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Research Article

Introgression of SUB1 QTL into BR22 Using Marker Assisted Backcrossing

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Abstract

Submergence is one of the important abiotic stresses incurring substantial loss to rice production in Bangladesh. With a view to improving submergence tolerance in BR22 (popular name *Kiron*) is a promising antioxidant enriched, high yielding and strongly photosensitive transplanted Aman rice variety, BRRI dhan 51 was used as a donor parent to introgress the *SUB1* QTL conferring submergence tolerance. Molecular marker within submergence tolerant QTL *(SUB1)*, flanking markers and evenly distributed background markers were utilized in a marker aided selection program to develop advanced breeding lines with broad-spectrum tolerance to flash flooding submergence. Indel and simple sequence repeats (SSRs) markers were used in the marker assisted backcrossing scheme in BC_4F_1 and BC_5F_1 generation. Sub1C173 a gene specific indel marker *SUB1* QTL was used to select plants possessing the tolerant genes (foreground selection). The result of recombinant selection also revealed that the size of the *SUB1* QTL introgression from the donor parent was estimated as 2.5 Mb. The percentage of recipient genome recovery in the best plant viz. BR10190-3-1-20-3 was 95%. This research work illustrates the successful application of marker-assisted breeding to introgress the submergence tolerant QTLs into the genetic background of BR22.

INTRODUCTION

Rice is the staple food of about three billion people including eight countries in Africa, nine countries in North and South America and seventeen countries in Asia [1,2]. Approximately half the world's population depends on rice for survival [3]. Although rice is a crop that requires irrigated condition for cultivation, most rice varieties cannot survive if the plants remain submerged for more than one week [4]. The major QTL responsible for submergence tolerance was mapped successfully to chromosome 9 named *SUB1*, stated to account for almost 70% of the phenotypic variation under submergence [5]. This *SUB1* QTL has been introgressed through marker assisted selection programme into a few numbers of rice varieties.

Bangladesh Rice Research Institute has so far released two submergence tolerant rice varieties namely BRRI dhan 51 and BRRI dhan 52 and the Bangladesh Institute of Nuclear Agriculture (BINA) has also released two submergence-tolerant varieties namely BINA dhan 11 and BINA dhan 12 [6]. In many parts of submergence prone areas of Bangladesh including northern part the duration of flash flood submergence is more than three weeks; sometimes it remains within 3 to 4 weeks. The presently available flash flood tolerant varieties of Bangladesh cannot survive whenever the level of stress is more than three weeks.

BR22 (popular name *Kiron*) is a promising antioxidant enriched, high yielding, strongly photosensitive lowland rice variety having 150 days growth duration [7]. BRRI scientists reported that BR22 possessed the highest Hydroxyl Ion Scavenging Activity (HISA). BRRI scientists assumed that BR22 is potential to promote human health, due to its content of the Phenolic Compound (PC) which is able to inhibit the formation of reactive cell-damaging free radicals or reduce the concentrations of free radicals, thus reducing the risk of coronary heart disease, cancer and averting oxidative damage of lipid as well as low-density lipoprotein (LDL) [8-10]. The variety does not possess the submergence tolerant QTL-*SUB1*. Nonetheless submergence stress not only depends on duration of submergence but also on many other environment and agronomic factors like water temperature, turbidity, depth of flood water etc. With this context Mohanty et al. [11], stated that submergence is a polygenic trait and *SUB1* QTL does not alone completely represent the trait. Even with two to three weeks of submergence tolerance, the present submergence tolerant varieties are often damaged whenever the environmental factors other than duration of submergence go beyond the capability of tolerance of the existing varieties. This is the reason why new submergence tolerant varieties with higher levels of tolerance are required. *SUB1* QTL introgressed into different genetic background can result into producing a new submergence tolerant variety with more level of tolerance as the

QTL works through interacting with other QTLs in the genetic background.

Major thrust is that BR22 is a strongly photosensitive variety. In case of multiple flash floods, the duration of photo insensitive or weakly sensitive variety is so delayed to be affected by cold at the later part of the growth phase. The duration of a strongly photosensitive Sub1-variety is expected not be delayed. With this point of view, efforts have been undertaken to introgress *SUB1* QTL into BR22 utilizing marker assisted backcrossing technique. To convert the high yielding, slightly salinity tolerant BR22 into a submergence tolerant type by incorporating *SUB1* QTL from BRRI dhan 51 through marker assisted backcrossing (MABC) technique. In this scenario, three levels of selection approaches viz. foreground selection, recombinant selection, and background selection have been performed. MABC has three major advantages over conventional backcrossing. For instance, trait specific target gene can be identified through foreground selection, the linkage drag can be minimized through recombinant selection, and the recovery of recurrent parent genome can be predicted through background selection [12].

