Introgression of *Sub1* QTL into an elite rice (*Oryza sativa* L.) variety Jyothi through Marker Assisted Backcross Breeding

Deepa John and K.S. Shylaraj*

Rice Research Station, Kerala Agricultural University, Vyttila P.O.Cochin 682 019.Kerala, India.

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Abstract

Sub1 QTL for submergence tolerance was introgressed into the most popular rice variety of Kerala, Jyothi, from the donor parent Swarna-Sub1 using Marker Assisted Backcross Breeding. For the foreground and recombinant selection of the F_1 as well as the backcross populations, molecular markers linked to *Sub1*locus and markers flanking the locus, respectively, were used. Markers unlinked to the *Sub1*locus were used for background selection among the selected progeny. Selected BC_2F_2progeny were found to have recurrent parent genome recovery in the range of 83.4 - 85.9%. The size of the introgressed donor fragment into the selected lines was estimated to be 1.6 - 5.6 Mb. Analysis of the selected BC_3F_2progeny showed the recipient parent genome recovery varying from 92.1 - 98.9% with the introgressed*Sub1* donor fragment size ranging from 4.2 - 5.6 Mb. The survival score of the developed BC_2F_2 and BC_3F_2 lines were same as that of the donor parent, confirming the introgression of *Sub1* QTL.

Keywords: Flood tolerance, Introgression, Marker Assisted Backcross Breeding (MABB), *Sub1* QTL, Submergence tolerance.

Introduction

Rice production in India was 104.32million tonnes during the year 2015-16 (Department of Agriculture, Cooperation and Farmers Welfare, 2017). India is likely to be the most populous country in the world by 2030 and would then require 120 million tons of rice (CRRI, 2011). Hence, the production of rice needs to be increased to meet the ever increasing population.Agricultural production is heavily affected by various abiotic stresses and climate change. Submergence is considered to be the third most important abiotic stress influencing rice production, next to drought and salinity. The potential areas of paddy cultivation in Kerala are situated below the mean sea level and have serious problems of water-logging (Jayan and Nithya, 2010). In coastal areas, crop loss due to submergence caused by flash flood of 7-14 days during monsoon season is very common,as crop establishment is affected and results in reduction of total rice production. The only solution to this is to develop submergence tolerant elite rice varieties. Submergence tolerance in rice is mainly controlled by *Sub1*, a single major Quantitative Trait Locus (QTL) fine mapped to chromosome 9 in the submergence tolerant donor cultivar FR13A (Xu and Mackill, 1996; Nandi et al., 1997; Xu et al., 2000). *Sub1* region encodes three transcription factors (*Sub1A*, *Sub1B*and *Sub1C*) belonging to the B-2 subgroup of the ethylene response factors (ERFs)/ ethylene-responsive element binding proteins (EREBPs)/apetala 2-like proteins (Xu et al., 2006). Tolerant cultivars have strong expression of *Sub1A* in response to submergence, whereas susceptible cultivars have weak or no expression.

Marker Assisted Backcrossing Breeding (MABB) is an effective way to introgress the desired trait

*Author for correspondences: Phone: 91-9846789150, Email: shylarajka@gmail.com

while retaining the essential characters of elite varieties (Neeraja et al., 2007; Iftekharuddaula et al., 2011). The main objective of MABB is to transfer a specific allele/gene at the target locus from a donor line to recipient line while selecting against donor introgressions across the rest of the genome (Tanksleyet al.,1989; Hospital, 2003). Maximum reduction of the size of the introgressed donor genome, limiting to the region responsible for the target trait, into the recurrent parents genetic background is the ultimate objective (Xi et al., 2008). Using MABB, the number of backcrosses required to transfer a target gene can be reduced from six to three generations (Frisch et al., 1999; Frisch and Melchinger, 2005).

Considerable progress has been made by International Rice Research Institute (IRRI) in developing several submergence tolerant rice varieties like MR219-Sub1 (Malaysian high yielding rice variety). Pooja-Sub1 and Pratikshya-Sub1, high yielding rice varieties in Eastern Region of India and rice variety Amara-Sub1 of Andhra Pradesh were developed through MABB using Swarna-Sub1 as the donor (Ahmed et al., 2016; Pragnya et al., 2015; Girijarani et al., 2015). In these works, SSR markers like ART5 Sub1BC2, IYT1 and RM464A were used for foreground selection.These varieties are submergence tolerant and can be successfully cultivated in flood-prone areas (Ismail et al., 2013; Mackill et al., 2012; Septiningsih et al., 2013). The present study was carried outwith the objective to introgress submergence tolerance into the most popular rice variety of Kerala, Jyothi, through introgression of *Sub1*QTL through MABB.

