“MARKER ASSISTED SELECTION IN ADVANCED BACKCROSS POPULATION OF RICE VARIETY, MTU1010 FOR BACTERIAL BLIGHT AND BLAST RESISTANCE”

ABSTRACT

The present study was aimed for identification of the bacterial blight and blast resistant lines in the advanced back cross population of MTU-1010 using marker assisted foreground selection andalso for evaluation of agro-morphological characters. The M-16-59 introgressed line (derived from an intercrossing of BC2F1 plants of MTU-1010 x GPP2 and MTU-1010 x NLR 145) is developed under ongoing DBTDBSRR subproject -IV possessing *Xa21*, *xa13*, *Pi1* and *Pi54* resistance genes having broad spectrum resistance to bacterial blight and blast is used as a donor parent and MTU-1010 was used as a recurrent parent for the back cross. Before attempting the backcross both the parents were verified for the target genes along with the original donors GPP2 and NLR 145 using gene specific/linked molecular markers *viz.*, *xa13-promo* for *xa13* gene, *pTA248* for *Xa21* gene, RM 224 for *Pi1* and *Pi54 MAS* for *Pi*54. In F_1 generation (equal to BC3F1 as two backcrosses were completed earlier), 120 plants were screened and 16 plants were confirmed for *xa13*,*Xa21*, *Pi1* and *Pi54* genes in heterozygous condition. These confirmed plants were evaluated for agro-morphological characters *viz.*, yield, grain characters and plant type. The results revealed that the confirmed heterozygous plants were on par with MTU-1010 and advanced for further selection and evaluation.

Key words: Bacterial leaf Blight, Blast, Molecular markers, Foreground selection, Background selection, recurrent parent genome, Molecular breeding, MTU1010, *xa13*, *Xa21*, *Pi54* and *Pi1* genes.

Introduction

Rice is the staple food for more than half of the world’s population, and global rice demand is estimated to rise from 6.76 × 108 t in 2010 to 8.52 × 108 t in 2035 (Khush, 2013). To produce 1.76 × 108 t additional rice, it is need to increase the yield and also minimize the yield loss caused by various diseases and insect pests. Among the biotic stresses, bacterial blight (BB) and blast are important diseases that results in significant yield reduction worldwide. BB is caused by a bacterium, *Xanthomonas oryzae pv.oryzae*, which is a serious problem in irrigated and shallow lowland conditions in India causing yield losses ranging from 74 to 81% based on severity of the disease (Srinivasan and Gnanamanickam, 2005). Rice blast disease, caused by *Magnaporthe oryzae,* is one of the most serious diseases of rice. While it is present nearly everywhere rice is grown, blast is more of a problem in the temperate flooded and tropical upland cropping systems, marked by cooler climates (Scardaci *et al.,* 2000). In Andhra Pradesh and Telangana yield losses are very high especially in Nellore, West Godavari and Rangareddy districts (Rajarajeshwari *et al.,* 2006). Breeding and the development of resistant cultivars carrying major resistance (R) genes have been the most effective and economical strategy to control BB disease to have a neutral effect on the environment (Huang *et al.,* 1997; Singh *et al.,* 2001 and Jena and Mackill, 2008). However, cultivars undergo rapid breakdown in their resistance mainly by the mergence of new pathotypes, due to the high instability in the genome of the pathogen (Dean *et al*., 2005). Therefore, bringing together multiple genes conferring resistance to more than one pathotype into one genetic background is necessary for durable resistance. However, conventional breeding methods to improve rice cultivars for BB resistance have not found much success (Shin *et al.,* 2011).

To date, atleast 40 BB resistance (Kim *et al.,* 2015) genes conferring host resistance against various strains of *Xanthomonas oryzae pv. oryzae (Xoo* have been identified. Out of this 40, 29 dominant and 11 recessive genes have been identified and registered (Ranjith. Ellur K *et al*., 2015). Using MAS breeding approaches three or more BB genes, like *xa5, xa13, Xa21* have been successfully pyramided in diverse elite rice varieties like IR64, PR106, Pusa Basmati 1, Lalat, Tapaswini, Swarna, IR64 and Samba Mahsuri (Sundaram *et al*., 2014). Using the gene pyramid approach, a three-gene combination appeared to be the most effective with *Xa21* contributing the largest component of resistance (Pradhan *et al.,* 2015). Globally, 100 rice blast major resistance genes (R-genes) have been identified (Devanna *et al.,* 2014)), out of which 19 blast resistance genes have been cloned and over 50 major rice blast R genes have been mapped (Hayashi, 2005; Chen *et al.,* 2006). The most of identified blast R genes were found in a cluster on chromosome 6, 11 and 12 (Yang *et al.*, 2008). Recently the *Pi1* leaf blast resistance gene has been introgressed into the D521 line derived from the donor line BL122 (Fu *et al.*, 2012).

M-16-59, a gene stacked line developed from the ongoing DBTDBSRR sub project IV funded by DBT, Government of India at Institute of Biotechnology possessed two BB resistance genes (*xa13* and *Xa21*) and two blast resistance genes (*Pi 54* and *Pi1*). This line was developed by intercrossing of BC$_2$F$_1$ plants of MTU-1010 x GPP2 and MTU-1010 x NLR 145. M-16-59 is carrying the four genes with 85% recurrent parent genome (MTU 1010) and was recovered from the ICF$_2$ segregating population. Though this introgressed line is having four genes with 85% recovery it still resembles its donor. So, in the present study an attempt was made to introgress BB and blast resistant genes *xa13, Xa21, Pi54 and Pi1* to further increase the resistance and also to improve the recovery of MTU-1010. Resistance genes linked/gene specific molecular markers were used for foreground selection while polymorphic primer pairs that are spread all over rice genome were used for background selection to carry out MAS. In our study MTU-1010 is used as recurrent parent and M-16-59 is used as donor parent.

