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Marker assisted introgression of bacterial blight resistance in Ranjit, a popular rice variety of Assam

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Abstract
Bacterial Leaf Blight (BLB) is one of the devastating diseases of rice. BLB causes grain yield reduction upto the tune of 20-50%. *Xanthomonas oryxae pv.oryzae* is the pathogen responsible for BLB. In this study, the necessary BLB resistance genes was identified to be deployed in the popular variety, Ranjit. The genes required are *xa13* and *Xa21*. For these, the donor variety Improved Samba Mahsuri (ISM) was selected as there is similarity in genealogy with the variety Ranjit. By using ISM we can reduce the linkage drag. Further, identified the marker *xa13* promoter for the gene *xa13* and pTA248 for the gene *Xa21* for foreground selection. Screened 150 markers for background selection and identified 61 polymorphic markers to carry out background selection.

Keywords: Bacterial Leaf Blight, *Xa4, xa5, xa13 and Xa21* genes, foreground selection, background selection

Introduction
Rice is one of the most staple cereal foods for over half of the world's population. Among the rice growing countries in the world, India has the largest area under rice cultivation and ranks second in production next to China. Asia occupies an area of 137 m ha of rice cultivation wherein India has a lion's share of 44.6 m ha (23.3% of gross cropped area of the country) with an annual production of 106.1 million tonnes and contributes 25% to agricultural GDP (IIRR, 2015) with an average productivity of 23.80 q/ha. However, the production of rice is constrained by various fungal, bacterial and viral diseases. Bacterial Leaf Blight caused by *Xanthomonas Oryzae. pv.oryzae,* is a destructive disease of Rice. BLB disease can cause yield loss typically ranging from 20-30% but in severe cases, it can cause as high as 80% yield reduction. However, it depends on rice growth stages, geographic locations or seasonal conditions (Ou, 1985; Singh *et al*., 2007) [5]. In Assam and NE India, rice is the principal food crop. More than 90% population of the region depends on rice for the caloric requirement. BLB was not a major constrain in Assam upto 20th century. However, with higher adaptation of high yielding and hybrid varieties along with higher application of nitrogenous fertilizers, BLB is also causing a serious concern of our state. The most popular rice variety of our region – Ranjit is also becoming highly infested by the disease. However, the gene conferring its resistance to BLB has not been identified yet. Therefore to combat this disease and increase the productivity of the rice cultivation, the introgression of resistance genes of BLB in the popular rice varieties of Assam is of utmost importance. A number of rice genes have been identified along with its closely linked molecular markers that confer resistance against *X. oryzae pv.oryzae*. Out of the resistant genes - *Xa4, xa5, xa13* and *Xa 21* have been introgressed successfully in different combinations in the background of popular rice varieties of our country. For the introgression of the resistant gene, identification of parental polymorphism between the recipient variety and the donor is very important.

Materials and method
Plant materials: To conduct the experiment for identification of the polymorphic markers for BLB, two donor varieties *viz*., Ranjit and Improved Samba Mahsuri were considered to see the resistance ability of the above variety against BLB pathogen. The varieties were transplanted during kharif season at RARS, Titabar, AAU. At the age of maximum tillering and booting stage, plants were inoculated with BLB pathogen following Clip inoculation method. Leaf samples of these varieties were taken for DNA extraction and PCR analysis.

Genomic DNA of all the parents and F1 lines were extracted from the fresh leaf samples. The leaf samples of 10 cm length were collected from tender shoots of 15-30 days old seedlings. The DNA was extracted by following Dellaporta protocol as described by Dellaporta *et al*. (1983) [4] with minor modifications. The quality of DNA was checked by agarose gel electrophoresis and quantified by Nanodrop Spectrophotometer. Quality and quantity of genomic DNA was estimated based on agarose gel electrophoresis and spectral analysis respectively. The isolated genomic DNA of each genotype was subjected to agarose gel electrophoresis, as described below, to judge their integrity

