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Marker assisted selection for identification of recombinants for bacterial blight and blast resistance in segregating populations of Cottondora Sannalu

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ABSTRACT

The present investigation has been initiated to combine bacterial blight (BB) and blast resistance with the high yield potential of a short duration rice variety, MTU1010 (Cottondora Sannalu) of India. B95-1 carrying BB resistance genes (xa13 and Xa21) and NLR145 carrying blast resistance genes (Pi54 and Pi1) were selected for making crosses. B95-1 was validated for the presence of target genes, xa13 and Xa21 by using primers viz., xa13 promotor (functional marker-FM) and pTA 248 (STS marker), while NLR145 was validated by using primers viz., Pi54 MAS (functional marker-FM) and RM 224 (gene linked marker-GLM). These primers were also used to study polymorphism between resistant (B95-1 & NLR145) and susceptible (MTU1010) parents for the target genes. Two F2 segregating populations viz., MTU1010 x B95-1 and MTU1010 x NLR145 were evaluated during dry season, 2012. Foreground selection was carried out and the plants carrying the target genes in homozygous condition were identified. Genotyping revealed that BB and Blast resistance genes exhibited Mendelian pattern of segregation in 1:2:1 ratio and exhibited goodness of fit. Phenotypic studies for BB and Blast progenies in two F3 populations resulted in identification of resistant plants.

Key words: *rice, bacterial blight, blast, marker assisted selection*

Rice production is limited by various biotic factors; bacterial blight (BB) and blast being the two major diseases. Host plant resistance has been considered as the most economical and eco-friendly strategy for management of biotic stresses (Hulbert *et al*., 2001). Molecular markers are widely applied in agriculture, and their application in rice improvement has been recently reviewed (Mackill and McNally, 2004, Jordan *et al*., 2004, Xu *et al*., 2004, Toojinda *et al*., 2005, Liu *et al*., 2006 and Mackill, 2007). Kalaichelvan, 2009 used 78 SSRs for varietal identification and also assessed the genetic relationship among the elite rice cultivars using morphological and molecular markers. The marker used in the selection must have tight linkage with the target gene in order to have relatively high selection efficiency (Yunbi, 2010).

MAS has also been employed for moving genes from pyramided lines into new plant type (Sanchez *et al*., 2000), as well as into improved varieties grown in India (Singh *et al.,* 2001). Development of broad spectrum durable resistance through gene pyramiding or gene stacking for biotic stress resistance can be accelerated through the process of marker assisted selection (MAS) (Joshi and Nayak, 2010). Balachiranjeevi *et al*., 2012 introduced two major dominant genes each for BB *(i.e.Xa21* and *Xa33)* and blast *(i.e.Pi2* and *Pi54)* into the maintainer line (DRR17B) through molecular marker-assisted backcross breeding.

BB is caused by the *Xanthomonas oryzae pv. oryzae* and is one of the devastating diseases of rice causing yield losses ranging from 74% to 81% (Srinivasan and Gnanamanickam, 2005) in severe conditions. Till date 34 BB genes (Chen *et al*., 2011) have been identified in rice and a number of them have been deployed into breeding lines but disease breakdown

has resulted due to significant shift in pathogen-race frequency (Mew *et al*., 1992). Such breakdown can be delayed by marker assisted gene pyramiding. The *xa13* gene is fully recessive, conferring resistance only in the homozygous status (Khush and Angeles, 1999). Perumalsamy *et al*., 2010, introgressed three BB resistance genes *xa5*, *xa13* and *Xa21 into* two high yielding BB susceptible *indica* rice cultivars, 'ADT43' and 'ASD16' from isoline IRBB60 and F_2 populations were screened for the presence of all the three resistance genes by using functional markers. These pyramided genotypes with two or three resistance genes exhibited high levels of resistance against two predominant *Xanthomonas oryzae* isolates of South India. The broad spectrum BB resistance gene *Xa21* is expressed in dominant condition and was introgressed from a wild species *O. longistaminata* onto *O. sativa* chromosome 11 through conventional breeding (Khush *et al*., 1989). Basavaraj *et al*., 2010 also used markers *RG 136* and *pTA 248* linked to BB resistance genes *xa13* and *Xa21*, respectively for foreground selection to improve Pusa 6A by using improved Pusa 6B as donor for *xa13* and *Xa21*.

Rice blast, caused by the fungus *Magnaporthe grisea*, is one of the most devastating diseases of rice worldwide, and a major threat to rice cultivation leading to significant yield losses (Reddy, 2009). Till date 85 blast resistance genes have been identified (Sharma *et al*., 2010). *Pi54* is one of the major blast resistance genes and Sharma *et al*., 2005 reported the molecular mapping and cloning of a dominant gene (*Pi54*) present in Tetep. Ramkumar *et al*., 2011 developed PCR-based co-dominant molecular marker '*Pi54 MAS'* which can perfectly co-segregate with blast resistance in a mapping population with no recombinants and suggested for routine deployment in MAS of *Pi54* in breeding programs. *Pi1(t)* is another blast resistance gene and Alvarez *et al*., 2007 identified microsatellite markers *RM 1233*I* and *RM 224* linking rice blast gene *Pi1(t)* in cultivar C101LAC and successfully introgressed in to F_2 and F_3 segregating populations mapped at a distance of 0.0 cM. Vanisree *et al*., 2012 reported the presence of *Pi1* gene in Tetep and NLR145, is similar to that present in C101LAC

