	9	3.04	Light red	125
C-111	105	5	2.88	brown	130
C-112	97.5	6	3	red	130
C-113	85	8	2.26	Brown	135
C-118	88.75	13	2.88	Red	132
C-119	87.5	10	3.12	Brown	130
C-120	87.5	11	2.79	Light red	130
C-121	75	7	3.01	Light red	126
C-122	87.5	7	2.42	Brown	125
C-124	85.5	6	2.26	Brown	122
C-126	75	6	2.76	Light red	130
C-127	79.5	7	3.3	Light red	135
C-128	80	5	3.5	Red	125
C-129	87.5	6	3.52	Light red	132
C-130	70	8	3.35	Red	135
C-131	88	6	3.03	Brown	122
C-135	80	5	3.53	Light red	128
C-136	78.75	6	3.13	Brown	130
C-137	85	8	3.94	Light red	125
C-140	80	4	2.19	Light brown	132
C-143	77.5	7	3.01	Red	135
C-144	107.5	6	2.42	Brown	120
C-145	86.25	7	2.26	Light red	128
C-153	77.5	4	2.88	Light red	136
C-166	70	10	2.71	Brown	129
C-167	87.5	8	3.25	Red	130
C-175	80	9	3.33	light red	125
C-176	82.5	11	2.98	brown	122
C-178	82.5	14	3.04	Light red	130
Aiswarya	98.00	8	2.69	Red	120
PR-114	108.00	10	3.57	White	150
Mean	89.19±1.05	8.44±0.34	2.95±0.05	-	128.83±0.55

C-137(3.94) had a higher length/breadth ratio of grain than the donor parent and 31 plants exhibited higher or equal length /breadth ratio to the recurrent parent.

4.6.2.4. Kernel Colour

Aiswarya and PR-114 had contrasting red and white kernel colour respectively. BC_1F_1 plants exhibited wide range of kernel colour such as red to light red, brown to dark brown and white. Forty three BC_1F_1 plants showed light red to red colour kernel which was characteristic colour of recipient parent Aiswarya. Two BC_1F_1 plants such as C-7 and C-32 showed white kernel colour.

4.6.2.5 Days to Maturity

Days to maturity in the parental genotypes Aiswarya and PR-114 was recorded 120 and 150 days respectively. Maturity days in BC_1F_1 plants ranged between115 to 138 with average value of 128.83±0.55. Plant C-93 showed delayed maturity of 138 days and plant C-85 matured early in 115 days compared to recurrent parent. None of the BC_1F_1 plants took more than 150 days to mature. Four BC_1F_1 plants (C-45, C-77, C-101 and C-144) matured in 120 days just like the recurrent parent.

4.6.3 Prathyasa x ISM Derived Backcross Generation

4.6.3.1 Plant Height

Prathyasa x ISM (*xa13* and *Xa21*)

Plant height of the parental genotypes Prathyasa and ISM were 94 cm and 100 cm respectively. Plant height of the BC_1F_1 plants ranged between 70 and 105 cm with an average value of 82.73±1.03. Two BC_1F_1 plants (D-39 and D-72) had plant height of 95 cm which was more or less equal to recurrent parent Prathyasa and remaining plants recorded lower plant height

Table16. Phenotypic data of the BC_1F_1 plants of Prathyasa with the genes *xa13* and *Xa21*

Plant No.	Plant Height(cm)	Productive Tillers	L/B ratio	Kernel Colour	Days to Maturity
D-2	82.5	8	3.38	Light red	115
D-6	87.5	10	2.37	Brown	115
D-7	75	9	3.71	Light red	119
D-8	90	8	2.77	Light red	121
D-12	83.75	6	2.14	Light red	112
D-13	78	8	2.26	Red	115
D-22	80	10	2.87	Brown	118
D-24	85	11	3.34	Light red	125
D-28	88	7	2.10	Light red	128
D-30	77.5	12	2.12	Brown	120
D-32	80	13	2.87	Light red	129
D-33	78.75	8	3.25	Light red	130
D-36	76.5	5	2.80	Red	126
D-39	95	8	3.06	Light red	129
D-40	77.5	7	3.77	Red	128
D-54	85	10	2.80	Brown	130
D-56	80	9	2.36	Light red	128
D-60	87.5	7	2.86	Brown	121
D-61	90	11	2.02	Light red	115
D-67	75	10	2.97	Brown	126
D-70	88	13	2.47	Red	129
D-72	95	6	2.80	Brown	117
D-76	80	10	2.94	Light red	122
D-77	70	11	2.61	Light red	118
D-81	85	8	2.60	Brown	131
D-83	80	9	2.12	Red	118
D-84	77.5	7	3.17	Brown	125
D-85	79	11	3.23	Light red	128
D-91	105	12	3.15	Light red	128
D-99	87.5	6	3.08	Light red	121
D-106	75	8	2.75	Light red	115
D-107	77.5	9	3.42	Light red	120
D-114	77.5	11	2.98	Red	129
D-116	80	8	2.33	Brown	130
D-125	90	6	2.33	Light red	126
D-126	83.75	7	2.58	Light red	119
D-127	73.75	11	3.28	Brown	121
D-136	80	8	2.75	Brown	120
D-138	80	12	2.58	Red	112
D-140	85	13	3.55	Brown	125
D-143	85	10	2.16	Light red	120
D-150	87.5	9	3.42	Light red	119
Prathyasa	94.00	10	2.17	Red	110
ISM	100	12	2.86	White	150
Mean	82.73±1.03	9.1±0.33	2.81±0.07	-	122.45±0.86

Prathyasa x ISM (*Xa*33)

In this BC_1F_1 plant height ranged from 70 cm to 100 cm with average value of 83.70±1.61. Plant E-48 recorded highest height of 100 cm. Plants E-16, E-23, E-43 and E-57 exhibited lower plant height of 70cm. Two BC_1F_1 plants such as E-14 and E-38 showed height of 95 cm which was similar to that of Prathyasa.

4.6.3.2. Number of Productive Tillers Plant^{-1}

Prathyasa x ISM (*xa13* and *Xa21*)

Number of productive tillers plant^{-1} of the parental genotypes Prathyasa and ISM was 10 and 12 respectively. Among BC_1F_1 plants the minimum number of productive tillers (5) was observed in D-36. Plants D-32, D-70 and D-140 produced maximum number of productive tillers. The average value was 9.1±0.33. Six BC_1F_1 plants viz., D-6, D-22, D-54, D-67, D-76 and D-143 showed 10 numbers of productive tillers which was similar to that of Prathyasa variety.

Prathyasa x ISM (*Xa33)*

The number of productive tillers in BC_1F_1 plants ranged from 4 (E-37) to 16 (E-12) with an average value of 9.63±0.33. Six BC_1F_1 plants viz., E-16, E-22, E-31, E-57, E-62 and E-66 had 10 numbers of tillers which was similar to that of the recurrent parent.

4.6.3.3. Length and Breadth Ratio of Grain

Prathyasa x ISM (*xa13* and *Xa21*)

Length/breadth ratio of grain in the parental genotypes Prathyasa and ISM was 2.17 and 2.86 respectively. Among BC_1F_1 plants the Length/breadth ratio varied from 2.02(D-61) to 3.77 (D-40) with mean value of 2.81±0.07. D-12(2.14),

Table 17. Phenotypic data of the BC_1F_1 plants of Prathyasa with the gene *Xa*33

Plant No.	Plant height(cm)	Productive tillers	L/B ratio	Kernel Colour	Days to Maturity
E-1	87.5	12	3.33	Light red	125
E-5	75	11	2.72	Variegated Brown	128
E-6	75	8	2.52	Light red	130
E-12	88	16	2.85	Variegated Brown	125
E-14	95	11	3.23	Brown	132
E-15	80	13	3.05	White	130
E-16	70	10	2.12	Light brown	125
E-18	87.5	12	2.48	Dark red	120
E-22	91.25	10	3.12	Light red	140
E-23	70	8	3.23	Brown	122
E-24	72.5	6	2.51	Dark brown	130
E-29	90	11	2.86	Light red	130
E-31	100	10	3.37	Light brown	122
E-32	87.5	6	3.08	Light red	126
E-33	75	8	2.29	Dark brown	125
E-37	88	4	3.45	Light red	130
E-38	95	7	2.73	Red	119
E-39	80	6	3.67	Brown	120
E-40	87.5	7	2.41	Light red	119
E-41	91.25	7	2.48	Light red	116
E-43	70	8	3.12	Brown	118
E-44	72.5	13	3.08	White	120
E-45	90	11	3.12	Light brown	125
E-48	100	15	3.32	Dark Brown	122
E-55	87.5	15	2.70	Light red	129
E-57	70	10	2.48	Light red	125
E-58	72.5	6	3.12	Dark brown	130
E-62	90	10	2.72	Light brown	129
E-63	80	11	2.52	Light red	125
E-65	87.5	8	2.85	Dark brown	130
E-66	82.5	10	2.70	Light red	129
E-67	90	8	2.69	Red	125
Prathyasa	94	10	2.17	Red	110
ISM	100	12	2.86	White	150
Mean	83.70±1.61	9.63±0.33	2.87±0.07	-	125.66±0.90

D-30 (2.12), D-83(2.12), D-143(2.16) BC_1F_1 plants showed grain length and breadth ratio almost similar to recipient parent.

Prathyasa x ISM (*Xa33)*

Length/breadth ratio of grain in BC_1F_1 plants ranged from 2.12(E-16) to 3.67 (E-39) respectively with mean value of 2.87±0.07. Fourteen plants showed grain length and breadth ratio more than 3.00.

4.6.3.4. Kernel Colour

Prathyasa x ISM (*xa13* and *Xa21*)

Parents Prathyasa and ISM had contrasting colour red and white respectively. Twenty nine BC_1F_1 plants showed red to light red colour of kernels which was characteristic colour of Prathyasa .Thirteen plants exhibited brown color of kernels. None of the plant had white kernel colour.

Prathyasa x ISM (*Xa33)*

Among BC_1F_1 plants fifteen genotypes had red to light red, 15 plants had brown to light brown and only two plants exhibited white colour of kernel.

4.6.3.5. Days to Maturity

Prathyasa x ISM (*xa13* and *Xa21*)

Parental genotypes Prathyasa and ISM recorded 110 and 150 days to maturity respectively. The range of maturity in BC_1F_1 plants was 112 to 131 days with mean value of 122.45±0.86. Most of the plants recorded medium maturity duration in the range of 115-130.None of the plants matured late like donor parent.

Prathyasa x ISM (*Xa33)*

Maturity days in BC_1F_1 plants ranged from 116 to 140 days in E-41 and E-22 with mean of 125.66 ±0.90. Most of the plants had medium maturity duration with range of 120-130 days.

4.6.4 Prathyasa x PR-114(*Xa*38) Derived Backcross Generation

4.6.4.1 Plant Height (cm)

Parents Prathyasa and PR-114 had plant height 94 and 108cm respectively. The range of BC_1F_1 plants was from 70 cm to 105 cm with mean of 84.88±1.73. Minimum plant height (70 cm) was recorded in F-6, F-11, F-29, F-46, F-59 and maximum height of the plant (105cm) was observed in F-17. None of the plant exhibited plant height more than donor parent. BC_1F_1 plants F-4, F-24 and F-36, had plant height 95 cm which was more or less similar to the plant of recurrent parent Prathyasa.

4.6.4.2 Number of Productive Tillers Plant^{-1}

Number of productive tillers plant $^{-1}$of the parental genotypes, Prathyasa and PR-114 was 10. Among BC_1F_1 plants maximum numbers of productive tillers plant $^{-1}$ (16) were observed in plant F-3 and F-23 with average value of 10.81±0.48. Minimum numbers of productive tillers (6) were observed in F-12, F-37 and F-60 BC_1F_1 plants. Plants such as F-6, F-9, F-29, F-59 and F-64 had 10 productive tillers and were similar to the recipient parent for a trait.

4.6.4.3 Length and Breadth Ratio of Grain

Length/breadth ratio of grain in the parental genotypes Prathyasa and PR-114 was 2.17 and 3.57 respectively. Among BC_1F_1 plants the minimum length/breadth ratio of 2.19 was found in F-17 and F-60. Maximum length/breadth ratio (3.94) was observed in F-58. The average value was 3.01±0.08.

Table18. Phenotypic data of the BC_1F_1 plants of Prathyasa with the genes *Xa38*

Plant No.	Plant height(cm)	Productive tillers	L/B ratio	Kernel Colour	Days to Maturity
F-3	88	16	3.50	Light brown	122
F-4	95	11	3.51	Brown	124
F-5	80	13	3.34	Light red	125
F-6	70	10	3.03	Dark brown	128
F-8	87.5	12	3.53	Light red	132
F-9	91.25	10	2.62	Light red	135
F-11	70	8	3.73	light red	122
F-12	72.5	6	3.09	brown	128
F-14	90	11	2.30	Light red	130
F-16	77.5	14	3.36	light brown	125
F-17	105	14	2.19	Brown	132
F-18	80	14	2.31	Dark red	130
F-19	87.5	12	2.55	Light red	135
F-21	102.5	14	2.74	dark brown	136
F-22	75	8	2.89	Red	140
F-23	88	16	3.46	Light Red	135
F-24	95	11	2.21	Light red	130
F-27	80	13	3.05	Brown	130
F-29	70	10	3.52	Light red	135
F-36	95	7	2.63	Brown	132
F-37	80	6	2.76	brown	130
F-42	87.5	7	3.30	dark brown	130
F-45	91.25	7	3.50	Brown	126
F-46	70	8	3.51	Light red	125
F-48	77	8	3.03	Brown	130
F-52	72.5	13	3.34	Light red	122
F-55	90	11	3.03	Dark red	130
F-56	100	15	3.53	Light red	135
F-58	87.5	15	3.94	dark brown	132
F-59	70	10	2.28	light red	136
F-60	72.5	6	2.19	brown	120
F-64	90	10	2.32	Red	134
F-68	100	8	2.55	Light brown	130
F-70	87.5	12	2.74	Brown	125
F-72	102.5	11	2.89	Light red	130
F-75	75	12	3.46	Light red	136
F-78	87.5	11	3.45	Light red	132
Prathyasa	94	10	2.17	Red	110
PR-114	108	10	3.57	White	150
Mean	84.88±1.73	10.81±0.48	3.01±0.08	-	129.97±0.81

4.6.4.4 Kernel Colour

Parental genotypes Prathyasa and PR-114 showed contrasting kernel colour red and white respectively. 20 BC_1F_1 plants recorded red colour kernel which was the characteristic colour of recipient parent Prathyasa. Among BC_1F_1 plants, 17 plants were brown kernel colour.

4.6.4.5 Days to Maturity

Parental genotypes Prathyasa and PR-114 matured in 110 and 150 days. The variation of maturity among BC_1F_1 plants from 120 days to 140 days and recorded in F-60 and F-22 respectively with mean value of 129.97±0.81. Most of BC_1F_1 plants recorded medium maturity duration and none of the plant matured late compared to PR-114.

4.7 BACKGROUND MARKER ANALYSIS

4.7.1 Identification of Polymorphic Loci between Donor and Receipt Panels.

4.7.1.1 Microsatellite Analysis of Four Parental Lines Using 270 Loci:

The study deals with four varieties; two resistant gene donors Improved Samba Mahsuri (DP_1) and PR 114 (DP_2) and two recurring parents Aiswarya (RP_1) and Prathyasa (RP_2). The four varies were genotyped with 270 microsatellite loci (Appenix 1). Of the 270 loci, 180 yielded discrete, unambiguous amplification products in the four varieties. Of the 180 loci, 101 were polymorphic in at least one of the four varieties. The chromosome-wise distribution of polymorphic loci are as follows:

Chromosome 1- 10 loci; chromosome 2 - 07 loci: chromosome 3 - 04 loci; chromosome 4 - 09 loci; chromosome 5 - 10 loci; chromosome 6 - 11 loci; chromosome 7 – 09 loci; chromosome 8 – 09 loci: chromosome 9 – 10 loci; chromosome 10 – 03 loci; chromosome 11- 11 loci and chromosome 12- 08 loci.

4.7.1.2 Polymorphic Microsatellites in Four Donor X Recipient Panels

In the four panels of parental combinations Aiswarya (RP_1) X PR 114 (donor) ; Aiswarya (RP_1) X Improved Samba Mahsuri (donor) ; Prathyasa (RP_2) x PR 114 (donor) ; Prathyasa (RP_2) X Improved Samba Mahsuri (donor) panels 30 markers, 39 markers, 33 markers and 41 markers were polymorphic between the parental lines (Table 19).

At least one polymorphic marker per chromosome is there in each of the four panels except chromosome 3 in Prathyasa (RP_2) x PR 114 (donor). Number of polymorphic markers per chromosome arm varies from one marker to eight markers. Of the 24 chromosome arms, 21arms have polymorphic markers in Aiswarya (RP_1) x PR 114 (donor); 20 arms have polymorphic markers in Aiswarya (RP_1) x Improved Samba Mahsuri (donor); 19 arms have polymorphic markers in Prathyasa (RP_2) x PR 114 (donor) and 21 arms have polymorphic markers in Prathyasa (RP_2) x Improved Samba Mahsuri (donor).

4.7.2 Background Marker Analysis in BC_1F_1 Plants

The background selection was carried out on altogether 279 plants derived from four BC_1F_1 populations using altogether 143 polymorphic loci in total, ranging from 30 loci in back cross population derived from [(Aiswarya x PR114) x Aiswarya] to 41 loci in the population derived from [(Prathyasa x Improved Samba Mahsuri) X Prathyasa]. Thus allele profile was examined in individual plant. The number of homozygous loci and heterozygous loci produced by the individual marker were scored. Then the percent of recurrent genome recovered in individual BC_1F_1 plants of the six populations was calculated.

4.7.2 .1. Assessment of Recurrent Parent Genomic Contribution

SSR marker data was scored for recurrent parent homozygous loci as X and heterozygous loci as Y for each primer-genotype combination. The assessment of

Table 19. Simple sequence repeats markers that are polymorphic between donors x recipient panels

Aiswarya x ISM		Aiswarya x PR-114		Prathyasa x ISM		Prathyasa x PR-114	
Sl No	Loci (Chro. No.)	Sl No	Loci (Chro. No)	Sl No	Loci (Chro. No)	Sl No	Loci (Chro. No)
1	RM212 (1)	1	RM23 (1)	1	RM312(1)	1	RM212 (1)
2	RM312 (1)	2	RM449 (1)	2	RM431(1)	2	RM431 (1)
3	RM449 (1)	3	RM583 (1)	3	RM449(1)	3	RM583 (1)
4	RM583 (1)	4	RM240 (2)	4	RM583(1)	4	RM452 (2)
5	RM300 (2)	5	RM517 (3)	5	RM300(2)	5	RM623 (3)
6	RM450 (2)	6	RM255 (4)	6	RM450(2)	6	RM241 (4)
7	RM517 (3)	7	RM471 (4)	7	RM452(2)	7	RM252 (4)
8	RM520 (3)	8	RM440 (5)	8	RM520(3)	8	RM255 (4)
9	RM267 (5)	9	RM509 (5)	9	RM623(3)	9	RM451 (4)
10	RM509 (5)	10	RM162 (6)	10	RM252 (4)	10	RM470 (4)
11	RM548 (5)	11	RM190 (6)	11	RM451 (4)	11	RM518 (4)
12	RM190 (6)	12	RM454 (6)	12	RM470 (4)	12	RM13 (5)
13	RM204 (6)	13	RM214 (7)	13	RM518 (4)	13	RM267 (5)
14	RM225 (6)	14	RM445 (7)	14	RM13 (5)	14	RM413 (5)
15	RM253 (6)	15	RM264 (8)	15	RM413(5)	15	RM440 (5)
16	RM454 (6)	16	RM310 (8)	16	RM509(5)	16	RM509 (5)
17	RM469 (6)	17	RM433(8)	17	RM538(5)	17	RM111 (6)
18	RM510 (6)	18	RM447 (8)	18	RM548(5)	18	RM162 (6)
19	RM584 (6)	19	RM502 (8)	19	RM111(6)	19	RM454 (6)
20	RM51 (7)	20	RM257 (9)	20	RM204(6)	20	RM10 (7)
21	RM172 (7)	21	RM278 (9)	21	RM225(6)	21	RM11 (7)
22	RM214 (7)	22	RM553 (9)	22	RM253(6)	22	RM223 (8)
23	RM420 (7)	23	RM484 (10)	23	RM469(6)	23	RM264 (8)
24	RM445 (7)	24	RM4 B (11)	24	RM510(6)	24	RM433 (8)
25	RM223 (8)	25	RM224 (11)	25	RM584(6)	25	RM447 (8)
26	RM447 (8)	26	RM287 (11)	26	RM10(7)	26	RM502 (8)
27	RM502 (8)	27	RM4 A (11)	27	RM11(7)	27	RM278 (9)
28	RM257 (9)	28	RM277 (12)	28	RM172(7)	28	RM271 (10)
29	RM278 (9)	29	RM313 (12)	29	RM420(7)	29	RM496 (10)
30	RM285 (9)	30	RM453 (12)	30	RM445(7)	30	RM4 B (11)
31	RM464 (9)			31	RM72(8)	31	RM116 (11)
32	RM553 (9)			32	RM447(8)	32	RM309 (12)
33	RM271 (10)			33	RM502(8)	33	RM511 (12)
34	RM484 (10)			34	RM107(9)		
35	RM4 B (11)			35	RM257(9)		
36	RM287 (11)			36	RM116(11)		
37	RM4 A (11)			37	RM120(11)		
38	RM277 (12)			38	RM254(11)		
39	RM313 (12)			39	RM287(11)		
				40	RM309(12)		
				41	RM453(12)		

the genomic contribution of the parents in the BC_1F_1 genotypes based on SSR marker data was carried out using the formula G = [(X + 1/2Y) x100]/N (Sundaram *et al.*,2008).

Where,

N = total number of parental polymorphic markers screened

X = number of markers showing homozygosity for recurrent parent allele

Y = number of markers showing heterozygosity for parental alleles

4.7.2 .2. Aiswarya x ISM Derived Backcross Generation Plants

Aiswarya x ISM (*xa*13 and *Xa*21)

The percentage genome of the recurrent parent Aiswarya assessed in 42 Aiswarya x ISM (*xa*13 and *Xa*21) backcross plants selected in foreground selection, ranged from 28.13 to 87.84 in A-113 and A-69 (Table 20). Sixteen plants showed ≥ 75 percentage (Expected percentage contribution of recurrent parent genome in BC_1F_1 plants) percentage of recovery of recurrent parent genome. Five BC_1F_1 plants *viz.*, A-7 A-69, A-76, A-101 and A-148 exhibited highest percentage recovery of recurrent parent genome 85.14, 87.84, 84.72, 83.33, 84.29 respectively.

Aiswarya x ISM (*Xa33*)

The percent recovery of Aiswarya genome in BC_1F_1 plants ranged from 44.74 (B-45) to 81.82 (B-33) (Table 21). Twelve back cross plants *viz.*, B-17 (77.63%), B-24 (78.38%), B-33 (81.82%), B-43 (75%), B-47 (75%), B-61 (79.41%), B-63 (75.68%), B-72 (75%), B-82 (77.63%), B-90 (76.39%), B-92 (77.03%) and B-97 (79.17%) showed more or equal to 75 percentage genome recovery.

Table 20. Percentage of recurrent parent genome recovery in Aiswarya x ISM (*xa* 13 and *Xa*21) BC_1F_1plants

Genotype	Recurrent parent genome recovery (%)	Genotype	Recurrent parent genome recovery (%)
A2	58.57	A83	66.22
A8	75.00	A84	75.00
A17	85.14	A99	74.29
A22	75.00	A101	83.33
A23	76.32	A102	41.94
A27	68.42	A106	51.43
A32	68.42	A110	41.94
A33	70.27	A111	62.82
A35	55.41	A113	28.13
A38	63.51	A120	58.11
A46	75.00	A129	78.57
A47	77.63	A133	75.71
A60	62.16	A146	55.71
A62	62.16	A148	84.29
A65	47.06	A150	64.71
A66	79.17	A152	64.29
A69	87.84	A158	61.43
A71	75.00	A159	81.94
A72	72.86	A160	62.16
A74	67.14	A168	71.05
A76	84.72	A170	68.92

Table 21. Percentage of recurrent parent genome recovery in Aiswarya x ISM (*Xa*33) BC_1F_1 plants

Genotype	Recurrent parent genome recovery (%)	Genotype	Recurrent parent genome recovery (%)
B6	68.06	B49	50.00
B7	60.53	B50	57.14
B8	70.27	B51	54.69
B9	71.05	B54	58.57
B10	68.42	B61	79.41
B12	60.81	B63	75.68
B16	68.92	B64	63.89
B17	77.63	B68	71.05
B18	69.44	B69	60.53
B19	65.71	B71	60.53
B23	68.06	B72	75.00
B24	78.38	B73	68.92
B25	69.74	B79	66.22
B27	60.53	B80	71.62
B30	64.47	B82	77.63
B32	74.29	B90	76.39
B33	81.82	B92	77.03
B39	72.97	B97	79.17
B43	75.00	B112	65.71
B44	67.95	B114	55.88
B45	44.74	B116	62.82
B46	59.21	B117	48.61
B47	75.00	B118	68.92

4.7.2 .3 Aiswarya xPR-114 (Xa38) Derived Backcross Generation Plants

Recovery percentage of Aiswarya genome in the BC_1F_1 plants ranged from 33.33% (C-8 and C-121) to 80 % (C-178) (Table 22). Eight BC_1F_1 plants had more or equal to expected % of contribution of recurrent parent genome in BC_1F_1 plants and these plants were C-3, and C-49 (78.33%), C-12, C-18, C-54, C-86 (76.67%), C-176 (75%) and C-178(80%).

4.7.2 .4 Prathyasa x ISM Derived Backcross Generation Plants

Prathyasa x ISM (*xa*13 and *Xa*21)

Forty two BC_1F_1 plants identified through foreground markers were analysed by using polymorphic 41 markers. Among these BC_1F_1 plant D-72 had lowest percentage of Prathyasa genome 57.32 %.(Table 23). Back cross plant D-126 recorded highest recurrent parent genome 87.80%. Eleven plants showed highest percentage (≥80%) of Prathyasa genome and these plants were D-2 (84.29%), D-12(83.33%), D- 40(80.49%), D-61, D-67(82.93%), D-77(80%), D-83, D-91 (80.49%), D-126(87.80%), D-127 (85%) and D-138 (82.05%).

Prathyasa x ISM (*Xa*33)

In this backcross generation 32 plants selected through foreground selection were used 41 polymorphic background markers screening and percent of recovery of recurrent parent was analysed (Table24). The lowest percent recovery of Prathyasa genome recorded in E-39 (66.25%). Highest percent of genome recovery (82.50%) found in four BC_1F_1 plants (E-15, E-29, E-48 and E-62. Apart from these E-37(81.71%), E—31, E-63(81.25%) E-24 (80.77%), E-6, E-12, E-57 (80.49%) and E-66 (80%) plants exhibited more than 80% genome recovery.

Table 22. Percentage of recurrent parent genome recovery in Aiswarya X PR-114 (*Xa*38) derived BC_1F_1plants

Genotype	Recurrent parent genome recovery (%)	Genotype	Recurrent parent genome recovery (%)
C2	73.33	C85	66.67
C3	78.33	C86	76.67
C7	38.33	C88	70.00
C8	33.33	C90	58.33
C10	68.33	C91	70.00
C12	76.67	C93	46.67
C13	68.33	C94	68.33
C15	63.33	C96	41.67
C18	76.67	C101	35.00
C20	65.00	C103	48.33
C23	61.67	C106	50.00
C29	73.33	C109	48.33
C32	68.33	C111	58.33
C33	71.67	C112	36.67
C35	40.00	C113	35.00
C36	70.00	C118	35.00
C39	58.33	C119	71.67
C40	58.33	C120	53.33
C41	45.00	C121	33.33
C43	73.33	C122	61.67
C44	71.67	C-124	65.00
C45	40.00	C126	51.67
C46	71.67	C127	50.00
C47	71.67	C128	55.00
C49	78.33	C129	41.67

Table 22. (Continued ..)

Genotype	Recurrent parent genome recovery (%)	Genotype	Recurrent parent genome recovery (%)
C50	70.00	C-130	70.00
C52	63.33	C131	56.67
C54	76.67	C135	60.00
C60	45.00	C136	68.33
C62	66.67	C137	36.67
C63	50.00	C140	71.67
C67	63.33	C143	58.33
C68	70.00	C144	55.00
C70	71.67	C145	55.00
C71	50.00	C153	51.67
C72	61.67	C-166	60
C73	50.00	C-167	65.00
C74	71.67	C-175	70.00
C77	61.67	C-176	75.00
C78	60.00	C-178	80.00

Table 23.Percentage of recurrent parent genome recovery in Prathyasa x ISM (*xa*13 and *Xa*21) BC_1F_1 plants

Genotype	Recurrent parent genome recovery (%)	Genotype	Recurrent parent genome recovery (%)
D2	84.29	D72	57.32
D6	74.36	D76	63.16
D7	73.08	D77	80.00
D8	75.00	D81	76.83
D12	83.33	D83	80.49
D13	79.26	D84	73.75
D22	74.36	D85	78.75
D24	75.61	D91	80.49
D28	74.39	D-99	75.00
D30	74.39	D106	74.39
D32	64.47	D107	78.05
D33	78.05	D114	79.27
D36	79.27	D116	71.25
D39	78.05	D125	74.39
D40	80.49	D126	87.80
D54	76.83	D127	85.00
D56	79.27	D136	78.21
D60	58.33	D138	82.05
D61	82.93	D140	69.12
D67	82.93	D143	75.64
D70	71.95	D150	77.63

Table24. Percentage of recurrent parent genome recovery in Prathyasa x ISM (*Xa33*) BC_1F_1 plants

Genotype	Recurrent parent genome recovery (%)	Genotype	Recurrent parent genome recovery (%)
E1	70.27	E38	71.25
E5	79.27	E39	66.25
E6	80.49	E40	76.83
E12	80.49	E41	68.29
E14	68.29	E43	74.39
E15	82.50	E44	69.51
E16	79.49	E45	76.83
E18	78.75	E48	82.50
E22	78.95	E55	78.75
E23	78.75	E57	80.49
E24	80.77	E58	76.83
E29	82.50	E62	82.50
E31	81.25	E63	81.25
E32	73.17	E65	75.00
E33	71.95	E66	80.00
E37	81.71	E67	78.75

Table25. Percentage of recurrent parent genome recovery in Prathyasa x PR-114(*Xa38*) BC_1F_1plants

Genotype	Recurrent parent genome recovery (%)	Genotype	Recurrent parent genome recovery (%)
F3	50.00	F36	70.31
F4	57.69	F37	43.94
F5	59.38	F42	53.03
F6	62.50	F45	56.45
F8	45.31	F46	60.61
F9	61.29	F48	54.55
F11	53.23	F52	62.50
F12	56.45	F55	58.06
F14	70.31	F56	48.48
F16	48.44	F58	59.68
F17	54.55	F59	54.69
F18	57.81	F60	54.55
F19	78.79	F64	54.55
F21	54.55	F68	74.24
F22	46.88	F70	50.00
F23	48.48	F72	61.29
F24	63.64	F75	68.18
F27	56.90	F78	57.58
F29	68.18		

4.7.2.5 Prathyasa xPR-114 (Xa38) Derived Backcross Generation Plants

Thirty seven BC_1F_1 plants selected through foreground marker analysis were used for back ground marker analysis using 33 polymorphic markers and percent of recovery of recurrent parent was analysed (Table25). Among these plants F-37 exhibited lowest percent recovery of genome (43.94) and F-19 recorded highest percent of genome recovery (78.79). Three BC_1F_1 plants had more than 70% Prathyasa genome recovery and these were F-14, F-36 (70.31%) and F-68 (74.24%).