The glass casting plate, surround and comb were washed thoroughly with distilled water and then subsequently with ethanol. Agarose gel of 0.8 % strength was prepared by melting 0.8 gm of agarose in total volume of 100 ml 1X TBE by heating, and 10µl of ethidium bromide (10 mg/ml) was added, the solution was cooled down to 50°C. The mixture was poured into a leveled pre-set casting tray, fitted with clean comb and surround. After solidification, the comb and gel casting assembly were detached from the casting tray. The casting tray with the gel of approximately 10 cm length was mounted on a submarine electrophoresis chamber (BIO-RAD, USA). 4 µl of DNA of each sample were mixed with 2 µl of 6X loading buffer.

The DNA samples were loaded onto the wells of the gel. Lambda uncut DNA (330 ng/µl) was also loaded in one of the well to get an approximate estimate of the yield of DNA for each genotype. The lid was put over the tank and electrode was connected to the power supply unit, keeping the positive terminal away from the wells. The power supply was put on and the gel was run at 50 V (5 volts/cm). The run was stopped when bromophenol blue dye front reached 2/3rd of the gel length.

A photograph of the gel under the UV light was taken digitally in a gel documentation system (UVP, UK) to enable estimation of quantity of each sample by using LABWARE software. The DNA sample with strong band under the UV with minimum shearing was considered as good quality DNA for the present study.

Results and discussion

Identification of Polymorphic Marker for foreground selection

In the present study, functional and linked SSR markers were screened and identified the polymorphic markers for foreground selection. The polymorphism survey was carried out for the donor (ISM) and recurrent parent (Ranjit) for the target *genes Xa4*,*xa5*,*xa13* and *Xa21* using the markers RM144, Npb181 for *Xa4*, RM122 for *xa5*, xa13 promoter for *xa13*, pTa248- STS for *Xa21* for their use in foreground selection in the marker assisted backcross program.

Polymorphism in the PCR products was detected after electrophoresis. The PCR marker linked to *xa13, Xa21* and *Xa4* did not detect any banding pattern of resistance as ISM in both Ranjit and Ranjit Sub-1 and these two varieties did not have the resistance genes *xa13*, *Xa21* and *Xa4*, respectively. Amplification of genomic DNA of both Ranjit and Ranjit Sub-1 using marker linked to xa5 showed the same banding pattern as ISM conferring the presence of xa5 resistant gene in both the rice varieties. However, only the presence of xa5 is not sufficient to confer resistance to the pathovar available at Titabar, Assam. Therefore, it is essential to introgress more numbers of resistance gene in the popular variety, Ranjit to combat BLB.

Foreground selection for BLB resistant genes *Xa4, xa5, xa13* and*Xa21.*

Xa4: This marker *Xa4* linked with chromosome 4 produced a total of 2 bands all of which were polymorphic showing an absolute polymorphism percentage of 100 percent and the bands size ranged of 150 bp.

xa5: This marker locus linked with chromosome 5 produced a single monomorphic band and no polymorphism bands were produced. The band size was of the range from 240 bp.

xa13: This marker *xa13* linked with chromosome 8 produced a total of 2 bands all of which were polymorphic showing an absolute polymorphism percentage of 100 percent and the bands size ranged of 500 bp.

Xa21: This marker Xa21 linked with chromosome 11 produced a total of 2 bands all of which were polymorphic showing an absolute polymorphism percentage of 100 percent and the bands size ranged of 900 bp to 1000 bp.

Genotyping of parents for background selection of parents

In the present study, 150 SSR markers were used to study the SSR variation in Ranjit Sub-1 and ISM. Out of those primers, 61 primers were found to be polymorphic. The DNA amplification profile of 61 polymorphic primers used in the present study has been summarized and some representative gel picture of screening of rice genotypes with SSR markers are given in Plates 1 to 8.

RM 335: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 102 bp.

RM231: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 180bp.

RM304: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range is 160 bp.

RM327: This marker RM 518 is linked with Chromosome 2 that produced polymorphic bands with a polymorphism percentage of 100 per cent. The size range is 210 bp.