The present study was undertaken to identify plants showing high yielding ability, fine grain, short duration coupled with resistance to BB and blast. Two BB resistance genes *viz.*, *xa13* and *Xa21* from B95-1 and two blast resistance genes *viz.*, *Pi54* and *Pi1* from NLR145 were attempted to combine with high yield, short duration, fine grain qualities of MTU1010. Molecular markers *viz.*, *xa13 promotor* (FM for *xa13*), *pTA 248* (STS marker for *Xa21*), *Pi54 MAS* (FM for *Pi54*) and *RM 224* (GLM for *Pi1*) were validated in the resistant parents and the parental polymorphism was studied between susceptible and resistant parents for the target genes. The inheritance pattern of these genes was studied in the F_1 and F_2 population by using the selected markers along with phenotypic evaluation.

MATERIALS AND METHODS

Three genotypes *viz*., MTU1010, B95-1 and NLR145 were used. MTU1010 (Cottondora Sannalu), a short duration, high yielding, fine grain and BPH resistant rice variety released from Andhra Pradesh Rice Research Institute (APRRI), Maruteru, Andhra Pradesh in 1999 was found to be susceptible to both BB and blast. B95-1 and NLR145 were used as resistant parents. B95-1 (Improved Samba Mahsuri) was developed by Directorate of Rice Research, Rajendranagar, Hyderabad and it possesses BB resistance genes *viz.*, *xa13* and *Xa21* from SS1113 (Sundaram *et al*., 2008). NLR145 (Swarnamukhi) released from Agricultural Research Station, Nellore, A.P and it possesses blast resistance genes *viz.*, *Pi54* and *Pi1* genes from Tetep.

Genomic DNA was isolated from parents (MTU1010, B95-1 and NLR145) and source material (SS1113 and Tetep for the selected target genes), F_1 and F_2 plants following the mini preparation procedure (modified method of Zheng *et al.,* 1991). Quantification of the DNA samples was done by using 0.8% agarose gel electrophoresis with diluted uncut DNA ladder as standard and spectrophotometer (Thermo electronic corporation UV1) as per the procedure described by Sambrook *et al*., 2001.

PCR amplification was performed in 10 µl volume containing 50 ng of template DNA, 5 picomoles of each primer, 2 mM dNTPs, 10X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$ and 0.01 mg ml^{-1} gelatin) and 1U *Taq* DNA polymerase polymerase (Genei, Bangalore, India) on Applied Biosystems verity 96 well thermal cycler. The template DNA was amplified in PCR profile with initial

denaturation at 94° C for 5 min, denaturation at 94° C for 45 sec, primer annealing at 55° C (*xa13* promotor, *Pi54 MAS* and *RM 224*) and at 58° C (*pTA 248*) for 45 sec, extension at 72° C for 1 min, final extension at 72° C for 10 min, and cooling at 4°C for ∞. These steps were repeated for 35 cycles for amplification of DNA. The amplified products were then mixed with bromophenol blue and resolved electrophoretically in 2% (*xa13* promoter, *pTA 248*) and 3% (*Pi54 MAS* primers) agarose gels. 3% (1 Metaphor: 2 Seakem LE agarose) Metaphor agarose gel was used to resolve amplified products for *RM 224* primers along with the marker 50bp DNA ladder (Biolabs) for an hour in 1X·Tris–Acetic acid–EDTA (TAE) buffer. The resolved PCR bands were documented using Bio-Rad Molecular Imager Gel Doc XR System.

Two gene specific primer pairs *viz.*, *xa13* promoter and *pTA 248* for BB resistance genes *viz.*, *xa13* and *Xa21*, respectively were validated in B95-1 by comparing with the check material, SS 1113. Similarly, *Pi54 MAS* and *RM 224* markers were validated for the presence of blast resistance genes, *Pi54* and *Pi1* in NLR145 by comparing with the source material Tetep. Once these resistant parents were confirmed for the presence of resistance genes, these markers were used to survey the parental polymorphism (MTU1010 vs B95-1 and MTU1010 vs NLR145) for all four resistance genes. The molecular markers showing clear polymorphism between the parental lines were used for F_1 confirmation and co-segregation analysis in F_2 population (foreground selection).

Two F_1 crosses were made during dry season, 2010 *viz.*, MTU1010 x B95-1 and MTU1010 x NLR145. F_1 seeds were raised in the main field by planting single seedling hill^{-1} at a spacing of 20 x 20 cm during wet season, 2011. DNA isolated from all F_1 plants were used for genotyping in order to identify true hybrid plants. The seeds harvested from single hybrid plants carrying both *xa13* and *Xa21* genes in heterozygous condition *i.e. Xa13xa13/Xa21xa21* (MTU1010 x B95-1) and *Pi54* and *Pi1* genes in heterozygous condition *i.e. Pi54pi54/Pi1pi1* (MTU1010 x NLR145) were selfed and advanced to F_2 generation during dry season, 2011. Co-segregation analysis was carried out in F_2 populations of MTU1010 x B95-1 and MTU1010 x NLR145 for the resistance genes *xa13 and Xa21; Pi54 and Pi1*, respectively.

