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# Marker Assisted Selection for Sheath Blight, Blast and Bacterial Blight Resistance in Two Popular Rice Varieties

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**Major biotic stresses that affects rice production are sheath blight (ShB) caused by *Rhizoctonia solani* Kuhn, blast disease caused by fungal pathogen *Magnaporthe oryzae* and bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae*. To reduce the yield loss pyramiding of resistance (R) genes in an elite rice variety is the most effective method. Two high yielding rice varieties namely, ASD16 and ADT43 are extensively grown in South India. These varieties are susceptible to blast, bacterial blight and sheath blight diseases. Hence this study aims to improve these disease susceptible varieties to achieve higher yield in disease prone areas. Improved ASD16 (CB14004) harbouring BB and blast and improved ADT43 (CB14002) harbouring BB resistance genes were used in the present marker assisted backcross breeding program to introgress three sheath blight resistant QTLs (*qSBR 11-1, qSBR 11-2* and *qSBR 7-1*). In every backcross generation, PCR-based markers (RM224, RM209 and RM336), linked with sheath blight resistance QTLs, bacterial blight resistance genes (*xa5, xa13 and Xa21*) and blast resistance genes (*Pi54*) were utilized for foreground selection. In addition, a set of forty eight (CB14002 x tetep) and forty one (CB14002) parental polymorphic SSR markers were used for background selection and backcrossing was carried out until $BC_3$ generation. Background selection studies using polymorphic markers revealed that, an average recovery of 91.15% and the maximum of 92.68% recurrent parent genome in CB14004 x Tetep cross population. In CB14002 x Tetep an average of 90.20% and maximum of 92.70% has been recovered. These plants will be selfed to generate large number of segregating progenies to identify agronomically superior with good grain quality traits and multiple disease resistance lines in rice.**

**Key words:** Sheath blight, Bacterial blight, Blast, QTLs, R genes, Marker assisted selection, Tetep, CB14002, CB14002

The increase in rice production in India is primarily contributed by adoption of early high yielding semi-dwarf rice varieties with profuse tillering and short stature coupled with improved cultural technologies, which is associated with change from one rice crop season to two or even three crops seasons annually. Such extensive and intensive cultivation systems aggravates a shift in pest and disease problems of rice. Moreover compared with world average, rice yield in India is very low. One of the reasons for the low productivity in rice are various biotic factors affecting the crop. Among them, certain diseases attain epidemic proportion and cause serious crop losses. The diseases such as sheath blight, blast and bacterial blight are the most destructive and damaging (Singh *et al.*, 2014).

Sheath blight, second most destructive fungal disease of rice after blast caused by Rhizoctonia solani Kuhn is one of the major threat of rice growers in India. Though cultural, biological and chemical control methods are available, they are not a feasible method towards sheath blight infection because of the wide host range of the fungal pathogen and its agressiveness. The only effective way is to develop resistant cultivars through molecular breeding approach. ShB resistance in rice is typically quantitative trait controlled by polygenes (Zuo *et al.*, 2014). In rice, across all the 12 chromosomes a total of 50 ShB QTLs have been detected. Till date no rice lines with complete resistance to ShB have been reported. But some rice varieties like Tetep, Taduakan, Teqing, Jasmine etc. shows partial or moderate resistance to ShB. qSBR 11-1 is one of the major QTL providing resistance to ShB and it contains 11 tandem repeats with open reading frame encoding for proteins structurally similar to the plants class III chitinases, and those proteins believed to give resistance against ShB (Channamallikarjuna *et al.*, 2010). Over the past two eras, several ShB resistant QTLs have been mapped. Among these, three QTLs (qSBR 11-1, qSBR 11-2 and qSBR 7-1) mapped in Tetep were found consistently associated with ShB resistance across location and years (Channamallikarjuna *et al.*, 2010). Because of the unavailability of complete ShB resistant source, researches on ShB resistance were less. Hence, this study will add value to the science and farmer in terms of multiple disease resistance rice varieties. Through QTL mapping, Channamallikarjuna *et al*. (2010) identified eight QTLs that were mapped on

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six chromosomes (1, 3, 7, 8, 9 and 11) in Tetep under two locations and validated all the eight QTLs and selected three QTLs (qSBR 11-1, qSBR 11-2 and qSBR 7-1) for introgression.

In many countries, rice blast is the most destructive fungal disease caused by *Magnoporthae oryzae.* About the blast epidemics, its yield loss starts from 10-20% in regular seasons and as high as 100% yield loss in years. Introgression of single gene in the elite cultivars would lead to the development of new resistant rice genotypes and thereby the reduction in disease infestation. So far 100 blast resistant genes have been mapped and out of that 35 of them have been molecularly cloned, of which some R genes are *Pib, Pita, Pid2, Pi9, Piz-t, Pi36* and *Pi37* (Wang and Valent, 2017) . On chromosome 11 of *Oryza sativa* ssp. indica cultivar Tetep unique R-gene (Pi-$k^h$) was reported and which found to confer high degree of resistance to diverse isolates of *M. oryzae* (Rai *et al.*, 2011).