RM340: This marker locus produced a polymorphic bands were produced. The band size was of range of 210 bp.

RM204: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 160 bp.

RM 207: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 150 bp.

RM 31: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 150 bp.

RM 206: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 200 bp.

RM 169: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 220 bp.

RM 235: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 150 bp.

RM 517: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 260 bp.

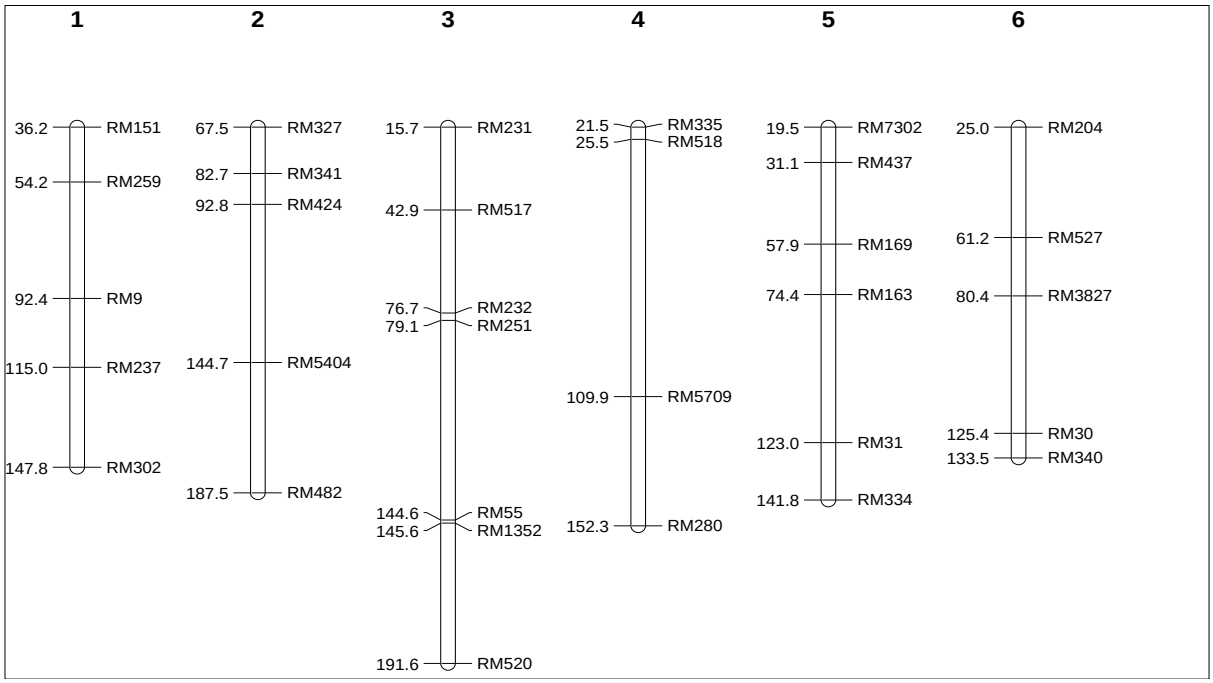
RM 21: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 160 bp.