A total of 420 F_2 plants from MTU1010 x B95-1 along with parents were genotyped to determine the inheritance of BB resistant genes *viz.*, *xa13* and *Xa21* using *xa13 promotor* and *pTA 248*. A total of 435 F_2 plants from MTU1010 x NLR145 along with parents were genotyped to determine the inheritance of two blast resistant genes *viz.*, *Pi54* and *Pi1* using *Pi54 MAS* and *RM 224* markers. Alleles at the SSR loci were detected on 2% and 3% agarose gel and 50bp or 100bp DNA ladder was added with the first load to confirm the allele sizes observed in the parental survey. Scoring of alleles was done to identify the plants carrying different genotypic combinations. The F_2 plants that showed a pattern similar to the susceptible parent alleles were scored as '1' and those with a banding pattern similar to the resistant parent alleles were scored as '2' and the plants with heterozygous allelic pattern were scored as '3'.

From F_2 generation, plants carrying target genes in homozygous condition were selected and advanced to F_3 generation for phenotyping of BB and blast diseases. Plants carrying target genes, *xa13* and *Xa21* in homozygous condition (*xa13xa13/Xa21Xa21*) were selected from MTU1010 x B95-1 F_2 population and were advanced to F_3 generation. In F_3 generation the progenies were inoculated with hyper-virulent isolate, DX-066 (collected from Raipur) of *Xanthomonas oryzae* pv. *Oryzae*. The plants were inoculated with bacterial culture at maximum tillering and flag leaf stages by using the leaf clipping method described by Kauffman *et al*., 1973. The inoculum was prepared by suspending bacteria, grown on Haywards agar media for 2 to 3 days at 28°C, in sterile distilled water at a final concentration of approximately 10^8cfu ml^{-1} (Preece, 1982). Inoculum density was adjusted to 10^7-10^8 (cfu ml^{-1}) and plant inoculation was carried out by clipping the tip (about 1 to 2 cm) of the fully expanded uppermost leaf with scissors that had been dipped into the inoculums. Disease scoring was done 15 days after inoculation. Five leaves plant^{-1} and 10 plants plot^{-1} were taken for scoring and the plant reaction was rated as per Standard Evaluation System (IRRI, 2002).

The homozygous resistant (*Pi54Pi54/Pi1Pi1)* F_2 plants were advanced to F_3 generation and tested in the Uniform Blast Nursery (UBN) for blast disease incidence at Maruteru and Nellore, which are hot spots for blast incidence. Disease development was ensured

by planting seedlings with spreader rows with susceptible variety (Fig. 3.4) and each test entry sown in a single row of 50cms long and 10cms apart. After every 10 test entries local susceptible variety (susceptible variety, NLR34242) was planted.

Excess nitrogen (>120 KgN ha^{-1}) was applied than recommended to enhance the disease. One-half of the nitrogen was applied as basal and the remaining half was applied 15 days after sowing (DAS). Other fertilizers were also applied as per the local recommendation. Farm yard manure was also applied before sowing. To create severe blast incidence additional inoculum was sprayed. For this diseased leaves were chopped into pieces of 3-6cm long and scattered them over the plot. Infected plants were also transplanted between the border rows. The test entries were scored based on leaf blast severity following Standard Evaluation Scale (SES). Two observations were taken on blast severity in entries 10 days intervals from 25 to 30 days after sowing (DAS). These seedlings were inoculated with *M. grisea* and disease scoring was done as per 0–9 scale (IRRI, 1996).

For inheritance studies of bacterial blight and blast resistance in the segregating population, the goodness of fit of expected genetic ratios were tested by $\div^2$–test (Singh *et al.*, 1977) using following formula:

$\div^2 = \sum(O-E)^2 / E$

where, O is observed value

E is expected value

$\sum$- Summation.

RESULTS AND DISCUSSION

A clear marker-trait association was established for both BB and blast. Hence it is possible to monitor the transmission of target genes *viz.*, *xa13, Xa21*, *Pi54* and *Pi1* using their respective markers, *viz.*, *xa13 promotor, pTA 248, Pi54 MAS* and *RM 224*.