It is expected that improved BR22-Sub1 lines with more submergence tolerance will be more adaptable under the submergence prone regions of Bangladesh, predominantly in the multiple flash flooding affected northern region of the country.

MATERIALS AND METHODS

Plant materials

The experiment was designed and carried out in order to introgress *SUB1* QTL into the genetic background of BR22 (recurrent parent). BR22 is a T. aman variety having 150 days growth duration, high yielding capability, strong photosensitivity, short and bold grain and does not possess *SUB1* QTL. Need to refer to this; cite appropriate reference, carrying the submergence tolerant QTL served as the donor parent during the cross. The parental crosses were initiated in T. Aman, 2011 (Kharif, 2011) season and crossing as well as marker assisted selection was attempted in every T. Aman season. This research work was started from BC_4F_1 to BC_5F_1 generation as a part of development of breeding pipelines of plant breeding division, Bangladesh Rice Research Institute, Gazipur-1701, using marker assisted selection techniques.

Genotyping approach

Leaf samples were collected following ten stick method (leaf picking-up from every ten plants tagged by sticks). DNA was extracted following modified Mini scale method [13] as well as CTAB method [14]. The DNA concentration of samples was adjusted to 25 ng/µl. PCR for Simple Sequence Repeats (SSRs) was performed in 10 µl reactions containing around 25 ng/µl of DNA template (3 µl DNA), 1 µl 10X TB buffer (containing 200 mM Tris–HCl pH 8.3, 500 mM KCl), 0.2 µl of 10 mM dNTP, 1.35 µl 25 mM $MgCl_2$, 0.5 µl each of 10 µM forward and reverse primers and 0.2 µl of *Taq* DNA polymerase (5 U/µl) using G-STORM thermal cycler [15,16] and Gene Atlas thermal cycler.

CR for STS (Sequence Tagged Sites) Primer *Sub1C173* was performed in 20 µl reactions containing around 25 ng/µl of DNA template (4 µl DNA with 10-20X dilution factor), 2 µl 10X TB, 2.7 µl 25mM $MgCl_2$, 0.4 µl of 10 mM dNTP, 1 µl each of 10 µM forward and reverse primers, 1 µl DMSO (Dimethyl Sulphoxide) and 0.4 µl of *Taq* DNA polymerase (5 U/µl) using G-STORM and Gene Atlas thermal cycler. The PCR products of SSR and STS markers were resoluted using Polyacrylamide gel electrophoresis. The size (in nucleotide base pairs) of the most intensely amplified band for each microsatellite marker was measured based on its migration in relation to a molecular-weight size marker (1 Kb^+ DNA Ladder) with Alpha Ease 4.0 software. The homozygous allele of the recipient parent for the particular SSR marker was scored as 'A'. Again, the homozygous allele of the donor parent for the particular SSR marker was scored as 'B'. However, heterozygous alleles were scored as 'H'. The molecular data was analyzed with Graphical Genotyper (GGT 2.0) software [17]. The software produced graphical sketch of the percentage of recurrent parent chromosomal segments in the selected backcross population.

Selection approaches of backcross population

Simple sequence repeats (SSRs) and indel markers were used for selection [18,19]. The different selection levels (Figure 1) followed in this backcross breeding is discussed as follows:

Foreground selection

In BC_3F_1 & BC_4F_1 generations of T. Aman 2014 & 2015 season, the foreground selection was done over 77 and 175 plants respectively using Sub1C173, an indel marker specific to *SUB1* QTL. After foreground selection, the heterozygous plants were marked by sticks in the field. Phenotypic selection was applied during active tillering stage based on resemblance to recipient parent BR22. Different characters considered during phenotypic selection were tiller numbers, tillering pattern, flag leaf angle, leaf shape, leaf length, leaf breadth, leaf color etc.

In this study, gene-based indel marker Sub1C173 specific to the putative gene of Sub1C of *SUB1* QTL was used in the foreground selection (Table 1). The individuals that were heterozygous for the foreground marker were selected in this selection step.