*Xanthomonas oryzae pv. oryzae (Xoo)* is one of the most devastating bacterium infecting rice crop and cause bacterial blight (BB). BB affects the entire rice acreages and causes serious yield losses depending on the cultivar susceptibility, environmental conditions, and stage of the crop. Severe infection at maximum tillering stage results in blighting of leaves, which ultimately causes significant yield losses in severely infected fields ranging from 20 to 30%, but this can reach up to 80% and grain quality also severely affected. Availability of gene-specific molecular markers linked to the resistance genes makes easy detection of plants with two or more R-genes (Singh *et al.,* 2001; Sundaram *et al.,* 2008). To date a total of 38 resistance gene has been identified against a range of strains of *Xoo*. It includes 11 recessive R genes *viz., xa5, xa5(t), xa8, xa13, xa15, xa19, xa20, xa24, xa28 (t), xa31 and xa32* and 27 dominant R genes *viz.Xa1, Xa2, Xa3, Xa4, Xa7, Xa10, Xa11, X12, Xa14, Xa16, Xa17, Xa18, Xa21, Xa22 (t), Xa23, Xa25 (t), Xa26, Xa27, Xa29, Xa30, Xa30 (t), Xa31 (t), Xa32 (t), Xa34, Xa35 (t), Xa36 (t), Xa38* (Baliyan *et al.,* 2018).

The disease resistance genes are successfully transferred into agronomically outstanding cultivars through marker assisted selection (Singh *et al.,* 2001; Sundaram *et al.,* 2008). Marker assisted gene pyramiding for major genes with high RpG recovery in order to maintain the original qualities of the elite cultivar could be an effective approach for rice improvement (Singh *et al.,* 2001; Sundaram *et al.*, 2008). MAS technology is very advantageous as compared to the conventional phenotyping (Collard and Mackill, 2008; Xu and Crouch, 2008). Functional markers play a major role in molecular breeding for host plant resistance to enhance the accuracy and precision for marker assisted selection of target gene(s) with minimum time, cost and effort (Ramkumar *et al.*, 2011). Keeping these points in view the present investigation was carried out to develop two multiple disease resistant rice genotypes through marker assisted back cross breeding.

## Material and Methods

### *Plant materials*

The experimental material consisted of Near isogenic lines (NILs) of two popular rice varieties. CB14004 (Improved ASD16), a high yielding bacterial blight and blast pyramided line, and CB14002 (Improved ADT43), a high yielding bacterial blight resistant lines developed in Department of Plant Biotechnology, Coimbatore (Perumalsamy *et al.*, 2010), but the improved CB lines found susceptible to sheath blight. Both the recurrent parents are advanced backcross progenies ($BC_3F_7$) of popular rice varieties ASD16 and ADT43, respectively. CB14004 shows stable resistance against BB and blast and CB14002 shows stable resistance against BB (Perumalsamy *et al.*, 2010). Tetep, a Vietnamese indica rice variety was used as the donor to introgress blast resistance gene *Pi54* as well as sheath blight QTLs, *qSBR7-1, qSBR11-1,* and *qSBR11-2.* The outline of the backcross breeding program is given in Figure 1.

### *DNA extraction*

The young leaves from 20-25 days old seedling were collected. Total genomic DNA was extracted using modified CTAB (Cetyl trimethyl ammonium bromide) method as described by Doyle and Doyle (1987). Isolated DNA was diluted with double distilled water and stored at -30ºC for subsequent analysis.

### *Pathological screening for sheath blight resistance in parents*

The parental lines CB14004, CB14002, Tetep, ASD16, ADT43, and IRBB60 were transplanted in to separate pots and kept under controlled condition. For artificial disease screening the method developed by Bhaktavatsalam *et al.* (1978) was adopted. Pathogen was multiplied in autoclaved stem pieces (2-3 inches in length) of water sedge (*Typha angustata*) soaked with 1% peptone solution for 8-10 days. Four to five stem bits colonized with fungal mycelia (and sclerotia) were then placed in between the tillers in the central region of the hill 5-10 cm above the water line and tied with rubber band to maintain high humidity in the microclimate. The observations were recorded and the entries were scored after 25 days after artificial inoculation, adopting the IRRI SES scale (IRRI, 2002).