Materials and Methods

Plant Material and Hybridization scheme

Swarna-Sub1 (IR49830-7 x Swarna), a submergence tolerant rice variety developed at IRRI, was used as the donor of *Sub1*. The recurrent parent Jyothi (PTB10 x IR 8) is the most popular and high yielding rice variety of Kerala but sensitive to submergence. MABB scheme followed in this breeding programme is given in Figure 1. The donor parent was crossed with recurrent parent to produce F_1 generation.Germinated F_1seedlings were transferred to trays and grown under natural environmental conditions. Fourteen day old seedlings were transplanted with four seedlings per labelled pot and standard crop management was followed (KAU, 2016).

The F_1 plants with the *Sub1* QTL were backcrossed with var. Jyothi to obtain BC_1F_1. The BC_1F_1 plants have been genotyped using foreground and recombinant markers and the heterozygous *Sub1* introgressed plants were selected. The details on these markers are given in Tables 1 and 2. These selected plants were subjected to background selection to identify individuals more similar to the recurrent parent. Selected BC_1F_1 plants (6) were backcrossed to raise BC_2F_1 and BC_3F_1 generations. Similar strategy was followed to select plants heterozygous for *Sub1* locus and highly similar to recurrent parent. Selected BC_2F_1(5) and BC_3F_1 (7) plants were selfed to produce BC_2F_2 and BC_3F_2 plants respectively. Finally BC_2F_2 and BC_3F_2 progeny were screened with foreground markers to select plants with *Sub1* locus in the homozygous state.

DNA isolation and quantification

DNA was extracted from young leaf tissues following CTAB method (Doyle and Doyle, 1987) and dissolved in 1X TE buffer. DNA was quantified spectrophotometrically using NanoDrop2000c (Thermo Scientific) and the concentration was adjusted to 25 ng μl^{-1} for use in polymerase chain reaction (PCR).

DNA amplification

PCR amplification was carried out in a total volume of 20 µl containing a final concentration of 25 ng of genomic DNA, 1X Taqbuffer with 2.5 m $MMgCl_2$, 400 µM of dNTPs, 1 unit of Taq polymerase enzyme and 0.4 µM each of forward and reverse primers. Some of the PCR amplifications were carried out using 25 ng of

*Table 1.*SSR markers used for foreground selection

Primer	Sequence	Position
IYT1F	TAGGGGCCCATGAGTACTTG	Sub1A promoter
IYT1R	TCAGACAGCTAGCTCGCAAC	
ART5F	CAGGGAAAGAGATGGTGGA	Sub1C promoter
ART5R	TTGGCCCTAGGTTGTTTCAG	
Sub1BC1F	CAATCGATGCGTGCTTCTT	Between Sub1B and Sub1C
Sub1BC1R	CGCAACAAGGCAGAAAAATA	
Sub1BC2F	AAAACAATGGTTCCATACGAGAC	Between Sub1B and Sub1C
Sub1BC2R	GCCTATCAATGCGTGCTCTT	
Sub1BC3F	CATGGGTAAAATTGCCATCC	Between Sub1B and Sub1C
Sub1BC3R	GCTTGAGGGTGAGTGGAGAG	

genomic DNA and 2XPCR Master Mix (Thermo Scientific), comprising of 1X Taqbuffer with 4mM $MgCl_2$, 0.4 mM of each dNTPs and 0.05 U/µl Taq polymerase enzyme. The PCR programme involved an initial denaturation at 94^{O} C for 5 min. followed by 31 cycles of denaturation (94^{O} C for 40 sec.), annealing at 55-65^{O} C depending on the GC content of the primers for 40 sec. and extension at 72^{O} C for 40 sec. Final extension was at 72^{O} C for 5 min. PCR reactions were carried out in a standard thermal cycler (Applied Biosystems, Veriti).

DNA visualization and documentation

The PCR amplified products were separated by electrophoresis on 8% polyacrylamide gels (PAGE).

The bands were visualized by silver staining (Benbouzaet al., 2006) and the images were documented (BioRad Gel Doc XR+). Since the PAGE gives better resolution which is generally required by the microsatellite markers, this electrophoresis strategy was followed. Additionally, PAGE gels can be stored and documented later as the developed colour does not disappear (Bassam and Gresshoff, 2007).

