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Rice Science, 2015, 22(1): 16−26

Development of New Submergence Tolerant Rice Variety for Bangladesh Using Marker-Assisted Backcrossing

Khandakar Md IFTEKHARUDDAULA, Helal Uddin AHMED, Sharmistha GHOSAL, Zakiah Rahman MONI, Al AMIN, Md Shamsher ALI

(*Plant Breeding Division, Bangladesh Rice Research Institute, Gazipur-1701, Bangladesh*)

Abstract: Submergence tolerant high yielding rice variety was developed using BR11 as a recipient parent applying foreground, phenotypic and background selection approaches. Recombinant selection was found essential to minimize linkage drag by BC_2F_2 generation. Without recombinant selection, the introgression size in the backcross recombinant lines (BRLs) was approximately 15 Mb on the carrier chromosome. The BRLs were found submergence tolerance compared to the check varieties under complete submergence for two weeks at Bangladesh Rice Research Institute, and produced higher yield compared to the isogenic Sub1-line under controlled submerged condition. The BRL IR85260-66-654-Gaz2 was released as BRRI dhan52 in 2010, which was the first high yielding submergence tolerant variety in Bangladesh. BRRI dhan52 produced grain yield ranging from 4.2 to 5.2 t/hm^2 under different flash flood prone areas of Bangladesh in three consecutive seasons. The study demonstrated the efficiency of recombinant selection and better adaptability of the newly released submergence tolerant high yielding variety in flash flood prone different areas of the country with respect to submergence tolerance and yield potential.

Key words: backcross recombinant line; marker-assisted backcrossing; recombinant selection; rice; submergence tolerance

By 2035, a 26% increase in rice production will be essential to feed the rising population (Seck et al, 2012). In addition to developing rice varieties for favorable ecosystem, improved high yielding rice cultivars for unfavorable ecosystems are also required. For further improvement in total rice production, rice breeders must develop high yielding varieties with tolerance against abiotic stress for unfavorable ecosystems. Among the abiotic stress, submergence is one of the important factors in the flash flood prone rice growing environment (Mackill, 1986).

IPCC (2007) provides scientific evidence of climate change that will increase the intensity and severity of drought and flood. Impact of climate change is now increasingly visible. The agroecosystems of Bangladesh are facing various environmental stress (Kabir, 2010). Flash flood submergence is an important hazard to the agriculture of Bangladesh related with climate-change. More than 2.0 million hectare areas of Bangladesh are affected by different grades of flash floods (Iftekharuddaula et al, 2009). Flash floods regularly affect rain-fed lowland rice (RLR) ecosystems in many parts of the country where flood water remains for around two weeks. The ecosystem affected by flash floods mainly constitutes low to medium lowland. *Kharif* season rice grown in Bangladesh often gets submerged during seedling and vegetative stages, and suffers substantial yield losses. Submergence is one of the most important technical constraints in Bangladesh, which accounts for 22% of all technical constraints (Dey and Upadhyaya, 1996).

The present study comprises all the selection levels of marker-assisted backcrossing (MABC) except recombinant selection. In contrast to our previous work to generate a converted mega-variety with *Sub1*, in this study, efforts were undertaken to develop

Received: 23 July 2014; **Accepted:** 11 September 2014

Corresponding author: Khandakar Md IFTEKHARUDDAULA (kiftekhar03@yahoo.com)

Peer review under responsibility of China National Rice Research Institute

http://dx.doi.org/10.1016/j.rsci.2015.05.003

transgressive backcross recombinant lines (BRLs) which will have improved phenotype so that the stress prone farmers can easily differentiate the newly developed stress tolerant variety from the original variety BR11, in other words, to develop transgressive backcross recombinant type BR11-Sub1 lines. As a result, a backcross recombinant line (using BR11 as the recipient parent) was developed using MABC and released as BRRI dhan52 in 2010 by the Bangladesh Rice Research Institute (BRRI) as a submergence tolerant high yielding variety. The present study reports the yield and adaptability tests of the released variety (BRRI dhan52) for flash flood prone areas of Bangladesh in three consecutive years.