4.8 SCREENING OF DONORS AND RECIPIENTS FOR LOCAL BB ISOLATES

BB inoculum *X. oryzae pv. oryzae* was collected from Rice Research Station, Moncompu, Kuttanad region(Plate 22) and was maintained in PSA media (Plate 23).

4.8.1 Pot Culture Study

The recipient parents (Aiswarya and Prathyasa) and donors (ISM (*xa13* and *Xa21*), ISM (*Xa33*) PR-114(*Xa38*)) were screened during month of July. These plants were inoculated with twenty four hours old isolate of the pathogen. Symptoms appeared on the inoculated plants fourteen days after inoculation. The length of lesion was recorded in each variety based on score chart (IRRI 2013). The disease severity was calculated and expressed in percentage (Table 26). The percentage of severity in ISM (*xa13* and *Xa21*) and ISM (*Xa33*) were zero and both were graded as resistant. There was no further progress in disease development in both these donors. The percentage of disease severity was 38% in PR-114 and was graded as moderately susceptible. The percentage of disease severity in Aiswarya and Prathyasa were 58.79% and 67.28% respectively and both were graded as susceptible (Plate 24).

Plate 22: *Xoo* strain isolated from RRS Moncompu Kuttanad region.

Plate23: Subcultured petri dish of *Xoo* strain in PSA media

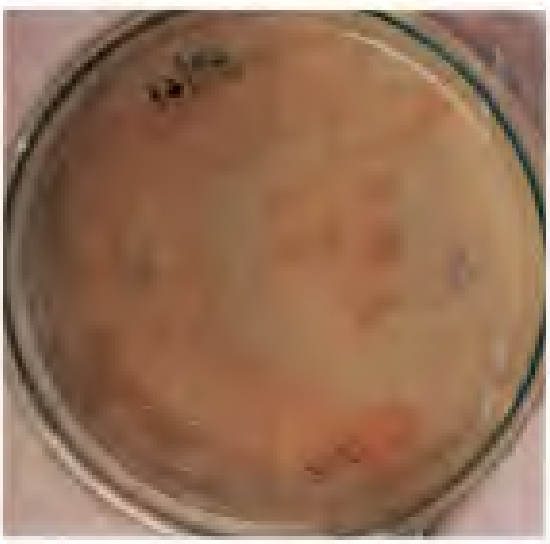
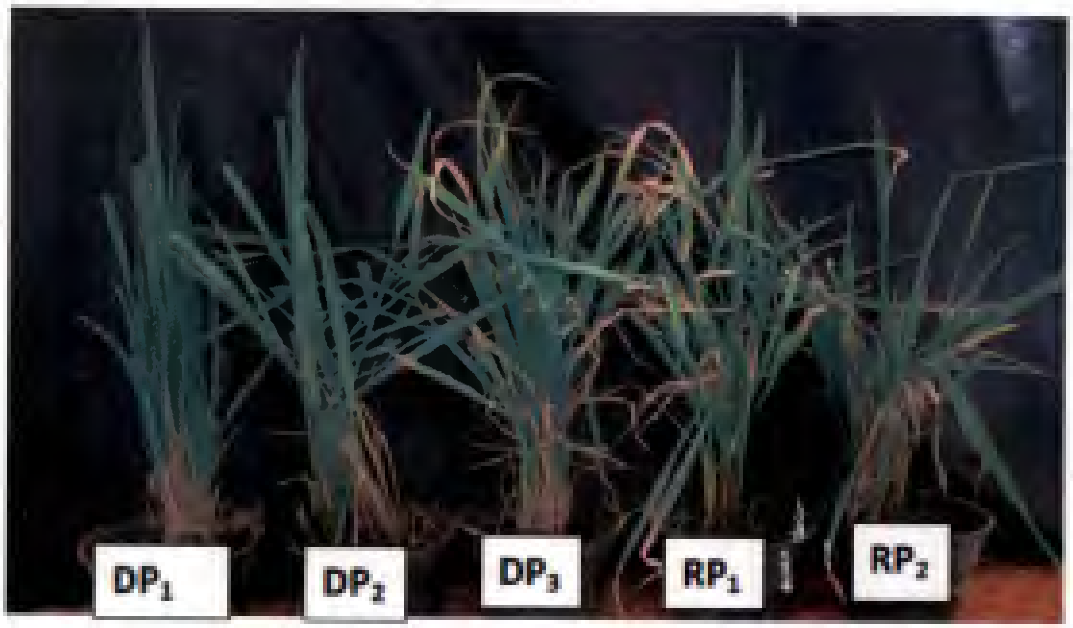


Plate.24. Disease severity in donors and recipients



DP_1 = ISM (*xa*13 and *Xa21*) DP_2= ISM (*Xa33*) and DP_3= PR-114(*Xa38*) RP_1=Aiswarya RP_2= Prathyasa

Table 26. Percentage of severity of bacterial blight in donors and recipient of parental genotypes .

Varieties	Mean percentage of BB severity	Score and host behaviour
Aiswarya (RP1)	58.5(49.88)	6- Susceptible
Prathyasa(RP2)	67(54.92)	6- Susceptible
ISM (*xa*13, *Xa*21) (DP1)	0(0)	1-Highly resistant
ISM (*Xa*33) (DP2)	0(0)	1-Highly resistant
PR-114(*Xa*38)(DP 3)	37.75(37.89)	5- Moderately susceptible
S.E(±)	0.32	
C.D	0.69	

*In parenthesis =angular transformed value

Plate 25. Field screening of donors and recipients for *Xoo*

Plate 26. Disease infection rate in donors

DP_1= ISM (*xa*13 and *Xa*21) DP2= ISM (Xa33) DP_3=PR-114(*Xa*38)

Table 27. Disease scoring at field level.

Parents (Donors and recipients)	Disease Incidence (observation in 5 plants)	Host behaviour
ISM (*xa13* and*Xa21)*	1/24,2/30,1/32,0/18,0/21 =3%	Highly resistant
ISM (*Xa33*)	0/20,1/18,2/16,3/24,3/32 =7.98%	Resistant
PR-114(*Xa38*)	10/19,6/27,12/32,5/36,12/39 =31.40%	Moderately Susceptible
Aiswarya	20/28,15/32,12/19,12/26,11/18 =57.70%	Susceptible
Prathyasa	19/32,12/26,18/29,15/26,15/30=55.00%	Susceptible

4.8.2 Field Screening of Bacterial Blight Disease

The recipient parents (Aiswarya and Prathyasa) and donors (ISM (*xa13* and *Xa21*), ISM (*Xa33*) PR-114(*Xa38*)) also screened in field at Moncompu against local isolate of *Xoo* (Plate 25). Percentage of disease incidence was recorded in each parental variety and presented in Table 27.

The percentage of disease of incidence in ISM (*xa13* and *Xa21*) and ISM (*Xa33*) were 3% and 7.98% and both were graded as highly resistant and resistant respectively. There was no further progress in disease development in both these donors. The percentage of disease incidence was 31.40% in PR-114 and was graded as moderately susceptible (Plate 26). The percentage of disease incidence in Aiswarya and Prathyasa were 57.70% and 55 % respectively and both were graded as susceptible.

4.9 INTERMATING BETWEEN BC_1F_1 AND IDENTIFICATION OF TWO OR THREE GENES COMBINATION IN BC_1F_1 INTERMATED LINES BY FOREGROUND SELECTION.

BC_1F_1 plants were selected based on foreground selection. Intermating was performed between BC_1F_1 plants having respective resistance genes to pyramid 2-3 genes combination. BC_1F_1 plants having *xa13* and *Xa21* genes were used as a female parent and BC_1F_1 plants with *Xa33* used a male parent (Table 28). Based on disease screening result, intermating was performed between BC_1F_1 plants (*xa13 & Xa21)* and BC_1F_1 plants (*Xa33)*. Based on background selection result seeds were harvested from female parents having highest number of homozygous loci. Around 100 -200 seeds were harvested from individual cross.

4.9.1 Foreground Selection in BC_1 F_1 Intermated Lines

Seeds of BC_1F_1 intermated lines selected based on background selection were kept in petri plates for germination and transferred to pots after 21 days for evaluation. DNA from all Aiswarya and Prathyasa derived intermated BC_1F_1 lines

Table 28. Intermating programme between BC_1F_1 plants

Sl. No	Female	Male	Seeds set	Pedigree
1.	BC_1F_1 plants with *xa13* and *Xa21* genes of the cross Aiswarya x Improved Samba Mahsuri (Population A)	BC_1F_1 plants with *Xa33* of the cross Aiswarya x Improved Samba Mahsuri (Population B)	200	ICAB
2.	BC_1F_1 plants with *xa13* and *Xa21* genes of the cross Prathyasa x Improved Samba Mahsuri (Population D)	BC_1F_1 plants with *Xa33* of the cross Prathyasa x Improved Samba Mahsuri (Population E)	120	ICDE

ICAB= Intercross between population A and population B
ICDE= Intercross between population D and population E

were isolated by using DNAeasy Mini kit. DNA from these lines was screened with polymorphic foreground markers and identified progenies with combination of 2-3 resistance genes (Table 29 and 30).

4.9.1.1 Aisawrya Derived BC_1F_1 Intermated Pyramided Lines

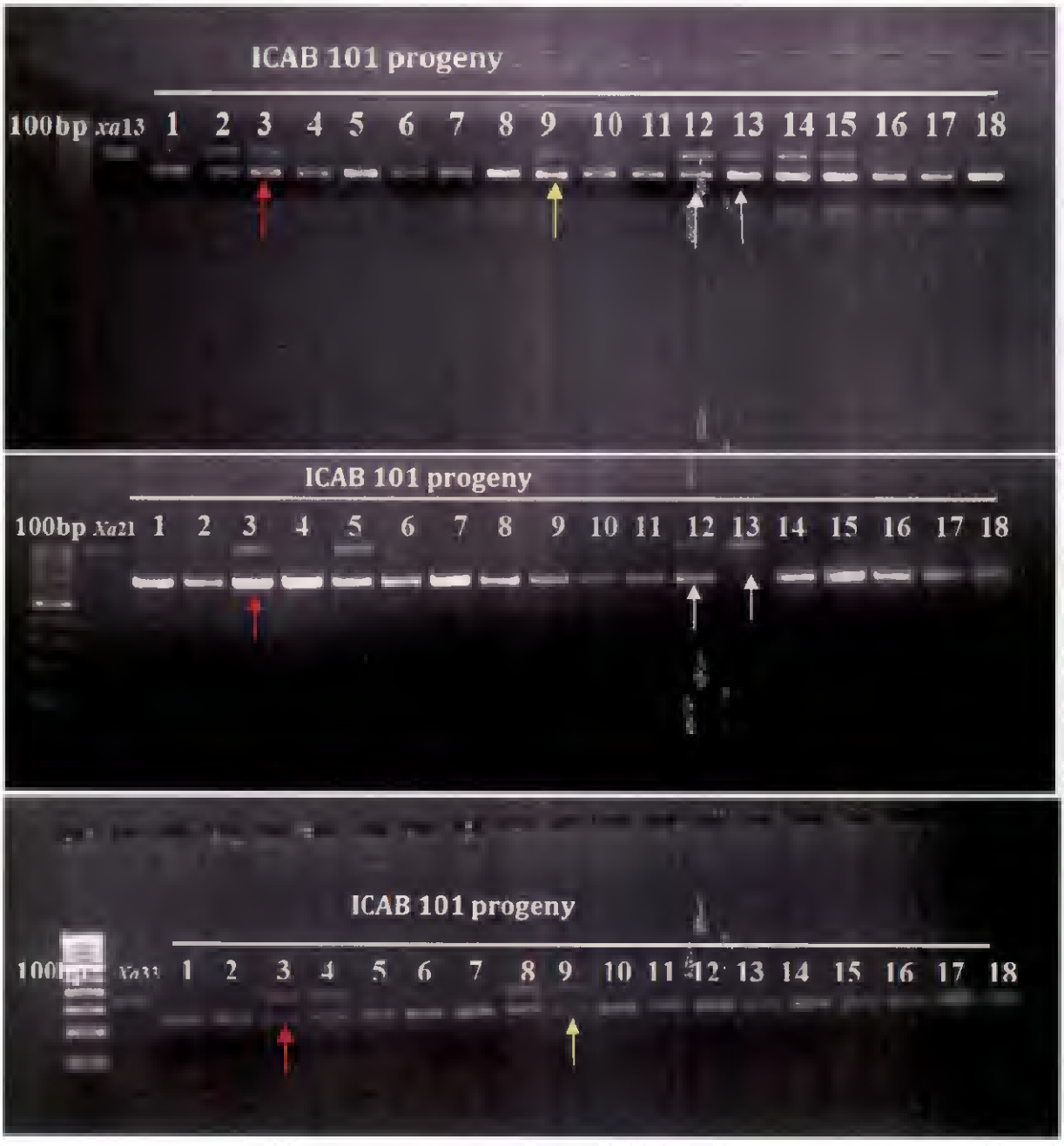
One hundred and forty nine BC_1F_1 intermated plants derived from seven plant progenies analysed for presence of two to three resistance genes combinations *Xa*21, *xa13* and *Xa33* (Plate27(a) to 27 (f)). Seven plants (ICAB 101-3, ICAB46-9, ICAB159-16, ICAB159-23, ICAB168-9, ICAB133-35 and ICAB133-50) from five progenies showed three genes combination *Xa*21+ *xa13* +*Xa33*. Seventeen plants from different progenies showed combinations of resistance genes *xa*13+*Xa*33. *Xa*21 + *xa*13 gene combination was found in 18 plant progenies. Nine plants *viz*., ICAB159-9, ICAB159-12, ICAB159-15, ICAB159-17, ICAB168-8, ICAB133-27, ICAB133-29, ICAB133-36 and ICAB133-48 from three progenies exhibited presence of *Xa*21 + *Xa*33 gene combinations (Fig.2).

4.9.1.2 Prathyasa Derived BC_1F_1 Intermated Pyramided Lines

Seventy seven intermated BC_1F_1 plants derived from eight progenies analysed with respective polymorphic markers such as pTA248, xa13 pro and RMWR 7.1 for *Xa21*, *xa13* and *Xa33* resistance genes (Plate28(a) to 28 (e)). Twelve plants *viz*., ICDE39-8, ICDE39-10, ICDE12-1, ICDE12-3, ICDE28-7, ICDE28-9 ICDE28-10 ICDE22-1, ICDE 8-1, ICDE8-2, ICDE8-4 and ICDE13-3 from six progenies had *Xa21+xa13* gene combinations. Nine plants viz., ICDE22-5, ICDE8-5, ICDE8-7 ICDE33-2, ICDE33-10, ICDE33-11, ICDE83-7, ICDE83-13 and ICDE83-14 from four progenies showed *xa*13 + *Xa*33 resistance gene combinations (Fig.3).

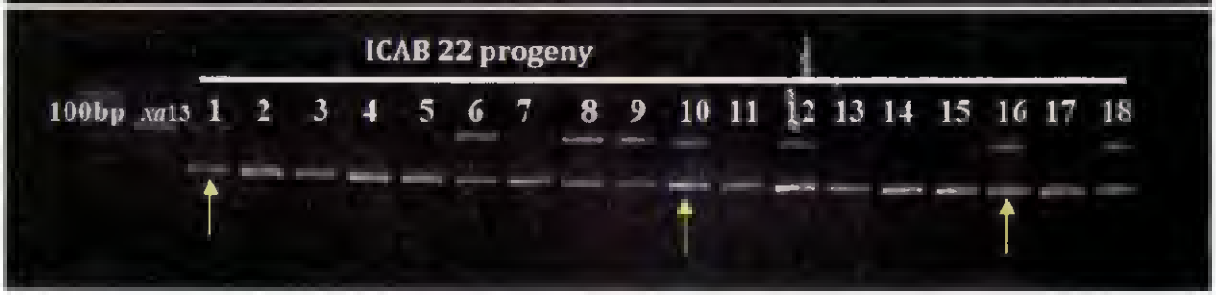
Plate 27. Foreground selection in Aiswarya derived pyramided lines using *xa*13 linked marker xa13 pro, *Xa*21 linked pTA248 and *Xa*33 linked with RMWR7.1

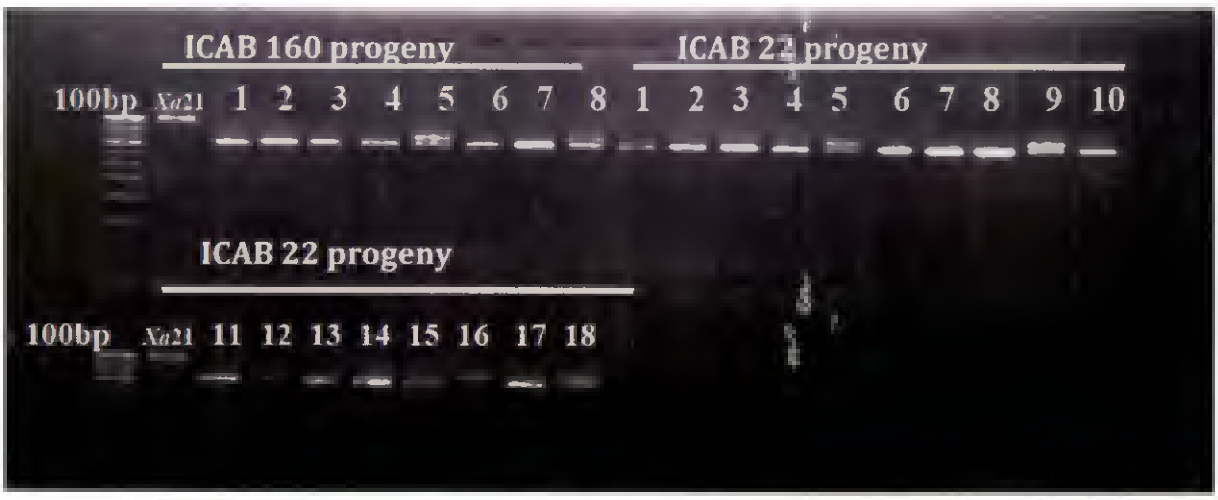
Plate 27a.

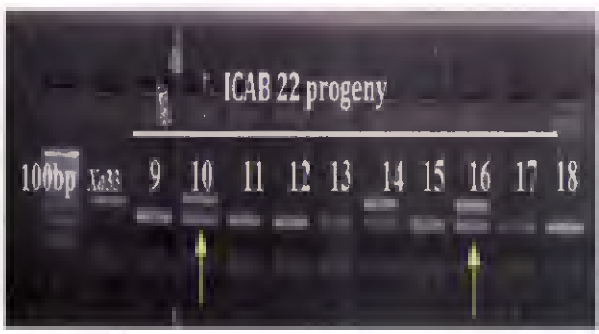


Red arrow=sample with *xa*13+*Xa*21+*Xa*33 resistance genes
Yellow arrow= sample with *xa*13 + *Xa*33 resistance gene
White arrow= sample with *xa*13+*Xa*21 resistance genes

Plate 27. (b)







Yellow arrow= sample with *xa*13 + *Xa*33 resistance genes

Plate 27. (c)

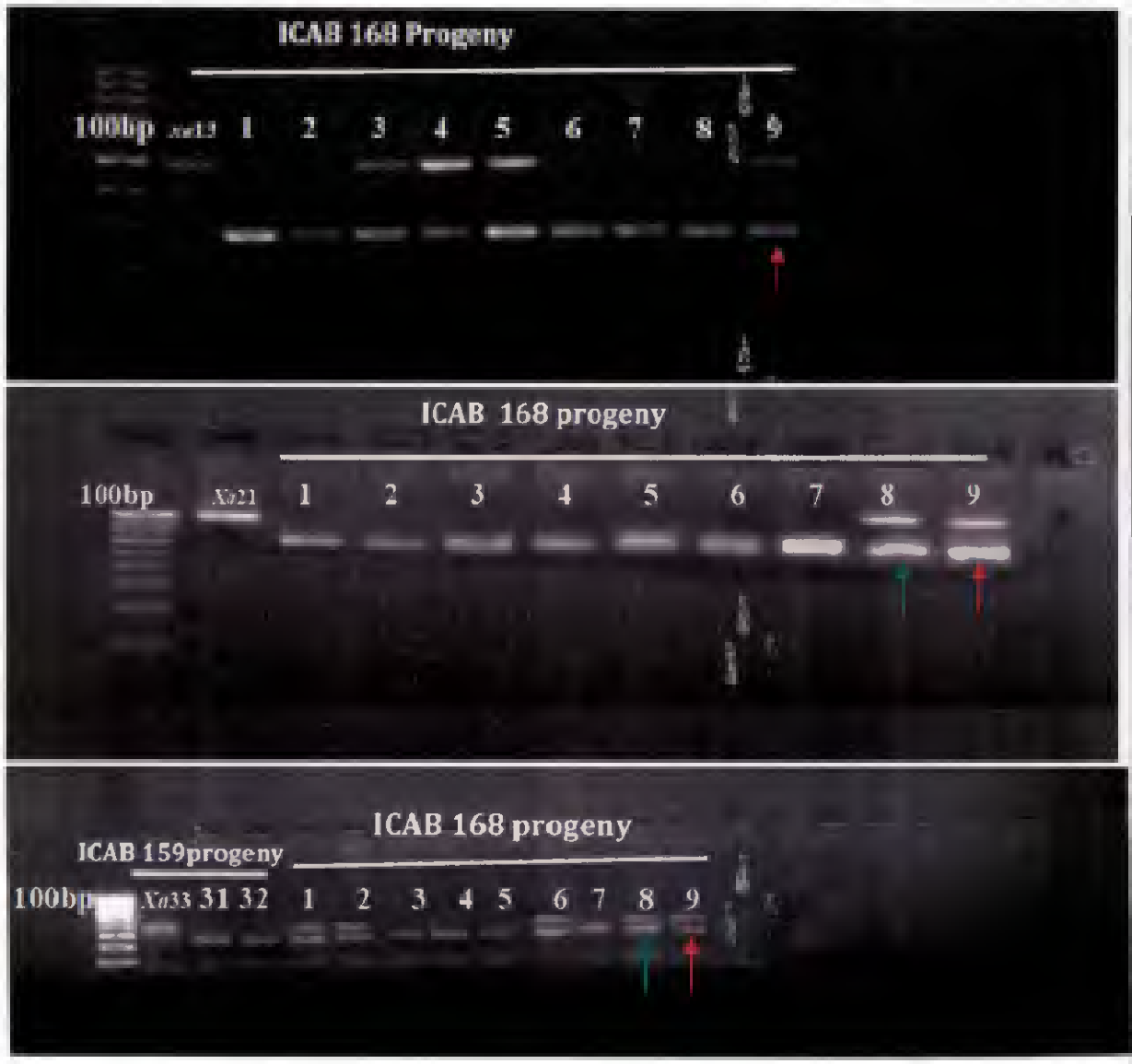
Red arrow= sample with *xa*13+ *Xa*21+ *Xa*33 resistance genes
Yellow arrow = sample with *xa*13 + *Xa*33 resistance genes
White arrow= sample with *xa*13+ *Xa*21 resistance genes

Plate 27. (d)

Red arrow= sample with *xa*13+ *Xa*21+ *Xa*33 resistance genes
Yellow arrow = sample with *xa*13 + *Xa*33 resistance genes
White arrow= sample with *xa*13+ *Xa*21 resistance genes
Green Arrow= sample with *Xa*21+*Xa*33 resistance genes

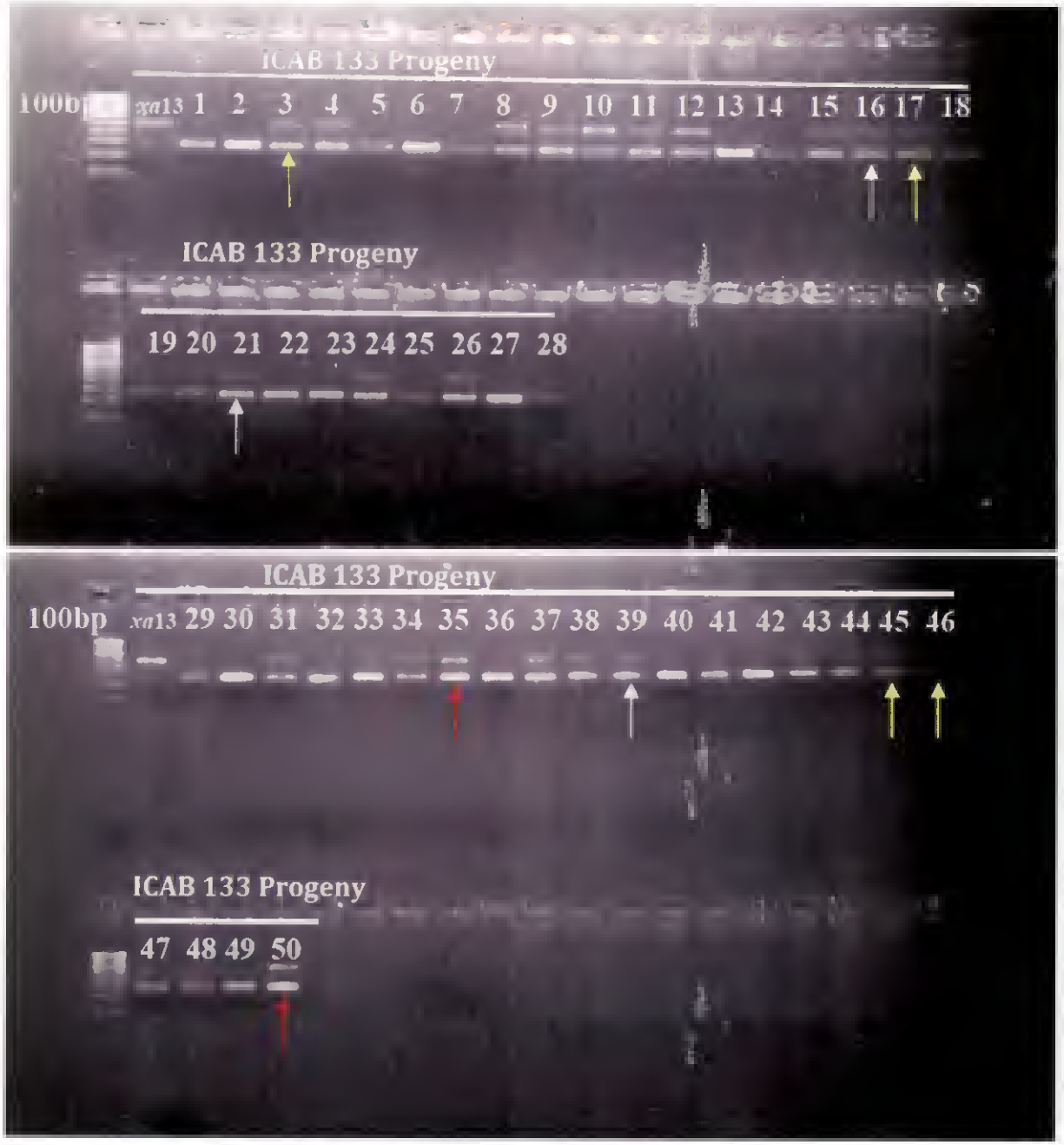
Plate 27 (d) (Continued....)

Plate 27. (e)



Red arrow= sample with *xa*13+ *Xa*21+ *Xa*33 resistance genes
Green Arrow= sample with *Xa*21+ *Xa*33 resistance genes

Plate 27. (f)



Red arrow= sample with *xa*13+ *Xa*21+ *Xa*33 resistance genes
Yellow arrow = sample with *xa*13 + *Xa*33 resistance genes
White arrow= sample with *xa*13+ *Xa*21 resistance genes
Green Arrow= sample with *Xa*21+*Xa*33 resistance genes

Plate 27. (f) (Continued.....)