Polymorphism survey and SSR marker selection

Foreground and recombinant selection

Primer combinations which have the capability to resolve different alleles of the gene, among the accessions under study are considered as polymorphic (Joshi et al.,1999) markers. A total of nine SSR markers tightly linked to *Sub1*named IYT1, IYT3, AEX, ART3, ART5, Sub1AB1, Sub1BC1, Sub1BC2 and Sub1BC3 were evaluated for their suitability in foreground screening (Septiningsih et al.,2009), out of which two,ART5 and Sub1BC2, showing clear polymorphism among parents were selected for further screening of backcross progeny. The markers flanking on either side of the *Sub1* locus were used for recombinant selection. Out of 15 flanking markers viz., RM219, RM464, RM23668, RM23679, RM8303, RM23770, RM23788, RM23778, RM23805, RM23887, RM23917, RM23922, RM23958, RM23928 and RM24005, six have been polymorphic between the parents and were used for recombinant selection of the backcross generations.

*Table 2.*SSR markers used for recombinant selection

Primer	Sequence (5'-3')	Position(Mb)
RM23679F	AGTGCATGTTGAGCTTGTGG	0.8
RM23679R	ACCTGGCAATGAGAACGAGT	
RM8303F	AGGGGAGAGGACACACACAC	2.3
RM8303R	GGATCCTCCTGCAAAATCAA	
RM23770F	GACCTTGTCCAGAGTGATTTTG	3.7
RM23770R	ATTTGAGAATAACTTTTCCTACTTCG	
RM23788F	ACCTTCACATAGCAGGGTTGAATC	4.2
RM23788R	ACTCTAAGCCCCTGGATAATCTGC	
RM23778F	ACACAGCCTAAAGGTGTTCTGAGC	3.9
RM23778R	GAGCTTCGGCCCTATAGTCTTCTC	
RM23805F	CACATAGTTTCCATGCTCGTTCAC	4.5
RM23805R	GGTAGAATCCATGACCGTCTCATC	
RM23887F	CAGTGTTGCTGCAAAAAGGA	6.5
RM23887R	AACATTGGTCGTGCTCAACA	
RM23922F	TGGAGGGAGTATCATTATTAGCCG	7.4
RM23922R	CTTGGATAGATTTGGTGGGATGAC	
RM23958F	GAGACAGATGTGTACGGTTTGGTG	7.9
RM23958R	TTGACAAGGGAATTGAAGGAGAAG	

Background Selection

A total of 600 equally spaced genome wide SSR markers unlinked to the target loci were used for polymorphism survey between recurrent parent Jyothi and donor parent Swarna-Sub1.The primer sequences for SSR markers were adapted from the GRAMENE database (http://www.gramene.org/). Of these, 133 markers were polymorphic among the two parents accounting for 22.16% of polymorphism. However, only 81 primers producing clear and reproducible polymorphic banding pattern were finally selected and further used for background selection of the backcross progeny. At least four markers per chromosome were used. The SSR markers that revealed fixed (homozygous) alleles at non-target loci at one generation were not screened at the next BC generation. Only those markers that were not fixed for the recurrent parent allele were analyzed in the following generations.

Statistical Analysis

The marker data from foreground and recombinant selection of *Sub1* introgressed BC_2F_2 and BC_3F_2 populations of Jyothi were analyzed with Graphical Genotyper (GGT2.0) software (Ralph, 2008). The homozygous recipient allele, homozygous donor allele and heterozygous allele were scored as 'A', 'B' and 'H' respectively. The Microsoft Excel file containing these data was imported into an Excel data exchange of GGT 2.0 software programme for further analysis. The size of *Sub1* donor regions in the progeny was estimated using the statistics function of GGT 2.0 software. Similar software analysis was used to calculate recurrent parent genome recovery in the selected *Sub1* introgressed BC_2F_2 and BC_3F_2 lines of Jyothi.

Screening for submergence tolerance

Fourteen day old seedlings of the selected BC_2F_2 and BC_3F_2 plants along with tolerant and susceptible check varieties, FR13A and IR64, respectively were sown in pots (three replications). Fourteen day old seedlings were completely submerged in 1 M deep tank filled with turbid water for 14 days following standard protocols (Neerajaetal., 2007). The survival percentage of plants was scored 14 days after de-submergence according to the IRRI Standard Evaluation System (IRRI, 1988) as shown in Table 3.