RM 8300: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 210 bp.
RM 324: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 300 bp.
RM 171: Two polymorphic bands were produced. The band size was of the range 320 bp.
RM 316: Two polymorphic bands were produced. The band size was of the range 200 bp
RM 1352: Two polymorphic bands were produced. The band size was of the range 220 bp.
RM 408: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 130 bp.
RM 280: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 155 bp.
RM 520: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 280 bp.
RM 149: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 260 bp.
RM 30: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 110 bp.
RM 19: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 220 bp.
RM 527: This marker produced 2 polymorphic bands and no monomorphic bands with absolute polymorphism percentage of 100 per cent, the band size range from 220 bp.
RM 167: This marker produced two polymorphic markers, size range is 120 bp.
RM 1235: This marker produced two polymorphic markers, size range is 120 bp.
RM 172: This marker produced two polymorphic markers, size range is 160 bp.
RM 237: This marker produced two polymorphic markers, size range is 130 bp.
RM 50: This marker produced two polymorphic markers, size range is 150 bp.
RM 518: This marker RM 518 is linked with Chromosome 4 that produced polymorphic bands with a polymorphism percentage of 100 per cent. The size range is 170 bp.
RM 424: This marker produced two polymorphic markers, size range is 240 bp.
RM 482: This marker produced two polymorphic markers, size range is 180 bp
RM 281: This marker RM 518 is linked with Chromosome 8 that produced polymorphic bands with a polymorphism percentage of 100 per cent. The size range is 140 bp.
RM341: This marker RM 518 is linked with Chromosome 2 that produced polymorphic bands with a polymorphism percentage of 100 per cent. The size range is 172 bp.
RM 334: This marker locus produced polymorphism and the band size is180bp.
RM 55: The primer amplified two fragments and all were polymorphic (100% polymorphism).The product size is 220 bp.
RM 19629: Two amplified fragments was observed for this primer and all were polymorphic (100% polymorphism) and the band size range is 320 bp.
RM 335: This marker locus produced polymorphism and the band size is 104 bp
RM 152: The primer amplified two fragments and all were polymorphic (100% polymorphism).The product size is 150 bp.
RM 259: The primer amplified two fragments and all were polymorphic (100% polymorphism).The product size is 162 bp.
RM 216: Two amplified fragments was observed for this primer and all were polymorphic (100% polymorphism) and the band size range is 146 bp.

Linkage Map

Using polymorphic bands generated by SSR markers, linkage map was developed.

Linkage map was constructed using Map Chart Software



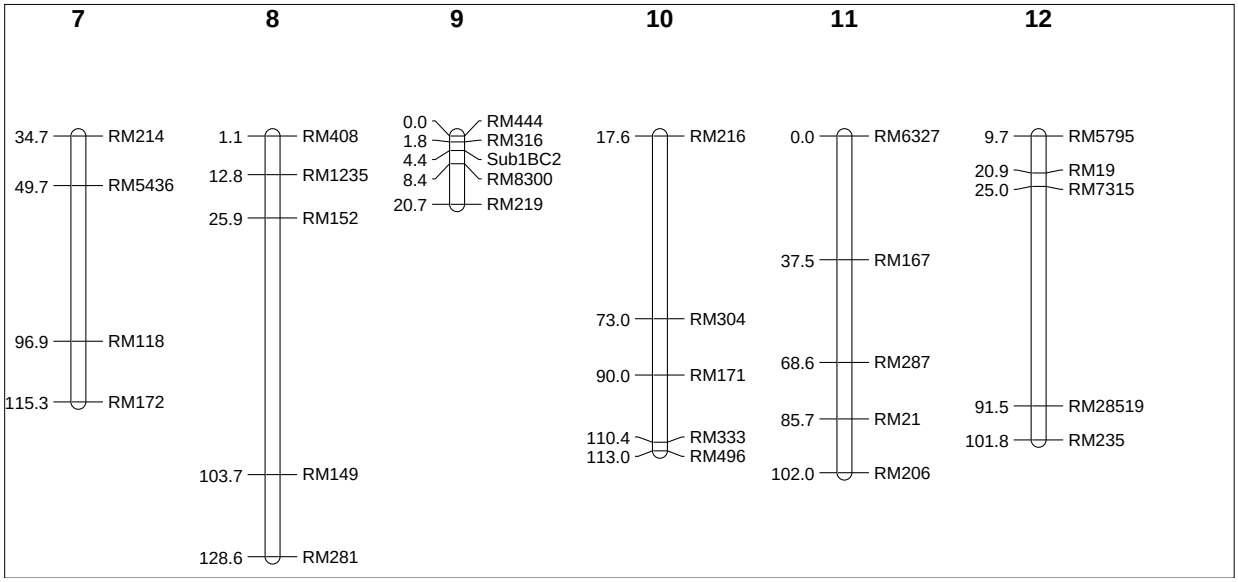


Fig 1: Linkage Map showing polymorphism in each linkage group.