The marker validation was done in the B95-1 (BPT5204 x SS1113) for *xa13* and *Xa21* genes *xa13 promotor* and *pTA 248* by comparing with SS1113, source material for BB genes. The results (Fig. 1.1) revealed that an allele of 500bp was amplified with *xa13 promoter* in the resistant parent and the marker *pTA 248* amplified an allele of 925bp (Fig. 1.2), in B95-1 which is exactly identical to the band that was amplified in the check material, SS1113, confirming that the resistant parent was carrying both *xa13* and *Xa21* genes. This is in confirmation with the results of Sundaram *et al.*, 2008. Another resistant parent NLR145 was also validated for the two blast resistant genes *viz.*, *Pi54* and *Pi1* using *Pi54 MAS* and *RM 224* by comparing with Tetep, the source material for blast. *Pi54 MAS* was developed by Ramkumar *et al.*, 2011 reported that the marker perfectly co-segregates with the blast resistance in mapping population with no recombinants. Therefore, *Pi54 MAS* marker was selected to screen *Pi54* gene in the present study. *Pi54 MAS* amplified an allele of 225bp in NLR145 (Fig.1.3), which was exactly identical to that of Tetep, confirmed that NLR145 posses *Pi54* gene and this is in confirmation with the findings of Ramkumar *et al.*, 2011. Similarly the marker *RM 224* amplified an allele

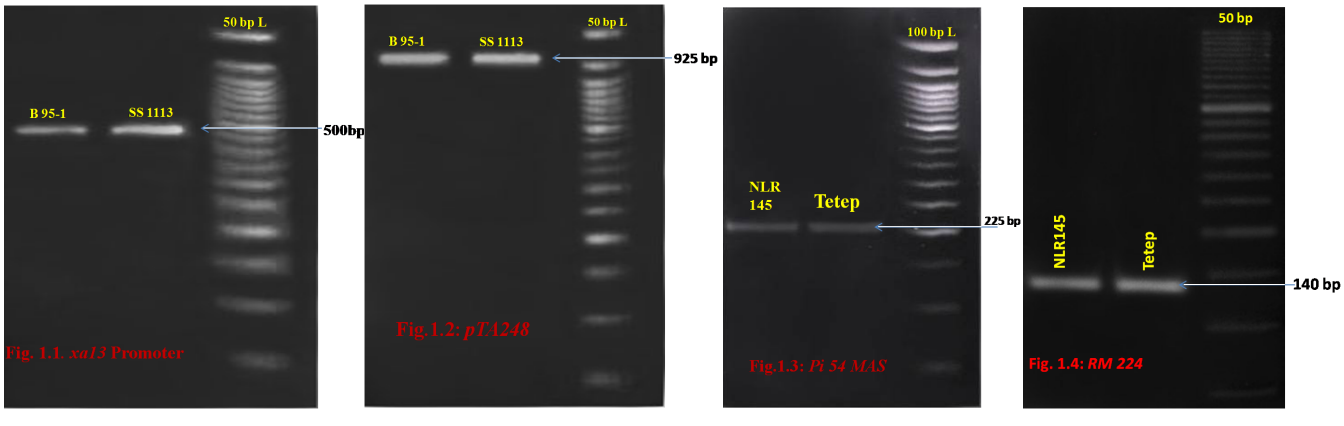


Fig. 1. Validation of Molecular Markers linked to the bacterial blight resistance genes viz., *xa13* (Fig. 1.1) and *Xa21* (Fig. 1.2) and blast resistance gene, *Pi54* (Fig. 1.3) and *Pi1* (Fig. 1.4) in resistant parents viz., B95-1 and NLR145.

of 140bp in NLR145 (Fig. 1.4), which was exactly identical to that of Tetep, confirmed that NLR145 posses *Pi1* gene and this result is in agreement with the findings of Prasad *et al.*, 2009. Vanisree *et al.*, 2012 also reported the presence of *Pi1* gene in NLR145 and Tetep.

Study of parental polymorphism is a prerequisite to begin marker assisted selection. Unless the parents are polymorphic for the traits of interest, the further selection of plants carrying the traits of interest is not possible in the progenies. SSR Markers can detect a significantly higher degree of polymorphism in rice (Okoshi *et al.*, 2004). In the present study, *xa13 promotor* amplified a clear band of 250bp in MTU1010 while another band of 500bp was amplified in the resistant parent, B95-1 (Fig. 2.1). Similarly, polymorphism was observed between B95-1 (925bp) and MTU1010 (730bp) when STS marker, *pTA 248* was used for amplification of *Xa21* gene (Fig. 2.2). Thus clear polymorphism was existed between the parents, MTU1010 and B95-1 for *xa13* and *xa21* genes. *pTA 248* (Huang *et al.*, 1997) and *xa13 promotor* (Sundaram *et al.*, 2008) were used as gene sequence based markers for BB resistance genes *viz.*, *Xa21* and *xa13* in marker assisted selection. *Pi54 MAS* and *RM 224* were used to study the polymorphism between MTU1010 and NLR145 for two blast resistance genes (*Pi54* and *Pi1*). *Pi54 MAS* linked to *Pi54* gene amplified a susceptible allele of 325bp in MTU1010, while the resistance allele of 225bp was observed in NLR145 (Fig.2.3). Similarly, a clear polymorphism was observed between NLR145 (140bp) and MTU1010 (155bp) when *RM 224* primer pair was used for amplification of *Pi1* gene (Fig. 2.4). The results showed clear polymorphism between parents. The present investigation clearly stated that two resistant genes *viz.*, *xa13* and *Xa21* for BB were present in B95-1, while blast resistance genes *viz.*, *Pi54* and *Pi1 were present in* NLR145. All the four corresponding susceptible alleles were present in the susceptible parent, MTU1010 (Table 3). Since the polymorphism was very clear among the parents for all four target genes, these markers were selected for foreground selection in the segregating generations (Table 1).