MATERIALS AND METHODS

Rice materials and crossing scheme

IR40931-33-1-3-2, one of the FR13A-derived submergence tolerant breeding lines (Mackill et al, 1993), was used as the donor of *SUB1*, with the moderate yield of 3–4 t/hm^2 and improved phenotype. The *SUB1* gene in this line was inherited from a landrace FR13A, a widely-used submergence tolerant donor with poor agronomic properties. The recipient variety was BR11, a widely grown cultivar in Bangladesh, which was derived from the cross IRRISail/IR5, and was originally designated BR52-87-1-HR88. The variety was released by BRRI in 1980 for the RLR season. The yield potential of this variety is 5.0 t/hm^2 under optimum management. For the MABC scheme (Fig. 1), BR11 was crossed with IR40931-33-1-3-2 to obtain F_1 seeds. F_1s were backcrossed with BR11 to obtain BC_1F_1 seeds.

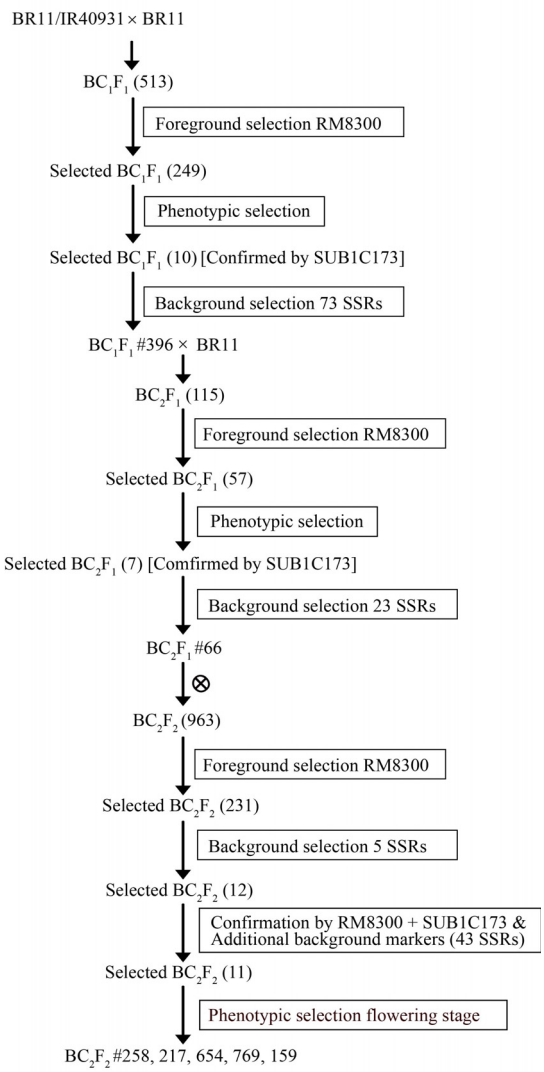

Fig. 1. Development of submergence tolerant backcross recombinant lines through marker-assisted backcrossing with details of markers used in each generation.

The number in parentheses indicates the total number of plants whereas the number followed by # indicates the individual plant number.

Molecular marker analysis

DNA was extracted from young leaves of 2-week-old plants using a protocol as described by Zheng et al (1995). PCR was performed in 10 μL reactions containing 25 ng of DNA template, 1 μL of 10 × TB buffer (containing 200 mmol/L Tris-HCl pH 8.3, 500 mmol/L KCl, 15 mmol/L $MgCl_2$), 1 μL of 1 mmol/L dNTPs, 0.50 μL each of 5 μmol/L forward and reverse primers and 0.25 μL of *Taq* DNA polymerase (4 U/μL) using a single 96-well thermal cycler (MJ Research Inc., Canada) or G-Storm thermal cycler (Gene Technologies Ltd., England). After initial denaturation for 5 min at 94 °C, each cycle comprised 1 min denaturation at 94 °C, 1 min annealing at 55 °C, and 2 min extension at 72 °C with a final extension for 5 min at 72 °C at the end of 35 cycles. The PCR products were mixed with gel loading dye and analyzed by electrophoresis on 8% polyacrylamide gel using mini vertical polyacrylamide gels for high throughput manual genotyping (CBS Scientific Co. Inc., CA, USA). As the standard marker, 1 kb$^+$ DNA ladder was used during electrophoresis to determine the amplicon size. The gels were stained in 0.5 mg/mL ethidium bromide, and photos were taken using Alpha Imager 1220 (Alpha Innotech, CA, USA). Microsatellite or simple sequence repeat (SSR) markers were used for selection (Temnykh et al, 2001; McCouch et al,

2002; IRGSP, 2005).