MATERIAL AND METHODS

Plant material : Cottondora Sannalu (MTU 1010) is an elite mega rice variety derived from the cross, Krishnaveni/ IR64 possessing short duration, high yielding ability and long slender grain quality was used as recurrent parent. M-16-59, an introgressed MTU1010 line possessing four [two BB (xa13 and *Xa21*) and two Blast(*Pi54* and *Pi1*)] biotic stress resistance genes with 85% MTU-1010 genome was used as the donor parent in the present study. The nucleus seed of MTU-1010 and NLR145 were obtained from APRRI, Maruteru respectively, while GPP2 seed which was used for positive check control were obtained from ICAR-IIRR, Rajendranagar, Hyderabad.

Molecular marker analysis

MTU-1010 and M-16-59 were verified for the target genes along with the original donors GPP2 and NLR145. This verification was carried out using gene specific molecular markers (Table 1) viz., xa13-promo, pTA248, Pi54 MAS for *xa13, Xa21, Pi54* genes respectively. While a gene linked marker RM224 was used for *Pi1* gene.

Genomic SSR markers for background selection

Parental polymorphism survey between donor and recurrent parents was carried out by using 354 genomic SSR markers covering all the twelve chromosomes, selected from gramene data base (www.gramene.org). Polymorphic markers were used for background selection in $F_{1,}$ BC_1F_1 and BC_1F_2 generations. In the earlier study of Aruna Kumari 2013, 616 SSR markers were tested covering all twelve chromosomes, while 108 markers showed polymorphism between recurrent parent (MTU-1010) and the two donor parents (GPP2 and NLR 145) during the development of M-16-59, an introgressed line of MTU-1010 carrying *xa13, Xa21, Pi54* and *Pi1* genes.

Generation of F_1 Material : Staggered sowings were taken up with 7 days interval to obtain synchrony between MTU-1010 and donor parent M-16-59 in order to make crosses. Twenty five days old seedlings were transplanted in two row plots with a spacing of 20 x 20 cm. Fertilizer application, inter cultivation, water management and plant protection measures were adopted as per the recommendation of PJTSAU. F_1 material was generated by making cross between MTU-1010 and M-16-59 during wet season, 2014 at ARI, Rajendranagar.

Generation of BC_1F_1, BC_1F_2 and BC_1F_3 along with parent material

F_1 seeds were raised at ARI, Rajendranagar during dry season, 2014-15. DNA was isolated from F_1 plants and were verified for the four target genes *xa13, Xa21, Pi54* and *Pi1* using the foreground markers viz., *pTA248, xa13 prom, Pi54-MAS* and RM224, respectively. The one true hybrid (*Xa13xa13, Xa21xa21, Pi54pi54, Pi1pi1*) plant was backcrossed with MTU-1010 (using the F_1 as male parent and MTU-1010 as female parent) to generate BC_1F_1 seeds. After foreground and background selection in BC_1F_1 generation, the BC_1F_1 plant with high recurrent parent genome (RPG) was selfed to generate BC_1F_2 generation. Foreground selection was carried out in 1060 BC_1F_2 population. Background analysis was carried out in 20 selected BC_1F_2 plants. In addition to twenty selected four and three gene BC_1F_2 plants, five plants with two gene combination were also selected based on their resistance to BB and selfed to generate BC_1F_3 generation.

DNA extraction and PCR analysis : DNA was isolated from the leaf samples according to Zheng et al., (1996). The quality and quantity of DNA was estimated in 0.8% agarose gel using 500ug/ml lamda (ë) Hind III DNA (New England Biolabs) as reference standard. PCR was carried out to detect the presence of four genes. PCR and gel electrophoresis protocols recommended by Sundaram *et al* (2008) and Ramkumar *et al* (2011) were adopted for marker-assisted selection of target genes *xa13, Xa21, Pi54* and *Pi1,*respectively.

Evaluation of agro-morphological characters : The F_1plants were transplanted in the main field at a spacing of 20 cm × 15 cm along with the donor and recurrent parents. Standard agronomic practices were followed to raise a healthy crop and agro-morphological characters like days to 50% flowering, days to maturity; plant height (cm), number of productive panicles per plant, panicle weight (g), panicle length (cm), grain yield per plant (g), 1000 grain weight (g) and grain type were recorded.