Results and Discussion

Foreground selection

Through parental polymorphism analysis, two foreground SSR markers (ART5 and Sub1BC2) with clear polymorphism were selected. Foreground selection uses markers those are tightly linked to the target gene or QTL (Hospital and Charcosset, 1997). True heterozygotes expressing both the alleles of 221 bp of the recurrent parent and 206 bp of the donor parent with respect to ART5 primer and alleles of size 286 bp of the recurrent parent and 238 bp of the donor parent with respect to Sub1BC2 primer were selected at each generation for further screening with flanking markers (recombinant selection). Figure 2 shows the banding pattern of BC_3F_1 progeny with marker $Sub1BC_2$. Selected BC_2F_2 and BC_2F_2 progeny expressed allele of the donor parent with the foreground markers.

Recombinant selection

Out of 15 flanking markers screened, six have been polymorphic among the parents and were used for recombinant selection of the backcross generations. With respect to recombinant selection, three markers (RM8303, RM23770 and RM23805) were used for proximal end whereas markers (RM23887, RM23922 and RM23958) were used for the distal end (Neeraja et al., 2007). The details of the primers are given in Table 4. Heterozygous progeny expressed both the donor and recurrent parent alleles of expected size as mentioned in Table 4.The selected BC_1F_1, BC_2F_1 progeny on screening with the six recombinant primers, showed presence of both the donor and recurrent parent alleles of expected size. When the selected BC_2F_2 progeny were screened with the six flanking markers, alleles of all the markers were of the donor type with expected band size (Table 3). However in plant no. 32-52-3, alleles of markers on the proximal end