Selection approach

In BC_1F_1 generation, foreground selection was initially carried out by the robust tightly-linked marker RM8300, and the individual plants that were heterozygous at the *SUB1* locus were identified reducing the population size for further selection, then were marked with sticks in the field. From these selected plants, 10 individuals with the phenotypic appearance closed to the recipient parent BR11 were selected visually at the vegetative stages (Iftekharuddaula et al, 2012).

Microsatellite markers unlinked to *SUB1* covering all the chromosomes, including the *SUB1* carrier chromosome 9, were used for background selection to recover the recipient genome. Out of 524 SSR markers surveyed, a total of 73 were used. Microsatellite markers that were not fixed for the recurrent parent allele were genotyped in the following generations. Background selection was carried out among the 10 phenotypically selected plants. Backcross seeds for the next generation were produced based on ranking of background selection. In all the cases, foreground selection was confirmed by gene-based marker genotyping. In the second backcross generation, the same strategy was followed for selection of individual plants with the desired allelic combination at the target loci. In the BC_2F_1 generation, a total of seven plants were phenotypically selected based on resemblance to the recurrent parent at the vegetative stages. Background selection was again carried out over the seven phenotypically selected plants and the plants having the highest rank according to background selection were self-pollinated to produce BC_2F_2 seeds. In the BC_2F_2 generation, plants that possessed the homozygous donor alleles for the tightly-linked marker were used for background selection with the remaining initial and additional background markers. Phenotypic selection was again carried out after background selection. A total of 11 homozygous plants were obtained, and five BRLs were selected and self-pollinated for further phenotypic adaptability. Fig. 1 illustrates the MABC process. Out of five lines, one line IR85260-66-654-Gaz2 was released as BRRI dhan52 in 2010 in Bangladesh.

Analysis of molecular data

The molecular weights of the different alleles were measured using the Alpha Ease FC 5.0 software. The marker data were analyzed using the Graphical Genotyper (GGT 2.0) (van Berloo, 2008). The homozygous recipient allele, homozygous donor allele and heterozygous allele were scored as 'A', 'B' and 'H', respectively. The percentages of markers homozygous for recipient parent and recipient alleles were calculated following the formulae described by Iftekharuddaula et al (2011).

Screening of Sub1-lines for submergence tolerance

Ten genotypes were evaluated along with two standard check varieties *viz*. BR11 and Swarna in the submergence pond No. 4 of deep water station of BRRI Gazipur during Aman season in 2009. Thirty-five-day-old seedlings were transplanted at 2 seedlings per hill with a spacing of 25 cm × 15 cm. The unit plot size was 5.4 m × 10 rows. The experiment was laid out in randomized completely block design with five replications. The seeding of the entries was done on 2 July 2009, transplanting on 5 August, submergence on 17 August and desubmergence on 1 September. The experimental plots were submerged for 15 d starting from 12 d after transplanting. The average depth of water was 93 cm. The recorded average water temperature was 32 °C, and water pH was 7.2. After complete submergence, water was completely drained out from the plot. Standard management practices were followed (M A Mazid, personal communication).

Adaptability test of BRRI dhan52 in multi-location trials

RLR season 2010

BRRI dhan52, submergence tolerant high yielding genotypes and standard check varieties were evaluated under natural flash flooding submergence condition at Lalmonirhat, Bangladesh. The experiment was laid out in RCB design with three replications. Thirty-five d old seedlings were transplanted using 2–3 seedlings per hill with the spacing of 20 cm × 15 cm. The unit plot size was 5.4 m × 12 rows. Fertilizer doses were 65:85:56:5 kg of triple super phosphate (TSP), muriate of potash (MP), gypsum and zinc sulphate per herctare. Total amount of TSP, gypsum and zinc sulphate and two-third MPs were applied at the time of final land preparation. Urea was applied in two splits. At first, 45 kg/hm^2 and one-third MP were applied at 10 d after desubmergence and secondly another 45 kg/hm^2 was applied at 20–25 d after first application of urea. Sanitation was done at 7 d after subsiding of water. Furadan 5G was applied twice along with first and

second top dress of urea fertilizer.