3.6.3. Evaluation of BC_1F_2 and BC_1F_3 Progenies for Agro-Morphological Parameters

The BC_1F_2 plants showing homozygosity for 2, 3 and 4 target genes were advanced to BC_1F_3 generation. 25 BC_1F_3 progenies along with MTU-1010 were grown during wet season, 2016 at ARI, Rajendranagar. The phenotypic data was recorded on twenty five BC_1F_2 plants possessing four target genes *viz.,xa13xa13,Xa21Xa21, Pi54Pi54,Pi1Pi1* (4 plants), 3 genes (16 plants) and 2 genes (5 plants) in different combinations in homozygous condition for Days to 50% flowering (DFF), Plant height (cm) and Grain type were recorded along with the recurrent parent MTU-1010. In $BC_1F_{3,}$ the material was raised in Randomized Block Design (RBD) with two replications. Each progeny was planted in 3 rows with a spacing of 20 X 15 cm. A healthy crop was raised by following standard agronomic practices recommended by PJTASU. Data was collected from five randomly selected plants from each replication for Days of 50% flowering (DFF), Plant height (cm), No. of panicles per plant, Number of filled grains per panicles, Panicle length (cm), Grain yield per plant (g), Thousand seed weight (g) and Grain type. Data on DUS characters *viz.*, Basal Leaf : Sheath Color, Leaf : Auricles, Leaf : Anthocyanin Colouration of auricles, Leaf : Shape of ligule, Leaf : color of ligule, Flag Leaf : Attitude of blade (Early observation),Time of heading (50% of the plants with panicles), Lemma : Anthocyanin coloration of area below apex, Stem length (excluding panicles; excluding floating rice), Stem : Anthocyanin coloration of nodes, Panicle : Length of main axis, Flag Leaf : Attitude of blade (late observation), Panicle : Curvature of main axis, Spikelet : Color of tip of lemma, Panicle : Awns, Panicle : Attitude of branches, Panicle : Exsertion, Sterile lemma : Color, Leaf : Senescence, Panicle : Presence of secondary branch, Lemma and palea : color was collected in comparison with recurrent parent. The data on DUS characters was recorded as per the guidelines (Subba Rao *et al.*, 2013).

Statistical Analysis

The data collected from BC_1F_3 progenies, which was raised in RBD design was subjected to analysis of variance (ANOVA), by using OPSTAT version 9.1 software.

RESULTS

In the present study to improve the recurrent parent genome recovery and development of resistant lines against bacterial blight and blast of MTU-1010 Marker assisted breeding has been successfully applied (Hari *et al.*, 2013, and Khanna *et al.*, 2015) as MAS saves time and offers a very simple efficient and accurate method (Singh *et al.*, 2012).

Verification of the parents for the resistance genes using gene specific/linked markers : Verification of the parents for the resistance genes using gene specific/ linked polymorphic markers is an important prerequisite before starting marker-assisted backcross breeding. A marker which is monomorphic bears no value in selection work because this type of marker cannot distinguish the two parental genotypes viz. MTU-1010, the recurrent or recipient parent and M-16-59, the donor parent of the MABC program. A total of 4 primers specific to *Xa21, xa13 BB* genes and *Pi1, Pi54* blast genes were surveyed for finding out polymorphic markers and all of them were found as polymorphic.

The results (Figure 1) revealed that 500bp resistance allele of *xa13* gene was amplified with *xa13 promoter* primer in M-16-59 line. This band was exactly identical to the band that was amplified in the check material, GPP2. The marker *pTA248* amplified 900bp resistance allele in M-16-59 line, which was similar with that of GPP2 confirming that the resistant parent was carrying *Xa21* gene. Magar *et al.* (2014), Hajira Shaik *et al.* (2014) and Balachiranjeevi *et al.* (2015) also utilized *xa13 promoter* and *pTA248* primers for validation of parents and foreground analysis in backcross derived population. In the similar way 250bp resistance allele of *Pi54* gene was amplified with *Pi54-MAS* in M-16-59 line and is similar to NLR145 which is used as the positive check control and also for *Pi1* gene in M-16-59 line the band is obtained at 150bp a resistant allele, when amplified with RM224, which is identical to that of original donor NLR145. These results confirmed that M-16-59 line was carrying *xa13, Xa21, Pi54* and *Pi1* genes. Jamal-oddin *et al.,* (2015) used the same primers *Pi54-MAS* and RM224 for *Pi54* and *Pi1* genes in MAS.

Discussion:

Foreground selection of BC_1F_1 progeny

Among 257 BC_1F_1 plants, 6 BC_1F_1 plants showed the presence of all four genes *xa13, Xa21, Pi54* and *Pi1* in heterozygous condition. This result is similar to previous reports on the successful utilization of MABC to transfer BB and Blast resistance genes into several elite rice varieties (Deng *et al.,* 2012; Suh *et al.,* 2013; Win *et al.,* 2013; Dash *et al.,* 2016 and Abhilash *et al.,* 2016). In the present study, MABC clearly overcome the obstacles when breeding for biotic resistance by conventional breeding method and demonstrated that MABC is generally an effective strategy for genes or QTL pyramiding. Identification of positive heterozygous plants for *xa13, Xa21, Pi54 and Pi1* genes in BC_1F_1 generation is very difficult and time consuming job, if done, based on phenotype based selection alone. Hence molecular markers used in the present study allowed precise selection of positive plants for four genes (Ribaut and Hoisington, 1998). MAS is particularly useful for identification of heterozygous individuals for recessive genes like *xa13*. In the absence of marker, identifying backcross plants that have this type of recessive genes would require progeny testing, which is an addition of one more generation study and cumbersome too (Sundaram *et al*., 2008). Like any other genetic markers, the PCR based DNA markers used in the present study (i.e. *xa13-prom, pTA248, Pi54 MAS* and RM224) are located very near to/within *xa13, Xa21, Pi54 and Pi1* genes (Sundaram *et al*., 2011; Ronald *et al*., 1992 , Ramkumar *et al*., 2011). Hence these markers can be used to complement classical breeding techniques in order to select segregating plants at early stage based on the DNA marker genotype rather than waiting to observe the phenotypic disease screening (i.e. rice blast and bacterial blight).