### *PCR for foreground and background selection*

Diluted 40 ng/µl DNA template was used for the downstream process. We used DNA markers for conformation of ShB resistant QTLs and gene specific markers for BB and blast resistant genes in each generation (Table 1). PCR was carried out in a thermo cycler (Eppendorf) and PCR amplification conditions for ShB QTLs is 94ºC for 4 min, followed by 35 cycles of 94ºC for 1 min, 55ºC for 1 min, 72ºC for 1 min and 72ºC for 7 min; for *Pi54* is 94ºC for 5 min, followed by 35 cycles of 94ºC for 1 min, 56ºC for 1 min, 72ºC for 1 min and 72ºC for 7 min; *xa13*

is 94ºC for 5 min, followed by 35 cycles of 94ºC for 1 min, 59ºC for 1 min, 72ºC for 90 sec and 72ºC for 7 min; *Xa21* is 94ºC for 5 min, followed by 35 cycles of 94ºC for 45 sec, 65ºC for 1 min, 72ºC for 90 sec and 72ºC for 7 min and *xa5* is 94ºC for 5 min, followed by 35 cycles of 94ºC for 1 min, 56ºC for 1 min, 72ºC for 90 sec and 72ºC for 7 min. To find *xa5* allelic pattern 10µl of PCR product were taken and incubated at 65ºC for 4 hr for restriction digestion using BSRI enzyme. To confirm ShB QTLs the PCR product was loaded on 3.5% agarose gel and BB and blast PCR products were separated on 1.5% agarose gel.

Marker assisted background selection in early backcross generations has been advocated for quick recovery of the RPG (Joseph *et al.*, 2004). In order to estimate the recovery of recurrent parent genome, background selection was done using SSR markers. For background selection, one hundred and fifty SSR markers were selected from gramene database (www.gramene.org) and polymorphic survey were carried out. Using SSR markers the polymorphism between donor parent (Tetep) and recurrent parents (CB14002 and CB14004) was identified. Markers were selected in such a way that it has to cover the entire 12 chromosomes. Forty eight markers were found to be polymorphic between Tetep and CB14002 and forty one markers were found to be polymorphic between Tetep and CB14004. List of polymorphic markers are given in Table 2a and 2b.

## Results and Discussion

### *Confirmation of resistance of parents through pathological screening*

In the present study, typha bits method (Bhaktavatsalam *et al.*, 1978) was followed with virulent, pure *Rhizoctonia solani* Coimbatore isolate. Relative lesion height (RLH) was recorded after 25 days of inoculation to classify the parental lines as resistant and susceptible (Table 3). Results of the artificial pathological screening shows that the donor parent, Tetep having moderate resistance and all other genotypes shows susceptible reaction to the sheath blight (Figure 2). According to Channamallikarjuna *et al*. (2010) also Tetep is having moderate resistance.

**Table 1. Details of the molecular markers used for marker-assisted backcross breeding**

| Target trait | Gene/ QTL | Marker | Primer sequence(5'-3') | Enzyme | AT (°C) | Chr | Product size(bp) | Reference |
|---|---|---|---|---|---|---|---|---|
| Bacterial Blight resistance | *xa5* | xa5 F | **F**-CGGATAGCAGCATTTCCAAGAG | BsrI | 56 | 5 | 299 | Iyer-Pascuzzi and Mccouch (2007) |
| | | xa5 R | **R**-GATTCCTTTAGCAAGGTGTG | | | | | |
| | xa13 | xa13 F | **F**-GAGCTCCAGCTCTCCAAATG | - | 59 | 8 | 500 | Chu *et al.* (2006) |
| | | xa13 R | **R**-GGCCATGGCTCAGTGTTTAT | | | | | |
| | *Xa21* | pTA248 | **F**-ATAGCTAGTTCATAGAGG<br>**R**-ACATCCGTCACTCTGCCA | - | 65 | 11 | 900 | Perumalsamy *et al.* (2010) |
| Blast resistance | *Pi54* | Pi54 MAS | **F**-CAATCTCCAAAGTTTTCAGG<br>**R**-GCTTCAATCACTGCTAGACC | - | 65 | 11 | 216 | Ramkumar *et al.* (2011) |
| | *qSBR11-1* | RM224 | **F**-ATCGATCGATCTTCACGAGG<br>**R**-: TGCTATAAAAGGCATTCGGG | - | 55 | 11 | 130 | Channamallikarjuna *et al.* (2010) |
| Sheath blight resistance | qSBR11-2 | RM209 | **F-** ATATGAGTTGCTGTCGTGCG<br>**R-** CAACTTGCATCCTCCCCTCC | - | 55 | 11 | 150 | Channamallikarjuna *et al.* (2010) |
| | qSBR7-1 | RM336 | **F-** CTTACAGAGAAACGGCATCG<br>**R**- GCTGGTTTGTTTCAGGTTCG | - | 55 | 7 | 200 | Channamallikarjuna *et al.* (2010) |

### *Confirmation of pyramided genes in CB14004 and CB14002*

CB14004 with both bacterial blight and blast resistance genes and CB14002 with bacterial blight resistance were developed through marker assisted selection by Perumalsamy *et al.* (2010). Polymerase chain reaction using functional markers for *xa5, xa13, Xa21* and *Pi54* were used to found out homozygosity of the above recipient parents. As expected, the banding pattern of the pyramided lines were found similar to that of resistant parents, IRBB60 for bacterial blight and Tetep for blast resistant gene. The plants homozygous for all the four genes were used as the recurrent parent for the backcrossing. The results are in line with Perumalsamy *et al.* (2010) results.