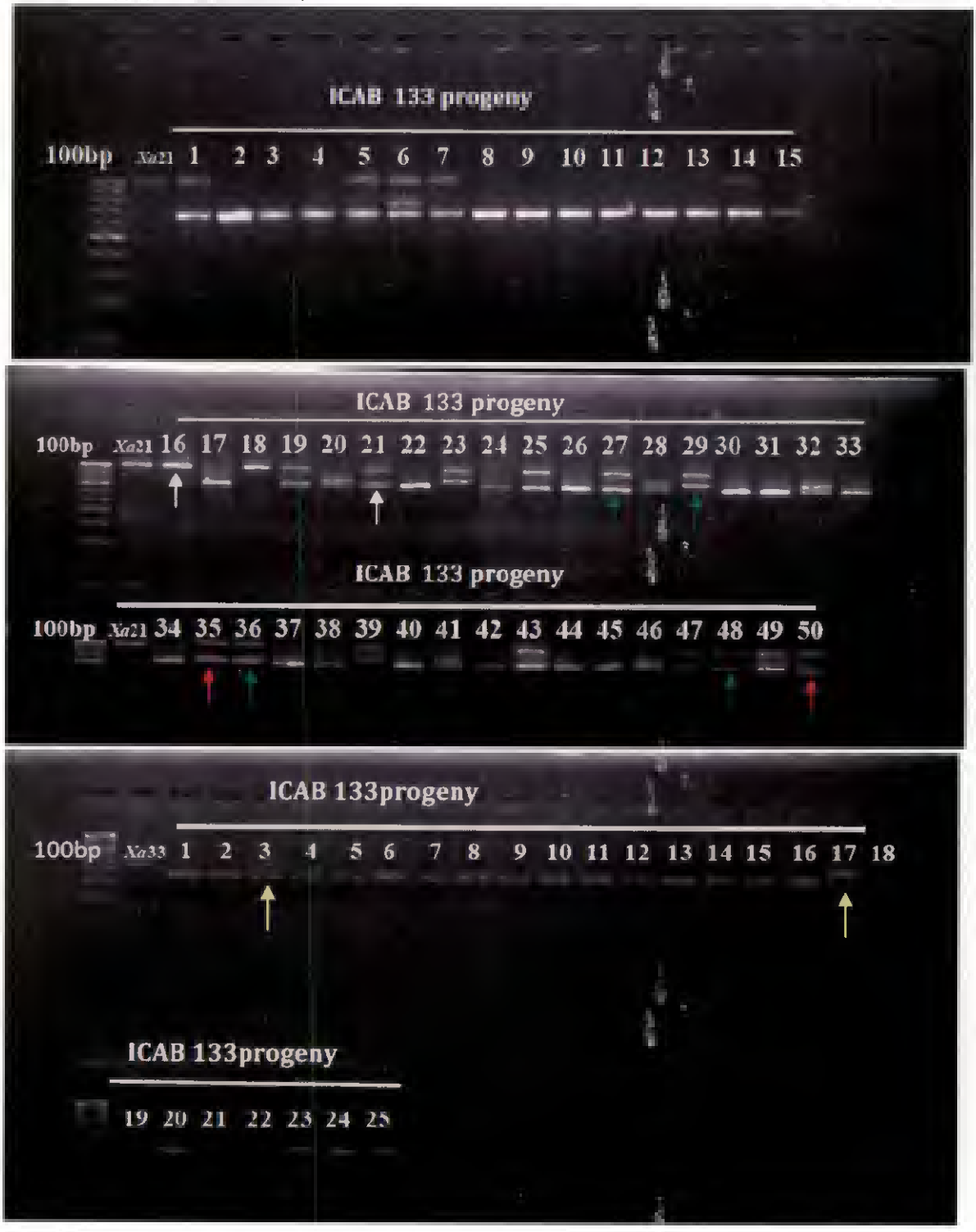
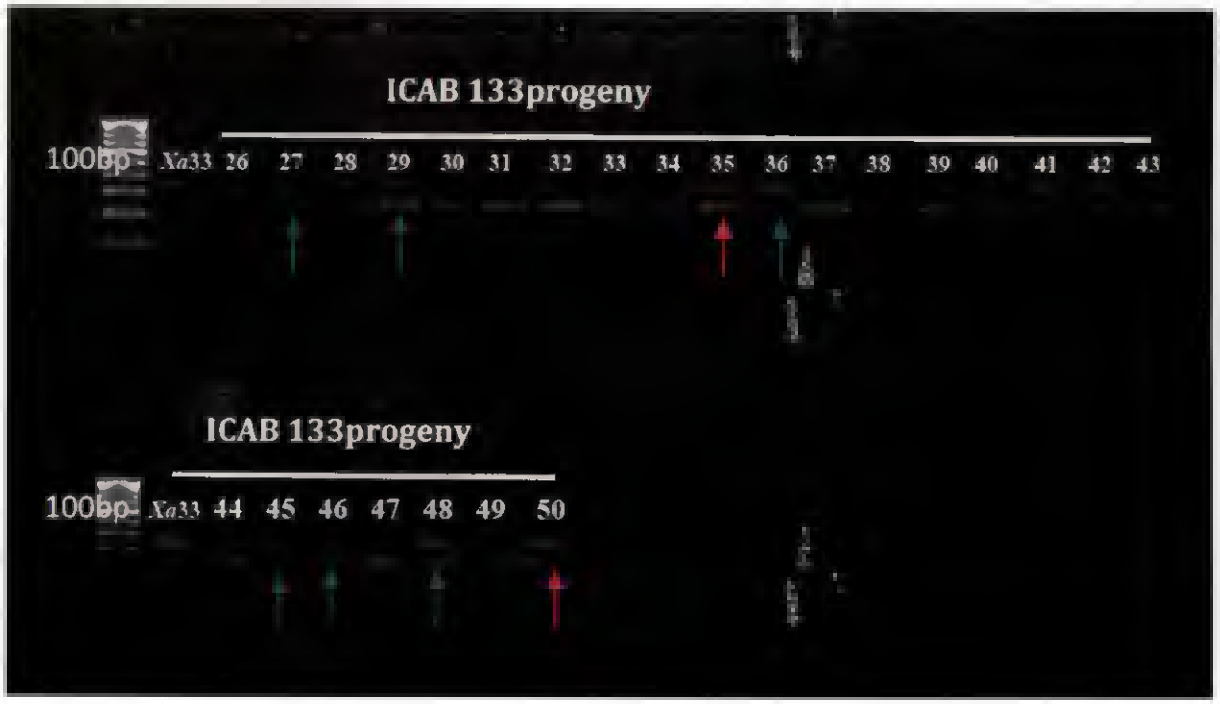


Plate 27. (f) (Continued.....)



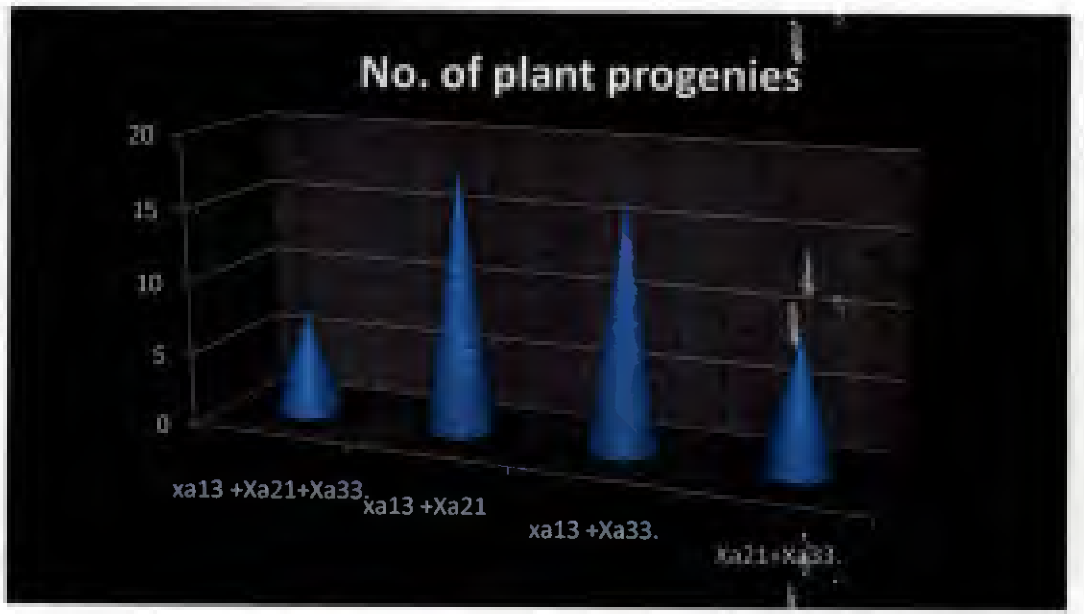


Fig.2. Graphical representation of number of Aiswarya derived intermated pyramided lines with 2 to 3 genes combination

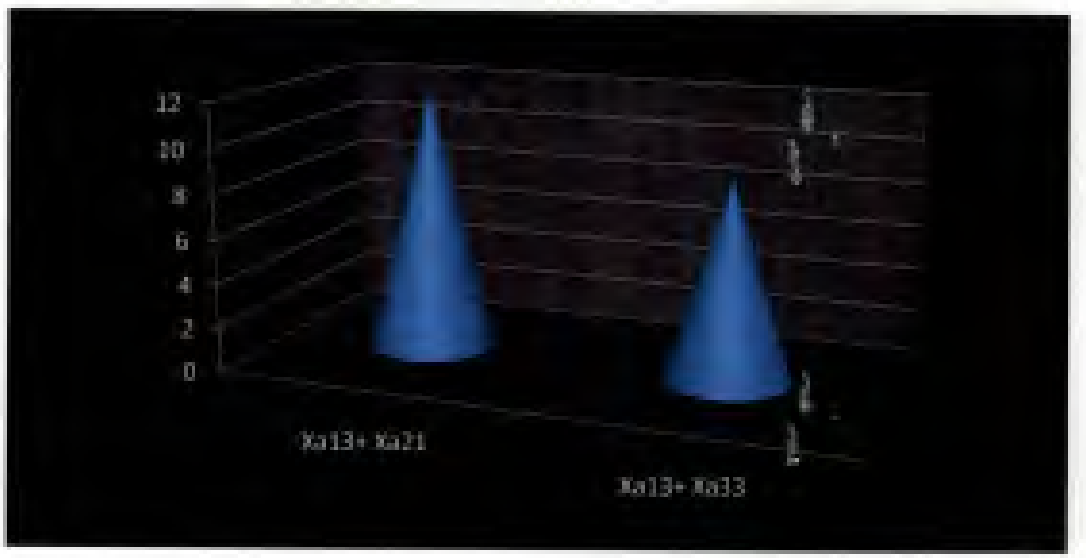


Fig. 3. Graphical representation of number of Prathyasa derived intermated pyramided lines with two genes combination

Table 29: Resistance genes combination in Aiswarya derived BC_1F_1 intermated pyramided lines

Sl.No	Progeny No.	Gene combinations	*xa*13	*Xa*21	*Xa*33
1	ICAB 101-3*	*xa13,Xa21* and *Xa33*	+	+	+
2	ICAB 101-9	*xa13* and *Xa33*	+	-	+
3	ICAB101-12	*xa13* and *Xa21*	+	+	-
4	ICAB101-13	*xa13* and *Xa21*	+	+	-
5	ICAB-160-5	*xa13* and *Xa33*	+	-	+
6	ICAB22-1	*xa13* and *Xa33*	+	-	+
7	ICAB22-10	*xa13* and *Xa33*	+	-	+
8	ICAB22-16	*xa13* and *Xa33*	+	-	+
9	ICAB46-1	*xa13* and *Xa33*	+	-	+
10	ICAB46-4	*xa13* and *Xa21*	+	+	-
11	ICAB46-5	*xa13* and *Xa21*	+	+	-
12	ICAB46-6	*xa13* and *Xa21*	+	+	-
13	ICAB46-7	*xa13* and *Xa21*	+	+	-
14	ICAB46-8	*xa13* and *Xa21*	+	+	-
15	ICAB46-9*	*xa13,Xa21* and *Xa33*	+	+	+
16	ICAB46-10	*xa13* and *Xa21*	+	+	-
17	ICAB159-2	*xa13* and *Xa21*	+	+	-
18	ICAB159-3	*xa13* and *Xa21*	+	+	-
19	ICAB159-5	*xa13* and *Xa33*	+	-	+
20	ICAB159-6	*xa13* and *Xa33*	+	-	+
21	ICAB159-9	*Xa21* and *Xa33*	-	+	+
22	ICAB159-10	*xa13 and Xa33*	+	-	+
23	ICAB159-12	*Xa21 and Xa33*	-	+	+
24	ICAB159-13	*xa13* and *Xa21*	+	+	-
25	ICAB159-14	*xa13* and *Xa21*	+	+	-
26	ICAB159-15	*Xa21* and *Xa33*	-	+	+
27	ICAB159-16*	*xa13,Xa21* and *Xa33*	+	+	+
28	ICAB159-17	*Xa21* and *Xa33*	-	+	+
29	ICAB159-18	*xa13* and *Xa21*	+	+	-
30	ICAB159-20	*xa13* and *Xa21*	+	+	-
31	ICAB159-21	*Xa13* and *Xa33*	+	-	+
32	ICAB159-22	*Xa13* and *Xa33*	+	-	+
33	ICAB159-23*	*xa13,Xa21* and *Xa33*	+	+	+
34	ICAB159-25	*xa13,Xa21*	+	+	-
35	ICAB159-27	*Xa13* and *Xa33*	+	-	+
36	ICAB159-30	*xa13* and *Xa33*	+	-	+

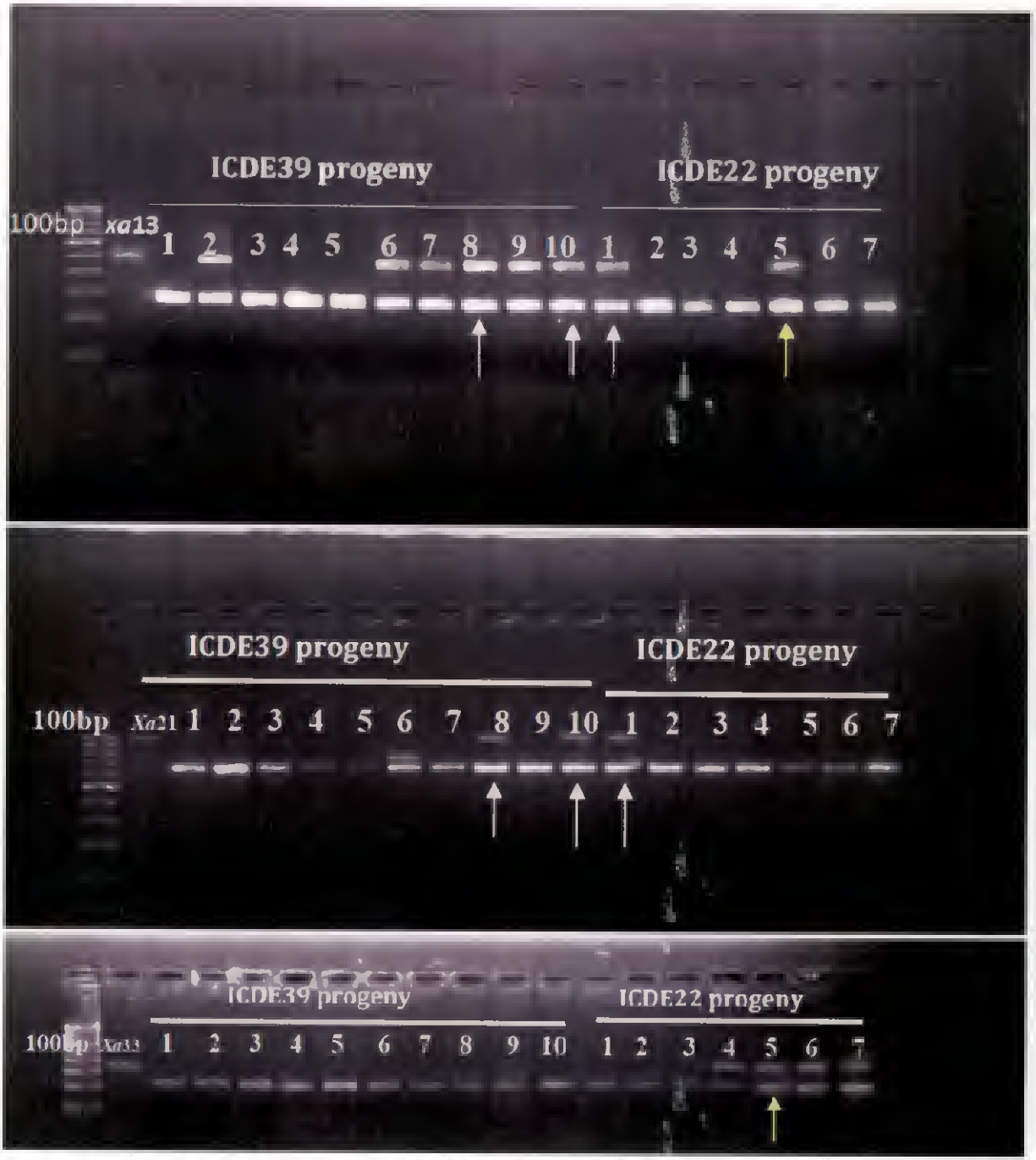
Table 29. (Continued...)

Sl.No	Progeny No.	Gene combinations	*xa*13	*Xa*21	*Xa*33
37	ICAB168-8	*Xa21* and *Xa33*	-	+	+
38	ICAB168-9*	*xa13,Xa21* and *Xa33*	+	+	+
39	ICAB133-3	*xa13* and *Xa33*	+	-	+
40	ICAB133-16	*xa13* and *Xa21*	+	+	-
41	ICAB133-17	*xa13* and *Xa33*	+	-	+
42	ICAB133-21	*xa13* and *Xa21*	+	+	-
43	ICAB133-27	*Xa21* and *Xa33*	-	+	+
44	ICAB133-29	*Xa21* and *Xa33*	-	+	+
45	ICAB133-35*	*xa13,Xa21* and *Xa33*	+	+	+
46	ICAB133-36	*Xa21* and *Xa33*	-	+	+
47	ICAB133-39	*xa13* and *Xa21*	+	+	-
48	ICAB133-45	*xa13* and *Xa33*	+	-	+
49	ICAB133-46	*xa13* and *Xa33*	+	-	+
50	ICAB133-48	*Xa21* and *Xa33*	-	+	+
51	ICAB133-50*	*xa13,Xa21* and *Xa33*	+	+	+

* Indicates three genes combination Presence(+) absence (-) of particular gene

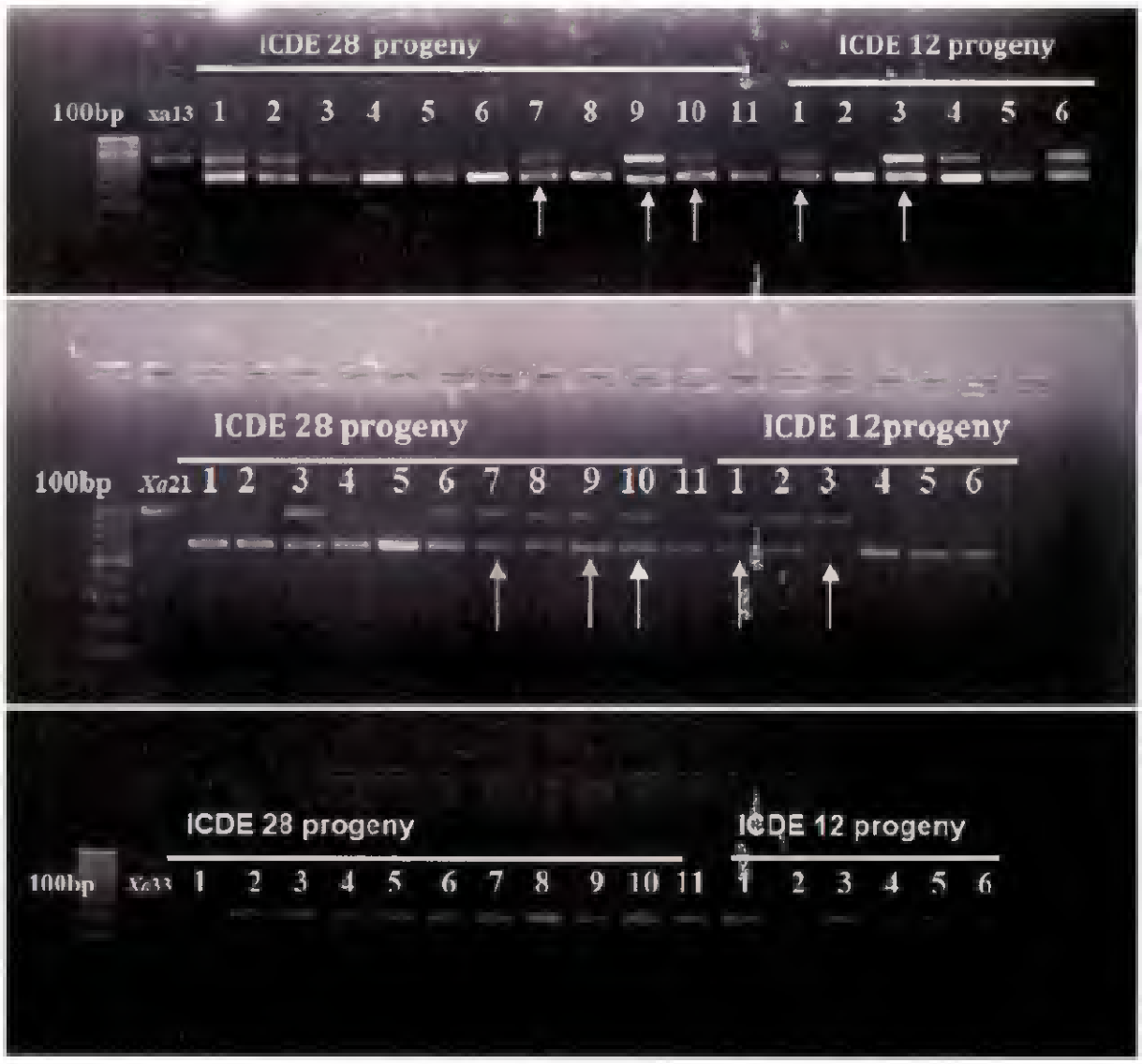
Plate 28. Foreground selection in Prathyasa derived pyramided lines using xa13 linked marker xa13 pro, *Xa*21 linked pTA248 and *Xa*33 linked with RMWR 7.1

Plate 28 (a)



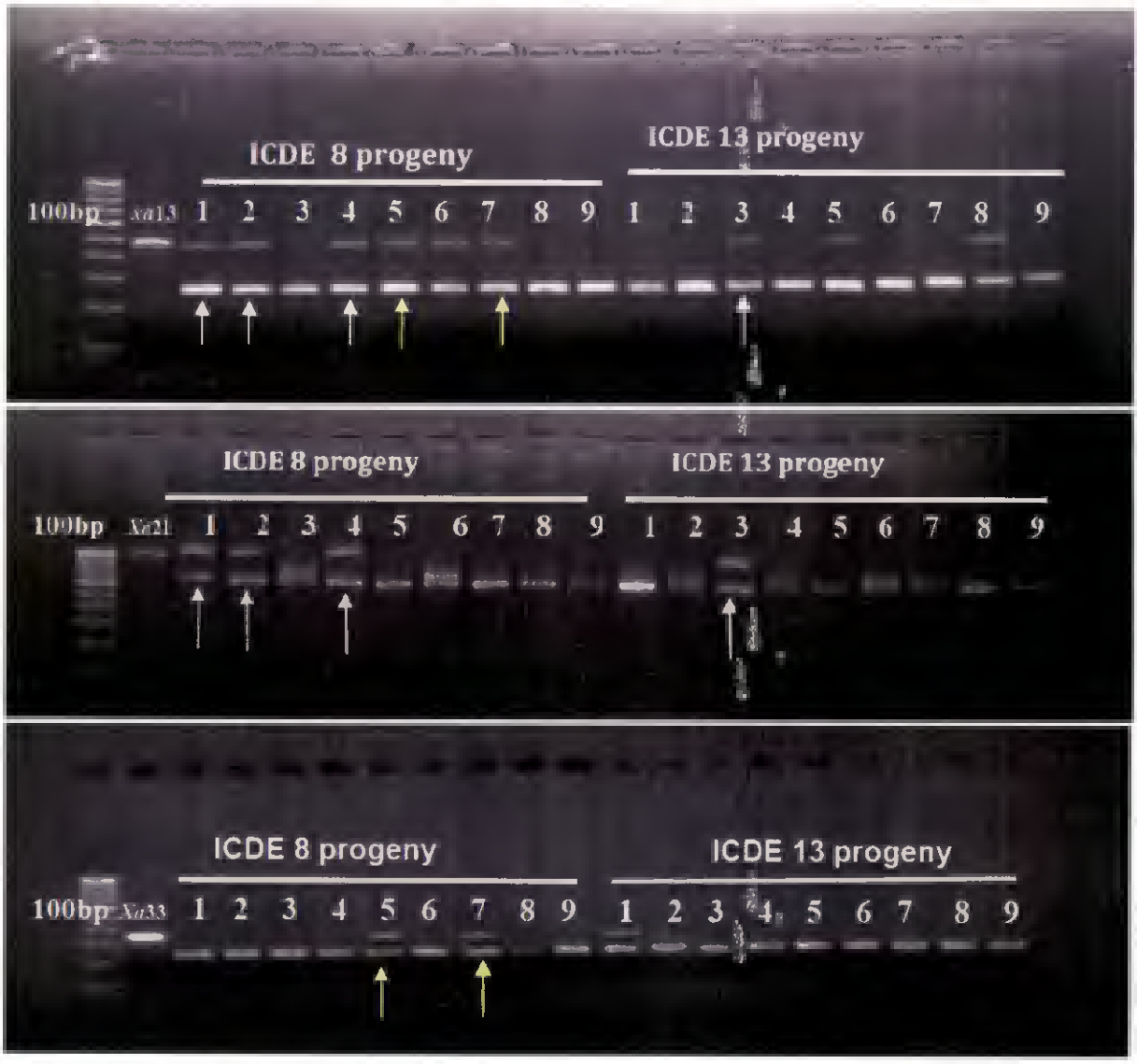
Yellow arrow = sample with *xa*13 + *Xa*33 resistance genes
White arrow= sample with *xa*13+ *Xa*21 resistance genes

Plate 28. (b)



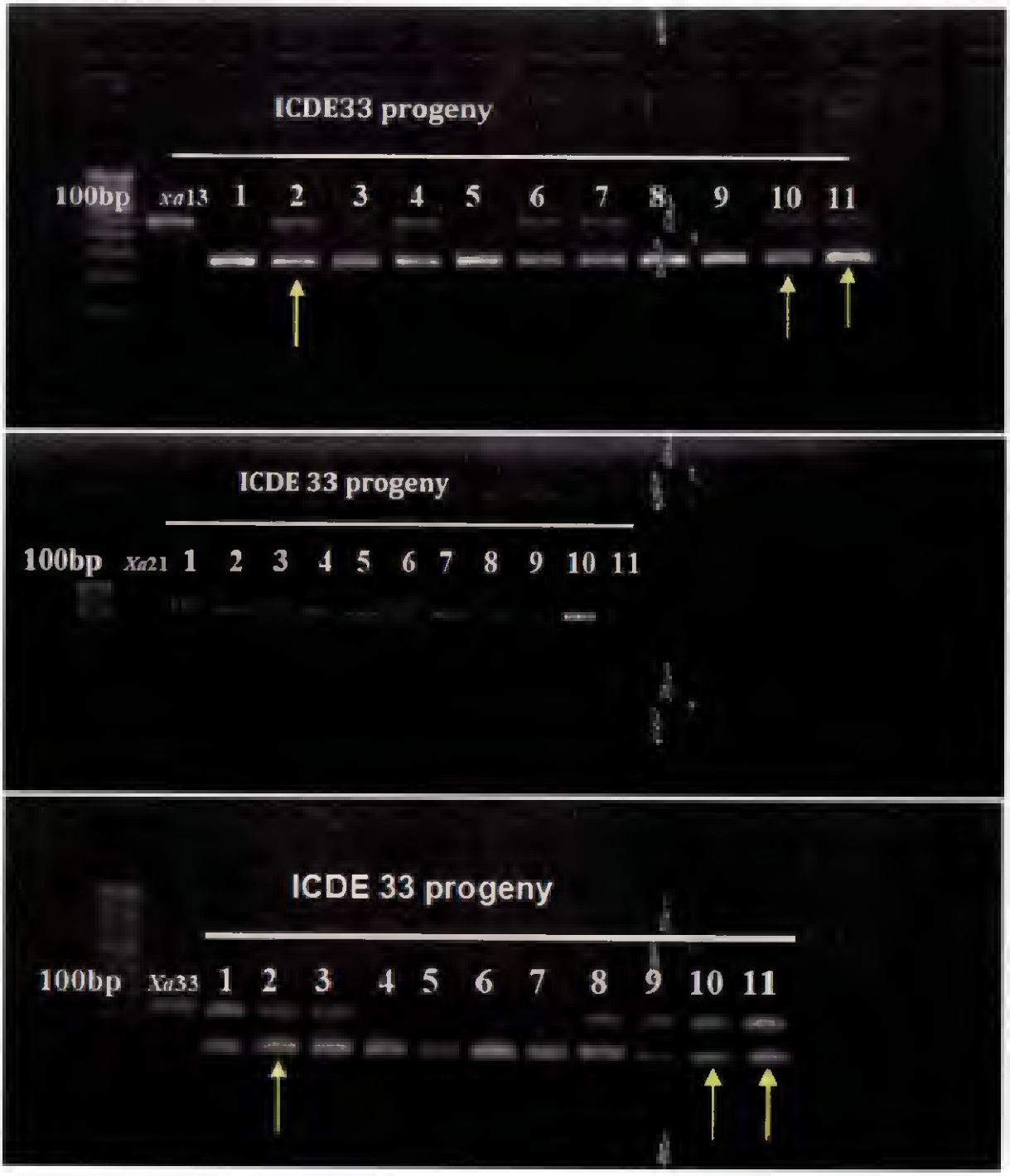
White arrow= sample with *xa*13+ *Xa*21 resistance genes

Plate 28. (c)



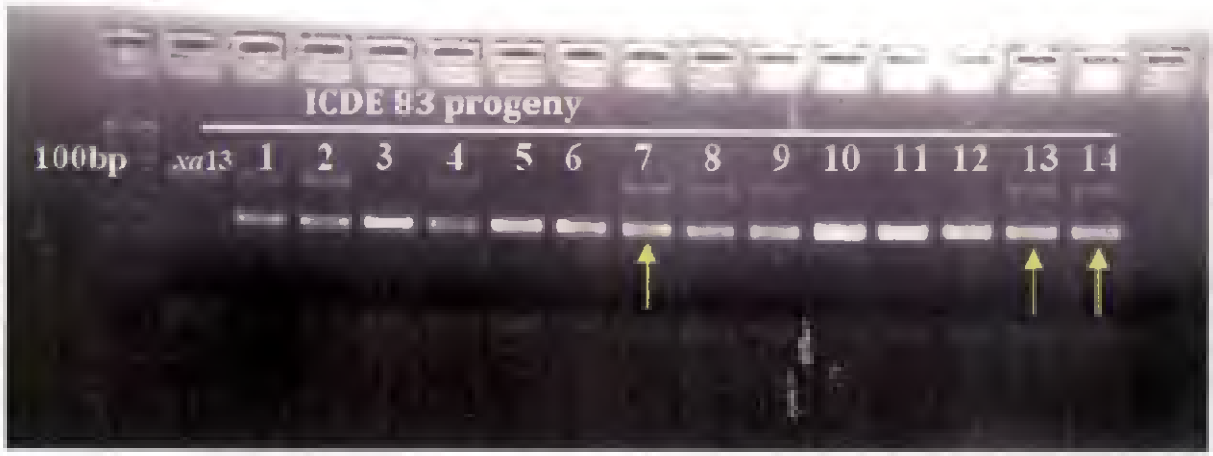
Yellow arrow = sample with *xa*13 + *Xa*33 resistance genes
White arrow= sample with *xa*13+ *Xa*21 resistance genes

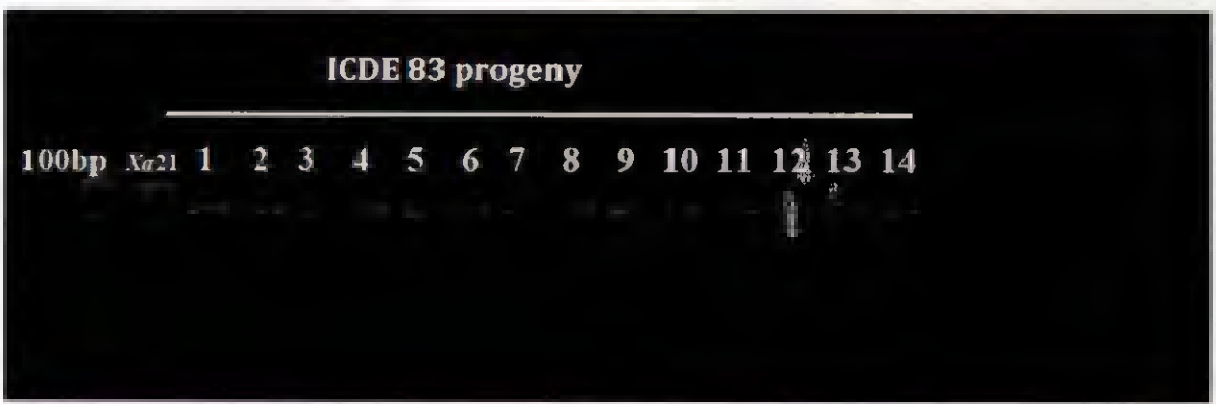
Plate 28. (d)