Background selection of BC_1F_1 progeny

The percent recurrent parent genome recovery observed in this study was identical to that of Sundaram *et al.* (2008) and also with Hasan *et al. (*2015). Polymorphic SSR markers used in the BC_1F_1 progeny and chromosome wise recovery of the recurrent parent genome (RPG) in the best line (BC_1F_1-198) is presented in table 1. Background screening with RM polymorphic SSR markers viz., RM12061, RM3472 is shown in Figures.The present BC_1F_1 is equal to BC_4F_1 as three backcrosses were completed earlier. In our study, we could recover 96.8% RPG as per the expectation of 96.875% of RPG in fourth backcross generation. Balachiranjeevi *et al.,* 2015 utilized marker assisted backcross breeding for recovering the plants with three biotic resistance (*xa13, Xa21* and *Pi54*) genes with maximum recurrent parent genome of DRR17B.

Foreground selection of BC_1F_2 population

Foreground selection of the BC_1F_2 population was carried out using PCR based gene specific and gene linked markers for the target genes. A total of 1060 BC_1F_2 plants were screened for homozygosity of all four target resistance genes. This BC_1F_2 population exhibited donor parent type, heterozygote and recurrent parent type alleles for all four markers.

Identification of homozygous BC_1F_2 plants is very important because if the selected BC_1F_2 plants contain one or more of the target genes in heterozygous condition, they will segregate in next generation. The plants carrying the donor parent alleles were selected. In the present study, only homozygous plants with the desirable gene combinations (i.e.*xa13xa13Xa21Xa21Pi54Pi54Pi1Pi1)* were selected for further advancement and evaluation.

Background Selection of BC_1F_2 gene positive plants

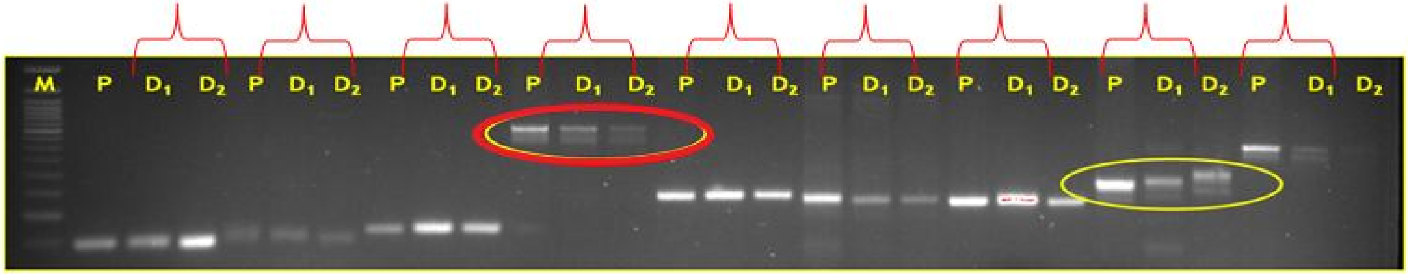
At BC_1F_2 generation (BC_1F_2 is equal to BC_4F_2 as three backcrosses completed earlier), the recovery of RPG was observed to be nearly equivalent to the theoretically expected value of 96.8%. In BC_1F_2 generation the recurrent parent genome recovery percentage was ranged between 96.0 and 97.8% (table 1). Identification of plants carrying more than one target gene with desired recurrent parent genome is extremely difficult through conventional breeding. In our study, we could identify the plants with four and three gene combinations with more than 96 % RPG, which is impossible through conventional breeding (Ahmed *et al.*, 2016ly, MR263-BR-3, MR263-BR-4, MR263-BR-13 and MR263-BR-26, which were developed through the incorporation of the blast resistance *Pi-7(t)*, *Pi-d(t)1* and *Pir2-3(t)* genes and qLN2 QTL into the MR263 background using an MABC breeding approach. They used simple sequence repeat (SSR) markers RM5961 and RM263 (linked to the blast resistance genes and QTL) for foreground selection and a collection of 65 polymorphic SSR markers for background selection in backcrossed and selfed generations. Background analysis (BC_2F_4 generation) revealed the highest rate of recurrent parent genome recovery of 96.1% in MR263-BR-4-3 and 94.3% in MR263-BR-3-2. In a similar study Tanweer *et al.*, 2015 introgressed blast resistance genes (Putative *Pi-b* and *Pi-54*) into elite rice cultivar MR219 through Marker-Assisted Selection. For background selection they used a total of 72 polymorphic markers. The minimum recovery of the recurrent parent genome in an improved line was 94% and the maximum recovery in an improved line was 97.5%. The percentage of chromosome segments derived from PongsuSeribu 2 was 2.5% and remained constant in all of the advanced improved lines. The average proportions of the recurrent parent genome in all 15 improved lines were 96.17%, showing the maximum similarity observed at the phenotypic level with the recurrent parent. Similarly, Basavraj *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 Pusal46 and 54 STMS markers polymorphic between PRR78 and Pusa1460. They recovered the recurrent parent genome ranging from 85.14 to 97.30% and 87.04 to 92.81% in the 10 selected BC_2F_5 families of Pusa6B and PRR78 respectively. 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. Sixteen BC_2F_3 progenies with nearly Type 3 Basmati seeds were finally selected for background profiling using 95 SSR and 12 ISSR markers. On the basis of SSR markers, these 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%).