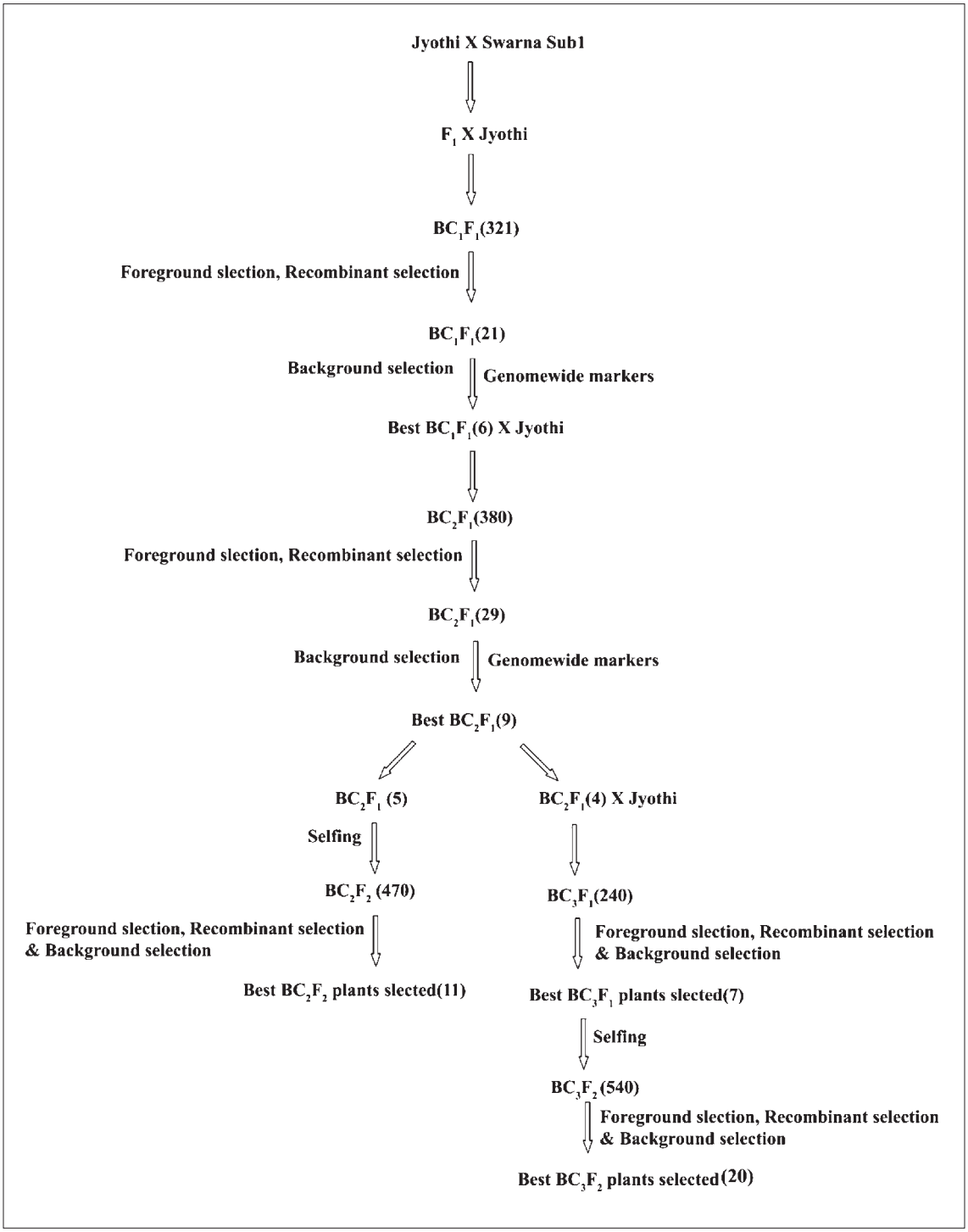

Figure1. Breeding strategy for Marker Assisted Backcross Breeding for introgressing*Sub1* QTL from var. Swarna to var. Jyothi

Table 3. Standard Evaluation System (SES) score for submergence tolerance in rice (IRRI, 1988)

Survival percentage	Score	Observation	Tolerance
100	1	Minor visible symptom of injury	Highly tolerant
95-99	3	Some visible symptom of injury	Tolerant
75-94	5	Moderate injury	Moderately tolerant
50-75	7	Severe injury	Susceptible
0-49	9	Partial to complete death	Highly susceptible

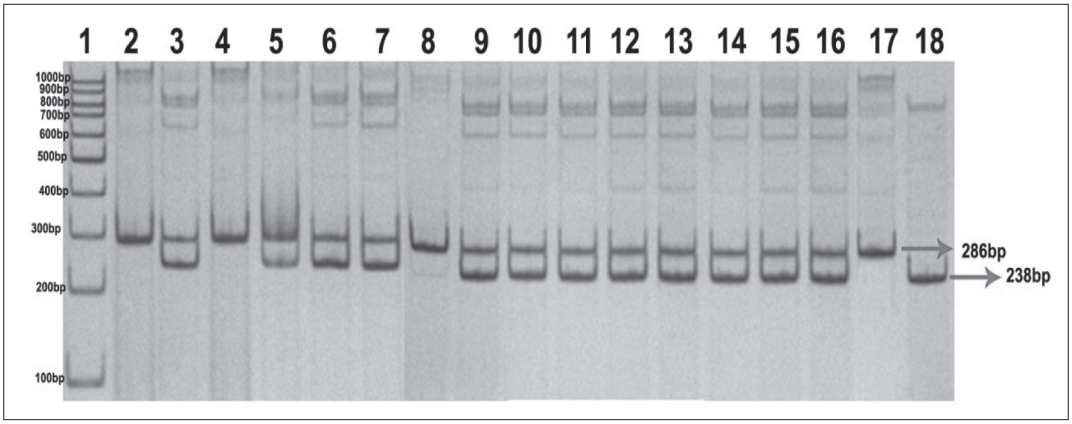

Figure2.Genotypic screening of BC_1F_1 individuals with foreground marker Sub1BC_2. Lane 1: 100bp Ladder, Lanes 2-16: BC_1F_1 individuals, Lane17: Jyothi, Lane18: Swarna- Sub1. Heterozgousprogeny are marked with red arrows

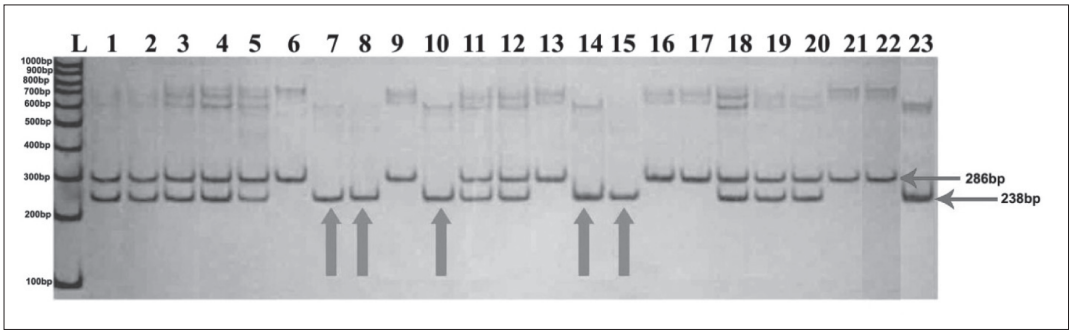

Figure3. Genotypic screening of BC_3F_2 individuals with foreground marker Sub1BC_2. Lane1: 100bp Ladder, Lanes 2-21: BC_3F_2 individuals, Lane22: Jyothi, Lane 23: Swarna-Sub1. Homozygous progeny are marked with red arrows (Lanes 7, 8, 10, 14 and 15).