Table 1. Polymorphism between resistant and susceptible alleles.

Trait	Gene	Primer	Resistant allele	Susceptible allele
Bacterial Blight Resistance	*xa13*	*xa13 promotor*	500bp	250bp
	Xa21	*pTA 248*	925bp	730bp
Blast Resistance	*Pi54*	*Pi54 MAS*	225bp	325bp
	Pi1	*RM 224*	140bp	155bp

Two F_1 crosses were made during dry season, 2010 *viz.*, MTU1010 x B95-1 and MTU1010 x NLR145. F_1 plants were raised in the field during wet season, 2011. Twenty five F_1 plants from the cross MTU1010 x B95-1 were genotyped for both *xa13* and *Xa21* genes. All twenty five plants were confirmed as true hybrids for *xa13* as co-dominant nature of alleles was observed with primers, *xa13 promotor* (Fig.3.1).

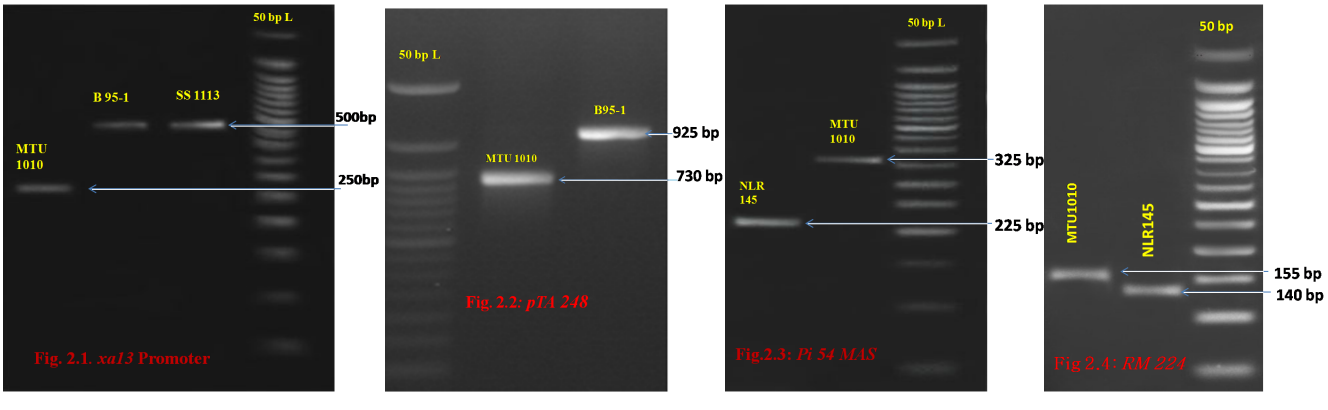
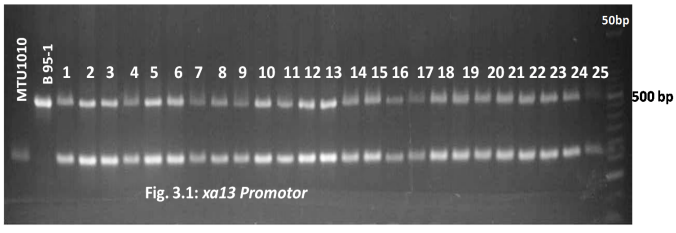


Fig. 2. Polymorphism between parental lines for four target genes viz., *xa13* (Fig. 2.1), *Xa21* (Fig. 2.2), *Pi54* (Fig. 2.3) and *Pi1* (Fig. 2.4) using xa13 promotor, pTA 248, Pi54 MAS and RM 224 markers, respectively.

Whereas, *pTA 248* amplified co-dominant bands in 15 out of 25 F_1 plants (Fig.3.2). Hence, these 15 F_1 plants were confirmed as true hybrids (*Xa13xa13/ Xa21xa21*) for both BB resistant genes (*xa13* and *Xa21*) and were allowed for selfing. The seed obtained from single F_1 plant was advanced to F_2 generation.

subjected to co-segregation analysis for the disease resistance genes *viz.*, *xa13, Xa21, Pi54* and *Pi1*. 420 F_2 populations derived from MTU1010 x B95-1 cross was analyzed with *xa13 promotor* (Fig.4.1). The result showed that 93 F_2 plants were identical to susceptible parent (250bp), while 101 F_2 plants were identical to