Table 1. Resistance and susceptible allele sizes of target gene specific / linked markers

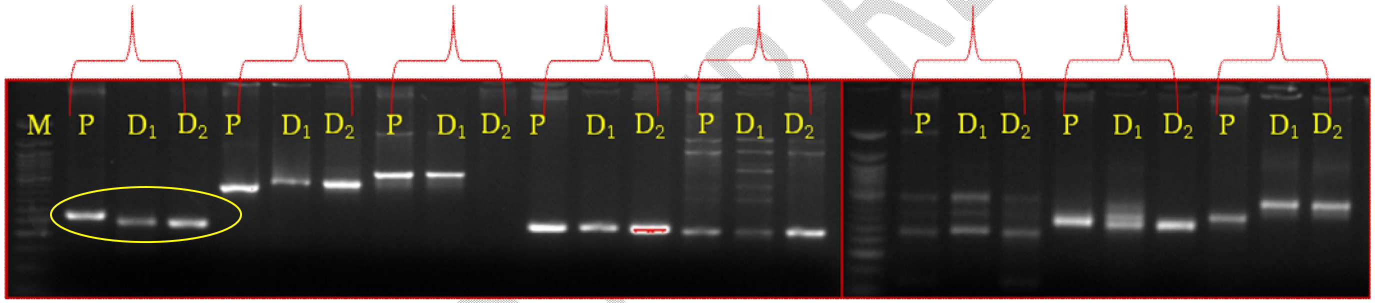
Gene	**Markers**	**Resistance allele**	**Susceptible allele**	**Reference**
xa13	*xa13-prom*	500bp	250bp	Sundaram*et al.*,2008
Xa21	*PTA248*	900bp	650bp	Ronald *et al.*, 1992
Pi54	*Pi54 MAS*	200bp	350bp	Ramkumar*et al.*, 2011
Pi1	RM224	130bp	150bp	Hittalmani*et al.*, 2000

Fig. 1. Parental polymorphism between recurrent parent and donors with genomic SSR markers

RM20495 RM228 RM581 RM124 RM434 RM18405 RM529 RM19629 RM7018



RM19472 RM25173RM11997 RM17721RM25178 RM26939 RM25969 RM17263

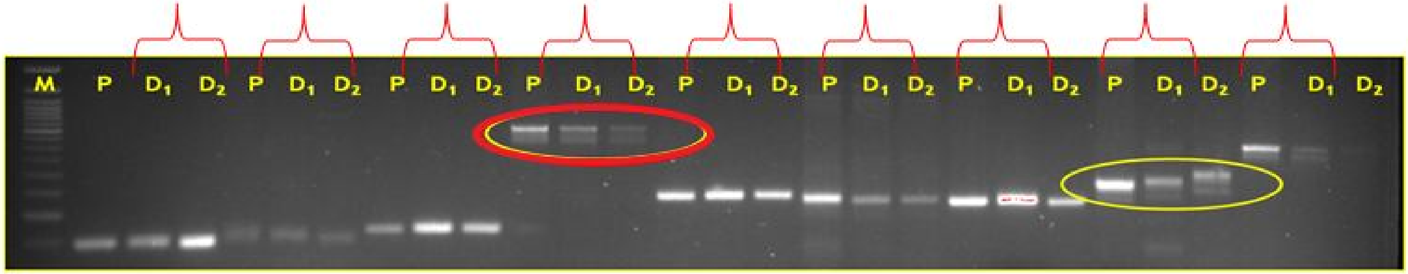


M : 50 bp LadderP: MTU1010
D_1 : GPP2
D $_2$: NLR145

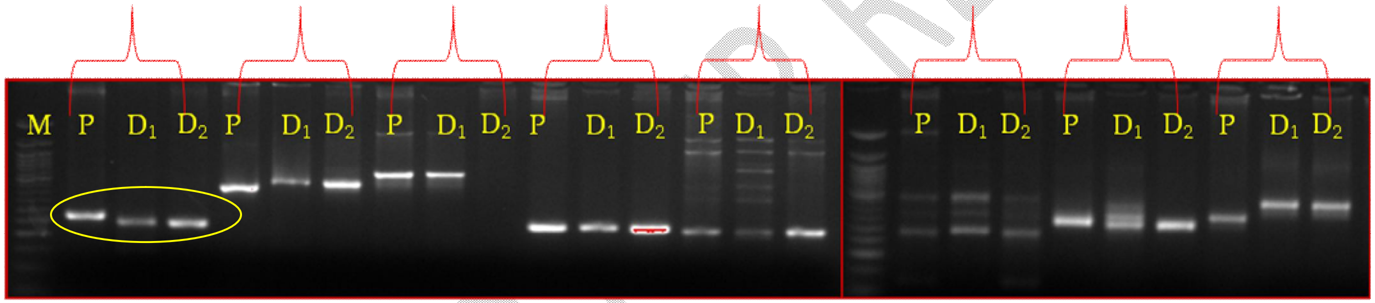
Red color ring indicates parental monomorphism with primer RM124 and yellow color rings indicate parental polymorphism with primer RM19629 and RM19472.