were heterozygous and alleles of all the markers on the distal end were of donor type. Plant no. 32-61-6 showed heterozygous alleles with markers RM23922 (7.4 Mb) and RM23958 (7.9 Mb) whereas, alleles of all the remaining markers were of the donor type. All these marker data were analyzed with Graphical Genotyper (GGT 2.0) software and the size of the introgressed*Sub1* region was found to be 1.6 - 5.6 Mb in the selected BC_2F_2 populationas estimated by Graphical Genotyper (GGT 2.0) software. Similarly, on screening the selected BC_3F_2 progeny with the six flanking markers, alleles of all the markers were of the donor type with expected band size (Table 3). However, plant no. 32-57-8-1 showed heterozygous alleles with marker RM8303 (2.3 Mb) and donor type alleles with the remaining markers. Plant no.32-57-9 showed heterozygous alleles with marker

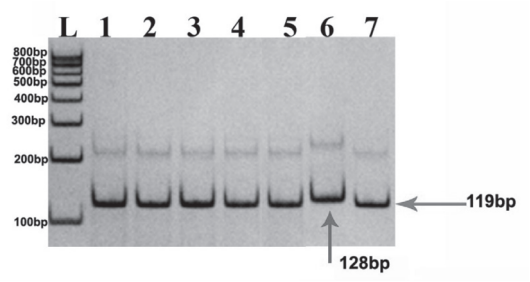

Figure 4. Genotypic screening of selected BC_3F_2 individuals with recombinant marker RM8303. Lane1: 100bp Ladder, Lanes 2-5 BC_3F_2 individuals, Lane6: Jyothi, Lane7: Swarna- Sub1.

RM23770 (3.7 Mb) and donor type alleles with the remaining markers. Similarly, with plant no. 12-3-3-3, alleles from markers RM23922 (7.4 Mb) and RM 23958 (7.9 Mb), were heterozygous and alleles of all the remaining markers were of donor type. Three progeny, plant no. 12-6-2-1, plant no. 12-6-2-7 and plant no. 12-6-2-8 showed recurrent parent allele of the expected size with markers RM23922 (7.4 Mb) and RM23958 (7.9 Mb) whereas, alleles of all the remaining markers were of donor type. On analysis of all these marker data with Graphical Genotyper (GGT 2.0) software,the size of the introgressed *Sub1* region was found to be 4.2-5.6 MB in the selected BC_3F_2 population.Gel images showing foreground selection with Sub1BC2 and recombinant selection with marker RM8303 of BC_3F_2 plants are shown in Figure 3 and Figure4, respectively. Recombinant selection uses markers flanking the target locus and involves selection of BC progeny with the target gene and recombination events between the target locus and linked flanking markers. Recombinant markers are useful in minimizing linkage drag (Collard andMackill, 2007). The size of *Sub1* region in the selected BC_2F_2 population was smaller compared to previous works such as that in the BC_2F_2 population of Swarna-Sub1 (6.5-7.3 Mb) (Neeraja et al., 2007). However, the size of the donor segment with the *Sub1* QTL was a little larger in our BC_3F_2*Sub1* introgressed lines of Jyothi in contrast to the limited size of 2.3-3.4 Mb donor fragment in the developed Swarna-Sub1 rice variety (Neeraja et al., 2007). The genetic background of Swarna-Sub1 around the *Sub1* QTL in the introgressed lines however, did not affect the tolerance level to submergence stress as they scored similar to the donor parent after phenotypic screening. Swarna-Sub1 is a high yielding rice variety with favourable agronomic characters and therefore, the small genetic background of Swarna-Sub1 in the introgressed lines may not affect their phenotype. Similar results were achieved by Ara et al. (2015) for the development of BRdhan49-Sub-1.

Background Selection

The next level of MABB involved background selection where the screening of backcross generations with markers those are unlinked to the target locus was done to select the plants with larger proportion of RP genome. Background markers are used to accelerate the recovery of recurrent parent genome (Collard and Mackill, 2007; Hospital and Charcosset, 1997). The present study used a total

Table 4. Polymorphic SSR markers used for foreground and recombinant selection

Marker	Reference	Type of marker	Chrom	Position (Mb)	Expected Band size (bp)	
					Donor parent allele	Recurrent parent allele
Sub1BC2	Septiningsih et al., 2009	Foreground	9	6.3	238	286
ART5	Septiningsih et al., 2009	Foreground	9	6.3	206	221
RM8303	Neeraja et al., 2007	Flanking	9	2.3	119	128
RM23770	Neeraja et al., 2007	Flanking	9	3.7	303	270
RM23805	Neeraja et al., 2007	Flanking	9	4.5	244	300
RM23887	Neeraja et al., 2007	Flanking	9	6.5	247	230
RM23922	Neeraja et al., 2007	Flanking	9	7.4	323	220
RM23958	Neeraja et al., 2007	Flanking	9	7.9	160	150