### *Marker assisted foreground selection*

DNA markers linked to the ShB QTLs RM224, RM209 and RM336 were used for foreground selection in F1 to BC3F1 generation. Ten plants out of ninety eight were found to be heterozygous for all three SSR markers (RM224, RM209 and RM336) in BC1F1 progenies from the background of CB14002 and out of twenty four BC1F1 plants, eight plants shows heterozygous for RM209. In BC2F1, out of

**Table 2a. List of polymorphic SSR markers used for background selection in CB14002 X Tetep cross**

| Chromosome locus | Number of polymorphic markers | Polymorphic markers |
|---|---|---|
| 1 | 4 | RM84, RM8051, RM493, RM488 |
| 2 | 4 | RM154, RM555, RM6165, RM3316 |
| 3 | 3 | RM520, RM545, RM6283 |
| 4 | 3 | RM5749, RM252, RM3843 |
| 5 | 5 | RM159, RM267, RM5140, RM163, RM538 |
| 6 | 4 | RM585, RM276, RM3183, RM461 |
| 7 | 5 | RM6697, RM473A, RM432, RM234, RM248 |
| 8 | 5 | RM310, RM6999, RM25, RM404, RM2339 |
| 9 | 4 | RM23788, RM24386, RM41, RM729 |
| 10 | 2 | RM474, RM3773 |
| 11 | 5 | RM26334, RM332, RM21, RM1233, RM144 |
| 12 | 4 | RM1302, RM28048, RM19, RM3813 |

thirty plants seven plants of CB14002 background were showing heterozygous bands for all the three QTLs and out of fifteen plants four plants of CB14004 background showing heterozygous bands for RM209.

In BC3F1, out of sixty six plants five plants of CB14002 background were showing heterozygous bands for all the three QTLs out of fifty six plants four plants of CB14004 background showing heterozygous

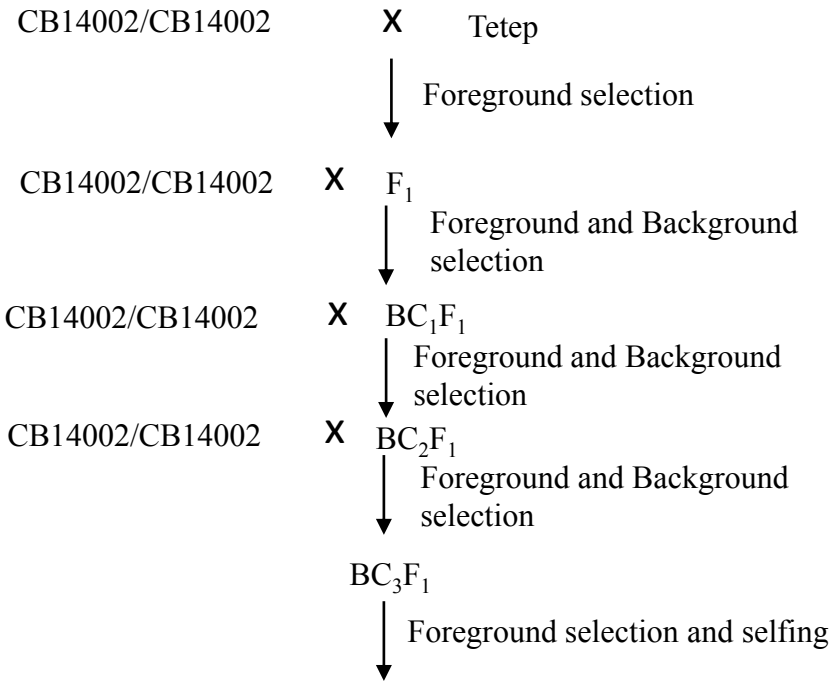


**Fig. 1. Outline of crossing scheme for development of backcross progenies**

bands for RM209. The identified heterozygous plants for all the QTLs was also confirmed for the presence of blast and bacterial blight in each generation (Figure 3).