Fig. 2. Parental polymorphism between recurrent parent and donors with genomic SSR markers

RM20495 RM228 RM581 RM124 RM434 RM18405 RM529 RM19629 RM7018



RM19472 RM25173RM11997 RM17721RM25178 RM26939 RM25969 RM17263

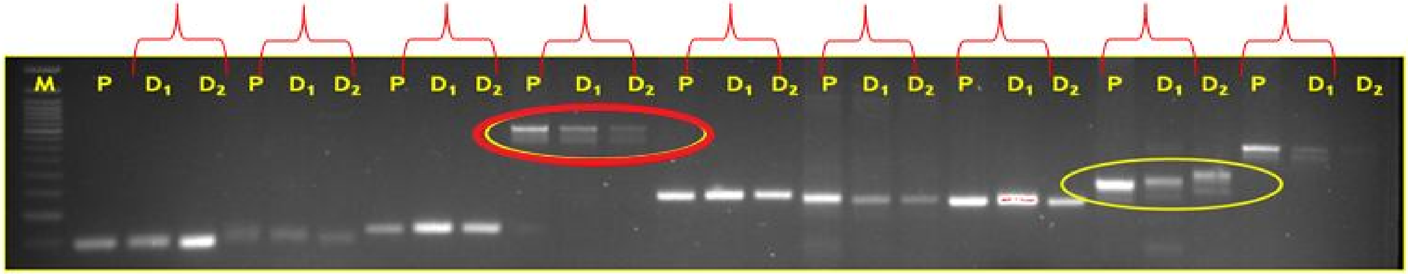


M : 50 bp LadderP: MTU1010
D_1 : GPP2
D $_2$: NLR145

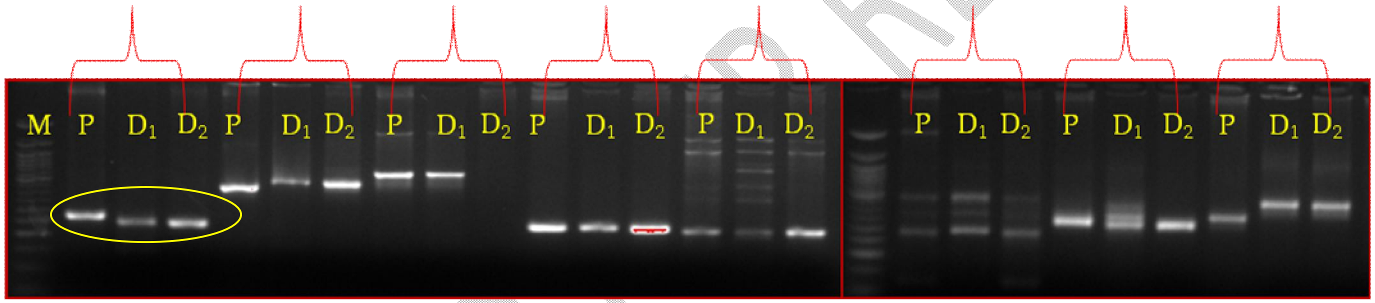
Red color ring indicates parental monomorphism with primer RM124 and yellow color rings indicate parental polymorphism with primer RM19629 and RM19472.

Fig. 3. Parental polymorphism between recurrent parent and donors with genomic SSR markers