Recombinant selection

In this selection level, individual plants that were homozygous for the recipient allele at the marker loci distally or proximally flanking the *Sub1* locus (i.e. recombinants) were identified. This was titled as "recombinant selection" [3]. The objective of this selection was to minimize the linkage drag. The flanking markers selected were as close as possible. Population sizes depend on distance of flanking markers from target locus. Flanking markers used for recombinant selection were selected from the tip of chromosome 9 around the *Sub1* region was targeted. In this study, four markers (Table 2) were selected as flanking markers like RM5799, RM23843, RM8300 and RM23915.

Background selection

Microsatellite or Simple Sequence Repeat (SSR) markers covering all the chromosomes that were polymorphic between the donor and recurrent parent were used for background selection to recover the recipient genome (Figure 5). Out of 403 SSR primers surveyed, a total of 97 microsatellite markers were used for background selection initially. Maximum number of background markers used was 10 for chromosome 1 and

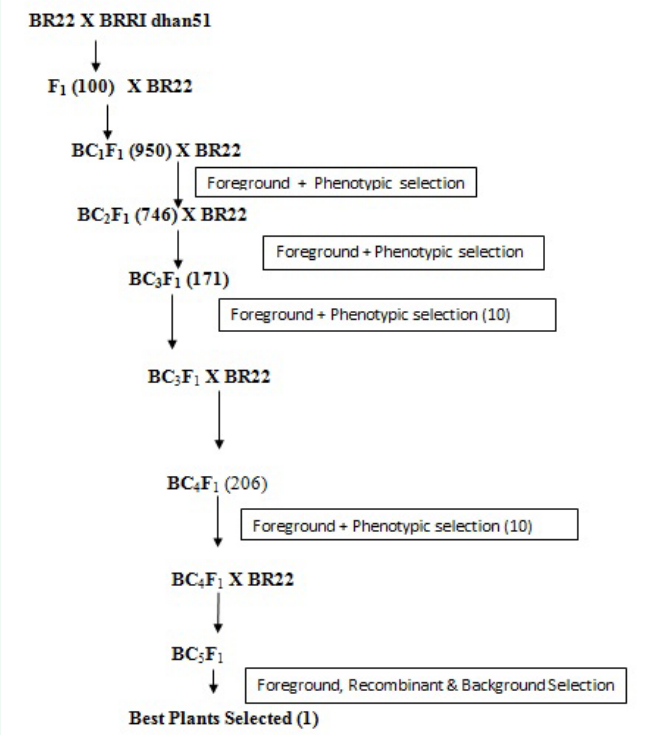

Figure 1 'Foreground, Phenotypic, Recombinant and Background' selection approach with selection details in each generation.

Table 1: Foreground marker used over two parents of MABC scheme.

SN	Primer	Position	Allele Size	Forward	Reverse	Repeat motif
1.	Sub 1C173	Exon of Sub1C	158 - 176	CTACTTCAATGTCACCAACG	TAGAAGATGGAAGACCTGAT	$(AGC)_{10}$

Table 2: Polymorphic flanking markers of *SUB1* QTL between recipient and donor parents on Chromosome 9.

SN	Primers	Position		Band Size (bp)	Forward Primer	Reverse Primer	Repeat motif
		cM	Mb				
1.	RM5799	15.0	3.7	146	CTTGCACAAGAGGCAACACTCC	GTTTGGTAGGTCGCATTGTTTGG	(AGC)9
2.	RM23843	22.2	5.6	628	CCTAGGCCATACATAATCTGACG	TTAGCGTGGAACTAAACACAGC	(AT)40
3.	RM83000	26.1	6.6	211-217	GCTAGTGCAGGGTTGACACA	CTCTGGCCGTTTCATGGTAT	(ACCATTAT)3
4.	RM23915	28.7	7.2	197	GAGGATCCTTACCATCAAACTTCG	CCAAGAACCTGCATTCTTCAAGG	(AC)15