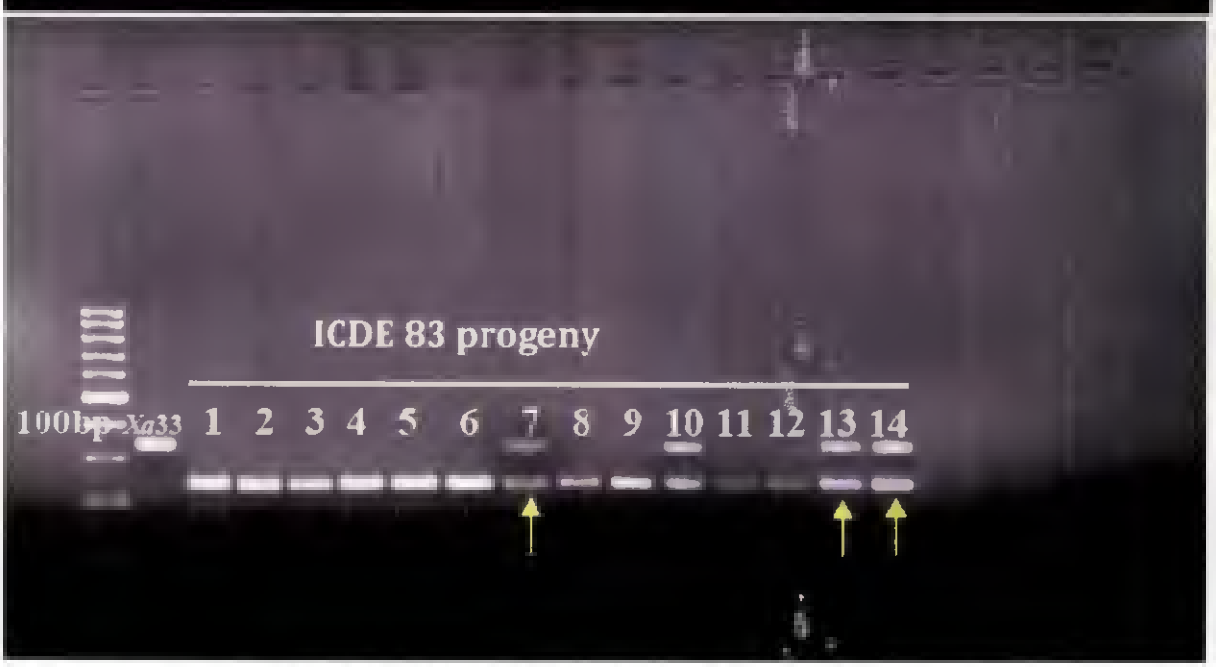


Yellow arrow = sample with *xa*13 + *Xa*33 resistance genes

Plate 28. (e)







Yellow arrow = sample with *xa*13 + *Xa*33 resistance genes

Table 30: Resistance genes combination in Prathyasa derived BC_1F_1 intermated pyramided lines

Sl.No.	Progeny No	Gene combinations	*xa*13	*Xa*21	*Xa*33
1	ICDE39-8	*xa13* and *Xa21*	+	+	-
2	ICDE39-10	*xa13* and *Xa21*	+	+	-
3	ICDE12-1	*xa13* and *Xa21*	+	+	-
4	ICDE12-3	*xa13* and *Xa21*	+	+	-
5	ICDE28-7	*xa13* and *Xa21*	+	+	-
6	ICDE28-9	*xa13* and *Xa21*	+	+	-
7	ICDE28-10	*xa13* and *Xa21*	+	+	-
8	ICDE22-1	*xa13* and *Xa21*	+	+	-
9	ICDE22-5	*xa13* and *Xa33*	+	-	+
10	ICDE 8-1	*xa13* and *Xa21*	+	+	-
11	ICDE8-2	*xa13* and *Xa21*	+	+	-
12	ICDE8-4	*xa13* and *Xa21*	+	+	-
13	ICDE8-5	*xa13* and *Xa33*	+	-	+
14	ICDE8-7	*xa13* and *Xa33*	+	-	+
15	ICDE13-3	*xa13* and *Xa21*	+	+	-
16	ICDE33-2	*xa13* and *Xa33*	+	-	+
17	ICDE33-10	*xa13* and *Xa33*	+	-	+
18	ICDE33-11	*xa13* and *Xa33*	+	-	+
19	ICDE83-7	*xa13* and *Xa33*	+	-	+
20	ICDE83-13	*xa13* and *Xa33*	+	-	+
21	ICDE83-14	*xa13* and *Xa33*	+	-	+

Presence(+) absence (-) of particular gene

4.9.2 Morphological Observation and Euclidean Distance of BC_1F_1

Morphological observations *viz.*, plant height(cm), number of productive tillers plant^{-1}, length / breadth ratio of grain, days to maturity and kernel colour of 51 Aiswarya and 21 Prathyasa derived pyramided backcross lines was recorded (Table 31 and 33). The distribution patterns of quantitative traits are presented in figures 4-5 and 7-8. Proximity dissimilarity matrix was calculated in intermated pyramided lines for above morphological observations except qualitative character kernel colour by Euclidean distance and made tables for comparing the individual pyramided lines with their respective recurrent parent (Table 32 and 34) .

4.9.2.1 Aiswarya Derived Pyramided Backcross Lines

4.9.2.1.1 Plant Height (cm)

Recurrent parent Aiswarya had plant height 98 cm. Among 51 intermated back cross pyramided lines minimum plant height (71cm) observed in ICAB133-16 and maximum height (110cm) found in ICAB159-9 with average value of 88.82cm (±1.36). Four pyramided lines from three progenies recorded plant height similar to recurrent parent and these were ICAB46-7 (98cm), ICAB46-8 (99cm), ICAB159-3(98cm) and ICAB168-9(99cm). Seven plants from three progenies *viz.*, ICAB46-4 (107), ICAB46-10(102), ICAB159-2(108), ICAB159-9(110), ICAB159-13(110), ICAB159-27 (101) and ICAB168-8 (102) were taller than the recurrent parent.

Proximity dissimilarity matrix was calculated by using Euclidean distance. The minimum coefficient of dissimilarity was 0.00 (ICAB46-7, ICAB159-3) and apart from this ICAB46-8, (1.00), ICAB168-9(1.00) plant progenies showed lesser coefficient of dissimilarity, when comparison made with recurrent parent Aiswarya. The maximum coefficient of dissimilarity was 27.00 and found in ICAB133-16.

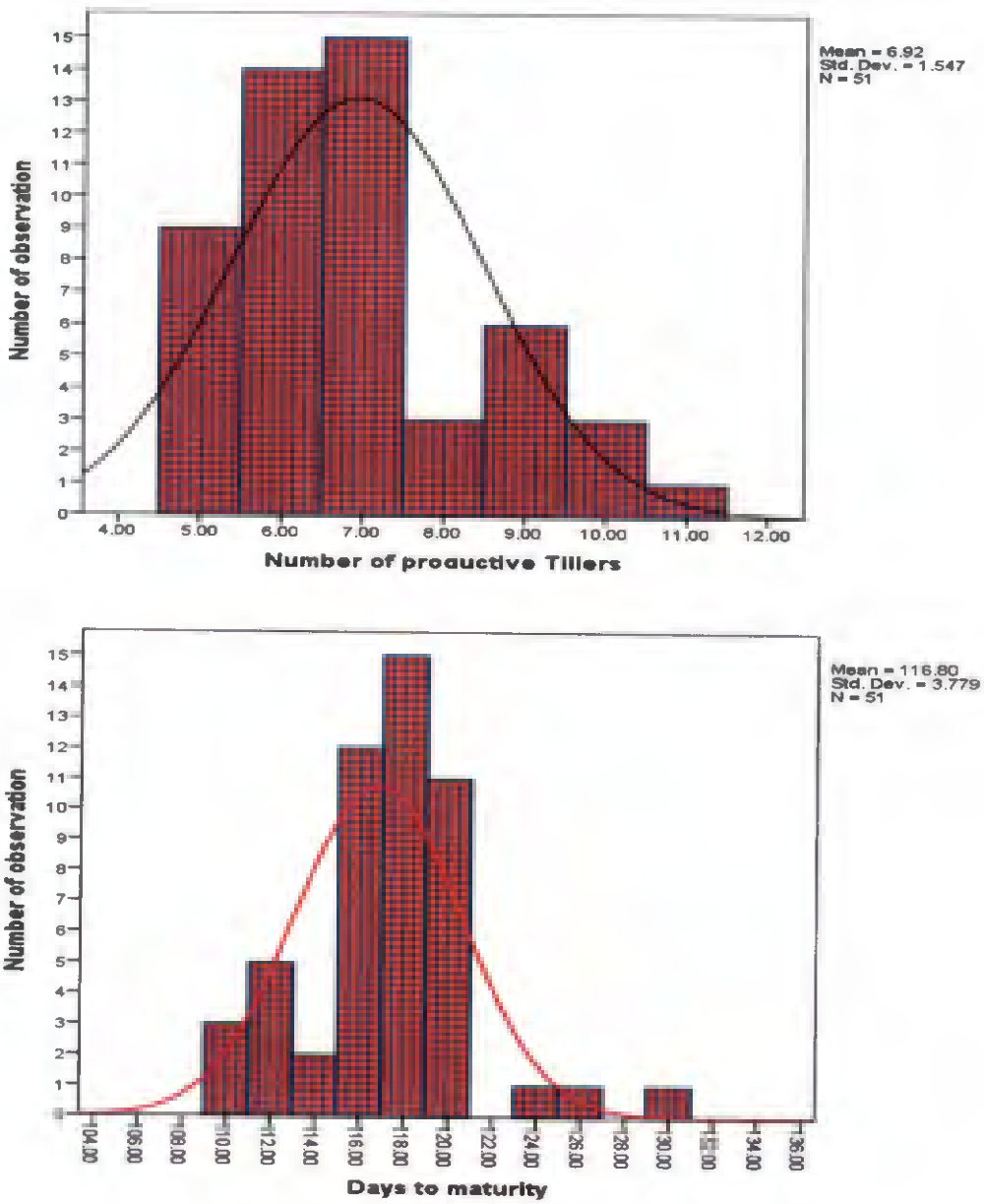


Fig. 4. Distribution pattern for number of productive tillers and days to maturity in Aiswarya derived pyramided lines

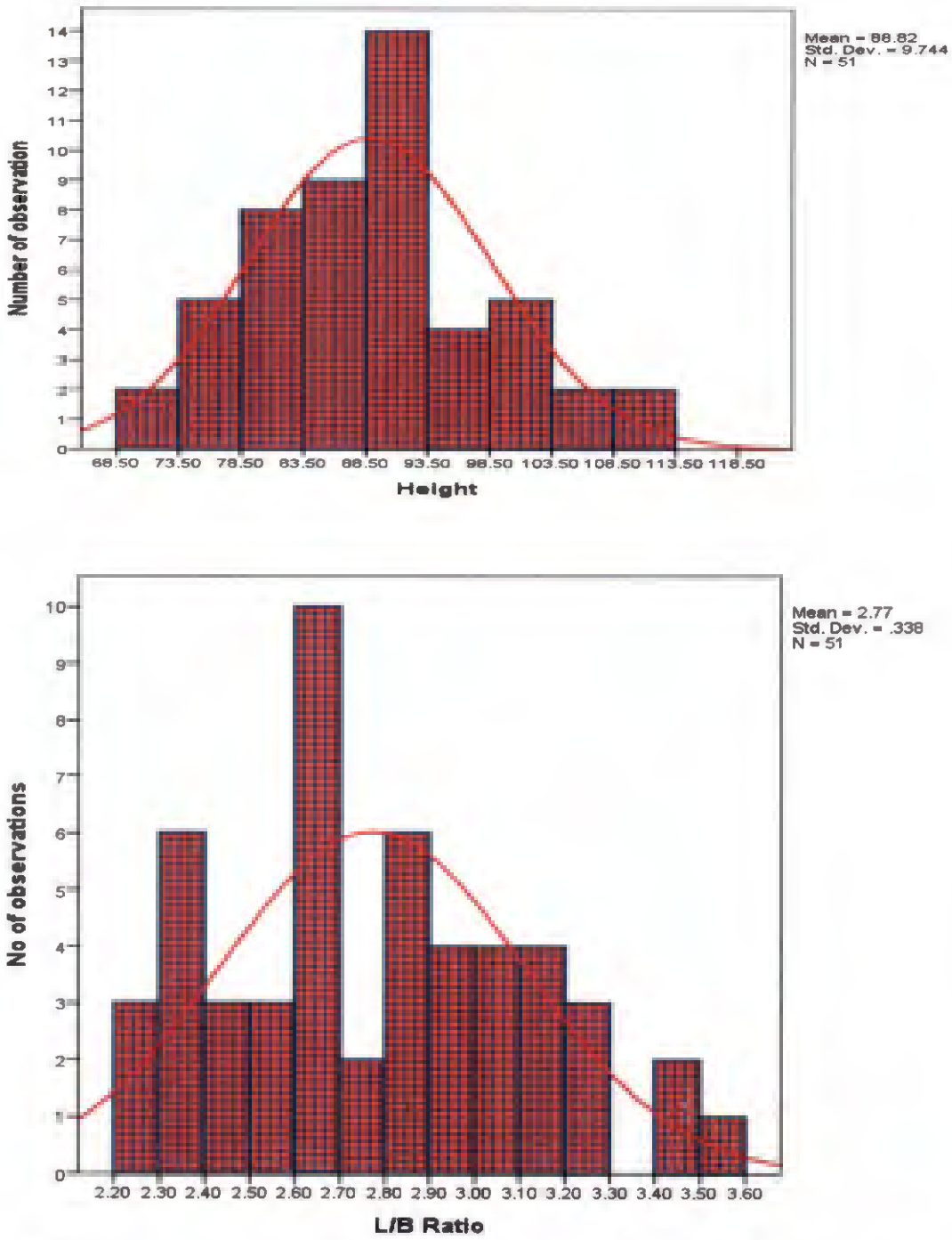


Fig. 5 .Distribution pattern for plant height and grain L/B ratio in Aiswarya derived pyramided lines

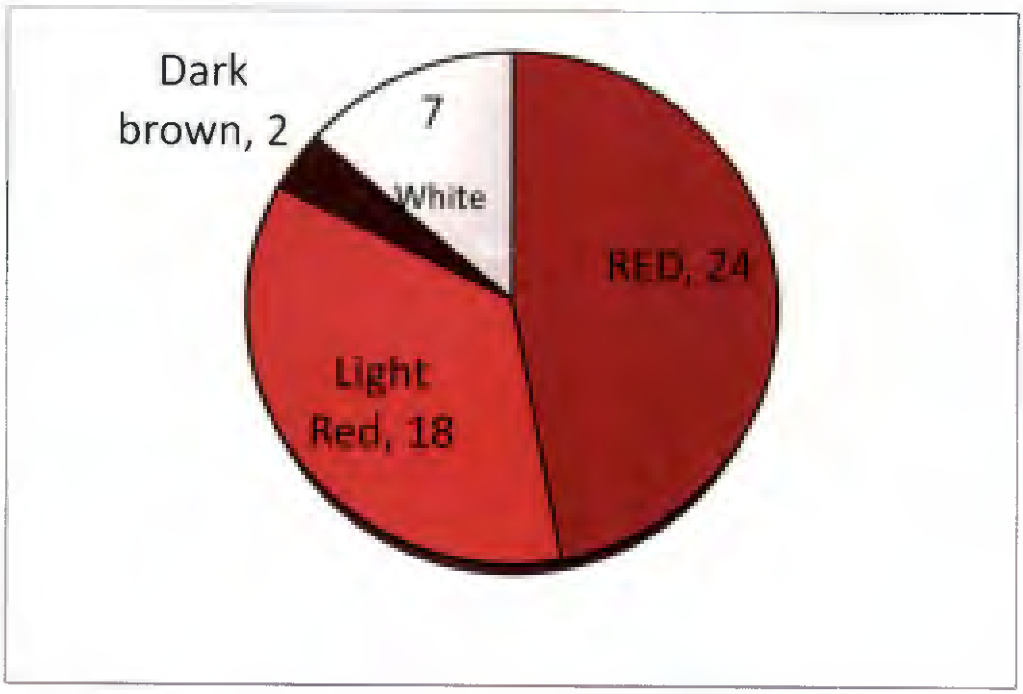


Fig.6 .Kernel colour in Aiswarya derived pyramided lines

Table31. Aiswarya derived pyramided backcross lines

Sl.No	Progeny	Plant Height(cm)	Productive Tillers	L/B ratio	Days to Maturity	Kernel Colour
1	ICAB 101-3	86	5	3.05	118	Light red
2	ICAB 101-9	90	6	2.86	130	Red
3	ICAB101-12	89	5	2.57	115	Light red
4	ICAB101-13	89	6	2.30	119	Red
5	ICAB-160-5	88	6	2.55	120	Red
6	ICAB22-1	90	5	3.26	117	Red
7	ICAB22-10	90	6	3.50	110	Red
8	ICAB22-16	77	5	2.89	112	White
9	ICAB46-1	75	9	2.96	115	Light red
10	ICAB46-4	107	7	2.30	112	Red
11	ICAB46-5	92	6	3.04	115	Light red
12	ICAB46-6	90	7	2.30	120	Light red
13	ICAB46-7	98	7	2.99	115	Dark brown
14	ICAB46-8	99	10	2.77	120	Red
15	ICAB46-9	92	7	2.64	120	White
16	ICAB46-10	102	7	2.91	117	Dark brown
17	ICAB159-2	108	9	2.54	120	White
18	ICAB159-3	98	7	2.47	115	White
19	ICAB159-5	91	8	2.67	115	Red
20	ICAB159-6	80	10	2.85	117	Red
21	ICAB159-9	110	6	2.28	115	Red
22	ICAB159-10	89	7	2.61	119	Red
23	ICAB159-12	95	6	2.44	111	Red
24	ICAB159-13	110	9	2.99	114	Light red
25	ICAB159-14	90	6	2.66	118	Light red
26	ICAB159-15	85	10	2.24	110	Red
27	ICAB159-16	86	9	2.71	112	Light red
28	ICAB159-17	81	8	2.29	120	Red
29	ICAB159-18	93	7	2.44	118	Light red
30	ICAB159-20	85	6	2.31	117	Light red
31	ICAB159-21	87	9	2.60	112	Light red
32	ICAB159-22	87	6	2.82	125	Red
33	ICAB159-23	88	8	2.36	120	Light red
34	ICAB159-25	96	6	2.82	114	Red
35	ICAB159-27	101	5	2.66	110	Light red
36	ICAB159-30	90	7	2.68	118	White
37	ICAB168-8	102	7	2.61	118	Red
38	ICAB168-9	99	11	3.42	117	Light red

Table 31. (Continued...)

Sl.No	Progeny	Plant Height(cm)	Productive Tillers	L/B ratio	Days to Maturity	Kernel Colour
39	ICAB133-3	72	6	3.10	115	Red
40	ICAB133-16	71	5	3.40	118	Red
41	ICAB133-17	75	5	3.29	117	White
42	ICAB133-21	80	9	2.37	115	Light red
43	ICAB133-27	80	7	2.84	123	Red
44	ICAB133-29	80	7	2.66	120	Light red
45	ICAB133-35	75	5	2.61	118	Red
46	ICAB133-36	90	6	3.17	115	Light red
47	ICAB133-39	85	7	3.12	120	Red
48	ICAB133-45	80	7	3.18	118	White
49	ICAB133-46	82	6	3.25	118	Light red
50	ICAB133-48	79	7	3.03	115	Red
51	ICAB133-50	76	5	3.02	115	Red
	Mean	88.82	6.92	2.77	116.51	
	Minimum	71.00	5.00	2.24	110.00	
	Maximum	110.00	11.00	3.50	130.00	
	Sd	9.74	1.55	0.34	3.71	
	S.E±	1.36	0.22	0.05	0.52	
	Aiswarya	98	8	2.69	120	Red

4.9.2.1.2 Number of Productive Tillers Plant $^{-1}$

Recurrent parent Aiswarya recorded eight productive tillers. Average number of productive tillers among BC_1F_1 intermated pyramided plants was 6.17(±0.22). The minimum number of productive tillers 5 and observed in nine pyramided lines *viz.,* ICAB 101-3, ICAB101-12, ICAB22-1, ICAB22-16, ICAB159-27, ICAB133-50, ICAB133-17, ICAB133-35 and ICAB133-16 derived from four progenies. Maximum number of productive tillers (11) found in ICAB168-9. Apart from this nine plant progenies *viz.*, ICAB159-15, ICAB133-21, ICAB159-21, ICAB159-16, ICAB159-13 ICAB159-6, ICAB159-2, ICAB46-8 and ICAB46-1 had more productive tillers (≥9). Three plants ICAB159-23, ICAB159-17 ICAB159-5 from ICAB159 progeny showed number of productive tillers (8) similar to Aiswarya parent.

Coefficient of dissimilarity was 0.00 in ICAB159-23, ICAB159-17, and ICAB159-23 plant progenies. The maximum dissimilarity (3.00) was found in ten pyramided lines.

4.9.2.1.3 Length and Breadth Ratio of Grain

Among BC_1F_1 intermated pyramided plants the lowest and highest grain length breadth ratio was found in ICAB159-15(2.24) and ICAB22-10(3.5) respectively with mean value of 2.77(±0.05). Ten plants *viz.,* ICAB46-9 (2.64), ICAB159-5 (2.67), ICAB159-10 (2.61), ICAB159-14 (2.66), ICAB159-16 (2.71), ICAB159-27(2.66), ICAB159-30(2.68), ICAB168-8(2.61), ICAB133-29 (2.66) and ICAB133-35 (2.61) from four progenies (ICAB46, ICAB159, ICAB168 and ICAB133) showed grain length/breadth ratio closer to Aiswarya (2.69).

Euclidean distance values ranged from 0.01 (ICAB159-30) to 0.81 (ICAB22-10). Plant progenies *viz.*, ICAB159-5(0.02) ICAB159-16, ICAB133-29(0.03) ICAB159-27(0.03) ICAB159-14 exhibited lesser Euclidean distance from recurrent parent Aiswarya.

Table 32. Proximity dissimilarity matrix of Aiswarya derived pyramided lines in comparison with Aiswarya by Euclidean distance

		Proximity dissimilarity matrix (Compared with Aiswarya)				
Sl.No	Progeny	Plant height	Productive tillers	L/B ratio	Days to Maturity	Overall
1	ICAB 101-3	12.00	3.00	0.36	2.00	12.54
2	ICAB 101-9	8.00	2.00	0.17	10.00	12.96
3	ICAB101-12	9.00	3.00	0.12	5.00	10.72
4	ICAB101-13	9.00	2.00	0.39	1.00	9.28
5	ICAB-160-5	10.00	2.00	0.14	0.00	10.20
6	ICAB22-1	8.00	3.00	0.57	3.00	9.07
7	ICAB22-10	8.00	2.00	0.81	10.00	12.99
8	ICAB22-16	21.00	3.00	0.20	8.00	22.67
9	ICAB46-1	23.00	1.00	0.27	5.00	23.56
10	ICAB46-4	9.00	1.00	0.39	8.00	12.09
11	ICAB46-5	6.00	2.00	0.35	5.00	8.07
12	ICAB46-6	8.00	1.00	0.39	0.00	8.07
13	ICAB46-7	0.00	1.00	0.30	5.00	5.11
14	ICAB46-8	1.00	2.00	0.08	0.00	2.24
15	ICAB46-9	6.00	1.00	0.05	0.00	6.08
16	ICAB46-10	4.00	1.00	0.22	3.00	5.10
17	ICAB159-2	10.00	1.00	0.15	0.00	10.05
18	ICAB159-3	0.00	1.00	0.22	5.00	5.10
19	ICAB159-5	7.00	0.00	0.02	5.00	8.60
20	ICAB159-6	18.00	2.00	0.16	3.00	18.36
21	ICAB159-9	12.00	2.00	0.41	5.00	13.16
22	ICAB159-10	9.00	1.00	0.08	1.00	9.11
23	ICAB159-12	3.00	2.00	0.25	9.00	9.70
24	ICAB159-13	12.00	1.00	0.30	6.00	13.46
25	ICAB159-14	8.00	2.00	0.03	2.00	8.49
26	ICAB159-15	13.00	2.00	0.45	10.00	16.53
27	ICAB159-16	12.00	1.00	0.02	8.00	14.46
28	ICAB159-17	17.00	0.00	0.40	0.00	17.00
29	ICAB159-18	5.00	1.00	0.25	2.00	5.48
30	ICAB159-20	13.00	2.00	0.38	3.00	13.50
31	ICAB159-21	11.00	1.00	0.09	8.00	13.64
32	ICAB159-22	11.00	2.00	0.13	5.00	12.25
33	ICAB159-23	10.00	0.00	0.33	0.00	10.01
34	ICAB159-25	2.00	2.00	0.13	6.00	6.63

Table 32. (Continued...)

		Proximity dissimilarity matrix (Compared with Aiswarya)				
Sl.No	Progeny	Plant height	Productive tillers	L/B ratio	Days to Maturity	Overall
35	ICAB159-27	3.00	3.00	0.03	10.00	10.86
36	ICAB159-30	8.00	1.00	0.01	2.00	8.31
37	ICAB168-8	4.00	1.00	0.08	2.00	4.58
38	ICAB168-9	1.00	3.00	0.73	3.00	4.42
39	ICAB133-3	26.00	2.00	0.41	5.00	26.56
40	ICAB133-16	27.00	3.00	0.71	2.00	27.25
41	ICAB133-17	23.00	3.00	0.60	3.00	23.40
42	ICAB133-21	18.00	1.00	0.32	5.00	18.71
43	ICAB133-27	18.00	1.00	0.15	3.00	18.28
44	ICAB133-29	18.00	1.00	0.03	0.00	18.03
45	ICAB133-35	23.00	3.00	0.08	2.00	23.28
46	ICAB133-36	8.00	2.00	0.48	5.00	9.66
47	ICAB133-39	13.00	1.00	0.43	0.00	13.05
48	ICAB133-45	18.00	1.00	0.49	2.00	18.14
49	ICAB133-46	16.00	2.00	0.56	2.00	16.26
50	ICAB133-48	19.00	1.00	0.34	5.00	19.68
51	ICAB133-50	22.00	3.00	0.33	5.00	22.76
	Aiswarya	0.00	0.00	0.00	0.00	0.00

4.9.2.1.4 Days to Maturity

Among pyramided lines the days to maturity varied from 110 cm (ICAB159-27, ICAB159-15, ICAB22-10) to 130cm (ICAB 101-9), with an average of 116.51 (±0.52). Nine plants *viz*., ICAB-160-5, ICAB133-29, ICAB133-39, ICAB46-8, ICAB46-6, ICAB46-9, ICAB159-2, ICAB159-17 and ICAB159-23, from four progenies matured in 120 days which is similar to recurrent parent Aiswarya. ICAB133-27(123), ICAB159-22 (125), ICAB 101-9 (130cm) plant progenies recorded delayed maturity compared to Aiswarya.

Euclidean dissimilarity coefficient varied from 0.00 to 10.00 in pair wise comparison between 51 intermated pyramided lines and Aiswarya. Plant progenies *viz.,* ICAB-160-5, ICAB46-6, ICAB46-8, ICAB46-9, ICAB159-2, ICAB159-17, ICAB159-23, ICAB133-29 and ICAB133-39 showed dissimilarity coefficient as zero and matured in 120 days like recurrent parent.

4.9.2.1.5 Kernel Colour

The variation of the kernel colour varied from red, brown to white. Recurrent parent Aiswarya had characteristic red kernel colour. In Fifty one intermated plants the distribution of kernel colour was in the ratio of 24 red: 18 light red: 7 white: 2 brown (Fig.6). Majority of the plants had red kernel colour which is characteristic colour of recurrent parent.