RM20495 RM228 RM581 RM124 RM434 RM18405 RM529 RM19629 RM7018



RM19472 RM25173RM11997 RM17721RM25178 RM26939 RM25969 RM17263



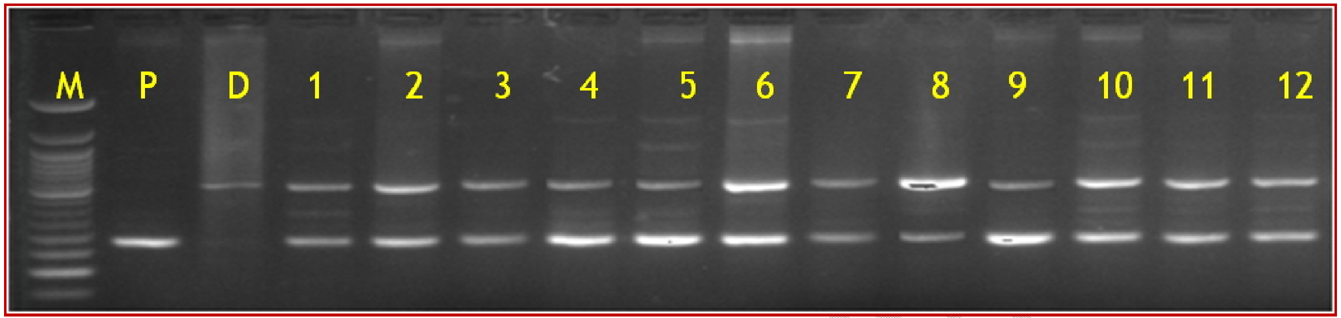
M : 50 bp LadderP: MTU1010

D_1 : GPP2

D $_2$: NLR145

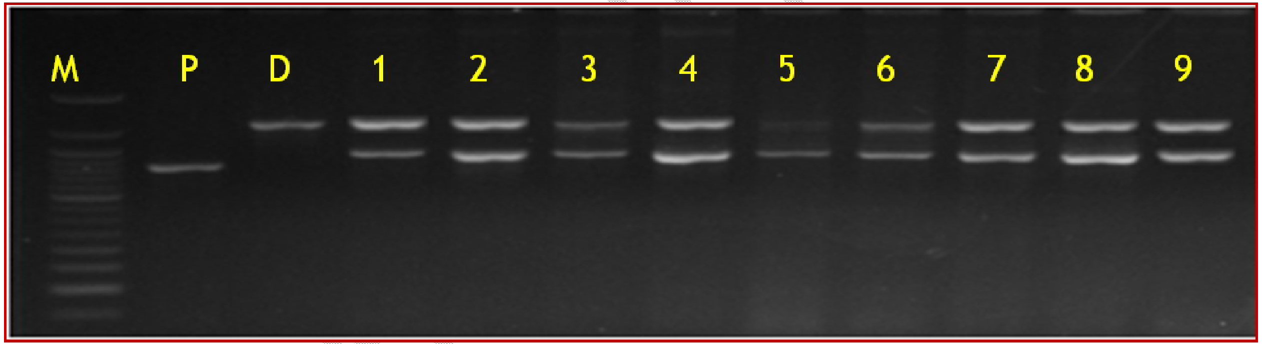
Red color ring indicates parental monomorphism with primer RM124 and yellow color rings indicate parental polymorphism with primer RM19629 and RM19472.

Fig. 4 Foreground analysis for confirmation of F_1s using *xa13-prom* for *xa13* gene



Note: Lane M: 50 bp ladder, Lane p : Recipient MTU1010, Lane D:Donar M-16-59, 1-12 are F_1 plants in heterozygous condition

Fig. 5. Foreground analysis for confirmation of F_1s using *PTA248* for *Xa21* gene



Note: Lane M: 50 bp ladder, Lane p : Recipient MTU1010, Lane D:Donar M-16-59, 1-9 are F_1 plants in heterozygous condition

Fig. 6 .Background analysis for recurrent parent genome recovery of F_1 plants using RM 19629

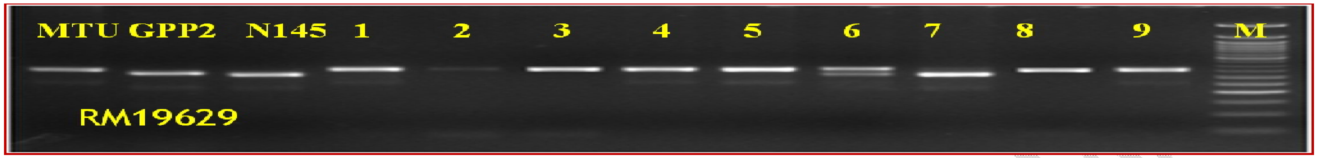


Fig. 7. Background analysis for recurrent parent genome recovery of F_1 plants using RM 22552

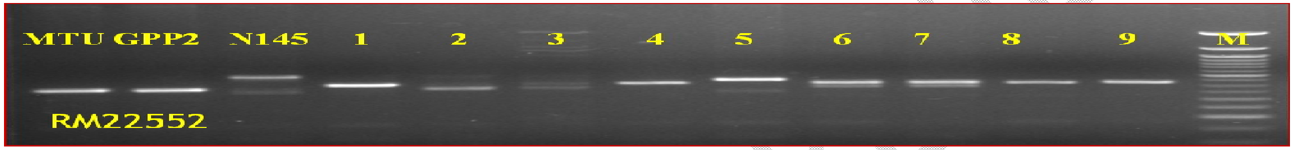


Fig.8 .Background analysis for recurrent parent genome recovery of F_1 plants using RM 21539

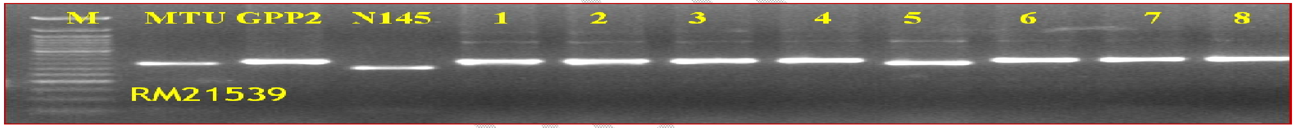
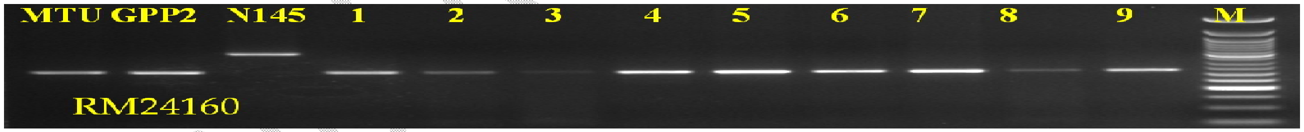


Fig.9. Background analysis for recurrent parent genome recovery of F_1 plants using RM 24160



Note: 1to 9 equals to F_1 – 5, F_1-17, F_1 - 19, F_1 - 27, F_1 - 51, F_1 - 63, F_1 – 65, F_1 - 69, F_1 -77plants, respectively

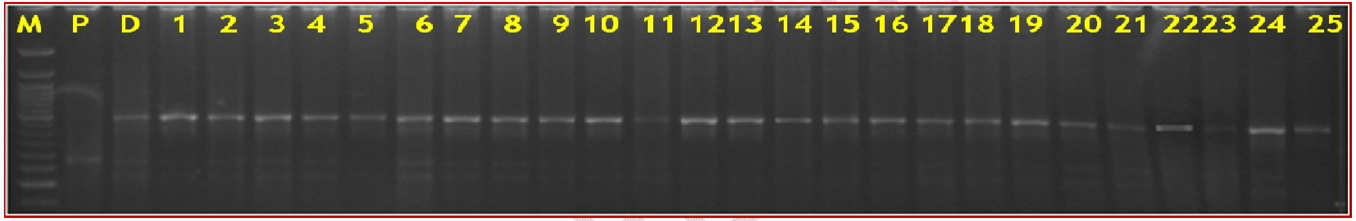
The plants possessing MTU1010 allele were selected