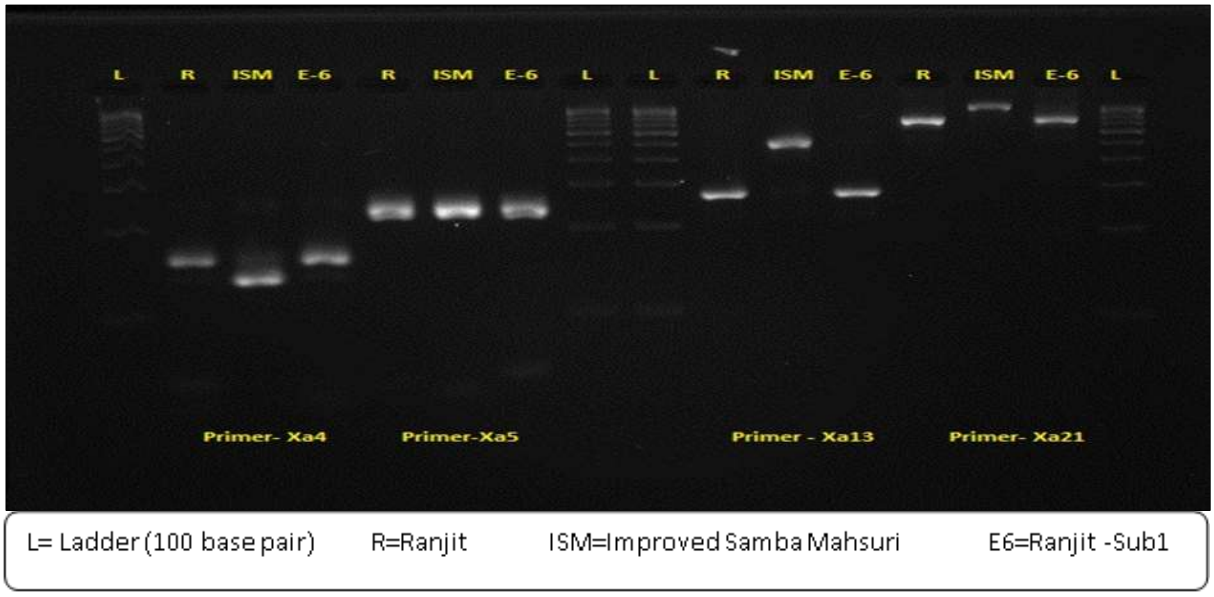


Plate 1: PCR products of Ranjit(R), Improved Samba Mahsuri(ISM), E-6(Ranjit-sub1) using *Xa4,xa5,xa13 and Xa21*.

Plate 2: PCR products of Ranjit- Sub 1 and ISM showing similar banding pattern of 240 bp using xa5.

Plate 3: Amplification with RM302, RM335, RM5709, RM231, RM304, RM327 in sample of Ranjit- Sub1 and ISM.

Plate 4: Amplification with RM 340, RM204, RM207, RM31, RM206, RM169, RM235in sample of Ranjit -Sub1 and ISM.