RLR season 2011

BRRI dhan52, IR64-Sub1, BRRI dhan51 and BRRI dhan33 were evaluated in submergence prone farmer's field at Faridpur, Bangladesh. The experiment was laid out in RCB design with three replications. Similar cultivation practices of RLR season 2010 were followed.

RLR season 2012

BRRI dhan51, BRRI dhan52, and six submergence-cum-stagnant flood tolerant high yielding genotypes were evaluated in this trial under rain-fed and real submergence and/or medium stagnation prone environments of the farmers' field in Kurigram, Bangladesh. The experiment was laid out in RCB design with three replications. Similar cultivation practices of RLR season 2010 were followed.

RESULTS

Foreground selection

In the BC_1F_1 generation, foreground selection was performed on 513 plants using RM8300, a marker tightly linked to the submergence tolerance QTL *SUB1*. Out of 513 plants, 249 plants showed heterozygous alleles (score 'H'), 261 plants showed fixed recipient allele (susceptible allele) (score 'A') and only 3 plants showed fixed donor allele (resistant allele) (score 'B'). The three plants with 'B' score were produced due to accidental failure of backcrossing. The results fitted to the expected 1:1 ratio of this generation for the homozygous recipient allele and heterozygous allele groups with a non-significant chi-square value of 0.28 at a probability level of 0.05. The 249 plants with the 'H' score were subjected for foreground selection. Fig. 2 shows the partial view of the gel picture of the foreground selection with the RM8300 of BC_1F_1 generation (plant number 49 to 93).

In the BC_2F_1 generation, foreground selection was carried out over 94 of 115 plants using RM8300. Out of 94 plants, 57 plants showed heterozygous 'H' score, 36 plants showed 'A' score and only 1 plant showed 'B' score. The results fitted the expected 1:1 ratio of this generation with a non-significant chi-square value of 4.75. Obviously, 57 plants with the 'H' score for the tightly linked marker were subjected for phenotypic selection.

BC_2F_2 seeds were produced by self-pollination of the best plant with number 396-66 of BC_2F_1 generation where foreground markers RM8300 and SUB1C173 were in the heterozygous state. Out of 963 plants, 496 plants showed 'H' score, 236 plants showed 'A' score and 231 plants showed 'B' score. The results fitted the expected 1:2:1 ratio of this generation (chi-square value of 0.9, $P > 0.05$). Obviously, 231 plants with the 'B' score for the tightly linked marker was subjected for background selection.

Phenotypic selection

In the first backcross generation, from the plants that were selected for *SUB1*, 10 individuals with the phenotypic appearance closed to the recipient parent BR11 were selected visually at the vegetative and flowering stages. The 10 selected plants were ranked based on their degree of phenotypic resemblance with BR11, and backcross seeds were produced from the three individuals (plant number 396, 14 & 445) with the highest phenotypic rankings. In the second backcross generation, the same strategy was followed for selection of individual plants with *SUB1*. In total, seven plants were selected and ranked based on visual phenotypic performance compared to recipient parent BR11 as before. BC_2F_2 seeds were produced from the plants having the highest phenotypic ranking.

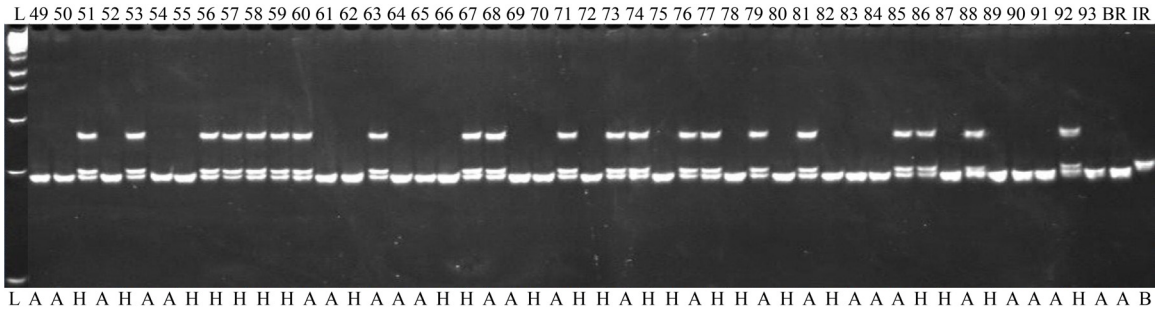

Fig. 2. Partial view of the gel picture of the foreground selection with the tightly linked marker RM8300, BC_1F_1 generation.