Representation of graphical genotype of selected BC_1F_2 plants in the genomic region around *xa13* (on Chromosome 8), *Xa21*, *Pi54* and *Pi1* (on Chromosome 11) based on analysis with parental polymorphic SSR markers

Chromosome – 8 **Chromosome - 11**

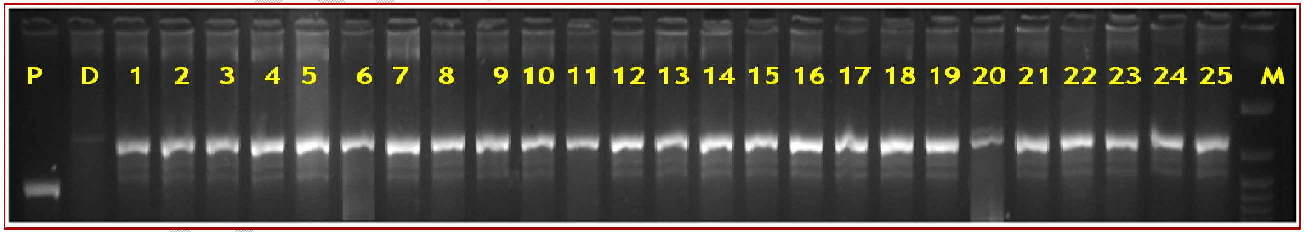
Note: R – Recipient (MTU1010), D- Donor (GPP2- *xa13* and *Xa21*) / (NLR145- *Pi54* and *Pi1*), 1: BC_1F_2-198-317, 2: BC_1F_2-198-52, 3: BC_1F_2-198-581, 4: BC_1F_2-198-620

Fig.10. Foreground analysis for confirmation of BC_1F_3s using *xa13-prom* for *xa13* gene



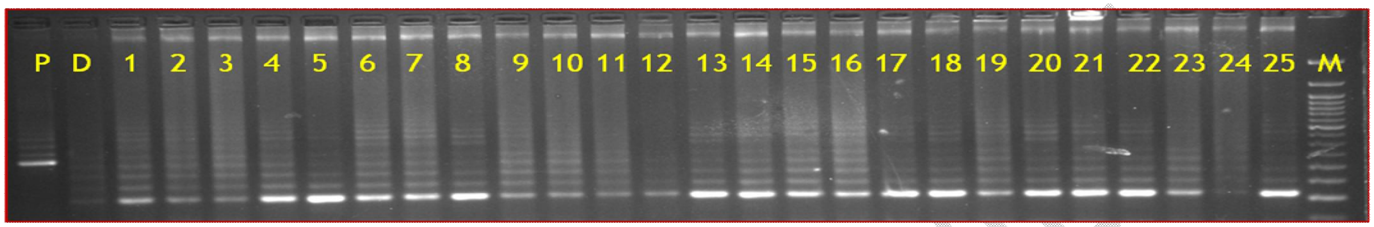
Note: Lane M: 50 bp ladder, Lane p : Recipient MTU1010, Lane D:Donar M-16-59, 1-25 are BC_1 F_3 plants

Fig. 11. Foreground analysis for confirmation of BC_1F_3s using *PTA248* for *Xa21* gene



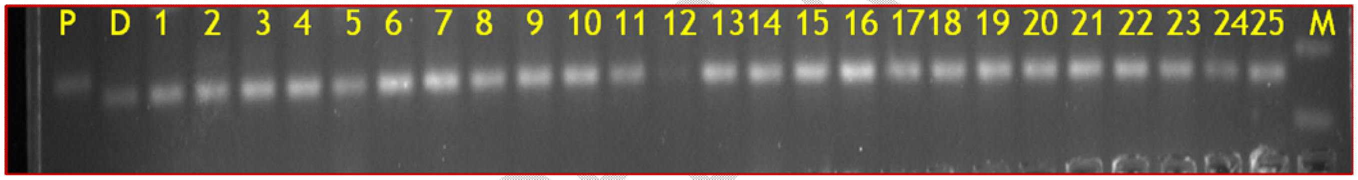
Note: Lane M: 50 bp ladder, Lane p : Recipient MTU1010, Lane D:Donar M-16-59, 1-25 are BC_1 F_3 plants

Foreground analysis for confirmation of BC_1F_3s using *Pi54 MAS* for *Pi54* gene



Note : Lane M: 50 bp ladder, Lane p : Recipient MTU1010, Lane D:Donar M-16-59, 1-25 are BC1 F3 plants

Fig. 12. Foreground analysis for confirmation of BC_1F_3s using RM224 for *Pi1* gene



Note : Lane M: 50 bp ladder, Lane p : Recipient MTU1010, Lane D:Donar M-16-59, 1-25 are BC_1 F_3 plants

Fig. 13. Field level screening of BC_1F_3 progenies against BB resistance with IIRR isolate *(DX-020)*. Arrows indicates the plant showing


MTU1010
GPP2
BC_1F_3 – 198 -52
BR 158
BR 160

Fig. 14. Blast nursery screening of BC_1F_3 progenies at IIRR, Rajendranagar, Hyderabad

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