***Marker assisted background selection***

Background selection was started from BC1F1 to BC3F1 generation and in each step genotype possessing highest genome content of the recipient parent was selected to hybridize for next backcross. In BC1F1 progenies of CB14002 x Tetep, three plants showed high parental genome recovery of 77.8 % (28), 75% (53) and 75% (72), respectively. In BC2F1 progenies of cross CB14002 x Tetep, plant number 10 and 12 showed highest recovery of recurrent parent genome (88.54%) and forwarded to produce next generation. In BC3F1 progenies of cross CB14002 x Tetep, plant number 49 (92.7%) and 61(91.66%) showed highest recovery of recurrent parent genome. Similarly, BC1F1 progenies of CB14004 x Tetep, plant number 6 and 17 showing the highest recurrent parent genome recovery of 76.82%. Hence, those plants were forwarded for subsequent generation. In BC2F1 of CB14004 x Tetep, plant number 2 and 4

**Table 2b. List of polymorphic SSR markers used for background selection in CB14004 X Tetep cross**

| Chromosome locus | Number of polymorphic markers | Polymorphic markers |
|---|---|---|
| 1 | 2 | RM84, RM443 |
| 2 | 4 | RM109, RM279, RM555, RM6165 |
| 3 | 3 | RM520, RM1002, RM251 |
| 4 | 2 | RM252, RM3843 |
| 5 | 5 | RM159, RM267, RM5140, RM163, RM274 |
| 6 | 3 | RM469, RM3183, RM461 |
| 7 | 4 | RM473A, RM21976, RM445, RM18 |
| 8 | 4 | RM152, RM25, RM404, RM339 |
| 9 | 4 | RM23865, RM41, RM13912, RM72 |
| 10 | 3 | RM7217, RM3773, RM228 |
| 11 | 5 | RM26334, RM332, RM287, RM21, RM254 |
| 12 | 2 | RM1302, RM19 |

shows highest recovery of recurrent parent genome recovery of 87.8% and 89.02%, respectively and forwarded to produce next generation. Similarly, plant number 34 (92.68%), 57 (91.46%) and 58 (91.46%) showed highest recovery of recurrent parent genome in BC3F1 of CB14004 x Tetep. The number of triple

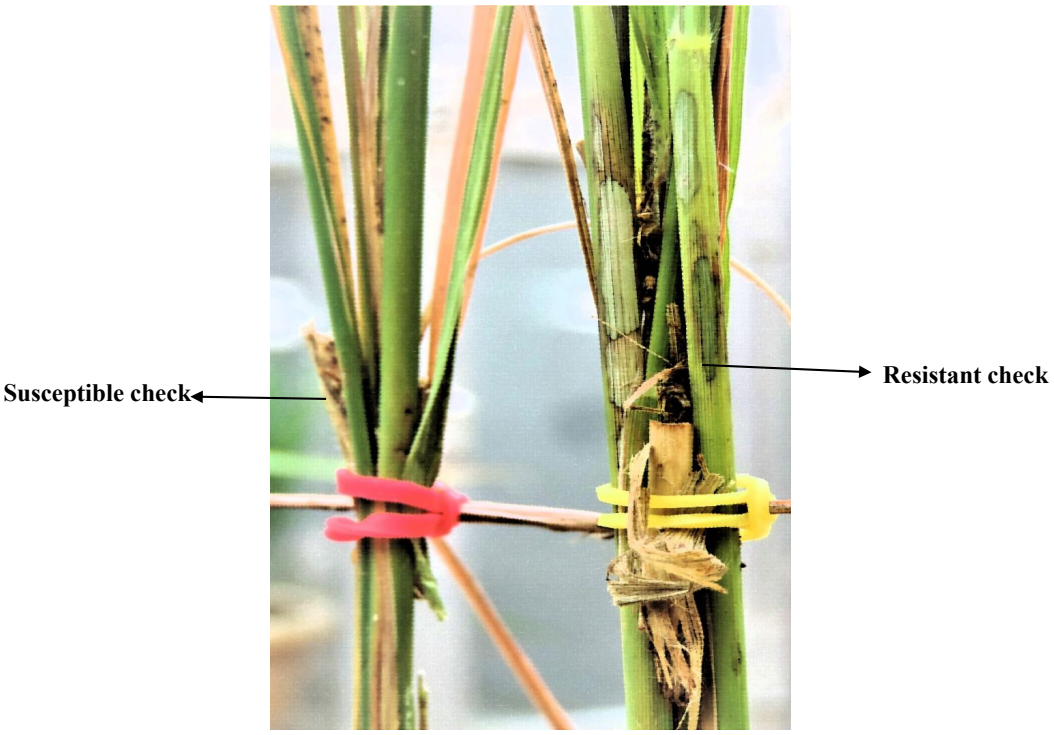


**Fig. 2. Susceptible and resistant plant reaction against sheath blight pathogen**

heterozygotes for ShB QTLs, BB and Blast resistant backcross progenies with the highest RpG recovery is given in the Table 4. Graphical representation of the RpG recovery of heterozygous progenies were done in Graphical genotype ver. 2 (GGT) (Figure 4).