4.9.2.2 Prathyasa Derived Intermated Pyramided Backcross Lines

4.9.2.2.1 Plant Height (cm)

Twenty one pyramided lines with combination of two resistance genes had varied plant height from 78cm (ICDE33-11) to 110cm (ICDE12-3) with an average value of 90.67±1.92 cm. Five plants *viz*., ICDE39-8 (104cm) ICDE12-3 (110 cm), ICDE22-1 (98 cm), ICDE22-5 (108 cm) and ICDE83-13

Table33. Prathyasa derived intermated pyramided backcross lines

Sl. No	Progeny	Plant Height(cm)	Productive Tillers	L/B ratio	Days to Maturity	Kernel Colour
1	ICDE39-8	104	6	3.24	115	White
2	ICDE39-10	92	9	2.29	108	Light red
3	ICDE12-1	85	5	3.96	124	White
4	ICDE12-3	110	7	2.93	115	Light red
5	ICDE 28-7	90	8	3.52	115	Light red
6	ICDE28-9	82	5	2.28	100	Red
7	ICDE28-10	80	6	2.78	110	Red
8	ICDE22-1	98	5	2.53	110	Light red
9	ICDE22-5	108	7	2.41	115	Red
10	ICDE 8-1	86	8	2.53	110	Light red
11	ICDE8-2	90	8	3.12	119	Light red
12	ICDE8-4	92	8	3.01	112	Red
13	ICDE8-5	83	6	2.82	110	Light red
14	ICDE8-7	90	6	2.20	105	Red
15	ICDE13-3	92	9	2.61	115	Light red
16	ICDE33-2	84	7	2.69	115	Light red
17	ICDE33-10	87	8	2.49	108	Red
18	ICDE33-11	78	10	2.73	116	Light red
19	ICDE83-7	87	11	2.78	115	Red
20	ICDE83-13	100	12	2.71	125	Light red
21	ICDE83-14	86	10	2.43	112	Light red
	Mean	90.67	7.67	2.77	113.05	
	Minimum	78	5	2.20	100	
	Maximum	110	12	3.96	125	
	Sd	8.82	1.98	0.43	5.74	
	S.E±	1.92	0.43	0.09	1.25	
	Prathyasa	94	10	2.17	110	Red

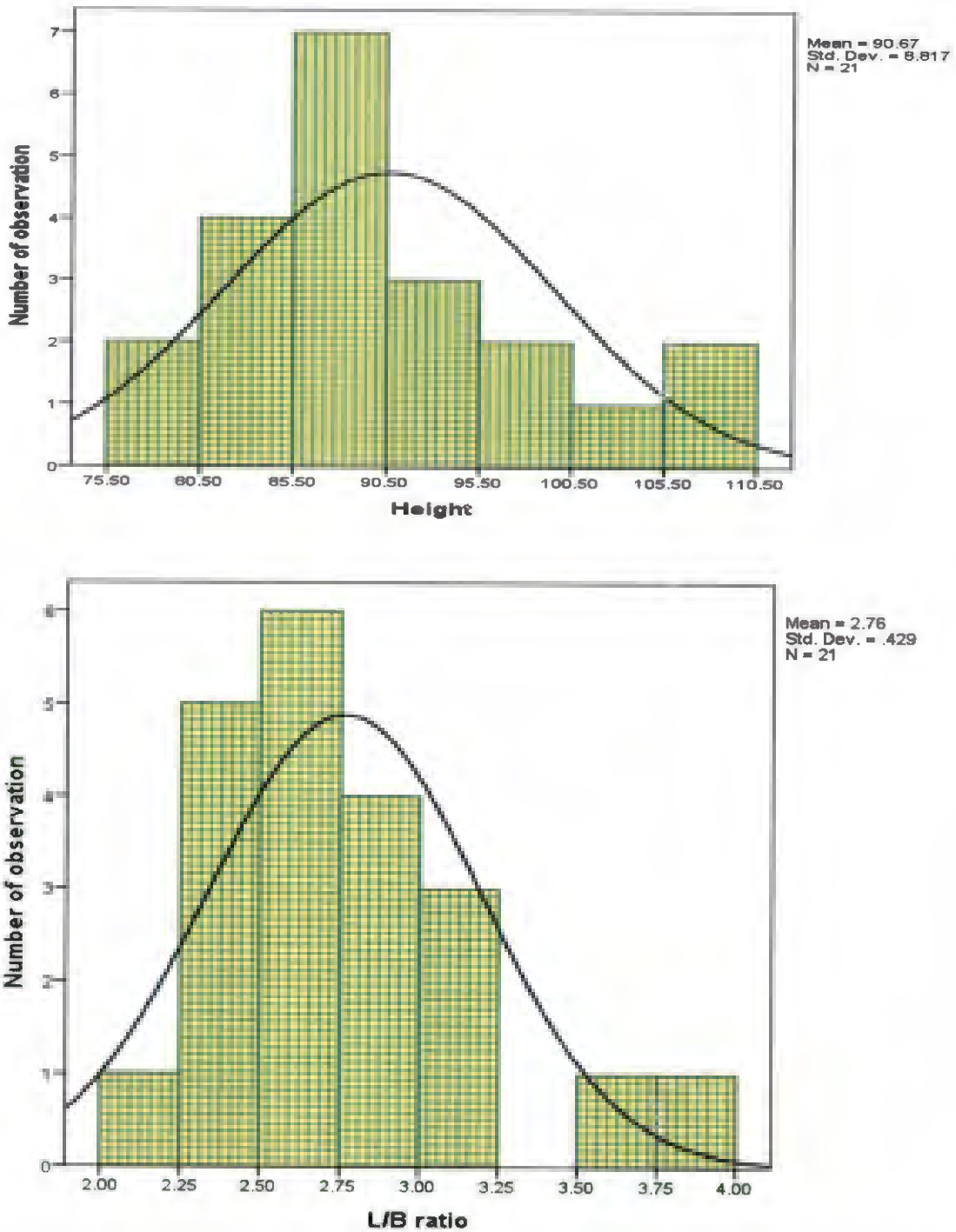


Fig.7 .Distribution pattern for plant height and grain L/B ratio in Prathyasa derived pyramided lines

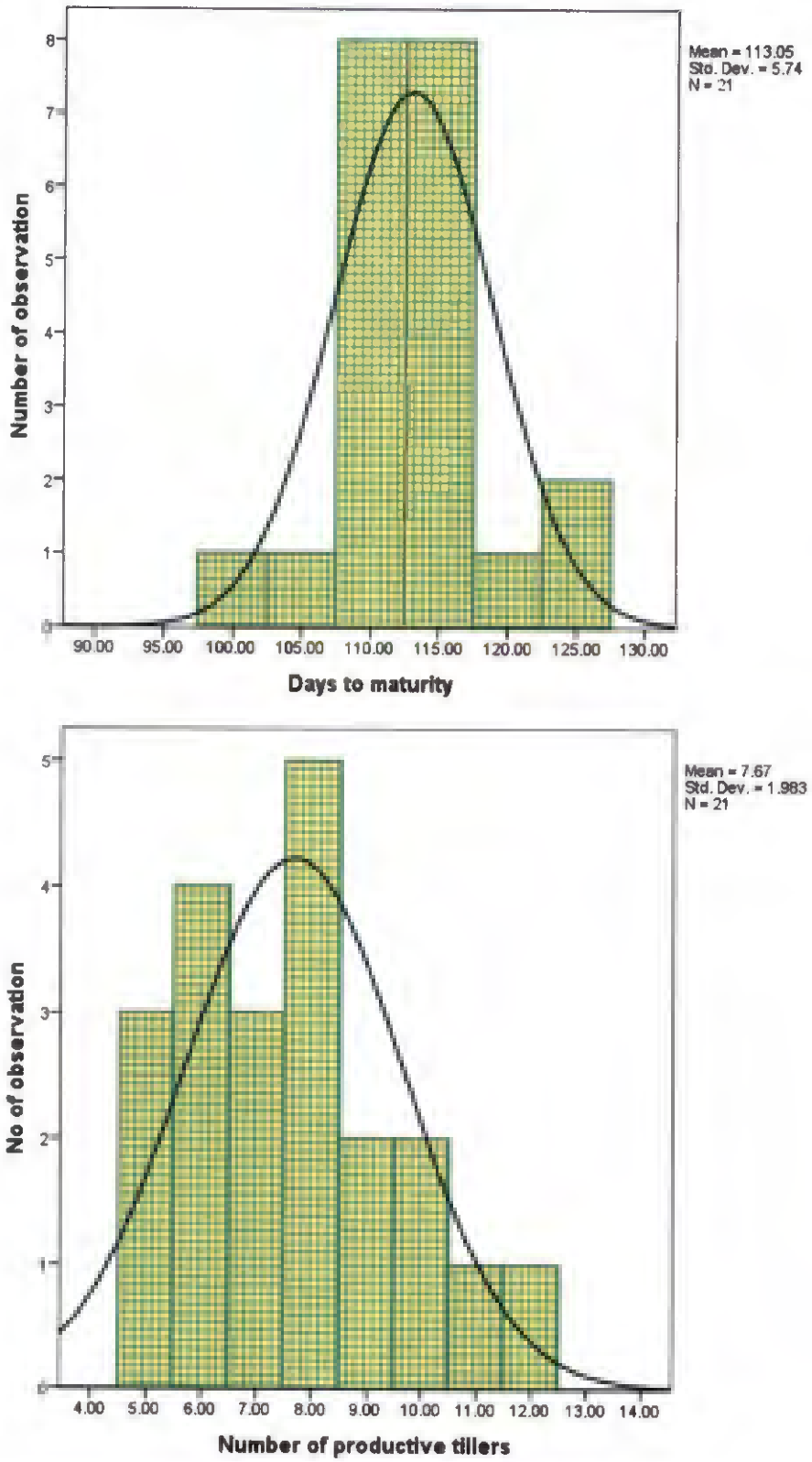


Fig.8. Distribution pattern for number of productive tillers and days to maturity in Prathyasa derived pyramided lines

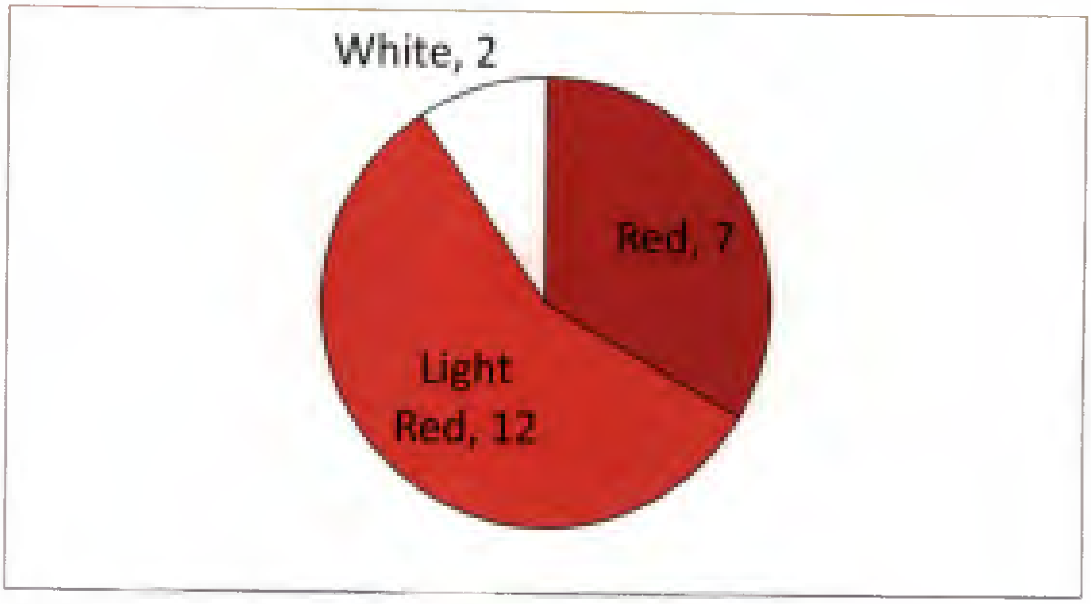


Fig. 9. Kernel colour in Prathyasa derived pyramided lines

Table 34. Proximity dissimilarity matrix of Prathyasa derived pyramided lines in comparison with Prathyasa by Euclidean distance

		Proximity dissimilarity matrix (Compared with Prathyasa)				
Sl.No.	Progeny	Plant height	Productive tillers	L/B ratio	Days to Maturity	Overall
1	ICDE39-8	10.00	4.00	1.07	5.00	11.92
2	ICDE39-10	2.00	1.00	0.12	2.00	3.00
3	ICDE12-1	9.00	5.00	1.79	14.00	17.47
4	ICDE12-3	16.00	3.00	0.76	5.00	17.05
5	ICDE 28-7	4.00	2.00	1.35	5.00	6.84
6	ICDE28-9	12.00	5.00	0.11	10.00	16.40
7	ICDE28-10	14.00	4.00	0.61	0.00	14.57
8	ICDE22-1	4.00	5.00	0.36	0.00	6.41
9	ICDE22-5	14.00	3.00	0.24	5.00	15.17
10	ICDE 8-1	8.00	2.00	0.36	0.00	8.25
11	ICDE8-2	4.00	2.00	0.95	9.00	10.09
12	ICDE8-4	2.00	2.00	0.84	2.00	3.56
13	ICDE8-5	11.00	4.00	0.65	0.00	11.72
14	ICDE8-7	4.00	4.00	0.03	5.00	7.55
15	ICDE13-3	2.00	1.00	0.44	5.00	5.49
16	ICDE33-2	10.00	3.00	0.52	5.00	11.59
17	ICDE33-10	7.00	2.00	0.32	2.00	7.56
18	ICDE33-11	16.00	0.00	0.56	6.00	17.10
19	ICDE83-7	7.00	1.00	0.61	5.00	8.68
20	ICDE83-13	6.00	2.00	0.54	15.00	16.29
21	ICDE83-14	8.00	0.00	0.26	2.00	8.25
	Prathyasa	0.00	0.00	0.00	0.00	0.00

(100cm) from four progenies were taller than recurrent parent Prathyasa (94cm). Three plant progenies ICDE39-10, ICDE8-4 and ICDE13-3 had plant height 92cm which was almost similar to recurrent parent height of Prathyasa.

The highest coefficient of dissimilarity (16.00) was observed in ICDE12-3 and ICDE33-11 and the lowest coefficient of dissimilarity (2.00) was observed ICDE39-10, ICDE8-4 and ICDE13-3 in pair wise comparison with recurrent parent Prathyasa. ICDE 28-7, ICDE22-1, ICDE8-2 and ICDE8-7 pyramided plants recorded coefficient of dissimilarity was 4.00.

4.9.2.2.2 Number of Productive Tillers Plant $^{-1}$

For this trait recurrent parent Prathyasa recorded 10 numbers of productive tillers. Among pyramided lines the minimum number of productive tillers (5) was found in ICDE12-1, ICDE28-9 and ICDE22-1 plant progenies. The maximum number of productive tillers /plant (12) was found in ICDE83-13. The average number of productive tillers among pyramided lines was 7.670 ±0.43. Two plant progenies were ICDE83-14 and ICDE33-11 recorded number of productive tillers which was similar to recurrent parent.

Euclidean distance was calculated between 21 pyramided lines with 2 resistance genes combination and recurrent parent Prathyasa. Coefficient of dissimilarity 0.00 was observed in two plant progenies ICDE83-14 and ICDE33-11. Plant progenies *viz.*, ICDE12-1, ICDE28-9, and ICDE22-1 had highest Euclidean distance 5.00.

4.9.2.2.3 Length and Breadth Ratio of Grain

Twenty one plants showed presence of two resistance gene combination. Among these the minimum L/B ratio was 2.20 in ICDE8-7 and other two plant progenies ICDE28-9 (2.28) and ICDE39-10 (2.29) showed length/breadth ratio closer to recurrent parent Prathyasa (2.17). The average value for this trait was 2.77±0.09. Maximum L/B ratio was 3.96 showed in ICDE12-1. Apart from this

ICDE 28-7 (3.52), ICDE8-2 (3.12), ICDE8-4(3.01) and ICDE39-8(3.24) plant progenies also showed higher L/B ratio of grain.

Among pyramided lines the lowest coefficient of dissimilarity (0.03) was recorded by plant progeny ICDE8-7. Apart from this two more plant progenies ICDE28-9 (0.11) ICDE39-10 (0.12) exhibited lower dissimilarity coefficient. Plant progeny ICDE12-1 showed highest coefficient of dissimilarity of 1.79.

4.9.2.2.4 Days to Maturity

The range for days to maturity in intermated plants was from 100 days (ICDE28-9) to125 days (ICDE83-13) with an average value of 113.05 ±1.25. Four plant progenies *viz.,* ICDE28-10, ICDE22-1, ICDE 8-1 and ICDE8-5 matured in 110 days which was similar to recurrent parent Prathyasa. Four plant progenies matured earlier and thirteen plant progenies showed delayed maturity compared to recurrent parent.

The Euclidean distance was assessed among BC_1F_1 pyramided lines in comparison with recurrent parent Prathyasa. ICDE28-10, ICDE22-1, and ICDE 8-1, ICDE8-5 pyramided lines showed dissimilarity coefficient zero. Plant progeny ICDE83-13 had maximum Euclidean distance of 15.00. Apart from this ICDE12-1, ICDE28-9 ICDE8-2 recorded dissimilarity coefficient 14.00, 10.00 and 9.00 respectively.

4.9.2.2.5 Kernel Colour

The variation of the kernel colour varied from light red to white. Recurrent parent Prathyasa had characteristic red kernel colour. Two plant progenies ICDE39-8 and ICDE12-1 exhibited white kernel colour. Among 21 intermated plants the distribution of kernel colour in the ratio of 7 red: 12 light red: 2 white (Fig.9). Majority of the plants had red kernel colour which is characteristic colour of recurrent parent.

Discussion

5. DISCUSSION

Rice *(Oryza sativa* L.) is an important food crop that serves as a major carbohydrate source for nearly half of the world's population. In India, it is grown in 43 million hectares accounting for 42 percent of food grain production and 55 percent of cereal production. In order to sustain self-sufficiency and meet the future food grain requirement, India has to produce 135–140 million tonnes of rice by 2030.

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most destructive diseases of rice in different rice growing areas in Asia. The disease is a major problem in *kharif* season (wet season) crop in rice growing regions of Punjab, Haryana, Uttaranchal, Bihar, West Bengal, Tripura, Assam, Tamil Nadu, eastern Uttar Pradesh and Andaman and Nicobar islands, coastal areas of Andhra Pradesh and Kerala and parts of Maharashtra, Chhattishgarh, Gujarat, Himachal Pradesh and Karnataka (Laha *et al.,* 2009). In some areas of Asia, it can reduce crop yield by up to 50 percent (Khush *et al.*, 1989) or even up to 80 percent (Singh *et al.*, 1977). This affects photosynthetic areas and reduces the yield drastically and produces partial grain filling and low quality fodder yield. Since chemical control is ineffective, development and deployment of BB resistant cultivars is the only practical strategy for managing the disease.

Till date, more than 40 BB resistance genes have been identified from diverse sources (Laha *et al.,* 2009; Kim *et al.,* 2015). Even though a majority of the BB resistance genes have been identified and derived from cultivated rice *(i.e. Indica / Japonica* genotypes), a few like *Xa21, Xa23, Xa27, Xa29t* and *Xa30t* have been derived from wild species of the genus *Oryza* (Song *et al.* 1995, Zhang *et al.,* 2001; Gu *et al.,* 2005; Lee *et al.,* 2003; Tan *et al.,* 2004; Cheema *et al.,* 2008). Most of the BB resistance genes are dominant in nature (*i.e. Xa4*, *Xa7*, *Xa21, Xa23, Xa26, Xa27* etc), while some are recessive (*e.g., xa5, xa8, xa13*). Some of the resistance genes are effective only in adult stage of the plants (*i.e. Xa21)* while others do not seem to be developmentally regulated. Sirisha *et al.* (2004) reported

that the dominant gene, *Xa21* confers a high and broad level of resistance to most of the pathotypes in India and elsewhere. The resistance gene *Xa3* is typically effective only in adult plants, but against at least one race (Philippine race 3) it gives resistance at all growth stages (Sun *et al.,* 2004). Some of the 'R' genes provide wide spectrum resistance against multiple isolates/strains (e.g. *xa13, Xa21, Xa23)* while others are narrow in their protective activity (*i.e. Xa1).*

A few of the 'R' genes have been introgressed into the background of the susceptible *Indica* cultivar IR24 and are available as near isogenic lines (NILs) carrying single BB resistance genes. Some of the effective gene combinations like *Xa21+xa13+xa5* or *Xa21+xa13* have been pyramided in the genetic background of elite rice varieties like Samba Mahsuri (Sundaram *et al.,* 2008) and Pusa Basmati1 (Joseph *et al.,* 2004) in India. Additionally the dominant gene, *Xa21,* originally derived from a wild species *O. longistaminata* has been widely deployed in hybrid rice parental lines with another dominant gene *Xa7* (Zhang *et al.,* 2006). Abhilash (2016b) transferred four major resistance genes (*i.e Xa*21and *Xa*33 for BB resistance and *Pi2* and *Pi54* for blast resistance) to RPHR-1005 using RPBioPatho-1(possessing *Xa21+Pi2*), RP Bio Patho2 (possessing *Xa21+Pi54*) and FBR1-15EM (possessing *Xa33*) as the donors. In view of these, present investigation was carried out to introgress the bacterial blight resistance genes *Xa21*, *xa*13 *Xa*33 and *Xa*38 into BB susceptible varieties Aiswarya and Prathyasa using marker assisted selection.

5.1 VALIDATION OF BB RESISTANCE GENES IN THE PARENTAL GENOTYPES

5.1.1. PCR Amplification Conditions

The PCR conditions need to be well defined to obtain reproducible patterns of DNA amplification. The effect of various concentrations of genomic DNA (100 ng, 150 ng and 200 ng), primer of each forward/reverse (10pmol/µl), $MgCl_2$ (25 mM, 10 mM), and Taq DNA polymerase (1.0 unit, 2.0 units and 3.0

units) in a reaction volume of 25 µl were investigated on amplified products. Analysis of the amplified products on agarose gel electrophoresis revealed that the DNA amplification was influenced by all these factors i.e. concentration of template DNA, $MgCl_2$, Taq DNA polymerase and the annealing temperature. Samples were run at different gradient temperature to find out annealing temperature. In low concentrations of DNA template, a few numbers of bands were observed, whereas, the high concentration of DNA template resulted in some non-reproducible bands. By changing the concentration of template DNA, both the yield and profile of PCR amplified products varied. High annealing temperature of 65° C and lower temperature 50° C gave products with faint bands or no amplification. . However, the annealing temperature of 55- 58° C was found to be optimum for generating clear and reproducible bands. Reproducible and clear banding patterns were obtained in a reaction mixture of 25µl containing 50 ng template DNA, 10 mM of dNTPs mix, 10pmol/µl of each primer (Forward/Reverse), 25 mM of $MgCl_2$ and 2.0 unit of Taq DNA polymerase with annealing temperature of 55 °C for 1 minutes.

Several other workers optimized concentration of various reagents and conditions for polymerase chain reactions in rice. Ramalingam *et al.* (2001) got the best banding pattern in the PCR reaction mixture of 20 µl containing 25–50 ng template DNA, 5 pmoles of each primer, 1X PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2 and 0.01% gelatin) and 1 unit of Taq polymerase. One minute primer annealing at 55 °C was the optimum for PCR amplification. Similarly, Basavaraj *et al.* (2010) performed PCR reactions using a reaction volume of 25 µl and obtained the most consistent and reproducible results using 50 ng template DNA, 5 pmol of each primer, 0.05 mM dNTPs, 1X PCR buffer,1.8 mM $MgCl_2$ and 0.5 U of Taq DNA. However, Sundaram *et al.* (2008) observed good amplification pattern by carrying out PCR using 5 ng DNA as template for amplification, 5 pmoles of each primer, 0.05 mM dNTPs, 1X PCR buffer 100 mM dUTP (Flourescein) and 1 U Taq DNA polymerase in a total volume of 12.5 µl. Primer annealing at 55°C for 30 seconds was found to be

optimum for their PCR amplification reactions. In a similar study, Kottapali *et al.* (2010) performed PCR using 50–100 ng template DNA, 50 ng of each primer, 0.05 mM dNTPs, PCR buffer (10 mM Tris/HCl. pH 8.4, 50 mM KCl, 1.8 mM MgCl2 and 0.01 mg gelatin/ml) and 0.6 U Taq DNA polymerase in a reaction mixture volume of 20 µl. Primer annealing was carried out at 55°C for 30 seconds to obtain good amplification pattern. Huang *et al.* (2012) also obtained clear.DNA banding pattern in PCR reaction mixture containing 50 ng template DNA, 50 ng of each primer, 0.05 mM dNTPs, 1X PCR buffer (10 mM TRIS, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$ and 0.01 mg/ml gelatin) and 1 unit Taq DNA polymerase in a volume of 20 µl.

5.1.2. Parental Polymorphism for Resistance Genes

DNA markers are widely used in marker-assisted breeding/selection. In MAS, markers are used at two levels, that is, foreground selection and back-ground selection. For foreground selection, gene-specific or tightly linked markers of target traits are used. A recent review by Jena and Mackill (2008) provided the list of DNA markers that are tightly linked with some major quantitative trait loci (QTLs) or genes relating to agronomic traits.

A clear marker-trait association was established for BB and so it is possible to monitor the transmission of trait genes viz., *xa13, Xa21, Xa33* and *Xa38* via closely linked makers (Fig.10). Resistance breeding with marker aided selection has been employed to develop broad spectrum durable BB resistance in rice. Two gene specific markers viz., xa13 promoter and pTA 248 that are closely linked to the BB resistance genes viz., *xa13* and *Xa21*, respectively were verified in Improved Samba Mahsuri. Once the resistant parents were confirmed for the presence of resistance genes, the two gene specific primers viz., xa13 promotor and pTA 248 were used to survey the parental polymorphism (ISM vs Aiswarya and Prathyasa) for resistance genes. The results revealed that the amplification profile produced by the marker pTA248 specific to the gene *Xa21* showed that the donor parent Improved Samba Mahsuri produced a product at 1000bp which was

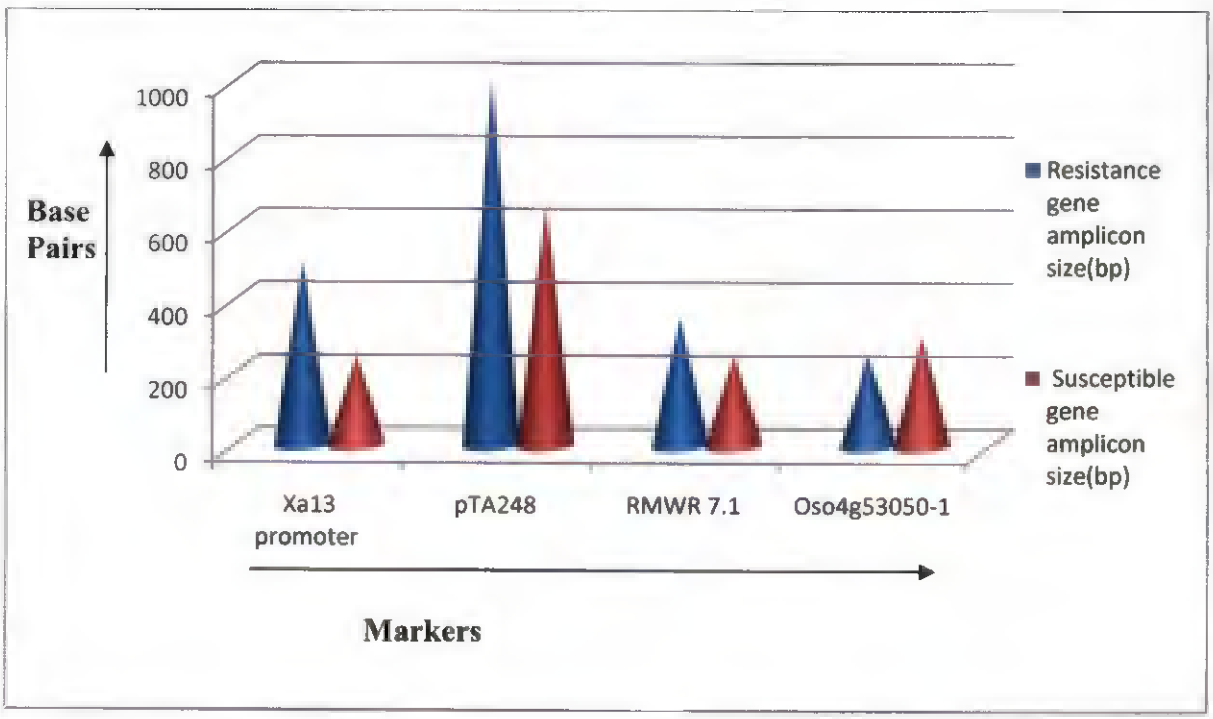


Fig. 10. Amplification pattern of BB resistance genes linked polymorphic markers in donor and recipient parents.

absent in both the susceptible parents Aiswarya and Prathyasa produced a product at 650bp. Functional marker xa13 pro linked with *xa13* gene was more accurate than earlier RG136, (CAPS marker is ~1.5 cM away from *xa13* gene). The amplification profile produced by the marker xa13 pro specific to the gene *xa*13 showed that the donor parent Improved Samba Mahsuri produced a product at 500bp which was absent in both the susceptible parents, Aiswarya and Prathyasa produced a product at 250bp. These results are in agreement with Sundaram *et al.* 2011, Kumar *et al.* 2013 Magar *et al.* 2014. The PCR-based DNA markers used in the present study (*i.e.* xa13- promo, pTA248) were tightly linked to *xa13 and Xa21* (Sundaram *et al.,* 2011; Ronald *et al.,* 1992) respectively, and hence, these markers showing clear polymorphism between the parental lines were used for F_1 confirmation and back cross generation (foreground selection).

The markers RMWR7.1 and RMWR7.6 are flanking to the novel BB resistance gene *Xa33* (Fig.11). Both these markers were used to evaluate the parental polymorphism in donors and recipients. RMWR7.1marker for the gene *Xa*33 amplified an allele of size ~350bp in donor parent and an allele of size ~250bp in recipient parents. Similarly marker RMWR7.6 produced an amplicon of size 190 bp and ~210 bp in donors and recurrent parents respectively. Among flanking markers, RMWR7.1 showed clear parental polymorphism compared to RMWR7.6 in the donors and recurrent parent combinations. So for further study RMWR7.1 was selected for foreground selection in segregation population. A set of very closely linked flanking markers RMWR7.1 and RMWR7.6 located on Chr.7, exhibited co-segregation with the trait phenotype with linkage distances of 0.92 and 1.15 cM, respectively, from the novel gene *Xa33*. Even though the two markers showed a few recombinants, they both are as such individually close enough for effective use in marker-assisted selection. Further, the fact that RMWR7.1 lie on one side of the gene and RMWR7.6 is located on the other side of novel gene locus (*i.e.* flanking nature of the SSR markers) helps in accurate prediction of the presence of *Xa33* with less than 1% error. (Kumar *et al.,* 2012).

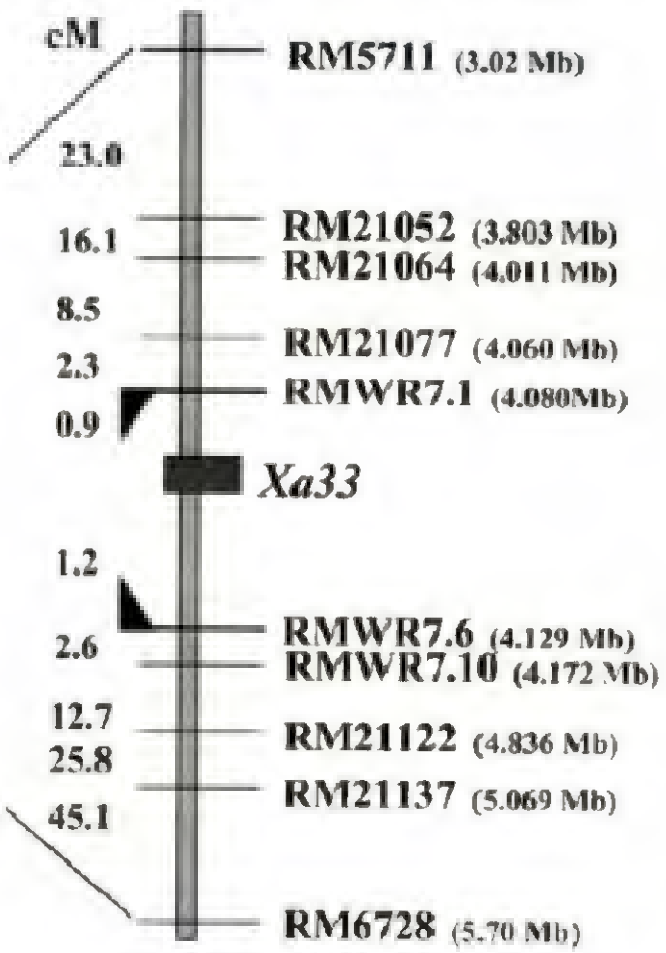


Fig. 11. Genetic linkage map of the genomic region in the vicinity of *Xa33* on chromosome7 (Kumar *et al.*, 2012).

The marker Oso4g53050-1 specific to gene *Xa*38 amplified an allele of size ~ 250bp in donor parent (PR-114) but not in its recipient parents (Aiswarya and Prathyasa). Oso4g53050-1 produced an allele of size 300-320bp in susceptible parents (Bhasin, *et al.,* 2012). Since the polymorphism was very clear among the parents for both the target genes, these markers were selected for foreground selection in the segregating generations. The closer the marker, the more reliable will be the selection. Functional markers are ideal for indirect selection and are highly predictive of phenotype. They target the functional polymorphism within a desired gene and overcome the problem of recombination/linkage (Andersen and Lubberstedt 2003; Iyer-Pascuzzi and McCouch 2007). Oso4g53050-1 is separated by approximately 19 kbp from Os04g53030, the most probable candidate for *Xa*38 and so can be clearly separated in agarose gel. Present analysis shows it to be a highly reliable marker for identifying the locus *Xa*38, as it would reduce effort, time and expense involved in transferring this important gene into new cultivars. Using agarose gel electrophoresis this gene could be pyramided with other BB resistance genes like *Xa*4 (Sun *et al.*, 2003), *xa*5 (Iyer-Pascuzzi and McCouch 2007), *xa*13 (Chu *et al.*, 2006) and *Xa21* (Huang *et al.*, 1997) to achieve durable resistance against bacterial blight in rice.