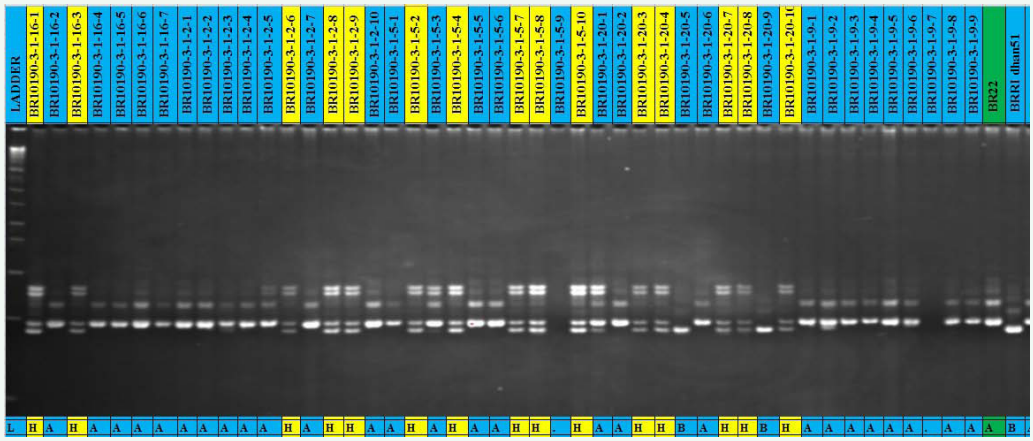

Figure 2 Partial view of gel picture of the foreground selection with the tightly linked marker Sub1C173 in BC5F1 generation.

chromosome 9. The minimum number of background markers used was 5 for chromosome 3. The average number of background markers over all the 12 chromosomes was 8.50 (Table 3). The microsatellite markers that revealed fixed (homozygous) alleles at non-target loci at one generation are not screened at the next BC generation. Only those markers that were not fixed for the recurrent parent allele are analyzed in the following generations. The segregates with fixed donor alleles were discarded from the selection in backcross-F_1 generation.

RESULTS AND DISCUSSION

Foreground selection

Foreground selection was performed among 77 BC_4F_1 plants and 175 BC_5F_1 plants in order to detect *SUB1* QTL. Foreground selection was carried out using Sub1C173. The size of the resistant allele of this marker, which was obtained from the donor of *SUB1* QTL viz. BRRI dhan 51, was 180 bp and that of susceptible allele obtained from BR22 was 196 bp. This marker produced very clear bands and it was possible to identify the genetic constitution of the Sub1-locus very easily using polyacrylamide gel electrophoresis. In BC_4F_1 plants, out of 77 plants, 35 plants were found showing the locus for the gene-based marker as heterozygous state (Score H), 43 plants were found with the locus fixed for recipient allele (susceptible allele) (Score A) and only 3 plants were found with the locus fixed for donor allele (resistant allele) (Score B) (Table 4). In BC_5F_1 plants, out of 175 plants, 87 plants had the heterozygous genotype (Score H), 74 plants were found with the susceptible allele (Score A) and only 14 plant were found with the locus fixed for donor allele (resistant allele) (Score B) (Table 4). In this marker assisted backcross breeding program, the allele fixed for recipient parent (BR22) for any marker has been scored as 'A', the allele fixed for donor allele as 'B' and that of with both the alleles of two parents, i.e. the heterozygous alleles as 'H'. The plants with B score were produced due to accidental failure of backcrossing.

Here, in BC_4F_1 generation, 31 plants showed 'H' score and 43 plants showed 'A' score which indicated that the results fitted to the expected 1:1 ratio of this generation with a non-significant chi square value of 0.82 at a probability level of 0.05. In BC_5F_1 generation, 87 plants showed 'H' score and 75 plants showed 'A' score which indicated that the results fitted to the expected 1:1 ratio of these generation with a non-significant chi square value of 0.90 at a probability level of 0.05. Iftekharuddaula [20] also obtained the similar results in foreground selection with *SUB1* QTL while introgressing this QTL into BR11 utilizing Marker Assisted Backcrossing technique.

Out of 87 plants, 10 plants were selected during maximum tillering stage based on resemblance to recipient parent BR22. It was expected that those 73 individuals possessed the submergence resistant allele, so these segregants were selected and promoted to further selection steps. Ten plants selected phenotypically were subjected for recombinant selection. The