A strict adoption of marker-assisted background selection in the carrier chromosomes for precise transfer of the ShB QTLs would have meant the removal of possible linkage drag. In addition to markers for foreground selection, polymorphic SSR markers were utilized for background selection in order to recover the recurrent parent genome in the shortest number of backcross generations as recommended by Hospital and Charcosset (1997) and also for estimation of the recurrent parent genome contribution at each generation of backcrossing. Sundaram *et al.* (2008) concluded that background selection with a limited number of polymorphic SSR markers in conjunction with four backcrosses is sufficient to recover the yield and quality characteristics of the recurrent parent while introgressing the target trait. In this study, we limited the number of backcrosses to only three and deployed a maximum of 48 parental polymorphic SSR markers

**Table 3. Phenotype screening for sheath blight resistance at 25 days after inoculation**

| Parents | Plant height (cm) | Lesion length (cm) | Relative lesion height (RLH) (%) | Disease scale |
|---|---|---|---|---|
| TN 1 (S) | 82 | 55.6 | 67.8 | 9 (HS) |
| Tetep | 142 | 32.9 | 23.1 | 3 (MR) |
| CB14004 | 98.4 | 46.7 | 47.45 | 7 (S) |
| CB14002 | 89.3 | 56.3 | 63.04 | 7 (S) |
| ASD16 | 94.8 | 58.2 | 61.39 | 7 (S) |
| ADT43 | 86.2 | 44.1 | 51.16 | 7 (S) |
| IRBB60 | 72 | 59.8 | 82.71 | 9 (H) |

for accelerating background genome recovery, and by the third backcross generation, we managed to identify plants possessing more than 90% recovery of CB14002 and CB14004 genome. Significantly, while carrying out background selection, we gave special emphasis deploying more polymorphic SSR markers

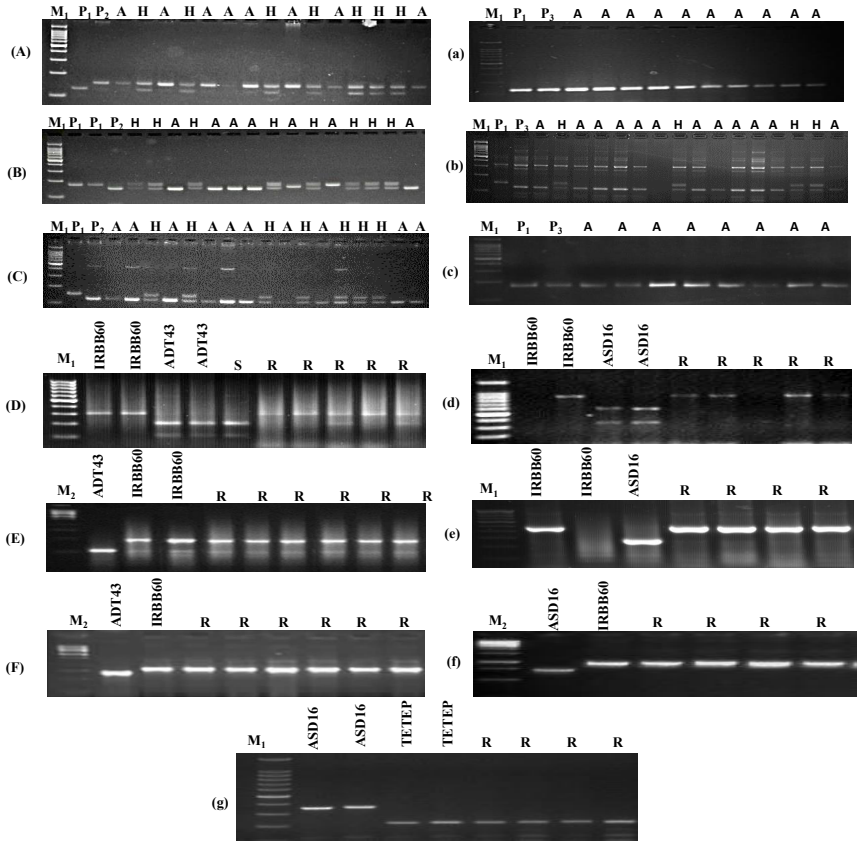


**Fig. 3. Agarose gel electrophoresis illustrating the presence of ShB QTLs (A,a) qSBR11-1, (B,b) qSBR11-2 and (C,c) qSBR7-1 and BB resistance genes (D,d) xa5., (E,e) xa13 ,(F,f) Xa21 and (g) Pi54 in BC2F1 progenies of CB14002 (right side) and CB14004 (left side). P1- Tetep, P2- CB14002, P2-CB14004, M1- 100bp ladder, M2 – 1kb ladder and IRBB60- Donor for BB resistance genes**

on chromosomes 7 and 11, on which target genes are located. This is because, the carrier chromosomes deserve special consideration in backcross programs, as they have greater selection pressure for the donor parent allele at the target QTLs (qSBR11-1, qSBR11-2 and qSBR7-1) in each backcross generation, with the rate of return to recipient genotype on target chromosomes slower than on non-carrier chromosomes (Hospital, 2001).