5.2 MARKER ASSISTED FOREGROUND SELECTION IN F_1 AND BC_1F_1 PLANTS

It is the first level of selection that involves the use of markers called 'foreground markers' that are linked to the gene for a particular trait and are used to screen the target trait. These markers are useful for traits that have laborious phenotypic screening procedures or governed by recessive alleles. The already cloned and characterized bacterial blight resistance genes *Xa21, xa13 and Xa33* and *xa38* were used in research for the introgression into bacterial blight susceptible varieties Aiswarya and Prathyasa. *Xa21* is a dominant resistance gene that encodes a receptor kinase containing NBS-LRR domains (Song *et al.*, 1995). The *xa*13 resistance gene is recessive in nature and has been shown to be a

mutation in the promoter region of a gene that is a homolog of the nodulin MtN3 (Chu *et al.*, 2006). In rice lines containing the dominant (susceptibility) allele of the gene, the expression of the nodulin homolog is up regulated upon infection with *Xoo*. It appears that the increased expression of this gene is necessary for *Xoo* to grow on rice. This up regulation does not occur in rice lines containing the resistance (recessive) *xa*13 allele (Yang *et al.*, 2006). The apparently different modes of action of the three resistance genes used in our work might contribute to make the resistance in the two and three-gene pyramid lines quite durable.

The genomic region of the Japonica rice flanked by the closely linked SSR markers RMWR7.1 and RMWR7.6 was downloaded and analyzed for putative candidate genes by using the software FGENESH (http://www. softberry.com). In total, eight putatively expressed genes were identified in the region analyzed and, among these; a gene encoding serine-threonine kinase appears to be the most probable candidate for *Xa33*. Bacterial blight (BB) of rice managed largely through the deployment of resistance genes. *Xa*38, a BB resistance gene identified from *Oryza nivara* acc. IRGC 81825, was mapped on chromosome 4L in a 38.4-kb region. The closely linked markers for this gene, identified earlier, were simple sequence repeat marker RM17499 and sequence-tagged site markers developed from loci Os04g53060 and Os04g53120. Marker Os04g53060 is dominant while the other two markers show smaller size differences difficult to resolve accurately on agarose gel. Based on gene annotation, three nucleotide binding site–leucine-rich repeat genes present in the target region were cloned from *O. nivara* and sequenced. One of the loci, LOC-Os04g53050, had a 48-base-pair deletion in *O. nivara* acc. IRGC 81825 compared to the cultivated rice. Primers were designed around the deletion and the resulting marker is codominant and easy to score in agarose gel (Bhasin *et al.*, 2012). These microsatellite markers pTA248, xa13 pro, and RMWR 7.1 and Os04g53050-1 showed highly effective results for the identification of resistant alleles of *Xa21*, *xa*13 and *Xa*33 and *Xa*38 genes in the study.

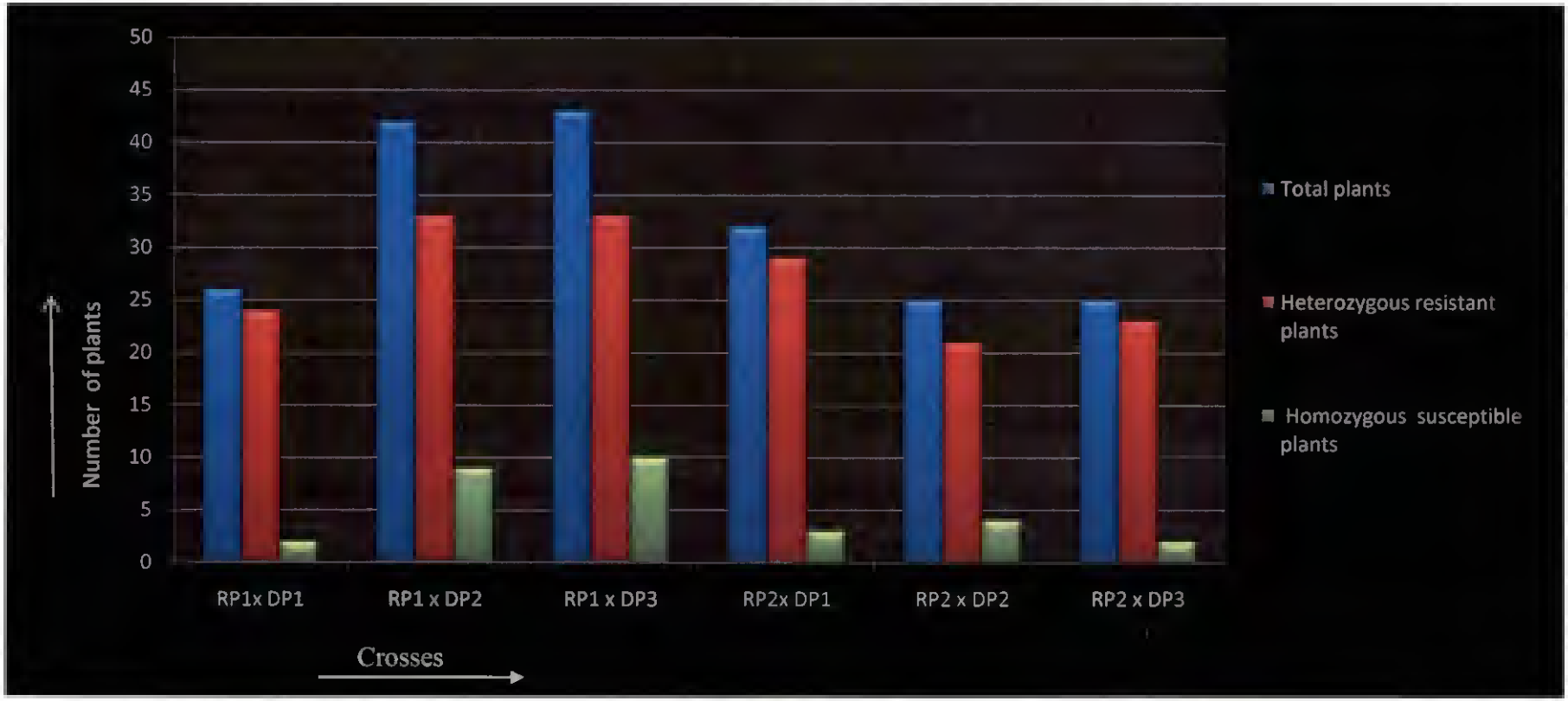


Fig. 12. Graphical representation of heterozygous and homozygous loci in F_1 plants of different crosses

RP_1= Aiswarya, RP_2=Prathyasa, DP_1=ISM (*xa*13 and *Xa21*), DP_2= ISM (*Xa*33) DP_3= PR-114(*Xa*38)

Clipping method of emasculation was followed in crossing programme. Three donors with respective resistance genes and two recipients were crossed to transfer genes to the recurrent parents. The F_1 seeds from resultant six crosses were harvested. The range of crossed seeds was 100-150. . These F_1 plants were checked for the presence of BB resistance genes *viz.*, *Xa21*, *xa*13, *Xa*33 and *Xa*38 using a set of four PCR based markers pTA248, xa13 promoter, RMWR7.1 and Oso4g53050-1 for carrying out foreground selection. These polymorphic markers showed highly effective results for the identification of resistant alleles of *Xa21*, *xa*13, *Xa*33 and *Xa* 38 genes in the study.

A range of 21-33 plants in individual cross had BB resistance genes in heterozygous resistance loci (Fig12). The F_1 plants having respective BB resistance genes (*xa13*and *Xa21, Xa33,* and *Xa38*) and the recurrent parents Aiswarya, Prathyasa sown on different staggered dates. One set of early sowing of recurrent parent was also done. The positive F_1 plants of respective cross and their recurrent parents *viz.*, Aiswarya and Prathyasa had flowering simultaneously. So backcrosses of F_1 plants were made with recurrent parents and BC1F1 seeds were harvested. The number of BC_1F_1 seeds ranged from 120 to 545 in cross Prathyasa x Improved Samba Mahsuri (*Xa*33) and Prathyasa x Improved Samba Mahsuri (*xa*13 and *Xa21*) respectively.

5.2.1 Aiswarya and Prathyasa Derived Back Cross Plants

Foreground selection was performed in Aiswarya and Prathyasa derived back cross plants by using respective polymorphic markers.

5.2.1.1 Foreground Selection for *xa*13 and *Xa21* Genes

The foreground selection was carried out in twenty five F_1 plants of cross Aiswarya x ISM (*xa*13 and *Xa21*) using the specific STS markers pTA248 and functional marker *xa*13 pro to detect the presence of both resistance genes *Xa21* and *xa13*, respectively. A total of twenty one F_1 plants were found to have both the BB resistance genes *Xa21* and *xa*13.These plants were backcrossed with

respective recurrent parent .Out of 172 BC_1F_1 plants screened, only forty two plants had both genes in heterozygous condition for both loci.

A total of twenty one out of twenty five F_1 plants of cross Prathyasa x ISM (*xa*13 and *Xa21*) were found to have both the BB resistance genes *Xa21* and xa13 in heterozygous condition. 178 backcross plants were screened for foreground selection by marker pTA248 and xa13 pro and forty two BC_1F_1 plants were selected for the presence of both the genes in the heterozygous condition for both loci. These plants were female in the intermating crossing programme.

Pradhan *et al.* (2015) raised 360 BC_1F_1 seeds for further backcrossing with Jalmagna. 93 BC_1F_1 plants showed the presence of *Xa21* resistance gene specific bands (1000 bp) while 91 plants showed the presence of *xa*13 resistance gene specific bands (490 bp and 530 bp). 116 BC_1F_1 plants showed the presence of xa5 resistance gene specific bands (160 bp). Based on the amplification of resistance specific bands, 31 BC_1F_1 plants showed the presence of *Xa21* and xa13 resistance genes. Dokku *et al.* (2013) screened 187 BC_1F_1 plants by using PCR primers pTA248 and RG136 for presence of *xa*13 and *Xa* 21 and found 53 had resistance genes in heterozygous condition. Sundaram *et al.* (2008) made crosses between SS1113 (having BB resistance genes *Xa21*, *xa*13 and *xa*5) and Samba Mahsuri and carried out foreground selection using the same set of STS markers. The positive F_1 plants were backcrossed with Samba Mahsuri. A total of 11/145 BC_1F_1 plants were found to have all the three resistance genes. The resulting BC_1F_1 lines were checked for presence of the marker linked to *Xa21* and xa13 resistance allele in a heterozygous condition and the process was continued up to BC_4F_1 stage. Similarly, Kottapali *et al.* (2010) used the same three STS markers i.e. pTA248 linked to *Xa21* gene, RG136 linked to *xa*13 gene and RG556 linked to *xa*5 gene for carrying out the foreground selection. A total of 200 F_1 plants were found positive for three resistance genes *xa*5, *xa*13 and *Xa21*. Pandey *et al.* (2012) also carried out foreground selection of F_1 plants of the crosses between Taraori Basmati x Improved Samba Mahsuri (having *Xa21* and *xa*13 BB genes) and Basmati 386 x Improved Samba Mahsuri using STS marker pTA248 linked to

Xa21 and *xa*13-pro linked to *xa*13 gene and backcrossed those using Basmati varieties as a female parent.

However, Suh *et al.* (2013) used different set of STS markers MP1 + MP2, T10Dw and U1/I1 linked with resistance genes *Xa*4, *xa*5 and *Xa21*, respectively for the similar type of study and obtained F_1 plants (Mangeumbyeo (BB susceptible) x IRBB57 (BB resistant) having the three BB resistance genes (*Xa*4, *xa*5 and *Xa21*). These F1 plants were backcrossed with the recurrent parent and 28/288 BC_1F_1 progenies having three BB resistance genes were obtained on the basis of molecular marker analysis and phenotypic selection. Shanti *et al.* (2010) carried out foreground selection of F_1 plants of the crosses Mahsuri/IRBB60 (donor for the four BB resistant genes), KMR3/IRBB60, PRR78/IRBB60, IR58025B/IRBB60, Pusa 6B/IRBB60 using three STS markers Nbp181, pTA248, RG136 tightly linked to *Xa*4, *Xa21* and *xa*13 gene respectively and a SSR marker RM122 tightly linked to *xa*5 gene. The BC_1F_1 plants having all the four BB genes were backcrossed with the recurrent parent and this was continued up to the BC_3F_1 generation.

5.2.1.2. Foreground Selection for Dominant Gene Xa33

Thirty three F_1 plants had presence of heterozygous loci for the marker linked to the gene *Xa*33 and these were backcrossed with Aiswarya. Seeds (122) germinated and later these plants were used for foreground selection. Forty six BC_1F_1 plants showed the presence of heterozygous loci for the gene specific marker. These plants were used as pollen parents in intermating programme. Chi square ratio 1:1 in this BC_1F_1 generation for single gene showed significant deviation. This deviation could be due to differential transmission of female gametes having *Xa33* or *xa33* or due to any other gene in the close vicinity of this resistance locus. Kumar *et al.* (2012) also reported segregation distortion for *Xa*33 gene in F_2 population. Earlier segregation distortion has been reported in rice by McCouch *et al.* (1988).

Twenty nine F_1 plants of this cross showed heterozygous loci for the gene specific maker. These resistant plants were backcrossed with recurrent parent Prathyasa and obtained 120 seeds. The plants were screened with foreground polymorphic marker RMWR7.1 and identified plants with heterozygous loci. Out of 72, only 32 plants had the resistance gene in heterozygous condition and remaining 40 plants were in homozygous susceptible indicating that a single dominant gene *Xa*33 could be controlling BB resistance ($\chi 2 = 0.89$, $P = 0.34$). It followed 1:1 Mendelian ratio and there is no significant deviation from the expected ratio. Similar result was reported by Kumar et *al.* (2012).

In the validation of marker in the parental lines RMWR 7.1 primer gave a marker which showed a difference of 100bp with the marker of susceptible parent. So polymorphic marker RMWR 7.1 used for marker assisted foreground selection. In contrast Abhilash *et al.*(2016 b) used marker RMWR7.6, for identification of 28 true"F_1s and they were backcrossed with RPHR-1005 to generate 175BC_1F_1. They observed that eighty-seven BC_1F_1 were heterozygous for *Xa33* gene .Selection of polymorphic markers from set of flanking markers depends on study of our donor and recipient polymorphism result.

5.2.1.3. Foreground Selection for Xa38 Resistance Gene

In the cross Aiswarya x PR-114 thirty three F_1s plants showed presence of heterozygous loci for the marker linked to resistance gene *Xa38* and these were backcrossed with Aiswarya and produced BC_1F_1 seeds. Out of 178 BC_1F_1 plants 80 plants had heterozygous loci to the specific marker gene. Chi- square test showed non significance deviation from expected ratio 1:1($\chi 2 = 1.82$, P=0.17). In the cross Prathyasa x PR-114 twenty three heterozygous plants were crossed with Prathyasa and produced BC_1F_1 seeds. Thirty seven BC_1F_1 plants had heterozygous loci for a gene *Xa*38. In this back cross population chi square tested for 1:1 segregation ratio ($\chi 2 = 0.21$ P= 0.64) and the segregation ratio for this marker did not deviate significantly from the expected 1:1 ratio since the calculated $\chi 2$ value was very less. The BC_1F_1 plants were selected based foreground marker

Os04g53050-1 and used as male parent in intermating programme. In both Aiswarya and Prathyasa derived BC_1F_1 with *Xa*38 gene showed 1: 1 segregation with respect to the marker linked to the gene. Similar result reported by Cheema *et al.* (2008). They reported that Xa38 locus showed 3:1 and 1:2:1 segregation ratio in the BC_2F_2 and BC_2F_3 generations, as expected of a single dominant gene. Ellur *et al.* (2016b) generated F_1s from the cross PB1121/PR114-*Xa38* was tested for hybridity using the marker-Os04g53050-1. A single plant confirmed for hybridity was backcrossed to recurrent parent and a total of 468 BC_1F_1 seeds were produced, out of which 223 plants were found to be heterozygous for *Xa38*.

5.3. MARKER ASSISTED BACKGROUND SELECTION IN BC_1F_1

Marker-assisted background analysis of the segregants/recombinants is useful in determining the relative contribution of the progenitor parents. Molecular marker analysis with SSR markers gives a quick evaluation of the genetic background of the recombinants. In the present study, the four varieties were genotyped with 270 microsatellite loci and of the 270 loci, 180 yielded discrete, unambiguous amplification products. Of the 180 loci, 101 were polymorphic in at least one of the four varieties to derive maximum genetic background of recurrent parents Aiswarya and Prathyasa.

5.3.1. Background Selection and Recovery Percentage of Recurrent Parent Genome (RPG)

The background selection was carried out on altogether 279 plants derived from four BC_1F_1 populations using altogether 143 polymorphic loci in total, ranging from 30 loci in back cross population derived from (Aiswarya x PR114) x Aiswarya to 41 loci in the population derived from (Prathyasa X ISM) x Prathyasa.

In Aiswarya x ISM (*xa*13 and *Xa21*) and Aiswarya x ISM (*Xa33*) backcross population the percentage of recovery of recurrent parent genome (RPG) was assessed using 39 parental polymorphic markers. In Aiswarya x ISM

(*xa*13 and *Xa21*) fourteen plants showed the recovery of RPG in the range of 70-80%.The highest RPG recovery (80-90%) was found in five plants. Similarly in the backcross of Aiswarya x ISM (*Xa33*) the percent recovery of Aiswarya genome by 14 BC_1F_1 plants ranged from 73.26 to 79.92.

In Aiswarya x PR-114 (*Xa*38) derived backcross generation, recovery of RPG was analysed by using 30 parental polymorphic markers in 80 plants. Eight plants had recovery of Aiswarya genome in the BC_1F_1 plants ranged from 75% to 80 %.

In Prathyasa derived backcrosses, two crosses which differed with respective resistance gene *viz,* Prathyasa x ISM ((*xa*13 and *Xa21*) and Prathyasa x ISM (*Xa*33) and in these population RPG recovery were assessed by using 41 parental polymorphic markers. Thirteen plants had 76 to 80% RPG and back cross plant D-126 recorded highest recurrent parent genome recovery 87.80%. In Prathyasa x ISM (*Xa*33) 19 plants had 78-83 percent recovery of RPG

Prathyasa x PR-114 (*Xa*38) population was analysed using 33 parental polymorphic markers. Four plants recorded RPG from 66-73% and two plants had high recovery from 73-79%.

Background genome recovery in six different cross combinations involving four different resistance genes in two recurrent parent backgrounds analysed. Theoretically *with* single backcross average background recovery should be 75% *(*Sundaram *et al.*, 2008) .In some BC_1F_1 plants the recovery of RPG was lesser than theoretical value. Rekha (2013) while studying the RPG recovery reported that it varied from 44.20 % to 78.90%. Reduced background recovery in some lines is largely due to linkage drag of the donor genotype on the carrier chromosomes around three target genes *Xa21*, *xa*13 and *xa*5. Similarly Sundaram *et al.* (2008) found that there is a variation from the theoretically expected 75% contribution from the recurrent parent genome in the BC_1F_1 plants. Some of the selected BC_1F_1 plants had a recurrent parent genome contribution that

was less than the expected 75%. They also found similar results in BC_1F_1 plants in another backcrossing program for introgression of *Xa21*, *xa*13 and *xa*5 from SS1113 into a rice cultivar called Triguna. This suggested that selection for introgression of the *Xa21*, *xa*13 and *xa*5 genes might be exercising a ''pull'' unknown mechanisms, which favours inheritance of additional unlinked loci from the donor genome in BC_1F_1 plants. In their study, a less than expected contribution from the recurrent parent was also observed in BC_2F_1 and BC_3F_1 generations. They also suggested that more experiments are needed to determine if such a ''pull'' does definitively exist. However, it is clear from their work that background selection with a limited number of polymorphic microsatellite markers (*50), in conjunction with four backcrosses is sufficient to recover the yield and quality characteristics of the recurrent parent. Background selection with markers, which are closely linked to the target traits, is probably helpful, but not essential, for recovery of recurrent parent characteristics following introgression of the *Xa21*, *xa*13 and *xa*5 genes in rice. Abhilash *et al.* (2016b) also analysed 43 plants using 59 parental polymorphic SSR markers and found that a solitary plant, (*i..e.,*#RPC-III- 38-27) possessing maximum RPG recovery of 78%.

Rajpurohit *et al.* (2010) also tested 209 rice SSR markers for background selection out of which a set of 95 markers showed polymorphism between the parents Type 3 Basmati and PR106-P2. They selected sixteen BC_2F_3 progenies with nearly Type 3 Basmati seeds for background profiling using 95 SSR and 12 ISSR markers. On the basis of SSR markers, 16 lines showed background recovery from 81.57% (41-3-40) to 92.10% (29-1-35). Pyramid line 29-1-35 recovered maximum recurrent parent genome (92.0%) followed by line 31-4-2 with RPG (91.05%). Similarly, Basavaraj *et al.* (2010) carried out marker assisted background selection in the 10 best BC_2F_5 families of Pusa6B and PRR78 using 74 STMS markers polymorphic between Pusa6B and Pusa146 and 54 STMS markers polymorphic between PRR78 and Pusa1460. They recovered 85.14 to 97.30% and 87.04 to 92.81% recurrent parent genome in the 10 selected BC_2F_5 families of Pusa6B and PRR78, respectively. Pandey *et al.* (2012) in a similar

study subjected the backcross-derived lines similar to their recurrent parents to background selection using 61 and 58 polymorphic SSR markers spanning all the 12 chromosomes for the breeding lines in the genetic background of Taraori Basmati and Basmati 386, respectively. Genome recovery ranged from 73.8 % (RP4693-47-4-4, RP4693-47-3-4) to 83.6 % (RP4693- 101-3-3) and 75.9 % (RP4694-157-1-2, RP4694-157-3-2) to 82.7 % (RP4694-157-2-1) in the genetic background of Taraori Basmati and Basmati 386, respectively.

However, Shanti *et al.* (2010) adopted conventional breeding strategy instead of using background markers for carrying out background selection of foreground selected pyramided plants. Chen *et al.* (2001), on the other hand, used 10 pairs of AFLP primers in contrary to SSR markers as used in this study, to select for the genetic background of the recurrent parent while incorporating *Xa21* in '6078', an elite restorer line. They developed an improved version of '6078 (*Xa21*)' with about 98.8% of the recurrent parent genome after three generations of backcrossing. Joseph *et al.* (2004) also used a total of 25 AFLP primer combinations to detect polymorphism between Pusa Basmati-1 and IRBB55 out of which eight primer pairs were found to be highly polymorphic between the parents. Genotyping of 21 BC_1F_3 plants revealed that percentage of recurrent parent genome varied from 80.4% in Pusa 32-2-6 to 86.72% in Pusa 75-4-14.

In the present study, the efficiency of the single backcross method using marker-assisted foreground and background selections along with phenotypic selection for various agronomic traits in rice was demonstrated. Similar strategy was followed by Joseph *et al.* 2004. Gopalakrishnan *et al.* (2008) also carried out marker-assisted selection for the two resistance genes in BC_1F_1, BC_1F_2 and BC_1F_3 generations. On background analysis using 252 polymorphic amplified fragment length polymorphism (AFLP) markers they detected 80.4 to 86.7% recurrent parent alleles in BC_1F_3 selections. However, in the studies by Singh *et al.* (2001); Narayanan *et al.* (2004); Perez *et al.* (2008), three backcrosses were attempted and selection of lines in the backcross generation was entirely based on foreground selection of target traits.

5.4 Morphological Traits Observation of Aiswarya Derived BC_1F_1 Plants

The morphological characters of some BC_1F_1 plants were similar or on par to the respective recurrent parents. These plants can be used for further back cross breeding programme.

5.4.1 Aiswaya x ISM Derived Back Cross Population

The plant height of parents Aiswarya and ISM were almost similar with difference of 2cm. Aiswarya x ISM (*xa13* and *Xa21*) derived six back cross plants showed height in the range of 95-100cm and which were closer to the parental lines. Four plants A- 72, A-111, A-129, and A-158 showed number of productive tillers similar to Aiswarya parent. Eight BC_1F_1 plant showed number of productive tillers (10) were intermediate between the two parents. Higher number of productive tillers is useful for obtaining higher yield. Grain length/breadth ratios of 4 parental lines were almost similar to the genotype Aiswarya. Parents Aiswarya and ISM had contrast red and white kernel colours. Most of BC_1F_1 plants (20) showed light red to red kernel colour. It indicated that red kernel character is dominant character over white. Red kernel colour rice is preferred by people of Kerala and most of the commercial varieties are red kernel rice. So in this programme, preference was given to phenotype kernel colour for selection. None of the plants exhibited higher maturity duration than donor parent ISM. BC_1F_1 plants A-22 and A-46 was taken time to matured similar to that of the Aiswarya. It indicated that some plants might have gained character of Aiswarya.

In Aiswarya x ISM (*Xa33*) derived nine BC_1F_1 plants height ranged from 95cm to 100cm and closer to the parental plant height. Six plants had eight numbers of productive tillers and which were more similar to Aiswarya. Some plants exhibited higher number of productive tillers than both the parents and selection preference might be given to these plants also to target higher yield. BC_1F_1 plants *viz.*, B-8, B-18, B-23, B-50, B-72, B-90, and B-112 recorded L/B ratio which were closer to recurrent parent value. Twenty one BC_1F_1 plants

exhibited light red colour of kernel. Twenty four plants had variegated brown to brown colour kernel and indicated large amount of variation among this population with respect to kernel colour. Most of the plants were medium matured and none of plant had taken long duration for maturity like long duration donor parent.

5.4.2 Aiswarya x PR-114 (*Xa38*) Derived Back Cross Population

Parents Aiswarya and PR-114 had plant height 98 cm and 108 cm respectively. Eight plants had height in the range of 95-100cm and were closer to plants height of Aiswarya parent. Eight numbers of productive tillers were observed in twelve plants and which were similar to recipient parent. In BC_1F_1 generation 31 plants exhibited higher or equal length /breadth ratio to the recipient parent. Aiswarya and PR-114 had contrasting red and white kernel colour respectively. BC_1F_1 plants exhibited wide range of kernel colour such as red to light red, brown to dark brown and white. Forty three BC_1F_1 plants showed light red to red colour kernel which is characteristic colour of recipient parent. None of the BC_1F_1 plants has taken time more than 150 days to mature which is maturity days of donor parent. Four BC_1F_1 plants matured in 120 days which is the maturity days of recipient parent.

5.4.3 Prathyasa x ISM Derived Backcross Generation

In Prathyasa x ISM (xa13 and Xa21) two BC_1F_1 plants (D-39 and D-72) had plant height of 95 cm which was more or less equal to recipient parent Prathyasa and remaining plants recorded lower plant height. Maximum numbers of tillers (13) were exhibited by plants D-32, D-70 and D-140. Six BC_1F_1 plants showed 10 numbers of productive tillers which was similar to Prathyasa variety for a trait and more number of productive tillers produced by these plants directly correlated with high yield. D-12, D-30, D-83, D-143 BC_1F_1 plants showed length and breadth ratio almost similar to recipient parents for the trait. Parents Prathyasa and ISM had contrasting kernel colour red and white respectively. Twenty seven

BC_1F_1 plants showed red to light red colour of kernels and which was characteristic colour of Prathyasa. Most of the plants recorded medium maturity duration in range of 115-130. None of the plant matured late like donor plant.

In Prathyasa x ISM (Xa33) derived backcross progenies, two BC_1F_1 plants such as E-14 and E-38 showed height of 95 cm which was similar height of Prathyasa. Six BC_1F_1 plants had 10 effective numbers of tillers which are similar to recipient parent for that trait. Among BC_1F_1 plants fifteen genotypes had red to light red kernel, 15 plants had brown to light brown and only two plants exhibited white colour of kernels. Most of the plants had medium maturity duration with range of 120-130 days. It indicated that more variation was present in this population.

5.4.4 Prathyasa x PR-114(Xa38) Derived Backcross Generation

None of the plant exhibited plant height more than donor parent. BC_1F_1 plants *viz.*, F-4, F-24 and F-36 had plant height 95 cm which was more or less similar to recipient parent Prathyasa. Maximum numbers of productive tillers per plant (16) were observed in plant F-3 and F-23 .Plants such as F-6, F-9, F-29, F-59 and F-64 had 10 productive tillers and which were similar to the recipient parent for a trait. Twelve BC_1F_1 plants recorded red colour kernel and which characteristic colour of recipient parent Prathyasa. Twenty BC_1F_1 plants recorded red colour kernel which is characteristic colour of recipient parent Prathyasa. Most of BC_1F_1 plants recorded medium maturity duration and none of the plant matured late compared to PR-114.

5.5 SCREENING OF DONORS AGAINST BB PRIOR TO INTERMATING

All the three donors *viz.,* ISM (*xa*13, *Xa21*), ISM (*Xa*33) and PR-114(*Xa*38) screened against local isolate of *Xanthomonas oryzae* pv.*oryzae* in order to pyramid the most effective resistance genes in the background of the recurrent parents Aiswarya and Prathyasa.

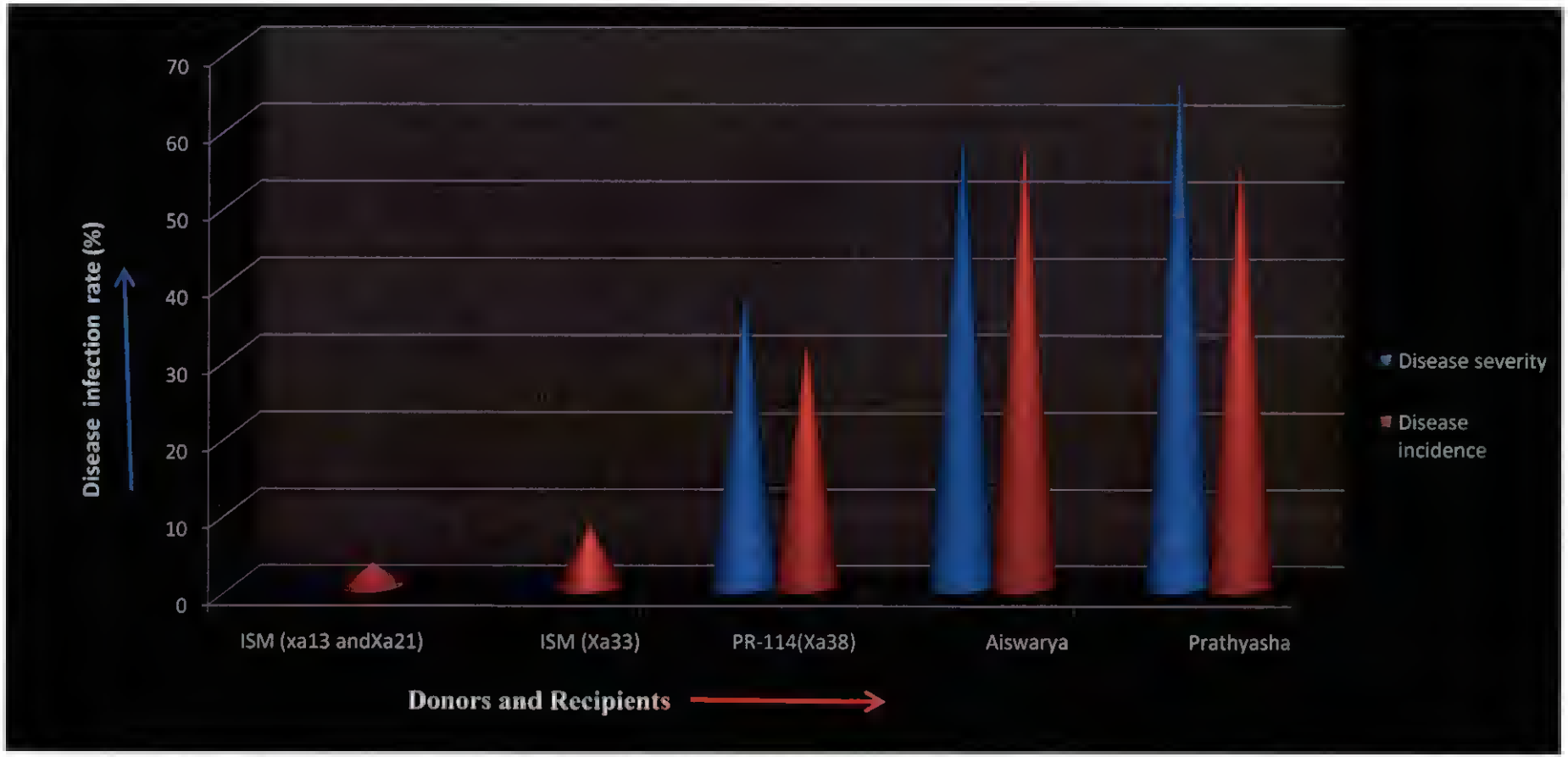


Fig. 13. Graphical representation of disease reaction against local *Xoo* isolate.