L, 1 kb$^+$ DNA ladder; BR, BR11, the recipient parent; IR, IR40931-33-1-3-2, the donor parent; A, Homozygous recipient allele; B, Homozygous donor allele; H, Heterozygous allele; 49–93, Plant number.

Table 1. Results of the background selection, BC_1F_1 generation.

Plant No.	A	H	B	Recipient allele (%)	Rank-background
14	46	26	0	81.9	2
59	26	46	0	68.1	7
73	23	41	8	60.4	9
86	29	43	0	70.1	5
145	34	38	0	73.6	4
235	18	43	11	54.9	10
248	26	46	0	68.1	8
396	48	24	0	83.3	1
445	44	28	0	80.6	3
496	27	45	0	68.8	6
Average		35		71.0	

A, Homozygous recipient allele; H, Heterozygous allele; B, Homozygous donor allele.

Background selection

In the BC_1F_1 generation, background selection was carried out over 10 phenotypically selected plants using 73 background markers. The highest percentage of recipient alleles was obtained in plant number 396 (83.3%), followed by plant number 14 (81.9%), plant number 445 (80.6%), and plant number 145 (73.6%). Therefore, plant number 396 was selected with the highest percentage of recipient alleles (Table 1).

In the second backcross generation, background selection was carried out over seven phenotypically selected plants. A total of 23 background markers remaining for this population were used. The percentage of recipient alleles was calculated in each selected plants considering the markers used in the BC_1F_1 generation, and the selected plants were ranked in ascending order. The highest percentage of recipient alleles was obtained in plant number 396-66 (96.1%), followed by plant number 396-26 (93.8%), plant number 396-18 (90.6%), plant number 396-2 and 396-66 (89.8%), respectively (Table 2). Plant number 396-66 was selected, and it had a total of five heterozygous background markers remaining. However, the average percentage of recipient alleles over 7 phenotypically plants was 90.8%, and average number of heterozygous alleles was 12.

Table 2. Results of the background selection, BC_2F_1 generation.

Plant No.	A	H	B	Recipient allele (%)	Rank-background
396-2	59	13	0	89.8	4
396-18	60	12	0	90.6	3
396-26	64	8	0	93.8	2
396-37	59	13	0	89.8	5
396-45	55	17	0	86.7	7
396-66	67	5	0	96.1	1
396-89	58	14	0	89.1	6
Average		12		90.8	

A, Homozygous recipient allele; H, Heterozygous allele; B, Homozygous donor allele.

In the BC_2F_2 generation, background selection was carried out over 231 segregants with the fixed donor alleles of *SUB1* QTL. A total of five background markers were remaining for the BC_2F_2 population. The remaining background markers were RM237 and RM486 in chromosome 1, RM154 and RM279 in chromosome 2 and RM566 in chromosome 9. Out of 231 plants, 10 plants *viz*. 396-158, 396-159, 396-217, 396-258, 396-294, 396-559, 396-607, 396-654, 396-664 and 396-771 possessed homozygous recipient alleles for four markers and homozygous donor alleles for one marker. Likewise, two plants *viz*. plant numbers 396-642 and 396-769 got homozygous recipient alleles for four markers and heterozygous alleles for one marker (Table 3).

Selection of backcross recombinant lines

It was not possible to recover the recurrent parent genome in these 12 selected plants completely. All the plants were homozygous for the *SUB1* QTL except plant number 396-642 and 396-769. The genetic constitutions of the plants were recombinant type compared to the genome of BR11 as the plants had a fixed chromosomal segment of the donor parent. These plants were termed as backcross recombinant lines, selection of them was one of the practical objectives of this approach with respect to varietal development. The phenotypes of the 12 selected plants

Table 3. Results of the background selection, BC_2F_2 generation.