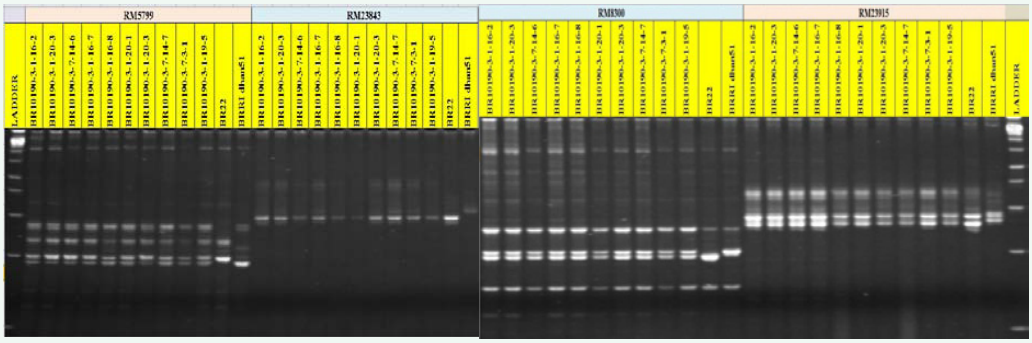

Figure 3 Gel picture shows the recombinant selection by four flanking markers.

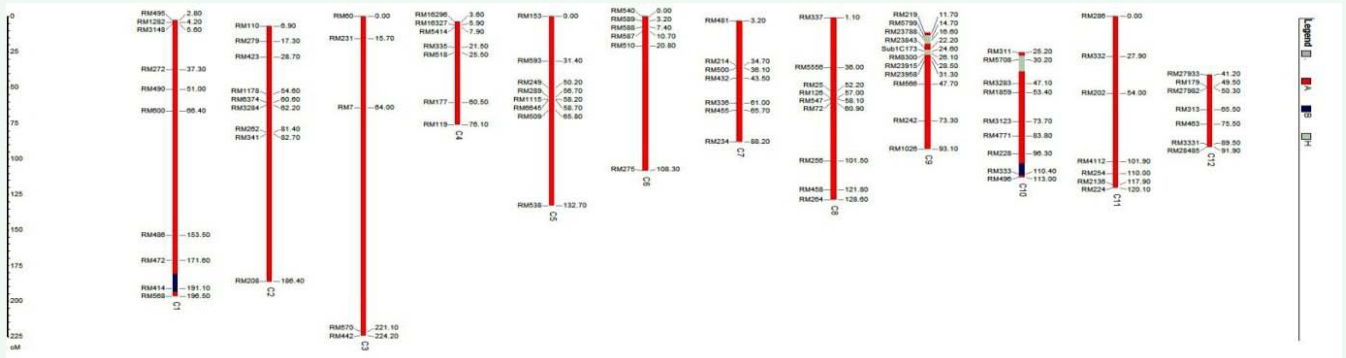

Figure 4 Graphical genotype of the selected best plant no. BR10190-3-1-20-3 of BC4F1 generation. The red colored regions on the chromosomes indicated homozygous region for the recipient genome while the gray colored regions indicated the heterozygous regions and the blue coloured regions indicated unknown region. The distances were represented in cM based on published map of Temnykh et al.

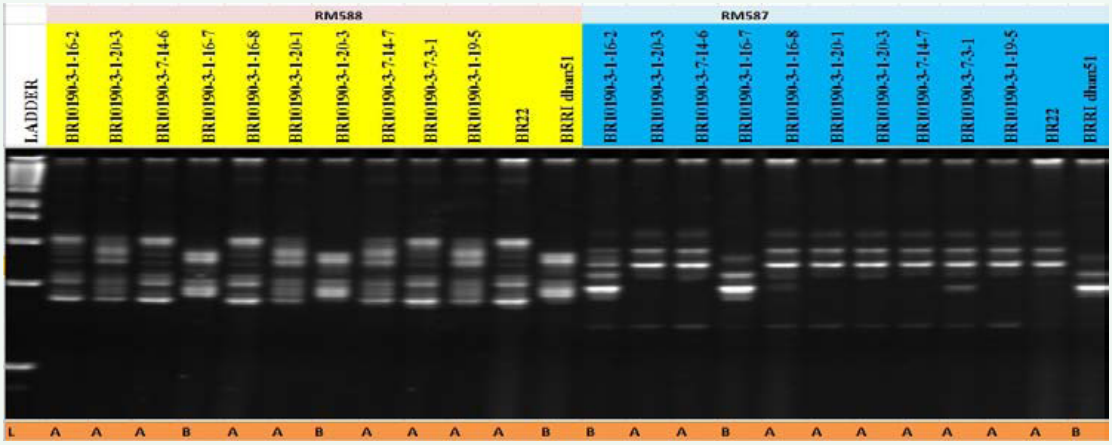

Figure 5 Partial view of the gel picture of the background selection using some background markers in selected best plants of BC_4F_1 generation..