In this screening study both the donors and recurrent parents were evaluated for their resistant or susceptible disease reaction against local isolate of *Xanthomonas oryzae* pv.*oryzae* obtained from the BB infected fields of RRS Mocompu Kuttanand region of Kerala.

The results of the study indicated that donors ISM (*xa13* and *Xa21*), ISM (*Xa33)* exhibited resistant disease reaction 14 days after inoculation and no further progress in disease development. Similarily Purushothaman and Niza (2013) found that the isolates *viz*., Kodallur (XKOR-3) and Moncombu (XMOU-1) showed resistant reaction on all six isogenic lines *viz*.,IRBB-4 (*Xa4*), IRBB-5 (*xa5*), IRBB-13 (*xa13*), IRBB-21 (*Xa21*) IRBB-57 (*Xa4/xa5/Xa21*) and (*Xa4/xa5/xa13/Xa21*) the three rice differentials *viz*., Ajaya, IR-8 and IR-24. *Xa*21 and *xa*13 are resistance genes which have been widely deployed by breeding methods for evolving BB resistant varieties. Chen *et al.* (2002); Wang *et al.* (2007); Ram *et al.* (2010); Sundaram *et al.* (2008) observed that the lines containing either of resistance gene *xa13* and *Xa21* exhibited high level of resistance. Kumar *et al.* (2012) observed that *Xa33* is the enhanced level of BB resistance as compared to *Xa21*. When they compared the average lesion length of IRBB21 (NIL possessing *Xa21*) and *O. nivara* (Acc. # 105710), it was observed that the backcross derived lines of Samba Mahsuri possessing *Xa33* displayed a higher level of resistance as compared to IRBB21.

However many further reports indicated that the dominant resistance gene *Xa*21 has broken down in India and other countries of Asia (Brindha and Gnanamanickam 1999). The transfer of a single resistance gene is not likely to be effective because large scale and long term cultivation of such resistant varieties may result in significant shifts in the virulence pattern of the pathogen population leading to breakdown of resistance (Mew *et al*., 1992). The deployment of rice cultivars that have multiple BLB resistance genes is expected to lead to more durable resistance. Studies conducted to identify the best gene combinations conferring broad spectrum resistance showed that a four gene (i.e., *Xa*4 + *xa*5 +*xa*13 + *Xa*21) combination was the most effective and did not show any sign of

breakdown of resistance to various strains of the pathogen (Shanti and Shenoy 2005; Nayak *et al.,* 2008).

However the percentage of BB disease severity was 38% in PR-114 and indicating susceptible reaction when artificially inoculated with Moncompu isolate of *Xoo*. In contrast to present finding Vikal *et al.* (2007) reported that the resistance imparted by *Xa38* gene implied that the gene may be effective against pathogenic races available in the major Basmati growing regions of India. In light of these results the line PR-114(*Xa*38) excluded from pyramiding programme.

Recipient parents Aiswarya and Prathyasa showed higher percent of disease severity and incidence (Fig 13). Both these parents were susceptible to the BB pathogen. Devika (2001) studied genetic analysis of bacterial leaf blight resistance in rice and reported that Aiswarya showed susceptible disease reaction against *Xoo* isolate of Moncompu, Kerala.

Marker-assisted gene pyramiding, two or more resistance genes, such as *Xa*21, *xa*13, *xa*5 and *Xa*4, have been incorporated in the genetic background of elite varieties (Huang *et al.*, 1997; Sanchez *et al.*, 2000; Singh *et al.*, 2001; Joseph *et al.,* 2004; Sundaram *et al.*, 2008). Even though gene pyramid combinations, such as, *Xa*21 + *xa*13 + *xa*5 or *Xa*21 + *xa*5 or *Xa*21 + *xa*13, have been observed to possess high level of resistance against multiple isolates of Xoo, the durability of resistance in such gene pyramid lines has not been validated so far because of continuous evolution of new pathogenic races of *Xoo*. In order to enhance the durability of resistance, it is desirable to identify and characterize new genes from wild relatives of rice so that they could be deployed along with *Xa*21 or *xa*13 or *xa*5 in elite rice varieties.

The pyramided lines (two gene or three gene combinations) had a higher level of resistance and broader spectrum of resistance than parental lines or lines with a single gene. This might be due to interaction and/or complementation between the resistance genes (Sanchez *et al.*, 2000).

5.6 INTERMATING AND MOLECULAR SCREENING OF PYRAMIDED LINES

Breakdown of resistance in varieties, a consequence of considerable shift in a predominant pathogen race in response to the deployment of resistant varieties having a single resistance gene has been reported in rice (Mew *et al.*, 1992). Pyramiding of multiple resistance genes into a single genetic background leading to the simultaneous expression of more than one gene in a variety is a strategy to prevent/delay the breakdown of resistance as the probability of simultaneous pathogen mutations for virulence to defeat two or more effective genes is much lower than for a single gene (Mundt 1990) So the study was carried out to deploy two or three effective resistance genes (*xa*13, *Xa21* and *Xa*33).These can achieve durable and broad-spectrum resistance in many BB prone rice growing areas in Kerala . The study clearly established the utility of MAS in pyramiding recessive genes *xa*13 with dominant genes *Xa21* and Xa33, to present a multiple gene barrier against one of the most destructive diseases on rice. The success may also stimulate several such studies to realize the potential of molecular plant breeding as the foundation for crop improvement in the 21st century.

In present study intermating was performed between BC_1F_1 plants having respective resistance genes to pyramid 2-3 genes combination based on disease reaction. BC_1F_1 plants having *xa13* and *Xa21* genes were used as female parents and BC_1F_1 plants with *Xa33* used as male parents. These crosses screened with foreground markers for presence of 2-3 resistance genes combination. Based on background selection result seeds harvested from female parent (ISM with *xa*13 and *Xa21*). Around 120 -200 seeds were harvested from individual cross.

The donor parent Improved Samba Mahsuri and recurrent parents Aiswarya and Prathyasa vary significantly with respect to grain type, ISM possessing medium-slender grain type with white kernel while the recipient parent bold grain type with red kernel. Hence, we resorted to phenotype-based

visual selection for light red to red kernel, starting from BC_1F_1 generation onwards, and due to a stringent selection involving screening a large number of backcross plants, we identified the plants that not only possessed BB resistance but also had light red to red kernel. Earlier, Joseph *et al.* (2004); Gopalakrishnan *et al.* (2008); Sundaram *et al.* (2008) ; Hari *et al.* (2011) adopted a strategy of morphology-based selection for grain type coupled with marker-based selection of target trait (*i.e.* bacterial blight resistance) while developing improved versions of Pusa Basmati-1, Samba Mahsuri and KMR-3R. A similar approach was adopted in the present study, and it was observed that the morphological traits of the improved lines were on par with recipient parent with marginal differences.

5.6.1 Aiswarya and Prathyasa Derived BC_1F_1 Intermated Pyramided Lines

In the background of Aiswarya seventeen plants showed combinations of resistance genes *xa*13+*Xa*33. In these, 4 lines (ICAB22-1 ICAB159-5, ICAB159-10 and ICAB159-30) showed very less dissimilarity coefficient with the recurrent parent. Calculated overall Euclidean distance based on four quantitative traits in comparison with recurrent parent Aiswarya was less than 10.00 (Fig.14). This indicates that these four lines are closer to the recurrent parent with respect to these characters. Remaining lines showed similarity with Aiswarya in one or other quantitative traits. Majority of lines showed red kernel colour which was major criteria for phenotypic selection in these lines. In the background of Prathyasa nine plants showed *xa*13 + *Xa*33 resistance gene combinations. ICDE8-7 and ICDE33-10 pyramided lines had lesser Euclidean coefficient of dissimilarity which was assessed based on four quantitative traits in comparison with the recurrent parent Prathyasa (Fig.15). Remaining pyramided lines had similarity with recurrent parent in one or other quantitative trait. For kernel colour trait, all nine pyramided lines showed light red to red. Different mechanisms in expressing defense pathways of dominant and recessive genes and therefore the interaction of recessive and dominant genes in a pyramided manner may show more durable and higher level of resistance compared with single gene resistance (Li *et al.*, 2006).

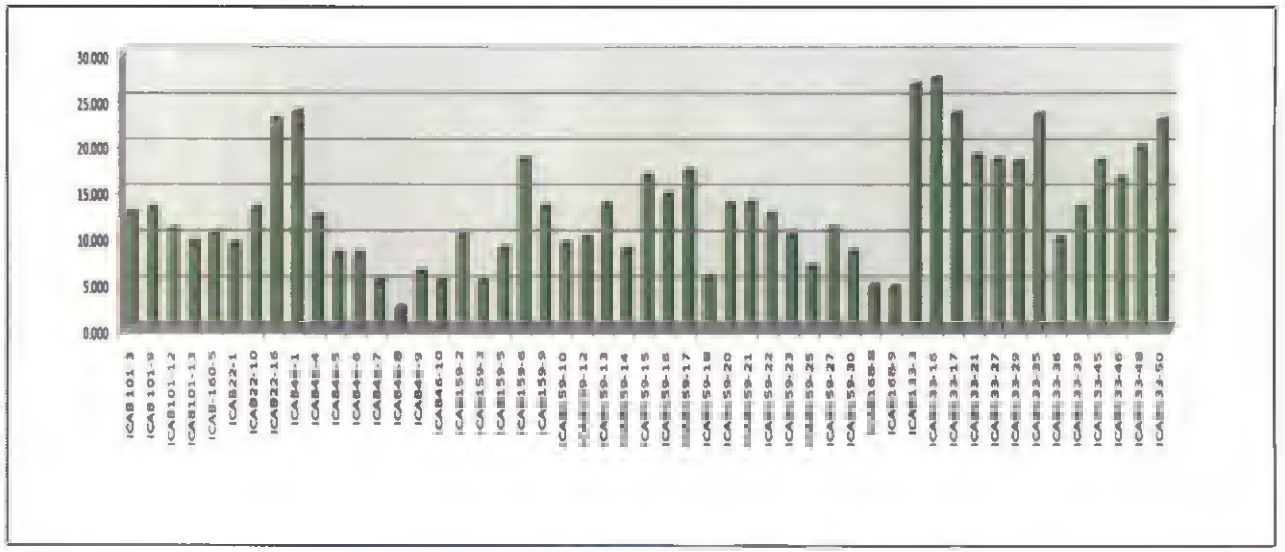


Fig.14. Overall Euclidean distance for quantitative traits in Aiswarya derived pyramided lines

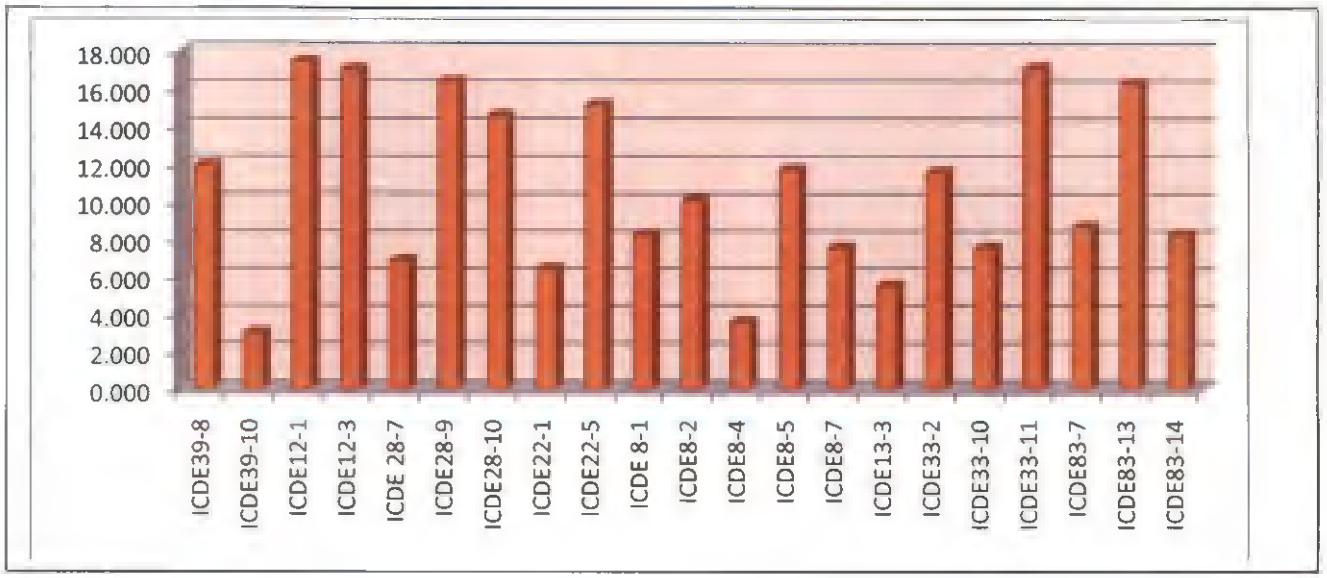


Fig.15. Overall Euclidean distance for quantitative traits in Prathyasa derived pyramided lines

PCR- based functional marker called pTA248, developed by Ronald *et al.* (1992) is available for marker-assisted selection of the gene. As far as choice of resistance genes for gene pyramiding along with *Xa21* is concerned, one of the recently identified, wild-rice derived genes, *Xa33* has attracted considerable attention. In our screening study it showed high level of resistance for local isolate of the BB pathogen and closely linked with available co-dominant molecular marker RMWR 7.1 for marker-assisted selection of the gene. Considering these facts, in the present study, combination of resistance genes *Xa21*+ *Xa*33 can be used for targeted improvement of recurrent parent. Nine plants were obtained with the resistance genes combination of *Xa21* + *Xa*33. Among these nine pyramided lines ICAB168-8 showed less dissimilarity coefficient (4.58) with Aiswarya parent. All nine pyramided lines had red kernel colour and could be used as pre breeding material in the development of essentially derived varieties for Kerala. Similar study was carried out by Abhilash *et al.* (2016 b) for improvement of RPHR-1005 for BB resistance. The BB resistance gene, *Xa21* to be highly effective under Indian conditions (Gopalakrishnan *et al.*, 2008; Sundaram *et al.*, 2008) and then newly identified wild rice derived BB resistance gene, *Xa33* has also been found to be effective against several Indian isolates of the pathogen (Kumar *et al.*, 2012). In contrast Li *et al.* (2006) reported that the MK-75 ILs with the *Xa21* and combinations such as xa5 + *Xa21* and *Xa21* + *xa*33 presented higher level of resistance than that without the *Xa21* within the combination (*xa*5 + *xa*33) against the virulence *Xoo* strain SK2-3. It suggested different mechanisms in expressing defense pathways of dominant and recessive genes and therefore the interaction of recessive and dominant genes in a pyramided manner may show more durable and higher level of resistance compared with single gene resistance. *Xa21* and *xa*5 are speculated to exert epistatic effects that lead to better response of xa33.

Eighteen plant progenies had *Xa21* + *xa*13 gene combination and among these 5 plant progenies *viz.*, ICAB46-7, ICAB46-8, ICAB46-10, ICAB159-3 and ICAB159-18, showed very less Euclidean distance (less than 6.00) and on par

with Aiswarya parent with four quantitative traits. Majority lines had red kernel, characteristic kernel colour of recurrent parent Aiswarya. In the background of Prathyasa, twelve plants had *Xa21+xa13* gene combinations. Among these ICDE39-10, ICDE13-3 and ICDE8-4 showed lesser overall Euclidian distance computed based on four quantitative traits and were closer to recurrent parent Prathyasa. These pyramided lines with *Xa21+xa*13 showed light red to red kernel colour. Similar findings were reported by Singh *et al.* (2001); Sanchez *et al.* (2000) ; Sridhar *et al.* (1999) ; Huang *et al.* (1997) and their results suggest that two gene combinations with *Xa21* + *xa*13 were most effective with shorter lesions lengths followed by *Xa21* + *xa*5 while lines with *xa*13 + *xa*5 were relatively less effective.

Seven plants showed three genes combination (*Xa21*+ *xa*13 + *Xa*33) and in them ICAB 46-9 and ICAB159-23 were on par with Aiswarya in terms of number of productive tillers, grain L/B ratio and maturity duration. Lines ICAB 168-9 and ICAB 46-9 showed very less dissimilarity coefficient, when overall Euclidean distance was under considerations, and indicating that these two lines can recover Aiswarya parent genome. Among the above, six pyramided lines had light red to red kernel. But line ICAB 46-9 showed white kernel. The six lines with red kernel can be used for further breeding programme to develop the essentially derived varieties in the background of Aiswarya. Shanti *et al.* (2010) also studied the agronomic and quality traits of the BC_2F_4 improved lines of Pusa6B and found that most of these lines were on par with Pusa6B with respect to yield and yield components. Significant variation was observed for various agro morphological traits among the pyramided lines. Similarly, Basavaraj *et al.* (2010) also observed significant variation for the agronomic traits among the ten BC_2F_3 improved lines of Pusa 6B and found that most of the families were on par with Pusa 6B with respect to yield and yield components.

The lines were pyramided with three resistance gene(*xa*13, *Xa21* and *Xa*33) based on the disease reaction of donors against local isolate of BB and it could be express higher levels of resistance in comparison to lines with two

resistance genes combination. Sanchez *et al.* (2000) reported that lines with *Xa21* in combination with *xa*5, *xa*13, have shown promise advocating the utility of *Xa21* in higher levels of resistance in rice suggesting that synergistic action and/or quantitative complementation between the resistant genes might have resulted in enhanced levels of resistance. In another study Win *et al.* (2013) reported that the MK-75 ILs with more BB genes introgressed showed higher level of BB resistance to a broader spectrum of *Xoo* strains (both Thai and Myanmar *Xoo* strains) than those with lesser number of genes introgressed. MK-75 ILs containing three *Xa* genes (*xa*5 + *Xa21* + *xa*33) exhibited higher level of BB resistance (shorter LL) than the MK-75 ILs containing two *Xa* genes (*xa*5 + *Xa21*, *xa*5 + *xa*33 and *Xa21* + *xa*33).

Rice lines with higher number of *Xa* genes tend to display higher levels and/or wider spectra of resistance to BB than rice lines with single *Xa* gene. Evidences suggested that the gene interaction or quantitative complementation between the resistance genes might play the key role in increasing the level of resistance against broader spectrum of *Xoo* strains (Yoshimura *et al.*, 1995; Huang *et al.*, 1997; Gopalakrishnan *et al.*, 2008).

Summary

6. SUMMARY

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most serious diseases in the rice growing tracts of the world. The hot and humid climate prevailing throughout the cropping period is quite conducive for the spread of the disease and yield losses up to 70-80% or more have been recorded due to heavy incidence of this disease. Host plant resistance offers the most effective, economical and environmentally safe option for management of disease as chemical control is not effective (Singh *et al.*, 1980). The pyramided lines with BB resistance genes showed wider spectrum and a higher level of resistance than the lines with single resistance gene (Huang *et al.*, 1997; Zhang *et al.*, 1998). The present investigation was, therefore, carried out to introgress BB resistance genes, *xa*13, *Xa*21, *Xa*33 and *Xa*38 obtained from BB resistant donor varieties Improved Samba Mahsuri (with *xa*13 and *Xa*21) Improved Samba Mahsuri (with *Xa*33) and PR-114(*Xa*38), into the, popular ,higher yielding but BB susceptible rice varieties of Kerala *viz.*, Aiswarya and Prathyasa, through marker assisted selection.

Validation of BB resistance genes was undertaken by PCR based detection method. Total genomic DNA was extracted from the young leaves of 3-4 week old seedlings of the parental genotypes using Qiagen Dneasy mini kit. DNA concentration was estimated by using UV spectrophotometer and its quality was checked by taking absorbance at 260 and 280nm, which were ranged from 1.8 to 2.00 for all the genotypes.

PCR amplification was carried out using the specific molecular markers pTA248, xa13 pro, Oso4g53050-1 linked to BB resistance genes *Xa*21, *xa*13 and *Xa*38, respectively, and flanking markers RMWR7.1 and RMWR7.6 linked to the BB resistance gene *Xa*33. A clear polymorphism was observed between donors and recipients for all molecular markers except RMWR7.6. So pTA248, xa13 pro, RMWR7.1 and Oso4g53050-1 linked markers were selected for foreground markers selection in F_1, BC_1F_1 and intermated plants.

Six crosses in all possible combination between three donors and two recipients were done and the F_1 seeds were collected. Foreground selection done in 163 F_1 plants obtained from six cross combinations using respective markers linked to resistance genes. The F_1 plants with heterozygous loci for genes studied were backcrossed with respective recurrent parents. The number of BC_1F_1 seeds ranged from 120 in the cross Prathyasa x ISM (*Xa*33) to 545 in the cross Prathyasa x ISM (*xa*13 and *Xa*21). These 776 BC_1F_1 plants were screened with four trait specific foreground markers and identified 279 plants with genes of interest. From among these, forty two plants in the two crosses Aiswarya x ISM (*xa*13 and *Xa*21) and Prathyasa x ISM (*xa*13 and *Xa*21) had both genes (*xa*13 and *Xa*21) in heterozygous condition. $\chi 2$ test was done with the genotypic data of BC_1F_1 plants with single gene *viz.*, *Xa*33 and *Xa*38. Segregation ratios in BC_1F_1 population from the crosses *viz.*, Prathyasa x ISM (*Xa*33), Aiswarya x PR-114(*Xa*38) and Prathyasa x PR-114(*Xa*38) did not show significant deviation from the expected 1:1 ratio. But BC_1F_1 plants with *Xa33* gene of the cross between Aiswarya and ISM showed significant deviation from 1:1 segregation ratio.

For background selection, a set of 270 SSR markers were used to find out the polymorphism between the four parental genotype panels. Totally 101 primers amplified polymorphic alleles between the donors and recipients and these were used to find out the recovery of recurrent parent genome in the BC_1F_1. The background selection was carried out on altogether 279 plants derived from four BC_1F_1 populations using altogether 143 polymorphic loci in total, ranging from 30 loci in back cross population derived from (Aiswarya x PR114) x Aiswarya to 41 loci in the population derived from (Prathyasa x Improved Samba Mahsuri) x Prathyasa.

In Aiswarya x ISM (*xa*13 and *Xa21*) fourteen plants showed the recovery of RPG in the range of 70-80%.The highest RPG recovery (80-90%) was found in five plants. Similarly in the backcross of Aiswarya x ISM (*Xa33*) the percent recovery of Aiswarya genome by 14 BC_1F_1 plants had ranged from 73.26 to 79.92. In Aiswarya x PR-114 (*Xa*38) derived backcross generation, recovery of

RPG was analysed by using 30 parental polymorphic markers in 80 plants. Eight plants had recovery of Aiswarya genome in the BC_1F_1 plants ranged from 75% to 80 %.

In Prathyasa x ISM ((*xa*13 and *Xa21*) and Prathyasa x ISM (*Xa*33) back crossed population Recurrent Parent Genome (RPG) recovery were assessed by using 41 parental polymorphic markers. In Prathyasa x ISM (*xa*13 and *Xa21*) backcross population thirteen plants had RPG recovery ranging from 76 to 80% and BC_1F_1 plant D-126 recovered highest RPG 87.80%. In Prathyasa x ISM (*Xa*33) backcross population, 19 plants had recovery of RPG ranging from 78-83 percent. Prathyasa x PR-114(*Xa*38) population when analysed using 33 parental polymorphic markers, showed that four plants recovered RPG by 66-73% and two plants had high recovery of 73-79%.

Morphological observations for quantitative traits varied substantially in 279 BC_1F_1 plants identified with genes of resistance. Some of the BC_1F_1 plants were similar or on par to the respective recurrent parents for morphological traits studied. Most of the BC_1F_1 plants had light red to red kernel colour.

When donors were screened with local isolate of Moncompu, the percentage disease severity was 38% in PR-114(Xa38) and was moderately susceptible. ISM (*xa13* and *Xa21*), ISM (*Xa33)* showed resistant disease reaction. Recipient parents Aiswarya and Prathyasa also showed higher percent of disease severity and incidence against local isolate of *Xoo*. PR-114 (*Xa*38) was excluded from the pyramiding programme due to its susceptibility.

Intermating was performed between BC_1F_1 plants having respective resistance genes to pyramid 2-3 genes combination based on disease reaction. In Prathyasa and Aiswarya derived backcross population, BC_1F_1 plants having *xa13* and *Xa21* genes were used as female parent and BC_1F_1 plants with *Xa33* used male parent. A total of 120-200 seeds were harvested from female parent (ISM with *xa*13 and *Xa21*) in the two crosses.

Ninety nine BC_1F_1 intermated plants derived from seven plant progenies were analysed for presence of two to three resistance genes combinations *Xa*21, *xa13* and *Xa33*. Seventeen plants showed combinations of resistance genes *xa*13+Xa33. In these, 4 lines (ICAB22-1 ICAB159-5, ICAB159-10 and ICAB159-30) showed very less dissimilarity coefficient and overall Euclidean distance based on four quantitative traits in comparison with recurrent parent. Nine plants *viz*., ICAB159-9, ICAB159-12, ICAB159-15, ICAB159-17, ICAB168-8, ICAB133-27, ICAB133-29, ICAB133-36 and ICAB133-48 from three progenies exhibited presence of *Xa*21 + *Xa*33 gene combinations. Among these nine pyramided lines ICAB168-8 showed less dissimilarity coefficient (4.58) with Aiswarya parent. All nine pyramided lines had red kernel colour. Eighteen Plant progenies showed *Xa*21 + *xa*13 genes combination. Among these 5 plant progenies *viz.,* ICAB46-7, ICAB46-8, ICAB46-10, ICAB159-3 and ICAB159-18, showed very less Euclidean distance(less than 6.00) from Aiswarya parent with respect to four quantitative traits.

Seven plants (ICAB 101-3, ICAB46-9, ICAB159-16, ICAB159-23, ICAB168-9, ICAB133-35 and ICAB133-50) from five progenies showed three genes combination *(Xa*21+ *xa13 +Xa33*). Among these two pyramided lines (ICAB46-9 and ICAB159-23) were on par with Aiswarya in terms of number of productive tillers, grain L/B ratio and maturity duration. Lines ICAB 168-9 and ICAB 46-9 showed very less overall Euclidean dissimilarity coefficient.

In the background of Prathyasa twelve plants had *Xa21+xa13* gene combinations. Among these ICDE39-10, ICDE13-3 and ICDE8-4 showed lesser overall Euclidian distance computed based on four quantitative traits and were closer to recurrent parent Prathyasa. These pyramided lines with *Xa21+xa*13 showed light red to red kernel colour. Nine plants showed *xa*13 + *Xa*33 resistance gene combinations. Among these pyramided lines ICDE8-7 and ICDE33-10 had lesser Euclidean coefficient of dissimilarity assessed on four quantitative traits in comparison with the recurrent parent Prathyasa.

These advanced breeding lines derived through Marker Assisted Selection (MAS) and phenotypic selection can be of practical value in providing durable resistance in the breeding programme for evolving BB resistant varieties suited for rice field of the Kerala. The lines obtained in the present study will be helpful for the development of potential BB resistant Essentially Derived Varieties (EDVs). The intermated BC_1F_1 pyramided lines possessing two to three resistance genes could be forwarded up to BC_2F_2 by backcross followed by selfing to obtain higher percentage recovery of recurrent parent genome. Foreground and background selection as well as assessment of the morphological traits of the pyramided BC_2F_2 plants having two to three resistance genes can be evaluated and used in the development of the EDVs.

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Appendices

Appendix 1. Details of microsatellites used in the study for background selection. * Successful loci; # loci polymorphic ; tm melting temperature.