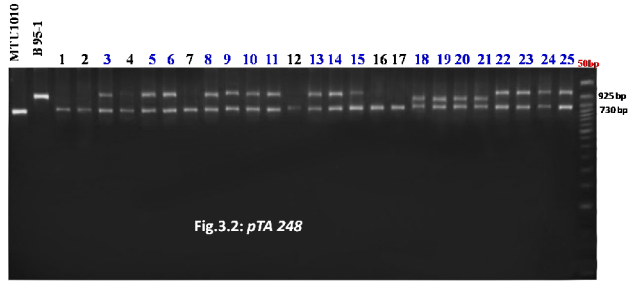
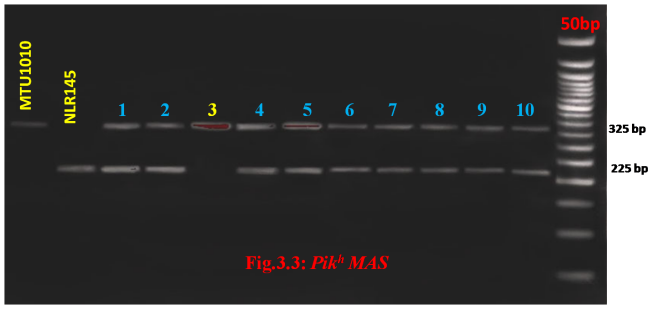


Fig. 3.1 and Fig. 3.2. Confirmation of F_1 plants for *xa13* gene and *Xa21* genes by using *xa13 promotor and pTA 248* markers, respectively.

Similarly, sixty F_1 plants obtained from the cross MTU1010 x NLR145 were genotyped using the *Pi54 MAS,* out of which 45 plants were found to be true hybrids on expression of co-dominant nature of alleles observed in the gel. The gel profile pertaining to ten F_1 plants are shown in Fig.3.3. Forty five plants were subjected to *RM 224* for *Pi1* gene out of which 27 plants were found to be true hybrids for *Pi1* gene. Seven F_1 plants were confirmed for both the *Pi54* and *Pi1* genes. The double heterozygous (*Pi54Pi54/Pi1pi1*) plants were allowed for selfing and seeds from single selfed plant was harvested, dried for 2-3 days and advanced to F_2 generation (Fig.3.4).

Two F_2 populations derived from crosses *viz.*, MTU1010 x B95-1 and MTU1010 x NLR145 were

resistant parent (500bp). 226 F_2 plants exhibited heterozygous nature for both the alleles. The ÷2-square analysis clearly indicated that the *xa13* gene segregated in a genotypic ratio 1*Xa13Xa13*: 2*Xa13xa13*: 1*xa13xa13* and exhibited a goodness fit to the expected segregation ratio for single gene model for *xa13* gene with ÷2-square value 2.73 at $p<0.05$. The study of F_1, F_2, F_3 and reciprocal backcross populations of crosses between resistant parents and the susceptible cultivar 'TNI' revealed that resistance in these cultivars and selections is under monogenic control (Petpisit *et al.*, 1977). Phenotypically it is not possible to differentiate all the genotypes. However, it is possible to make selection of plants carrying desirable gene combination *i.e. xa13xa13* by MAS. Out of 420 F_2 plants, *pTA 248*



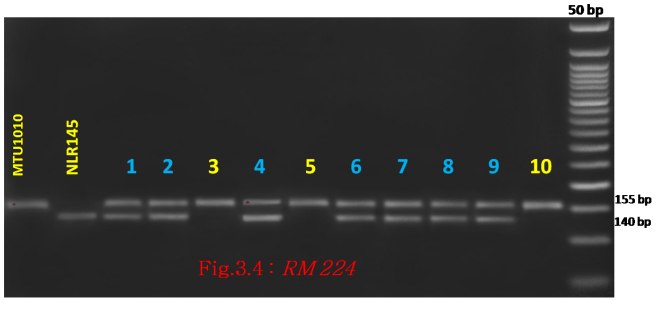


Fig. 3.3 and Fig. 3.4. Confirmation of F_1 plants for *Pi54* and *Pi1* genes by using *Pi54 MAS* and *RM 224* markers, respectively.

primer pair amplified anallele of 730bp in 109 F_2 plants identical to susceptible parent, an allele of 925bp in 94 F_2 plants identical to resistant parent and 217 F_2 plants exhibited heterozygosity (Fig.4.2). Genetic analysis of the result shows a good fit to the expected segregation ratio 1*Xa21Xa21*: 2*Xa21xa21*: 1*xa21xa21* for single gene model with ÷2-square value 1.53 at p<0.05. This result is in agreement with the results of Jiang *et al.*, 2004, for *Xa21* gene in the F_2 population of 'Minghui 63'.

genes segregate into nine distinct classes to be 1:2:2:4:1:2:1:2:1 out of which the seven resistant genotypic classes *viz.*, *xa13xa13/Xa21Xa21*, *xa13xa13/Xa21xa21*, *xa13xa13/xa21xa21*, *Xa13xa13/Xa21Xa21*, *Xa13xa13/Xa21xa21*, *Xa13Xa13/Xa21Xa21* and *Xa13Xa13/Xa21xa21* are expected to segregate in the ratio of 1:2:1:2:4:1:2. for the two gene combination.