Table 3: List of background markers of all 12 chromosomes used in the background selection.

Chr. 1	Chr. 2	Chr. 3	Chr. 4	Chr. 5	Chr. 6
RM495	RM110	RM60	RM16296	RM153	RM540
RM1282	RM279	RM231	RM16327	RM593	RM589
RM3148	RM423	RM7	RM5414	RM249	RM588
RM272	RM1178	RM570	RM335	RM289	RM587
RM490	RM6374	RM442	RM518	RM1115	RM510
RM600	RM3284		RM177	RM6645	RM275
RM486	RM341		RM119	RM509	RM461
RM472	RM262		RM16256	RM538	
RM414	RM208				
RM568					
Chr. 7	**Chr. 8**	**Chr. 9**	**Chr. 10**	**Chr. 11**	**Chr. 12**
RM481	RM337	RM219	RM311	RM286	RM27933
RM214	RM5556	RM5799	RM5708	RM332	RM179
RM500	RM25	RM23788	RM3283	RM202	RM27982
RM432	RM126	RM23843	RM1859	RM4112	RM313
RM336	RM547	Sub1C173	RM3123	RM254	RM463
RM455	RM72	RM8300	RM4771	RM2136	RM3331
RM234	RM256	RM23915	RM228	RM224	RM28485
	RM458	RM23958	RM333		
	RM264	RM566	RM496		
		RM242			

different characters considered during phenotypic selection were plant height, tillering pattern, leaf length, leaf width, leaf angle, etc. (Figure 2) shows the partial view of gel picture of the foreground selection with the gene-based marker Sub1C173 in BC_5F_1 generation.

Recombinant selection

The foremost objective of recombinant selection in BC_5F_1 generation was to obtain single recombinant type segregants at one side of the *SUB1* QTL. Recombinant selection was carried out among 10 plants having heterozygous alleles for the gene-based marker in foreground selection. Recombinant type segregates were those types of segregants which minimized linkage drag at both sides of the QTL. In other sense, single recombinant type segregants possessed the recipient allele for the flanking markers used at any sides of the QTL (Figure 3). These types of segregants are very rare in frequency. A total of four flanking markers from the sub-telocentric Sub1-region of chromosome 9 were used in recombinant selection in BC_5F_1 generation. Out of four flanking markers, two markers were taken from proximal end and other two markers were taken from distal end. The description of these flanking markers is presented in Table 5.

Frisch et al., stated that moderately small population sizes could be used for recombinant selection. For recombinant selection four flanking markers were used as RM5799, RM23843, RM8300 and RM23915. RM5799 and RM23843 were used for proximal end and RM8300 and RM23915 were used for distal end. The distance between the closest flanking marker and *SUB1* QTL was 0.6 Mb at proximal end and 0.4 Mb at distal end (Table 6). The scores for the flanking markers viz. RM23843 and RM23915 were 'A' (homozygous recipient allele) whereas these were 'H' (heterozygous alleles) for the other two flanking markers RM5799 and RM8300. The results indicated that it was possible to minimize linkage drag or to obtain recipient alleles for the two flanking markers with 'A' scores. However, it was not possible to avoid linkage drag for the other two flanking markers, which will be achieved in the next backcross generation. The results of recombinant selection also revealed that the size of the *SUB1* QTL introgression from the donor parent was estimated as 2.5 Mb. It was assumed that, the crossing over was taken place in the middle place of the markers.

Background selection

A total of 97 microsatellite markers were used for background selection over 10 BC_4F_1 plants resulting from foreground, phenotypic and recombinant selection. Maximum number of background markers was used 11 for chromosome 9. Minimum number of background markers was used 5 for chromosome 3 (Table 3). Hospital et al., and Visscher [21,22] reported that, as a general rule, two to four markers per 100 cM could be efficiently used to accelerate the recovery of the recurrent parent genome in the early generations such as BC_1F_1 or BC_2F_1. The reason for this was that in early generations, few recombinant events occurred so that donor chromosome segments were represented by a few long segments on each chromosome. Takeuchi et al. [23], used 116 RFLP markers covering 12 chromosomes in background selection in rice. Neeraja et al. [16], used 56 SSR markers as initial background markers for the development of Swarna-Sub1. However, the average number of background markers over all the 12 chromosomes was 7.83. The percent markers homozygous for the recipient parent ranged from 62.11% to 92.63% in those 10 plants (Table 7).