Sl.No	Loci	Chr	Forward primer	Reverse primer	Tm(^{0}C)
1	RM1*#	1	gcgaaaacacaatgcaaaaa	gcgttggttggacctgac	55
2	RM23*#	1	cattggagtggaggctgg	gtcaggcttctgccattctc	55
3	RM84*	1	taagggtccatccacaagatg	ttgcaaatgcagctagagtac	55
4	RM104*	1	ggaagaggagagaaagatgtgtgtcg	tcaacagacacaccgccaccgc	61
5	RM128*	1	agcttgggtgatttcttggaagcg	acgacgaggagtcgccgtgcag	55
6	RM129	1	tctctccggagccaaggcgagg	cgagccacgacgcgatgtaccc	55
7	RM212*#	1	ccactttcagctactaccag	cacccatttgtctctcattatg	55
8	RM226	1	agctaaggtctgggagaaacc	aagtaggatggggcacaagctc	55
9	RM237	1	caaatcccgactgctgtcc	tgggaagagagcactacagc	55
10	RM243*#	1	gatctgcagactgcagttgc	agctgcaacgatgttgtcc	55
11	RM246*	1	gagctccatcagccattcag	ctgagtgctgctgcgact	55
12	RM272	1	aattggtagagaggggagag	acatgccattagagtcaggc	55
13	RM283*	1	gtctacatgtacccttgttggg	cggcatgagagtctgtgatg	55
14	RM302*#	1	tcatgtcatctaccatcacac	atggagaagatggaatacttgc	55
15	RM312*#	1	gtatgcatatttgataagag	aagtcaccgagtttaccttc	55
16	RM315*	1	gaggtacttcctccgtttcac	agtcagctcactgtgcagtg	55
17	RM403*	1	gctgtgcatgcaagttcatg	atggtcctcatgttcatggc	55
18	RM428*	1	aacagatggcatcgtcttcc	cgctgcatccactactgttg	55
19	RM431*#	1	tcctgcgaactgaagagttg	agagcaaaaccctggttcac	55
20	RM449*#	1	ttgggaggtgttgataaggc	accaccagcgtctctctctc	55
21	RM472	1	ccatggcctgagagagagag	agctaaatggccatacggtg	55
22	RM486*#	1	ccccctctctctctctctc	tagccacatcaacagcttgc	55
23	RM488*	1	cagctagggttttgaggctg	tagcaacaaccagcgtatgc	55
24	RM490	1	atctgcacactgcaaacacc	agcaagcagtgctttcagag	55
25	RM495*	1	aatccaaggtgcagagatgg	caacgatgacgaacacaacc	55
26	RM583*#	1	agatccatccctgtggagag	gcgaactcgcgttgtaatc	55
27	RM109*#	2	gccgccggagagggagagagag	ccccgacgggatctccatcgtc	67
28	RM138	2	agcgcaacaaccaatccatccg	aagaagctgcctttgacgctatgg	55
29	RM154	2	accctctccgcctcgcctcctc	ctcctcctcctgcgaccgctcc	61
30	RM208*	2	tctgcaagccttgtctgatg	taagtcgatcattgtgtggacc	55
31	RM221	2	acatgtcagcatgccacatc	tgcaagaatctgacccgg	55
32	RM240*#	2	ccttaatgggtagtgtgcac	tgtaaccattccttccatcc	55
33	RM250*#	2	ggttcaaaccaagctgatca	gatgaaggccttccacgcag	55
34	RM266*#	2	tagtttaaccaagactctc	ggttgaacccaaatctgca	55
35	RM279	2	gcgggagagggatctcct	ggctaggagttaacctcgcg	55
36	RM300*#	2	gcttaaggacttctgcgaacc	caacagcgatccacatcatc	55
37	RM324*	2	ctgattccacacacttgtgc	gattccacgtcaggatcttc	55
38	RM423*	2	agcacccatgccttatgttg	ccttttcagtagccctccc	55
39	RM424*	2	tttgtggctcaccagttgag	tggcgcattcatgtcatc	55

Sl.No	Loci	Chr	Forward primer	Reverse primer	Tm(^{0}C)
40	RM450*#	2	aaaccacagtagtacgccgg	tccatccacatctccctctc	55
41	RM452*#	2	ctgatcgagagcgttaaggg	gggatcaaaccacgtttctg	55
42	RM482	2	tctgaaagcctgactcatcg	gtcaattgcagtgccctttc	55
43	RM498	2	aatctgggcctgctctttc	tcctagggtgaagaaagggg	55
44	RM525	2	ggcccgtccaagaaatattg	cggtgagacagaatccttacg	55
45	RM550	2	ctgagctctggtccgaagtc	ggtggtggaagaacaggaag	55
46	RM555	2	ttggatcagccaaaggagac	cagcattgtggcatggatac	55
47	RM7	3	ttcgccatgaagtctctcg	cctcccatcatttcgttgtt	55
48	RM16	3	cgctagggcagcatctaaa	aacacagcaggtacgcgc	55
49	RM22	3	ggtttgggagcccataatct	ctgggcttctttcactcgtc	55
50	RM85	3	ccaaagatgaaacctggattg	gcacaaggtgagcagtcc	55
51	RM132*	3	atcttgttgtttcggcggcggc	catggcgagaatgcccacgtcc	67
52	RM143*	3	gtcccgaaccctagcccgaggg	agaggccctccacatggcgacc	67
53	RM156*	3	gccgcaccctcactccctcctc	tcttgccggagcgcttgaggtg	67
54	RM231*	3	ccagattatttcctgaggtc	cacttgcatagttctgcattg	55
55	RM232	3	ccggtatccttcgatattgc	ccgacttttcctcctgacg	55
56	RM282*	3	ctgtgtcgaaaggctgcac	cagtcctgtgttgcagcaag	55
57	RM293	3	tcgttgggaggtatggtacc	ctttatctgatccttgggaagg	55
58	RM319*	3	atcaaggtacctagaccaccac	tcctggtgcagctatgtctg	55
59	RM338*	3	cacaggagcaggagaagagc	ggcaaaccgatcactcagtc	55
60	RM416	3	gggagttagggttttggagc	tccagtttcacactgcttcg	55
61	RM468	3	cccttccttgttgtggctac	tgatttctgagagccaaccc	55
62	RM487	3	tttctcgaacgcaggagaac	gctaggaacatcaacccgag	55
63	RM489*	3	acttgagacgatcggacacc	tcacccatggatgttgtcag	55
64	RM514*#	3	agattgatctcccattcccc	cacgagcatattactagtgg	55
65	RM517*#	3	ggcttactggcttcgatttg	cgtctcctttggttagtgcc	55
66	RM520*#	3	aggagcaagaaaagttcccc	gccaatgtgtgacgcaatag	55
67	RM545*	3	caatggcagagacccaaaag	ctggcatgtaacgacagtgg	55
68	RM623*#	3	ggtgaagttccaatccagatg	tgatgctgtacagtgtcttcg	
69	OSR13	3	catttgtgcgtcacggagta	agccacagcgcccatctctc	55
70	RM124*#	4	atcgtctgcgttgcggctgctg	catggatcaccgagctcccccc	67
71	RM131*	4	tcctccctcccttcgcccactg	cgatgttcgccatggctgctcc	61
72	RM142	4	ctcgctatcgccatcgccatcg	tcgagccatcgctggatggagg	67
73	RM177	4	ccctcttagacagaggccagaggg	gtagccgaagatgaggccgccg	61
74	RM185	4	agttgttgggagggagaaaggcc	aggaggcgacggcgatgtcctc	61
75	RM241*#	4	gagccaaataagatcgctga	tgcaagcagcagatttagtg	55
76	RM252*#	4	ttcgctgacgtgataggttg	atgacttgatcccgagaacg	55
77	RM255*#	4	tgttgcgtgtggagatgtg	cgaaaccgctcagttcaac	55
78	RM273	4	gaagccgtcgtgaagttacc	gtttcctacctgatcgcgac	55
79	RM280	4	acacgatccactttgcgc	tgtgtcttgagcagccagg	55

Sl.No	Loci	Chr	Forward primer	Reverse primer	Tm(^{0}C)
80	RM303*	4	gcatggccaaatattaaagg	ggttggaaatagaagttcggt	55
81	RM307	4	gtactaccgacctaccgttcac	ctgctatgcatgaactgctc	55
82	RM317	4	catacttaccagttcaccgcc	ctggagagtgtcagctagttga	55
83	RM348*	4	ccgctactaatagcagagag	ggagctttgttcttgcgaac	55
84	RM349*	4	ttgccattcgcgtggaggcg	gtccatcatccctatggtcg	55
85	RM417*	4	cggatccaagaaacagcag	ttcggtatcctccacacctc	55
86	RM451*#	4	gatcccctccgtcaaacac	cccttctcctttcctcaacc	55
87	RM470*#	4	tcctcatcggcttcttcttc	agaacccgttctacgtcacg	55
88	RM471*#	4	acgcacaagcagatgatgag	gggagaagacgaatgtttgc	55
89	RM518*#	4	ctcttcactcactcaccatgg	atccatctggagcaagcaac	55
90	RM537*#	4	ccgtccctctctctcctttc	acagggaaaccatcctcctc	55
91	RM551*	4	agcccagactagcatgattg	gaaggcgagaaggatcacag	55
92	RM559*	4	acgtacacttggccctatgc	atgggtgtcagtttgcttcc	55
93	RM567*	4	atcagggaaatcctgaaggg	ggaaggagcaatcaccactg	55
94	RM13*#	5	tccaacatggcaagagagag	ggtggcattcgattccag	55
95	RM26*#	5	gagtcgacgagcggcaga	ctgcgagcgacggtaaca	55
96	RM87	5	cctctccgatacaccgtatg	gcgaaggtacgaaaggaaag	55
97	RM122*	5	gagtcgatgtaatgtcatcagtgc	gaaggaggtatcgctttgttggac	na
98	RM146	5	ctattattccctaacccccataccctcc	agagccactgcctgcaaggccc	55
99	RM159	5	ggggcactggcaagggtgaagg	gcttgtgcttctctctctctctctctc	55
100	RM161	5	tgcagatgagaagcggcgcctc	tgtgtcatcagacggcgctccg	61
101	RM164*	5	tcttgcccgtcactgcagatatcc	gcagccctaatgctacaattcttc	na
102	RM169	5	tggctggctccgtgggtagctg	tcccgttgccgttcatccctcc	67
103	RM173	5	cctacctcgcgatccccccctc	ccatgaggaggaggcggcgatc	67
104	RM178*#	5	tcgcgtgaaagataagcggcgc	gatcaccgttccctccgcctgc	67
105	RM188	5	tccgcctctcctctcgcttccc	gcaacgcacaaccgaaccgagc	61
106	RM267*#	5	tgcagacatagagaaggaagtg	agcaacagcacaacttgatg	55
107	RM289*	5	ttccatggcacacaagcc	ctgtgcacgaacttccaaag	55
108	RM334	5	gttcagtgttcagtgccacc	gactttgatctttggtggacg	55
109	RM413*#	5	ggcgattcttggatgaagag	tccccaccaatcttgtcttc	55
110	RM421*	5	agctcaggtgaaacatccac	atccagaatccattgacccc	55
111	RM430*	5	aaacaacgacgtccctgatc	gtgcctccgtggttatgaac	55
112	RM437*	5	acaccaaccagatcagggag	tgctcgtcaatggtgagttc	55
113	RM440*#	5	catgcaacaacgtcaccttc	atggttggtaggcaccaaag	55
114	RM459	5	ctgcaatgctgcatgacc	cactttctctgcagcaccag	55
115	RM507*#	5	cttaagctccagccgaaatg	ctcaccctcatcatcgcc	55
116	RM509*#	5	tagtgagggagtggaaacgg	atcgtccccacaatctcatc	55
117	RM538*#	5	ggtcgttgaagcttaccagc	acaagctctcaaaactcgcc	55
118	RM548*#	5	tcggtgagaaactgagagtacg	aaggaggccatctcaatgtg	55
119	RM111*#	6	cacaacctttgagcaccgggtc	acgcctgcagcttgatcaccgg	55

Sl.No	Loci	Chr	Forward primer	Reverse primer	Tm(^{0}C)
120	RM115*#	6	ttgccgcagtggccgttaccac	aggaggcggcggaaatggaagg	61
121	RM121	6	accgtcgccttccactttcccc	ttcggggttgccggtgatgttg	55
122	RM133*	6	ttggattgttttgctggctcgc	ggaacacggggtcggaagcgac	61
123	RM136*	6	gagagctcagctgctgcctctagc	gaggagcgccacggtgtacgcc	55
124	RM141	6	caccaccaccaccacgcctctc	tcttggagaggaggaggcgcgg	55
125	RM162*#	6	gccagcaaaaccagggatccgg	caaggtcttgtgcggcttgcgg	61
126	RM170	6	tcgcgcttcttcctcgtcgacg	cccgcttgcagaggaagcagcc	55
127	RM176	6	cggctcccgctacgacgtctcc	agcgatgcgctggaagaggtgc	67
128	RM190*#	6	ctttgtctatctcaagacac	ttgcagatgttcttcctgatg	55
129	RM204*#	6	gtgactgacttggtcataggg	gctagccatgctctcgtacc	55
130	RM217	6	atcgcagcaatgcctcgt	gggtgtgaacaaagacac	55
131	RM225*#	6	tgcccatatggtctggatg	gaaagtggatcaggaaggc	55
132	RM253*#	6	tccttcaagagtgcaaaacc	gcattgtcatgtcgaagcc	55
133	RM345*	6	attggtagctcaatgcaagc	gtgcaacaaccccacatg	55
134	RM402	6	gagccatggaaagatgcatg	tcagctggcctatgacaatg	55
135	RM412	6	cacttgagaaagttagtgcagc	cccaaacacacccaaatac	55
136	RM435*	6	attacgtgcatgtctggctg	cgtacctgaccatgcatctg	55
137	RM439	6	tcataacagtccactccccc	tggtactccatcatcccatg	55
138	RM454*#	6	ctcaagcttagctgctgctg	gtgatcagtgcaccatagcg	55
139	RM461	6	gagaccggagagacaactgc	tgatgcggtttgactgctac	55
140	RM469*#	6	agctgaacaagccctgaaag	gacttgggcagtgtgacatg	55
141	RM494	6	gggaggggatcgagatagac	tttaaccttccttccgctcc	55
142	RM508*	6	ggatagatcatgtgtgggggg	acccgtgaaccacaaagaac	55
143	RM510*#	6	aaccggattagtttctcgcc	tgaggacgacgagcagattc	55
144	RM527	6	ggctcgatctagaaaatccg	ttgcacaggttgcgatagag	55
145	RM528*	6	ggcatccaattttacccctc	aaatggagcatggaggtcac	55
146	RM540*	6	gccttctggctcatttatgc	ctaggcctgccagattgaac	55
147	RM541	6	tataaccgacctcagtgccc	ccttactcccatgccatgag	55
148	RM549	6	acgaactgatcatatccgcc	ctgtggttgatccctgaacc	55
149	RM557	6	gtggcgagatctatgtggtg	gctttgtgtgtgtgtgtgtg	55
150	RM584*#	6	agaaagtggatcaggaaggc	gatcctgcaggtaaccacac	55
151	RM588*	6	gttgctctgcctcactcttg	aacgagccaacgaagcag	55
152	RM589*	6	atcatggtcggtggcttaac	caggttccaaccagacactg	55
153	RM597	6	cctgatgcacaactgcgtac	tcagagagagagagagagagag	55
154	RM2	7	acgtgtcaccgcttcctc	atgtccgggatctcatcg	na
155	RM10*#	7	ttgtcaagaggaggcatcg	cagaatgggaaatgggtcc	55
156	RM11*#	7	tctcctcttcccccgatc	atagcgggcgaggcttag	55
157	RM47	7	actccactccactccccac	gtcagcaggtcggacgtc	55
158	RM51*#	7	tctcgattcaatgtcctcgg	ctacgtcatcatcgtcttccc	55
159	RM82	7	tgcttcttgtcaattcgcc	cgactcgtggaggtacgg	55

Sl.No	Loci	Chr	Forward primer	Reverse primer	Tm(^{0}C)
160	RM118*	7	ccaatcggagccaccggagagc	cacatcctccagcgacgccgag	67
161	RM125*	7	atcagcagccatggcagcgacc	aggggatcatgtgccgaaggcc	55
162	RM172*#	7	tgcagctgcgccacagccatag	caaccacgacaccgccgtgttg	55
163	RM180*	7	ctacatcggcttaggtgtagcaacacg	acttgctctacttgtggtgagggactg	55
164	RM214*	7	ctgatgatagaaacctcttctc	aagaacagctgacttcacaa	55
165	RM234*	7	acagtatccaaggccctgg	cacgtgagacaaagacggag	55
166	RM248	7	tccttgtgaaatctggtccc	gtagcctagcatggtgcatg	55
167	RM295	7	cgagacgagcatcggataag	gatctggtggaggggagg	55
168	RM336	7	cttacagagaaacggcatcg	gctggtttgtttcaggttcg	55
169	RM346	7	cgagagagcccataactacg	acaagacgacgaggagggac	55
170	RM420*#	7	ggacagaatgtgaagacagtcg	actaatccaccaacgcatcc	55
171	RM427*#	7	tcactagctctgccctgacc	tgatgagagttggttgcgag	55
172	RM429*#	7	tccctccagcaatgtctttc	ccttcatcttgctttccacc	55
173	RM432	7	ttctgtctcacgctggattg	agctgcgtacgtgatgaatg	55
174	RM436*	7	attcctgcagtaaagcacgg	cttcgtgtacctccccaaac	55
175	RM445*#	7	cgtaacatgcatatcacgcc	atatgccgatatgcgtagcc	55
176	RM455*#	7	aacaacccaccacctgtctc	agaaggaaaagggctcgatc	55
177	RM481	7	tagctagccgattgaatggc	ctccacctcctatgttgttg	55
178	RM505	7	agagttatgagccgggtgtg	gatttggcgatcttagcagc	55
179	RM533*	7	gcaactgctctacgcctctc	cctgaggcttcacctactcg	55
180	RM542*	7	tgaatcaagcccctcactac	ctgcaacgagtaaggcagag	55
181	RM560	7	gcaggaggaacagaatcagc	agcccgtgatacggtgatag	55
182	RM72*#	8	ccggcgataaaacaatgag	gcatcggtcctaactaaggg	55
183	RM149*#	8	gctgaccaacgaacctaggccg	gttggaagcctttcctcgtaacacg	55
184	RM223*#	8	gagtgagcttgggctgaaac	gaaggcaagtcttggcactg	55
185	RM230	8	gccagaccgtggatgttc	caccgcagtcacttttcaag	55
186	RM264*#	8	gttgcgtcctactgctacttc	gatccgtgtcgatgattagc	55
187	RM284*	8	atctctgatactccatccatcc	cctgtacgttgatccgaagc	55
188	RM310*#	8	ccaaaacatttaaaatatcatg	gcttgttggtcattaccattc	55
189	RM337*	8	gtaggaaaggaagggcagag	cgatagatagctagatgtggcc	55
190	RM350	8	tgatcgtcgcgattcccggc	ccccaccctgcgcctctccc	55
191	RM404*#	8	ccaatcattaacccctgagc	gccttcatgcttcagaagac	55
192	RM407*	8	gattgaggagacgagccatc	ctttttcagatctgcgctcc	55
193	RM433*#	8	tgcgctgaactaaacacagc	agacaaacctggccattcac	55
194	RM447*#	8	cccttgtgctgtctcctctc	acgggcttcttctccttctc	55
195	RM477*	8	tctcgcggtatagtttgtgc	accactaccagcagcctctg	55
196	RM502*#	8	gcgatcgatggctacgac	acaacccaacaagaaggacg	55
197	RM515	8	taggacgaccaaagggtgag	tggcctgctctctctctctc	55
198	RM544	8	tgtgagcctgagcaataacg	gaagcgtgtgatatcgcatg	55
199	RM41	9	aagtctagtttgcctccc	aatttctacgtcgtcgggc	55

Sl.No	Loci	Chr	Forward primer	Reverse primer	Tm(^{0}C)
200	RM107*#	9	agatcgaagcatcgcgcccgag	actgcgtcctctgggttcccgg	67
201	RM108	9	tctcttgcgcgcacactggcac	cgtgcaccaccaccaccaccac	67
202	RM160*	9	agctagcagctatagcttagctggagatcg	tctcatcgccatgcgaggcctc	55
203	RM201*	9	ctcgtttattacctacagtacc	ctacctcctttctagaccgata	55
204	RM215*#	9	caaaatggagcagcaagagc	tgagcacctccttctctgtag	55
205	RM242*#	9	ggccaacgtgtgtatgtctc	tatatgccaagacggatggg	55
206	RM245	9	atgccgccagtgaatagc	ctgagaatccaattatctgggg	55
207	RM257*#	9	cagttccgagcaagagtactc	ggatcggacgtggcatatg	55
208	RM278*#	9	gtagtgagcctaacaataatc	tcaactcagcatctctgtcc	55
209	RM285*#	9	ctgtgggcccaatatgtcac	ggcggtgacatggagaaag	55
210	RM288*#	9	ccggtcagttcaagctctg	acgtacggacgtgacgac	55
211	RM296*	9	cacatggcaccaacctcc	gccaagtcattcactactctgg	55
212	RM316*#	9	ctagttgggcatacgatggc	acgcttatatgttacgtcaac	55
213	RM321	9	ccaacactgccactctgttc	gaggatggacaccttgatcg	55
214	RM328*	9	catagtggagtatgcagctgc	ccttctcccagtcgtatctg	55
215	RM409*	9	ccgtctcttgctagggattc	ggggtgttttgctttctctg	55
216	RM410	9	gctcaacgtttcgttcctg	gaagatgcgtaaagtgaacgg	55
217	RM434*	9	gcctcatccctctaaccctc	caagaaagatcagtgcgtgg	55
218	RM464*#	9	aacgggcacattctgtcttc	tggaagacctgatcgtttcc	55
219	RM553*#	9	aactccacatgattccaccc	gagaaggtggttgcagaagc	55
220	RM147	10	tacggcttcggcggctgattcc	cccccgaatcccatcgaaaccc	55
221	RM184	10	atcccattcgccaaaaccggcc	tgacacttggagagcggtgtgg	55
222	RM239*	10	tacaaaatgctgggtacccc	acatatgggacccacctgtc	55
223	RM269*	10	gaaagcgatcgaaccagc	gcaaatgcgcctcgtgtc	55
224	RM271*#	10	tcagatctacaattccatcc	tcggtgagacctagagagcc	55
225	RM294A	10	ttggcctagtgcctccaatc	gagggtacaacttaggacgca	55
226	RM304	10	tcaaaccggcacatataagac	gatagggagctgaaggagatg	55
227	RM467*	10	ggtctctctctctctctctctc	ctcctgacaattcaactgcg	55
228	RM474*	10	aagatgtacgggtggcattc	tatgagctggtgagcaatgg	55
229	RM484*#	10	tctccctcctcaccattgtc	tgctgccctctctctctctc	55
230	RM496*#	10	gacatgcgaacaacgacatc	gctgcggcgctgttatac	55
231	RM590	10	catctccgctctccatgc	ggagttggggtcttgttcg	55
232	RM591*	10	ctagctagctggcaccagtg	tggagtccgtgttgtagtcg	55
233	RM4A*#	11	ttgacgaggtcagcactgac	agggtgtatccgactcatcg	55
234	RM4B*#	11	ttgacgaggtcagcactgac	agggtgtatccgactcatcg	55
235	RM20B	11	atcttgtccctgcaggtcat	gaaacagaggcacatttcattg	55
236	RM116*#	11	tcacgcacagcgtgccgttctc	caagatcaagccatgaaaggaggg	55
237	RM120*#	11	cacacaagccctgtctcacgacc	cgctgcgtcatgagtatgta	na
238	RM139	11	gagagggaggaagggaggcggc	ctgccatggcagagaaggggcc	55
239	RM181	11	acgggagcttctccgacagcgc	tatgcttttgccgtgtgccgcg	67

Sl.No	Loci	Chr	Forward primer	Reverse primer	Tm(^{0}C)
240	RM187	11	ccaagggaaagatgcgacaattg	gtggacgctttatattatggg	55
241	RM209*	11	atatgagttgctgtcgtgcg	caacttgcatcctcccctcc	55
242	RM224*#	11	atcgatcgatcttcacgagg	tgctataaaaggcattcggg	55
243	RM254*#	11	agccccgaataaatccacct	ctggaggagcatttggtagc	55
244	RM260	11	actccactatgacccagag	gaacaatcccttctacgatcg	55
245	RM286*	11	ggcttcatctttggcgac	ccggattcacgagataaactc	55
246	RM287*#	11	ttccctgttaagagagaaatc	gtgtatttggtgaaagcaac	55
247	RM330A*#	11	caatgaagtggatctcggag	catcaatcagcgaaggtcc	55
248	RM332*	11	gcgaaggcgaaggtgaag	catgagtgatctcactcaccc	55
249	RM441*	11	acaccagagagagagagagagag	tctgcaacggctgatagatg	55
250	RM457*	11	ctccagcatggcctttctac	acctgatggtcaaagatggg	55
251	RM479*#	11	ccccttgctagcttttggtc	ccatacctcttctcctcccc	55
252	RM536*#	11	tctctcctcttgtttggctc	acacaccaacacgaccacac	55
253	RM552*#	11	cgcagttgtggatttcagtg	tgctcaacgtttgactgtcc	55
254	RM12*#	12	tgccctgttatttttcttctctc	ggtgatcctttcccatttca	55
255	RM17	12	tgccctgttatttttcttctctc	ggtgatcctttcccatttca	55
256	RM20A*#	12	atcttgtccctgcaggtcat	gaaacagaggcacatttcattg	55
257	RM83*	12	actcgatgacaagttgagg	cacctagacacgatcgag	55
258	RM101	12	gtgaatggtcaagtgacttaggtggc	acacaacatgttccctcccatgc	55
259	RM117	12	cgatccattcctgctgctcgcg	cgcccccatgcatgagaagacg	55
260	RM155	12	gagatggccccctccgtgatgg	tgccctcaatcggccacacctc	55
261	RM179	12	ccccattagtccactccaccacc	ccaatcagcctcatgcctcccc	61
262	RM277*#	12	cggtcaaatcatcacctgac	caaggcttgcaagggaag	55
263	RM309*#	12	gtagatcacgcacctttctgg	agaaggcctccggtgaag	55
264	RM313*#	12	tgctacaagtgttcttcaggac	gctcacctttgtgttccac	55
265	RM453*#	12	cgcatctctctcccttatcg	ctctcctcctcgttgtcgtc	55
266	RM463*#	12	ttcccctcctttatggtgc	tgttctcctcagtcactgcg	55
267	RM511*#	12	cttcgatccggtgacgac	aacgaaagcgaagctgtctc	55
268	RM512*	12	ctgcctttcttacccccttc	aacccctcgctggattctag	55
269	RM519*	12	agagagcccctaaatttccg	aggtacgctcacctgtggac	55
270	RM558A*	12	gaactcctcgaactcgatgc	aggcattcaacctgttcgac	55

Appendix 2.Composition of Peptone Sucrose Agar (PSA)

Component	Quantity
Sucrose	50gm
Beef extract	5g
Peptone	10g
Nacl	1g
Agar	20g
Distil.water	1000ml
pH	6.0

PYRAMIDING BACTERIAL LEAF BLIGHT RESISTANCE GENES INTO POPULAR RICE VARIETIES OF KERALA THROUGH MARKER ASSISTED SELECTION

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(2013 - 21 - 111)

ABSTRACT

of the thesis submitted in partial fulfilment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING AND GENETICS

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM – 695 522

KERALA, INDIA

2017

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ABSTRACT

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a devastating disease of rice in Asia and Africa . Major rice growing areas of Kerala are highly prone to this disease which results in damage upto 35%. Exploitation of host plant resistance is the only practical strategy for managing the disease in an ecofriendly manner. Till date, more than 40 resistance genes for BB have been identified from diverse sources and pyramiding of these resistance genes will impart durable resistance. So, present study entitled "Pyramiding Bacterial Leaf Blight resistance genes into popular rice varieties of Kerala through marker assisted selection" was undertaken at the College of Agriculture Vellayani Thiruvananthapuram, to pyramid the genes for resistance to bacterial blight (*xa*13, *Xa*21, *Xa*33, *Xa*38) into the popular rice varieties, Prathyasa and Aiswarya through foreground selection for the genes using molecular markers, to obtain lines with the two/three resistance genes combination in the background of above cultivars.

DNA markers closely linked to the BB resistance genes, such as pTA248 (*Xa*21 gene), xa13 pro (*xa*13 gene), RMWR7.1 (*Xa*33 gene) and Oso4g53050-1(*Xa*38 gene) were used for validation of the marker polymorphism in the donors of the genes for resistance to BB *viz*. Improved Samba Mahsuri with *xa13* and *Xa21*, Improved Samba Mahsuri with *Xa*33, and PR-114 with *Xa*38 and susceptible recipient parents Aiswarya and Prathyasa . This validation confirmed the absence of the genes in the recurrent parents chosen for the study. These polymorphic markers were also used for foreground selection in F_1 plants and backcross generations.

Initial hybridization was performed between two recipient parents and three donor parents to transfer the genes for resistance. Foreground selection was carried out in 193 F_1 plants from these six crosses using molecular markers specific to the genes. F_1 plants (163) with heterozygous loci for trait specific

marker were backcrossed with respective recurrent parent and obtained 776 BC_1F_1 plants in six cross combinations. These plants were screened with four trait specific foreground markers and 279 plants were identified with genes of interest. From among these forty two plants in the 2 crosses Aiswarya x ISM (*xa*13 and *Xa*21) and Prathyasa x ISM (*xa*13 and *Xa*21) had both genes (xa13 and Xa21) in heterozygous condition. $\chi 2$ test was done with the genotypic data of BC_1F_1 plants with single gene *viz. Xa*33 and *Xa*38. Segregation ratios in BC_1F_1 population from the crosses *viz*. Prathyasa x ISM (*Xa*33), Aiswarya x PR-114(*Xa*38) and Prathyasa x PR-114(*Xa*38) did not show significant deviation from the expected 1:1 ratio. But BC_1F_1 plants with *Xa33* gene of the cross Aiswarya x ISM showed significant deviation from 1:1 segregation ratio. This suggests the presence of segregation distortion in these segregants.

For the background selection four varieties were genotyped with 270 microsatellite loci and 180 discrete, unambiguous amplicons specific for the four varieties were identified. The background selection was carried out on altogether 279 plants from six BC_1F_1 populations using 143 loci polymorphic in the parents. The BC_1F_1 plants with highest percentage of recurrent parent genome recovery in the six populations were selected based on the background markers. These plants were used for intermating programme to pyramid the genes.

The disease screening of donors, Improved Samba Mahsuri(*xa13* and *Xa21*), Improved Samba Mahsuri (*Xa*33), and PR-114 (*Xa*38) against local isolate of *Xanthomonas oryzae* pv.*oryzae* conducted at RRS, Moncompu showed resistant disease reaction in the donors ISM (*xa*13 and *Xa*21) and ISM (*Xa*33). PR-114(*Xa*38) exhibited moderate susceptible disease reaction and so this donor was excluded from further breeding programme.

BC_1F_1 plants having *xa13* and *Xa21* genes were used as female parent and BC_1F_1 plants with *Xa33* used as male parent in intermating programme for pyramiding. Foreground selection was performed in the intermated plants using trait specific markers. Among Aiswarya derived BC_1F_1 intermated plants, seven

plants showed three resistance gene combination *(Xa21+ xa13 +Xa33)* and forty four plants showed two resistance gene combinations. Among the forty four plants with two resistance gene combination, seventeen had *xa*13+Xa33, 18 had Xa21 + *xa*13 and 9 had Xa21 + *Xa*33. In Prathyasa derived BC_1F_1 intermated plants 12 plants had *Xa21+xa13* gene combinations and nine had xa13 + *Xa*33 resistance gene combination.

Based on the four quantitative traits (plant height, number of productive tillers, grain L/B ratio and days to maturity) Euclidean coefficient of dissimilarity was assessed in comparison with the respective recurrent parents, in plants pyramided with resistance genes. In Aiswarya derived pyramided plants coefficient of dissimilarity with Aiswarya for the four quantitative traits varied from 2.24 to 27.25. Coefficient of dissimilarity with Prathyasa in the Prathyasa derived pyramided lines based on the four quantitative traits ranged from 3.00 to 17.47. The morphological traits of the two / three gene pyramided BC_1F_1 genotypes were found to be either superior or on par with the recurrent parents Aiswarya and Prathyasa. Majority of pyramided lines showed red kernel colour of the recurrent parents.

This research lead to development of plants pyramided with two /three genes for resistance to BB in the background of Aiswarya and plants with two resistance genes in the background of Prathyasa. These pyramided lines can be used for further breeding programme to develop Essentially Derived Varieties (EDVs) to tackle the Bacterial Leaf Blight (BB) disease.