In this study, no positive or negative interactions were observed between genes conferring resistance against bacterial blight and blast. Similar results were found by Sundaram *et al.* (2008) and Balachiranjeevi *et al.* (2015). Singh *et al*. (2014) also reported the incorporation of multiple disease resistance including BB, rice blast and ShB in Pusa 6B and developed the new stable improved lines of the Pusa 6B. In the present study it is to be noted that, for as yet unknown

**Table 4. Number of triple QTL heterozygotes identified and estimation of recurrent parent genome contribution**

| Generation | Crosses | Total number of plants scored | No. of plants that are triple heterozygotes in CB14002 and heterozygotes for RM209 in CB14004[a, b] | Estimated maximum % contribution of recurrent parent genome to selected[c] | Expected % contribution of recurrent parent genome to selected backcross plant[d] |
|---|---|---|---|---|---|
| $BC_1F_1$ | CB14004 X Tetep | 24 | 8 | 76.82 | 75 |
| | CB14002 X Tetep | 98 | 10 | 77.8 | |
| $BC_2F_1$ | CB14004 X Tetep | 15 | 4 | 89.02 | 87.5 |
| | CB14002 X Tetep | 30 | 7 | 88.54 | |
| $BC_3F_1$ | CB14004 X Tetep | 56 | 4 | 92.68 | 93.754 |
| | CB14002 X Tetep | 66 | 5 | 92.7 | |

**a** At each backcross generation, genomic DNA was isolated from derivative lines and genotyping was performed using primers that are linked to ShB QTLs as described in Materials and methods ; **b** At each backcross generation, fewer than expected triple heterozygotes were obtained. This is due to the fact that some of the putative backcross progeny were obtained by inadvertent selfing of CB14004 and CB14002 (the female parents in these backcrosses) ; **c** At each backcross generation, genomic DNA was isolated from derivative lines that are triple heterozygotes for 'ShB QTLs linked DNA markers. Microsatellite markers that are polymorphic between the parental lines were then used, as described in Materials and Methods, to identify the plant with maximum recurrent parent genome contribution ; **d** As per Mendelian ratios for independent gene action

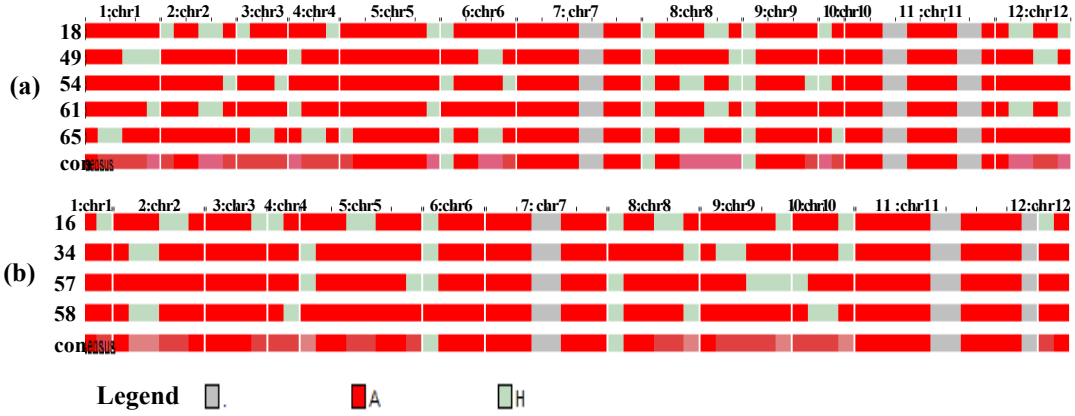


**Fig. 4. Graphical representation of background screening in Sheath blight resistant BC3F1 line of cross (a) CB 14002 X Tetep and (b) CB 14004 X Tetep. The profiling of alleles for all the 48 and 41polymorphic SSR markers respectively distributed across all the 12 chromosomes were graphically represented. The map which provides a view of percentage of recovery of recurrent parent genome in the sheath blight resistant backcross line**

reasons, the contribution of the recurrent parent to the genome of the BC3 progenies was little less than expected and can be overcome by one more backcross or incorporation of more polymorphic SSR markers in further experiments.