Marker	Plant number											
	396-158	396-159	396-217	396-258	396-294	396-559	396-607	396-642	396-654	396-664	396-769	396-771
RM237	A	A	A	A	A	A	A	A	A	A	A	A
RM486	A	A	A	A	A	A	A	A	A	A	A	A
RM154	A	A	A	A	A	A	A	A	A	A	A	A
RM279	A	A	A	A	A	A	A	A	A	A	A	A
RM566	B	B	B	B	B	B	B	H	B	B	H	B

A, Homozygous recipient allele; H, Heterozygous allele; B, Homozygous donor allele.

of BC_2F_2 population were also investigated thoroughly. Based on phenotypic performance and morphological variation *viz*. plant type, long panicle, dense grains, five plants were selected *viz*. plant number 396-66-159, 396-66-217, 396-66-258, 396-66-654 and 396-66-769, which were later designated as IR85260-66-159, IR85260-66-217, IR85260-66-258, IR85260-66-654 and IR85260-66-769 as per International Rice Research Institute nomenclature system.

Use of additional background markers

The twelve selected plants were tested with 43 additional background markers. Segregation was not observed in these five plants with the use of these additional background markers.

Confirmation of *SUB1* gene in the selected BRLs

Four selected BC_2F_2 BRLs were tested with the cleaved amplified polymorphic sequence (CAPS) marker specific to *SUB1A* which was the putative candidate marker for *SUB1* gene. Fig. 3 shows that the susceptible allele of BR11 was cut but the resistant allele of IR40931-33-1-3-2 was not cut. The tested four BRLs showed the same banding pattern of resistant allele of the donor of *SUB1* gene IR40931-33-1-3-2. Hence, it was possible to confirm that all the selected plants contained *SUB1* gene.

Monitoring introgression size

The size of the introgression of *SUB1* QTL was monitored in the selected best plant IR85260-66-654 and measured to be 60 cM or 15 Mb (Fig. 4) assuming that 1 cM is equal to 4 Mb.

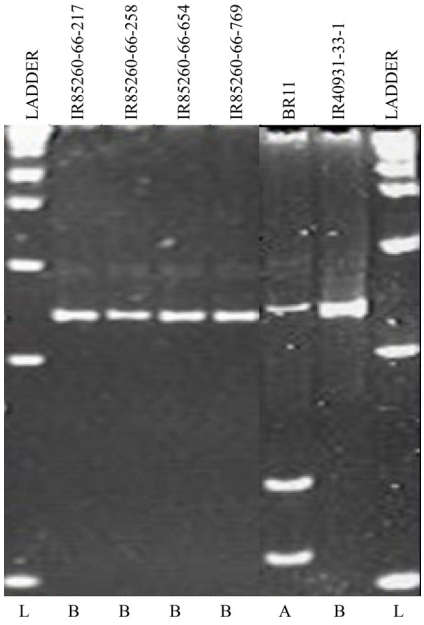

Fig. 3. Confirmation of *SUB1* allele in four backcross recombinant lines derived from BR11/IR40931-33-1-3-2 by GnS2 marker.
A, Homozygous recipient allele; B, Homozygous donor allele; L, 1 kb^+ DNA ladder.

Grain quality parameters

Based on kernel length (6.09 mm) and the ratio of kernel length and kernel breadth (2.20), the brown rice shape of IR85260-66-654-Gaz2 (BRRI dhan52) was classified as medium (both length and shape). Considering grain chemical properties, 29.5% amylose was obtained from BRRI dhan52 with medium gel consistency. Both the milling outturn and head rice recovery of the