435 F_2 populations developed from cross MTU1010 x NLR145 were screened for *Pi54* and *Pi1*

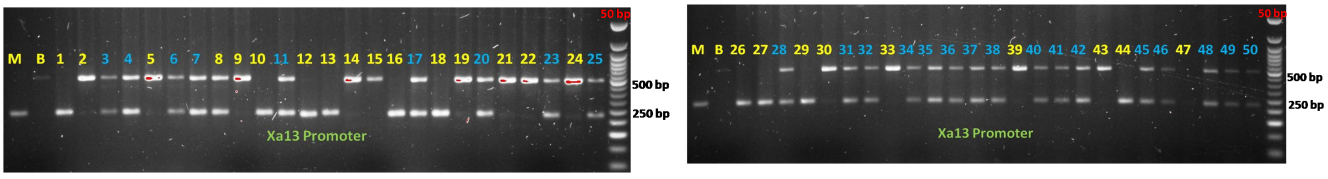


Fig. 4.1. Segregation of F_2 individuals derived from cross MTU1010 x B95-1for *xa13* gene for plants 1 to 50.

The *xa13* gene confers resistance only when present in the homozygous recessive condition whereas *Xa21* is dominant in nature and can be expressed even in the heterozygous condition governing resistance to multiple races of *Xoo*. The co-segregation analysis of two BB resistance genes *viz.*, *xa13* and *Xa21* together showed goodness of fit to the expected ratio 1:2:2:4:1:2:1:2:1, for two genes with high degree of significance (Table 2.). This result indicated that the two BB resistance genes *viz.*, *xa13* and *Xa21* followed Mendelian Inheritance with ÷2-square value 9.43 at p<0.05. Joseph *et al.*, 2004 also reported that the two genes. *Pi54 MAS* amplification (Fig.4.3) showed that 104 F_2 plants had the alleles similar to susceptible parent alleles, MTU1010 (325bp), while 120 F_2 plants had the alleles similar toresistant parent alleles, NLR145 (225bp) and 211 plants exhibited heterozygocity. The ÷2-square analysis indicated that the *Pi54* gene segregated in a genotypic ratio 1*Pi54Pi54*: 2*Pi54Pi54*: 1*Pi54Pi54* which shows a good fit to the expected segregation ratio for single gene model with ÷2-square value 1.55 at p<0.05. Ashkani *et al.*, 2011 reported segregation ratio for resistance and susceptibility allele in F_2 population derived from Pongsu Seribu 2 and

Table 2. Co-segregation analysis of two BB resistance genes *viz.*, *xa13* and *Xa21* in F_2 population of MTU1010 x B95-1

S.N.	Genotype	Observed value	Observed ratio	Expected ratio	Expected value	÷^{2} value
1	*Xa13 Xa13Xa21 Xa21*	26	0.99	1	26.25	0.02
2	*Xa13 Xa13Xa21 xa21*	48	1.82	2	52.5	0.38
3	*Xa13 xa13Xa21 Xa21*	47	1.79	2	52.5	0.57
4	*Xa13 xa13Xa21 xa21*	112	4.26	4	105	0.46
5	*xa13 xa13Xa21 Xa21*	21	0.8	1	26.25	1.05
6	*xa13 xa13Xa21 xa21*	59	2.24	2	52.5	0.80
7	*Xa13 Xa13xa21 xa21*	19	0.72	1	26.25	2.00
8	*Xa13 xa13xa21 xa21*	66	2.51	2	52.5	3.47
9	*xa13 xa13xa21 xa21*	22	0.83	1	26.25	0.68
Total		420	15.96	16	420	9.43**

The calculated ÷^{2} value, 9.43 less than tabulated value, 15.5 at df=8 and P=0.05 and 13.362 at P=0.1

Mashuri for resistance to blast pathotype P7.2 with 11 SSR markers showed a good fit to the expected segregation ratio 1:2:1. *RM 224* primer pair linked to *Pi1* gene amplified dominant allele (140bp) in 102 plants which is identical to resistant parent, while recessive allele (155bp) was observed in 96 plants and heterozygosity was observed in 237 plants (Fig.4.4). Genetic analysis showed a goodness of fit to the expected segregating ratio of 1*Pi1Pi1*: 2*Pi1pi1*: 1*pi1pi1* resistance in dominant condition and can be expressed even in the heterozygous condition governing resistance to multiple races of *Magnaporthe grisea*. The observed segregation ratio for two genes shows goodness of fit to the expected segregation ratio 1:2:2:4:1:2:1:2:1, for two genes with χ^2 value 6.59 at p<0.05. This signifies that the two blast resistant genes *viz.*, *Pi54* and *Pi1* followed the Mendelian Inheritance high degree of significance (Table 3).