## Conclusion

Introgression of sheath blight resistant QTLs (*qSBR11-1, qSBR11-2 and qSBR7-1)* along with bacterial blight (*xa5, xa13 and Xa21*) and blast *(Pi54*) resistance genes through marker assisted selection in the elite cultivars, ASD 16 and ADT 43 was successfully carried out. Through the stringent foreground and background selection, back cross lines were identified. Two plants in CB14002 x Tetep and three plants in CB14004 x Tetep were identified as positive for all the seven genes/QTLs targeted. These plants will be selfed to generate large number of segregating progenies to identify agronomically superior with good grain quality traits and multiple disease resistance lines in rice. Foreground and background selection in the progenies, along with pathological screening for ShB resistance, and stringent phenotypic selection will aid in development of genotypes with multiple disease resistance in short period of time. In addition to offering the potential for release as cultivars, the pyramided lines will serve as useful donor of gene(s) for BB, blast and ShB in future rice breeding programmes.

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## References

Balachiranjeevi, C., Bhaskar, N. S., Abhilash, V., Akanksha, S., Viraktamath, B., Madhav, M., Hariprasad, A., Laha, G., Prasad, M. and S. Balachandran. 2015. Marker-assisted introgression of bacterial blight and blast resistance into DRR17B, an elite, fine-grain type maintainer line of rice. *Mol breed.,* **35(7)**:151.

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

Bhaktavatsalam, G., Satyanarayana, K., Reddy, A. and V. John. 1978. Evaluation for sheath blight resistance in rice. Int Rice Res Newsl Int Rice Res Inst.

Channamallikarjuna, V., Sonah, H., Prasad, M., Rao, G., Chand, S., Upreti, H., Singh, N. and T. Sharma. 2010. Identification of major quantitative trait loci qSBR11-1 for sheath blight resistance in rice. *Mol Breed.,* **25(1):** 155-166.

Collard, B.C. and D.J. Mackill. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Phil. Trans. R. Soc. B*., **363(1491)**: 557-572.

Doyle, J. and J. Doyle. 1987. Genomic plant DNA preparation from fresh tissue-CTAB method. *Phytochem Bull*., **19(11)**:11-15.

Hospital, F. and A. Charcosset, 1997. Marker-assisted introgression of quantitative trait loci. Genetics, **147(3)**: 1469-1485.

Hospital, F. 2001. Size of donor chromosome segments around introgressed loci and reduction of linkage drag in marker-assisted backcross programs. *Genetics,* **158(3)**: 363.

IRRI. 2002. Standard evaluation system for rice (SES). Manila, Philippines: Internation Rice Research Institute, DAPO Box 7777

Joseph, M., Gopalakrishnan, S., Sharma, R., Singh, V., Singh, A., Singh, N. and T. Mohapatra. 2004. Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. *Mol Breed.,* **13(4):** 377-387.

Perumalsamy, S., Bharani, M., Sudha, M., Nagarajan, P., Arul, L., Saraswathi, R., Balasubramanian, P. and J. Ramalingam. 2010. Functional marker-assisted selection for bacterial leaf blight resistance genes in rice (*Oryza sativa* L.). *Plant breed.,* **129(4):** 400-406.

Rai, A. K., Kumar, S.P., Gupta, S. K., Gautam, N., Singh, N. K. and T.R. Sharma. 2011. Functional complementation of rice blast resistance gene Pi-k h (Pi54) conferring resistance to diverse strains of *Magnaporthe oryzae*. *J. Plant Biochem Biotechnol.,* **20(1)**: 55-65.

Ramkumar, G., Srinivasarao, K., Mohan, K. M., Sudarshan, I., Sivaranjani, A., Gopalakrishna, K., Neeraja, C., Balachandran, S., Sundaram, R. and M. Prasad. 2011. Development and validation of functional marker targeting an InDel in the major rice blast disease resistance gene Pi54 (Pik h). *Mole breed.,* **27(1)**: 129-135.

Singh, A. K., Singh, V. K., Singh, A., Ellur, R. K., Pandian, R. T. P., Gopala Krishnan, S., Singh, U. D., Nagarajan, M., Vinod, K. K. and K. V. Prabhu. 2014. Introgression of multiple disease resistance into a maintainer of Basmati rice CMS line by marker assisted backcross breeding. *Euphytica,* **203(1)**: 97-107.

Singh, S., Sidhu, J., Huang, N., Vikal, Y., Li, Z., Brar, D., Dhaliwal, H. and G. Khush. 2001. Pyramiding three bacterial blight resistance genes (xa5, xa13 and Xa21) using marker-assisted selection into indica rice cultivar PR106. *Theor Appl Genet.*, **102(6-7)**: 1011-1015.

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

Wang, G.-L. and B. Valent. 2017. Durable resistance to rice blast. Science, **355(6328)**: 906-907.

Xu, Y. and J. H. Crouch. 2008. Marker-assisted selection in plant breeding: from publications to practice. *Crop Science*, **48(2)**: 391-407.

Zuo, S., Zhang, Y., Yin, Y., Li, G., Zhang, G., Wang, H., Chen, Z. and X. Pan. 2014. Fine-mapping of qSB-9 TQ, a gene conferring major quantitative resistance to rice sheath blight. *Mol breed.,* **34(4)**: 2191-2203.

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