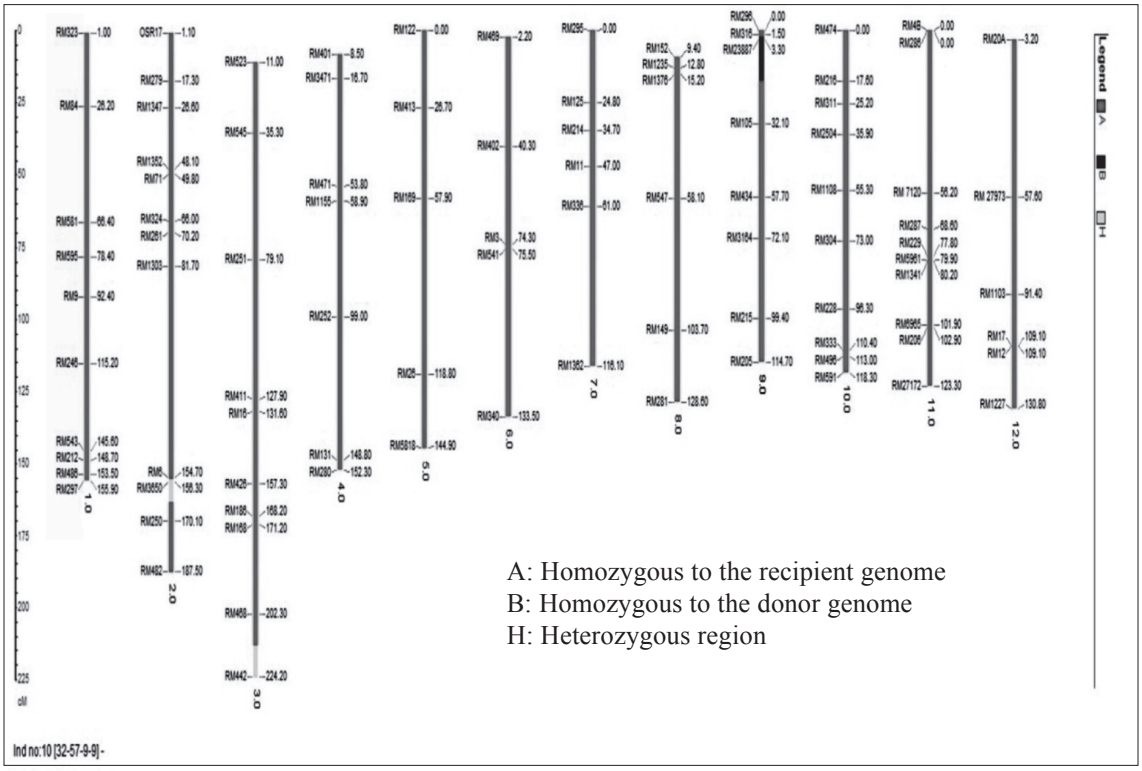

Figure 5. Graphical genotype of the best BC_3F_2 plant 32-57-9-9

Table 5. Background and introgressed segment analysis in selected lines of *Sub1* introgressed BC_3F_2 population

Plant No.	Recurrent parent(A %)	Donor parent (B %)	Heterozygous (H %)
32-57-8-1	93.6	0.9	5.5
32-57-8-4	95.5	3.0	1.5
32-57-8-6	94.7	2.4	2.8
32-57-8-8	97.0	1.0	2.0
32-57-9-1	98.0	0.9	1.1
32-57-9-3	98.0	0.9	1.1
32-57-9-5	98.0	0.9	1.1
32-57-9-7	98.0	0.9	1.1
32-57-9-9	98.0	0.9	1.1
32-57-18-1	97.1	1.5	1.4
32-57-18-4	97.1	1.5	1.4
32-57-18-5	97.5	1.0	1.4
12-3-3-3	95.9	1.0	3.0
12-3-3-9	95.9	3.7	0.3
45-65-1-1	97.6	2.4	0.0
45-65-1-4	94.8	2.4	2.8
45-65-1-10	93.6	3.6	2.8
12-6-2-1	92.1	2.5	5.4
12-6-2-7	95.6	2.8	1.6
12-6-2-8	92.1	3.1	4.8