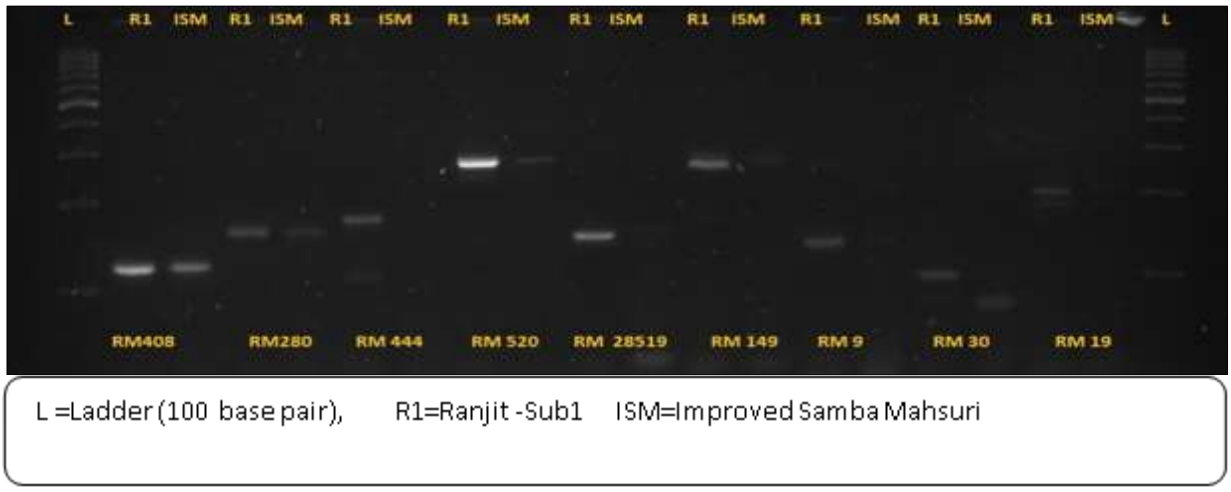


Plate 5: Amplification with RM408, RM280, RM444, RM520, RM28519, RM149, RM 9, RM 30, RM 19 in sample of Ranjit- Sub1 and ISM.

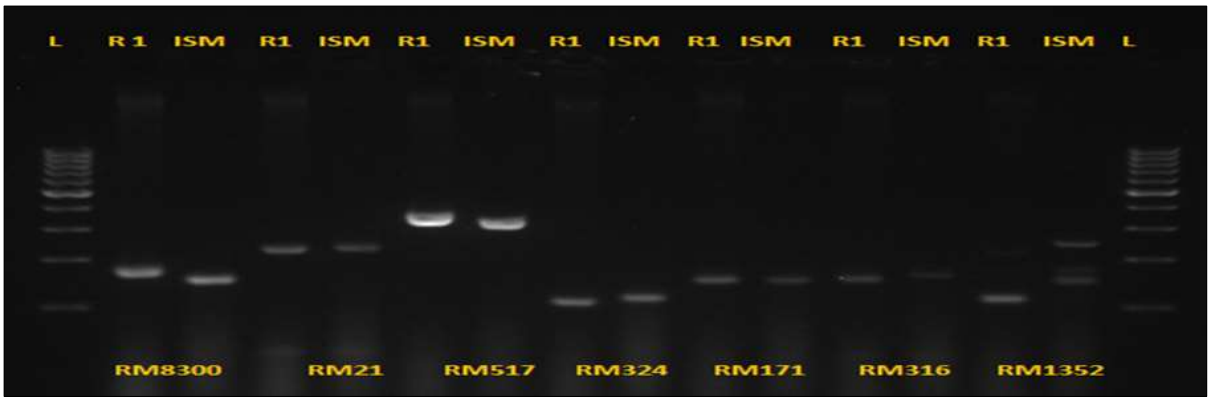


Plate 6: Amplification with RM8300, RM21, RM517, RM324, RM171, RM316, RM 1352 in sample Ranjit -Sub1 and ISM.