In the plant number BR10190-3-1-20-3 (Table 8) of BC_5F_1 generation, 88 markers out of 97 markers (92.63%) were like recipient parent type. But if the alleles of the homologous chromosomes were considered, the percentage of recipient alleles in plant number BR10190-3-1-20-3 was 95.26%. So, this plant had been considered as the first best plant of that population. Figure 4 shows the graphical genotype of the best plant (plant no. BR10190-3-1-20-3). The contribution of recipient genome among the 10 selected individuals varied widely. The percentage of recipient alleles recovered were 68.95%, 69.95%, 83.68%, 84.21%, 85.26%, 88.42%, 88.42%, 93.95%, 94.26% and 95.26% in the selected plants viz. plant number BR10190-3-1-16-7, BR10190-3-7-3-1, BR10190-3-1-19-5, BR10190-3-1-16-2, BR10190-3-1-16-8, BR10190-3-7-14-7, BR10190-3-1-20-3, BR10190-3-1-20-1, BR10190-3-7-14-6, BR10190-3-1-20-3 respectively (Table 8).

For available background markers, in the selected best plant (Plant No. BR10190-3-1-20-3), chromosome 1, 2, 3, 4, 5, 6, 7, 8, 11 & 12 were completely like the recipient type, whereas the recipient genome ranged from 81.8% (chromosome 9) to 93.8% (chromosome 10) in the remaining chromosomes (Table 8).

Table 4: Summary results of foreground selection, BC_4F_1 and BC_5F_1 generation, T. Aman and T. Aman.

SN	Marker Score	Number of plants
BC_4F_1		
1.	H	31
2.	A	43
3.	B	3
		Total = 77
BC_5F_1		
1.	H	87
2.	A	74
3.	B	14
		Total = 175

Table 5: Flanking markers used in recombinant selection in BC_5F_1 generation.

SN	Flanking Markers (RM Nmbers)	Position (Mb)	Positional type
1.	RM5799	3.7	Proximal end
2.	RM23843	5.6	do
	SUB1 QTL	6.2	
3.	RM83000	6.6	Distal end
4.	RM23915	7.2	do

Table 6: Results of the recombinant selection using flanking markers, BC_5F_1 generation.

Plant number selected	Markers			
	RM5799	RM23843	RM83000	RM23915
BR10190-3-1-16-2	H	A	H	A
BR10190-3-1-20-2	H	A	H	A
BR10190-3-7-14-6	H	A	H	A
BR10190-3-1-16-7	H	A	H	A
BR10190-3-1-16-8	H	A	H	A
BR10190-3-1-20-1	H	A	H	A
BR10190-3-1-20-3	H	A	H	A
BR10190-3-7-14-7	H	A	H	A
BR10190-3-7-3-1	H	A	H	A
BR10190-3-1-19-5	H	A	H	A

Seed production in backcross population

Totally 527 BC_4F_1 seeds were produced from 10 selected plants of BC_3F_1 generation (Table 9) and totally 632 BC_5F_1 seeds were produced from 10 selected plants of BC_4F_1 generation (Table 10).

In BC_5F_1 generation, foreground and background selection will again be applied to identify the best plant which will be possible to self-pollinate in order to obtain homozygosity in BC_5F_2 generation of T. Aman, 2016 season. In this way, BR22-Sub1 will be developed which will be reported elsewhere.

Table 7: Results of background selection, BC_5F_1 generation.

Items	Plant Numbers									
	BR10190-3-1-16-2	BR10190-3-1-20-3	BR10190-3-7-14-6	BR10190-3-1-16-7	BR10190-3-1-16-8	BR10190-3-1-20-1	BR10190-3-1-20-3	BR10190-3-7-14-7	BR10190-3-7-3-1	BR10190-3-1-19-5
A	74	78	83	59	73	81	88	78	61	71
H	13	13	12	14	17	12	7	13	12	18
B	10	6	2	24	7	4	2	6	24	8
%A	77.89	82.11	91.58	62.11	76.84	85.26	92.63	82.11	63.11	74.74
%Recipient allele	84.21	88.42	94.26	68.95	85.26	93.95	95.26	88.42	69.95	83.68
Rank			**2**			**3**	**1**			

Table 8: Chromosome-wise percentage of recipient alleles in the best plant (Plant no. BR10190-3-1-20-3), BC_4F_1 generation.