of 81 polymorphic markers to calculate the recurrent parent genome percentage in all the backcross generations. The recurrent parent genome recovery in the selected 21 BC_1F_1 individuals after foreground and recombinant selection ranged from 43.4-73.0%. The highest recurrent parent genome recovery was in plant no.12 (73%) followed by plants P_{32}, P_{45} and P_{30} with the recipient allele percentage as 72.1, 68.8 and 63.6%, respectively. All these selected plants were used to backcross with recurrent parent and develop BC_2F_1 plants. The BC_2F_1 plants showed recipient allele genome recovery in the range of 72.7- 90.9%. A total of 9 BC_2F_1 plants with higher recurrent parent genome recovery (78.6 - 90.5%) were selected to develop BC_2F_2 by selfing and BC_3F_1 plants by backcrossing with recurrent parent.

The selected 11 BC_2F_2 progeny showed recurrent parent genome recovery in the range of 82.8 - 95.9%

Table 6. Screening of BC_3F_2 lines for submergence tolerance

Plant No.	Total number of plants before submergence	Total number of plants after 14 days of submergence	Survival percent-age	Score
32-57-8-1	40	38	95	3
32-57-8-4	40	38	95	3
32-57-8-6	40	38	95	3
32-57-8-8	40	38	95	3
32-57-9-1	40	39	97.5	3
32-57-9-3	40	39	97.5	3
32-57-9-5	40	39	97.5	3
32-57-9-7	40	39	97.5	3
32-57-9-9	40	39	97.5	3
32-57-18-1	40	38	95	3
32-57-18-4	40	38	95	3
32-57-18-5	40	38	95	3
12-3-3-3	40	38	95	3
12-3-3-9	40	38	95	3
45-65-1-1	40	38	95	3
45-65-1-4	40	38	95	3
45-65-1-10	40	38	95	3
12-6-2-1	40	39	97.5	3
12-6-2-7	40	39	97.5	3
12-6-2-8	40	39	97.5	3
Jyothi	40	0	0	9
Swarna–Sub1	40	39	97.5	3

with the highest recipient genome percentage in plant no.32-52-3. The recovery percentage of recipient alleles in the BC_3F_1 population ranged from 89.4 - 97.5%. The maximum percentage of recipient alleles in the BC_3F_1 population was 97.5% found in plant no.32-57-9. Plant no.32-57-3 showed the minimum percentage of recipient alleles as 89.4%. Additionally chosen plants for selfing to raise BC_3F_2 generation were 32-57-1, 32-57-8, 32-57-48, 12-3-3, 32-65-1 and 12-6-2. The percentage of recurrent parent genome in these selected plants ranged from 90.2 - 95.5%. Plant no. 32-57-9 with the highest recurrent parent genome recovery showed complete recovery of recipient genome with respect to chromosomes 1, 4, 5, 6, 7, 8, 10, 11, 12. The remaining non-*Sub1*- carrier chromosomes had the recipient genome as 83.3% (chromosome 2) and 90% (chromosome3).

BC_3F_2 generation plants showed recipient parent genome recovery in the range of 92.1 - 98.0%. Plants 32-57-9-1, 32-57-9-3, 32-57-9-5, 32-57-9-7 and 32-57-9-9 had the maximum recurrent parent genome recovery of 98.0%. The percentages of markers homozygous for recipient parent (A%), the percent donor alleles (B%) and heterozygous (H%) of the selected *Sub1* introgressed BC_3F_2 lines are shown in Table 5. Graphical genotype of the best plant 32-57-9-9 with maximum recurrent parent genome recovery of 98.0% is shown in Figure 5.

Submergence tolerance screening

The selected BC_2F_2 and BC_3F_2 plants were subjected to fourteen days of submergence stress and all the plants scored 3 similar to the donor parent Swarna-Sub1 suggesting that they are tolerant to submergence stress (Table 6). The results confirmed the successful introgression of *Sub1* QTL in the selected lines. Similar findings were reported by Neeraja et al.(2007). Rice varieties expressing *Sub1* show quiescence strategy to survive submergence stress and recover by elongation of shoot and production of new leaves soon after the water recedes(Fukao and Bailey-Serres, 2008).

The QTL *Sub1* responsible for submergence tolerance has been successfully transferred into the genetic background of popular rice variety of Kerala, Jyothi,using MABB approach. The recovery of recurrent parent genome was accelerated with the use of background markers with 3 backcross generations. The newly developed submergence tolerant lines of Jyothi can be successfully cultivated in the flood prone areas of Kerala.

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