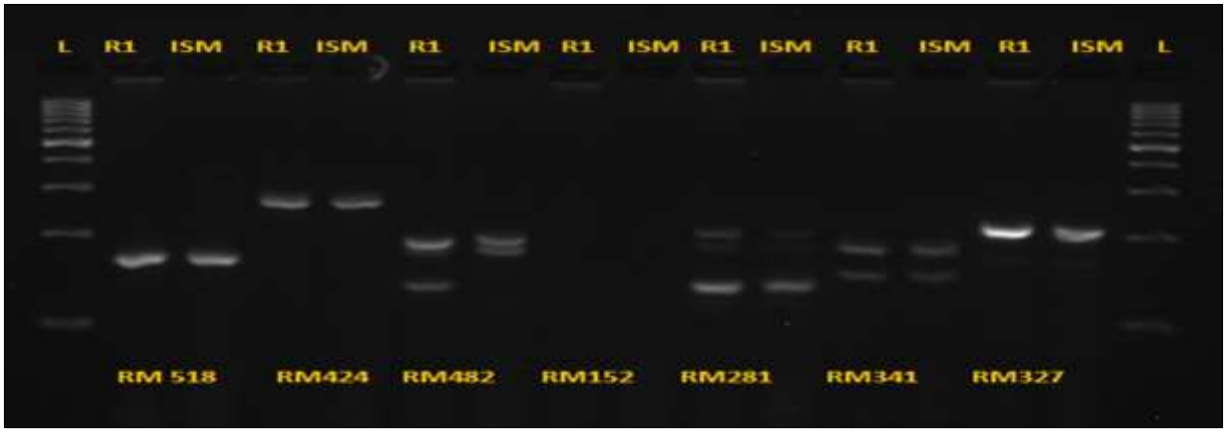


Plate 7: Amplification with RM518, RM424, RM482, RM152, RM281, RM341, RM327 in sample Ranjit -Sub1 and ISM.

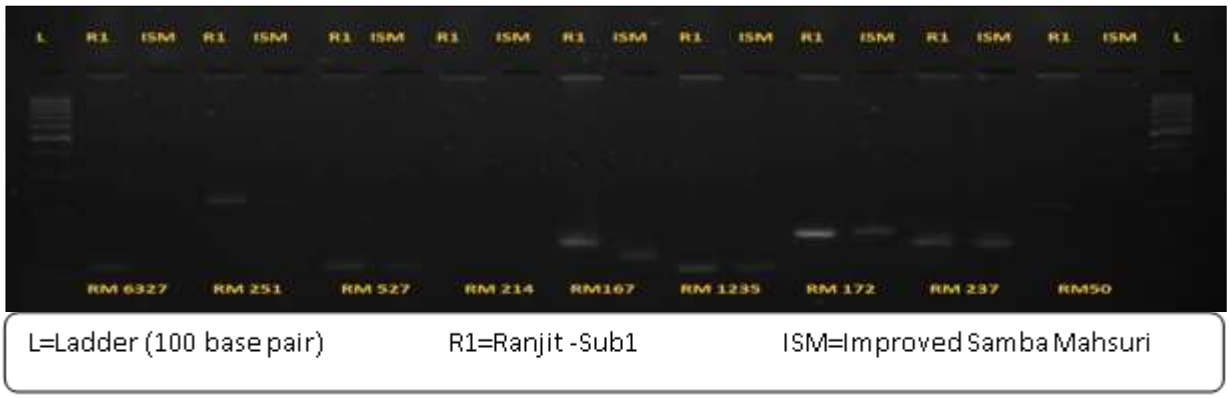


Plate 8: Amplification with RM 6327, RM251, RM527, RM 214, RM 167, RM 1235, RM 172, RM 237, RM 50 in sample Ranjit -Sub1 and ISM.

Conclusion

Introgression of resistance genes *xa5, xa13* and *Xa21* in Ranjit-Sub 1 will impart BLB resistance for the prevalent pathotypes in Assam. So, deployment of these genes will be sufficient for BLB resistance in Ranjit background. For xa5, Ranjit Sub 1 and ISM is showing similar banding pattern, the presence of xa5 gene. This may be due to the presence of xa5 in Ranjit sub 1.Howevever it needs further confirmation. Identified the marker xa13 promoter for the gene *xa13* and pTA248 for the gene *Xa21* for foreground selection. Screened 150 markers for background selection and identified 61 polymorphic markers to carry out background selection. The highest 17.21 % polymorphism was observed for the chromosome number 3, while, the lowest 9.8% was observed in chromosome number 7 which were sufficient to recover the whole chromosome content of recurrent parent.

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