Items	Chr 1	Chr 2	Chr 3	Chr 4	Chr 5	Chr 6	Chr 7	Chr 8	Chr 9	Chr10	Chr 11	Chr 12
A	9	9	5	7	8	6	7	9	7	7	7	7
H	0	0	0	0	0	0	0	0	4	1	0	0
Recipient allele (%)	100	100	100	100	100	100	100	100	81.8	93.8	100	100

Table 9: Number of BC_4F_1 seeds produced from selected BC_3F_1 plants, T. Aman season.

Backcross Combinations	Serial No.	Pedigree Line No.	BC_4F_1 Seeds Produced
BR22*4 / BRRI dhan51	1	BR10190-3-1-16	28
	2	BR10190-3-1-2	90
	3	BR10190-3-1-5	40
	4	BR10190-3-1-20	59
	5	BR10190-3-1-9	25
	6	BR10190-3-7-14	50
	7	BR10190-3-7-3	42
	8	BR10190-3-7-8	38
	9	BR10190-3-1-19	70
	10	BR10190-3-7-20	85
	Total		527

Table 10: Number of BC_5F_1 seeds produced from selected BC_4F_1 plants, T. Aman 2015 season.

Backcross Combinations	Serial No.	Pedigree Line No.	BC_5F_1 Seeds Produced
BR22*5 / BRRI dhan51	1	BR10190-3-1-16-2	85
	2	BR10190-3-1-20-3	10
	3	BR10190-3-7-14-6	128
	4	BR10190-3-1-16-7	24
	5	BR10190-3-1-16-8	38
	6	BR10190-3-1-20-1	165
	7	BR10190-3-1-20-3	10
	8	BR10190-3-7-14-7	42
	9	BR10190-3-7-3-1	70
	10	BR10190-3-1-19-5	60
	Total		632

The first target of this experimental work was to introgress the *SUB1* QTL into the genetic background of BR22 using marker-assisted backcrossing in order to maximize the recovery of the desirable traits of the recurrent parent. During this process, three levels of selection approaches of marker assisted backcross breeding viz. foreground, recombinant and background selections were followed. In this study foreground selection was done in BC_3F_1 generation to produce BC_4F_1 seed and foreground, phenotypic, recombinant and background selections were done in BC_5F_1 generation in order to introgress *SUB1* QTL into BR22. After performing all the levels of selections, backcrosses were done with the selected plants of BC_4F_1 generation to develop BC_5F_1 generation.

In Marker Assisted Backcrossing of the present study, foreground, phenotypic, recombinant and background selection steps were applied in BC_4F_1 generation in order to develop BR22-Sub1 variety. In foreground selection, Sub1C173, gene-based marker specific to putative Sub1C gene was used to select the backcross progenies with *SUB1* QTL. In this selection step, BC_4F_1 progenies segregated following 1:1 ratio which indicated that *SUB1* QTL followed Mendelian segregation.

It was possible to minimize linkage drag or to obtain recipient alleles for the two flanking markers with 'A' scores. However, it was not possible to avoid linkage drag for the other two flanking markers, which may be achieved in the next backcross generation. The results of recombinant selection also revealed that the size of the *SUB1* QTL introgression from the donor parent was estimated to be 2.5 Mb. The agronomically suitable genetic background of BRRI dhan 51 (proved earlier) nearby *SUB1* QTL will hopefully not affect the phenotype of the newly developed breeding lines.

In the present study 3 best plants were identified possessing 95.26%, 94.26% and 93.95% recipient parent genetic background along with target *SUB1* QTL in the heterozygous state. The rest of the genome was found segregating for the remaining heterozygous background markers. The recovery of recipient parent genome in the best plant of BC_4F_1 generation indicated that one more backcrossing and a final self-pollination in the BC_5F_1 generation would be required in order to develop BR22-Sub1 homozygous line. The results of the Marker Assisted Backcrossing works in BC_5F_1 generation indicated that the introgression work will be completed in BC_5F_2 generation.

The current output of Marker Assisted Backcrossing activities of this research work reflected that the approaches could routinely be used in order to introgress tolerant or resistant genes or QTLs, conferring tolerances or resistances to abiotic stresses, into the genetic background of high yielding varieties.

CONCLUSION

The newly developed BR22-Sub1 lines could be useful as a strongly photosensitive submergence tolerant rice line for the multiple flash flood affected greater Northern region of Bangladesh. However, the submergence tolerance, yield and other attributes of the newly developed BR22 line will be evaluated later.

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