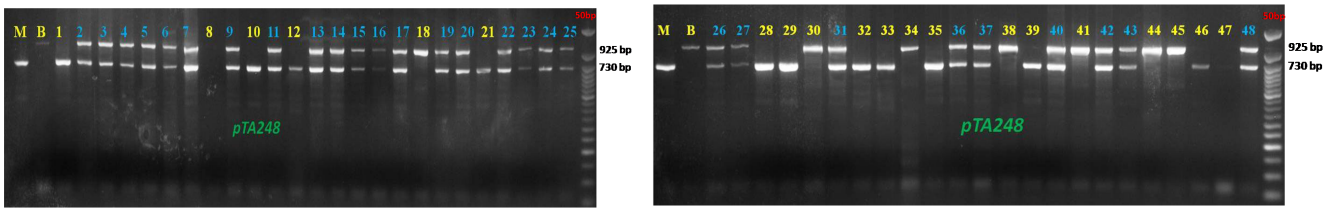


Fig. 4.2. Segregation of F_2 individuals derived from cross MTU1010 x B95-1 for *Xa21* gene for plants 1 to 48.

for single gene model gene with ÷2-square value 3.64 at p<0.05. Our result is in agreement with Prasad *et al.*, 2009, who analyzed F_2 population derived from cross C101LAC x BPT5204 by using SSR marker *RM 224* to the highly virulent blast isolate DRR001 and result show good fit to expected segregation ratio 1:2:1 for single gene model confirming the presence of a major gene *pi-1(t)*.

The F_2 population derived from the cross MTU1010 x NLR145 was also analyzed for the co-segregation of two genes governing blast resistance *viz.*, *Pi54* and *Pi1* together. Both genes confer

Twenty one and twenty four plants possessing BB and blast resistance genes respectively in homozygous condition (*xa13xa13/Xa21Xa21* and *Pi54Pi54/Pi1Pi1*) were advanced to F_3 generation and phenotyping for BB and blast were carried out during dry season, 2012-13. All 21 F_3 progenies showed resistance reaction for BB isolate, DX-066 (1-5% DLA). Similarly, higher levels of resistance in gene pyramid lines containing multiple BB resistance genes as compared to lines having single (or fewer) resistance genes have been reported earlier (Yoshimura *et al.*, 1996). Pandey *et al.*, 2013 also improved traditional BB-susceptible Basmati varieties (Taraori Basmati and

Table 3. Co-segregation analysis of two Blast resistance genes *viz.*, *Pi54* and *Pi1* in F_2 population of MTU1010 x NLR145

S.N.	Genotype	Observed value	Observed ratio	Expected ratio	Expected value	χ^2 value
1	*Pi54 Pi54Pi1 Pi1*	24	0.88	1	27.18	0.37
2	*Pi54 Pi54Pi1 pi1*	58	2.1	2	54.37	0.24
3	*Pi54 Pi54Pi1 Pi1*	47	1.72	2	54.37	0.99
4	*Pi54 Pi54Pi1 pi1*	113	4.15	4	108.75	0.16
5	*Pi54 Pi54Pi1 Pi1*	31	1.14	1	27.18	0.53
6	*Pi54 Pi54Pi1 pi1*	66	2.42	2	54.37	2.48
7	*Pi54 Pi54pi1 pi1*	22	0.81	1	27.18	0.98
8	*Pi54 Pi54pi1 pi1*	51	1.87	2	54.37	0.20
9	*Pi54 Pi54pi1 pi1*	23	0.84	1	27.18	0.64
Total		435	15.93	16	435	6.59**

The calculated χ^2 value, 6.59 less then tabulated value, 15.5 at df=8 and P=0.05 and 13.362 at P=0.1.

Basmati 386) by introgressing two major BB resistance genes, *Xa21* and *xa13*, coupled with phenotype-based selection. They reported improved lines possessing a single resistance gene (*i.e.* either *Xa21* or *xa13*) both in homozygous condition (*Xa21Xa21* or *xa13xa13*) displayed moderate resistance to BB, while lines possessing both *Xa21* and *xa13* in homozygous condition (*Xa21Xa21xa13xa13*) showed significantly higher levels of resistance equivalent to resistance with ISM and SS1113 possessing *Xa21*, *xa13* and *xa5*..

The donor parent NLR145, which possesses *Pi54* gene showed high level of resistance for rice blast with '3' disease score and the parent MTU1010 showed presence of disease lesions in more than 50% leaf area with disease score '7'. Among 24 F_3 progenies carrying resistance genes in homozygous condition (*Pi54Pi54*/*Pi1Pi1*) from the cross MTU1010 x NLR145, only 11 progenies showed resistance reaction for blast. The susceptibility of 13 gene positive plants to blast disease might be attributed due to occurrence of recombination between *Pi1* gene and selected marker, RM224. Narayanan *et al.,* 2002 pyramided two major blast resistance genes *Pi-1* and *Piz-5* by using RFLP markers r10 for *Pi-1* and a PCR-based SAP marker RG64 for *Piz-5*. Though *Pi54* gene gives broad spectrum disease resistance, the blast disease is controlled by nearly 80 genes (Costanzo and Jia, 2010) which could be the reason that no line showed '0' score for blast disease.

The present investigation indicated that the use of gene specific molecular markers or markers that are closely linked to traits of interest resulted in effective selection of desired combination of genotypes. Identification of desired genotypes possessing more than one gene is not possible through conventional method unless phenotyping is done for different strains of pathogens. MAS helps to shorten the development time of cultivar (Dwivedi *et al.* 2007). Molecular markers enable breeders to exercise precise selection on genotypic differences (*i.e.*, differences in DNA) rather than phenotypic differences, which has the potential to greatly increase selection efficiency and shorten the breeding cycle (Yencho *et al.* 2000).

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