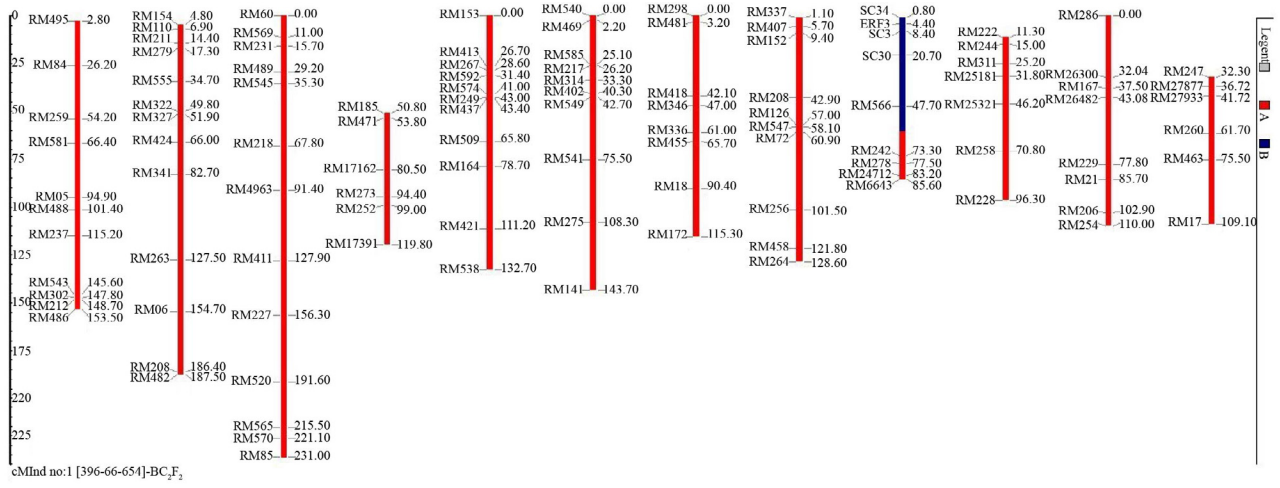

Fig. 4. Graphical genotype of plant number IR85260-66-654 of BC_2F_2 population.
The red colored regions on the chromosomes indicate homozygous regions for the recipient genome while the blue colored regions indicate the heterozygous regions. The distances were represented in cM based on published map of Temnykh et al (2001).

Table 4. Performances of Sub1-lines under controlled submergence condition, T. Aman 2009, BRRI, Gazipur.

Designation	Days to maturity (d)	Plant height (cm)	No. of panicles per plant	Panicle length (cm)	No. of grains per m^2	Survival rate (%)	Grain yield (t/hm^2)
BR11-Sub1	152	123	14.4	26.1	25 044	79.6	5.67
Swarna-Sub1	155	95	15.3	23.8	33 783	89.6	5.53
Sambha Mahsuri-Sub1	151	93	14.1	23.6	32 424	89.0	4.40
IR85260-66-217-Gaz2	153	127	11.8	25.7	27 303	90.8	5.80
IR85260-66-654-Gaz2 (BRRI dhan52)	152	127	11.8	26.5	26 909	93.4	6.13
IR85620-66-769-Gaz2	151	127	11.7	26.1	24 461	94.4	5.54
IR85620-391-217	152	116	10.6	25.7	25 402	88.2	5.17
IR85260-391-1192	152	120	9.8	25.3	26 278	87.8	5.86
IR85260-66-258-Gaz2	153	128	12.5	27.7	27 284	88.4	5.79
IR64-Sub1	135	102	17.8	25.6	22 942	92.6	3.92
Swarna (CK)	164	90	22.4	24.5	18 075	24.8	2.15
BR11 (CK)	164	125	19.4	27.6	17 982	30.6	3.67
LSD (0.05)	2.4	3.7	6.53	1.53	5 942	15.1	1.37

LSD (0.05), Least significant difference at 0.05 level of probability.

variety were 70%. However, the taste of the cooked rice was also found good and acceptable by the consumers of flash flood prone areas of Bangladesh.

Submergence screening, RLR season, 2009, BRRI, Gazipur

The highest yield performance was obtained from IR85260-66-654-Gaz2 (6.13 t/hm^2) followed by IR85260-391-1192 (5.86 t/hm^2) and IR85260-66-258-Gaz2 (5.79 t/hm^2). The highest yielding Sub1-line produced around 2.5 t/hm^2 more yield than the check variety BR11 and the Sub1-line was 12 days earlier than BR11, and the days to maturity of BRRI dhan52 was 12 d earlier than that of BR11. The highest survival rate (%) was obtained from IR85260-66-769-Gaz2 (94.4%), followed by IR85260-66-654-Gaz2 (BRRI dhan52) (93.4%) and IR64-Sub1 (92.6%). In comparison, Swarna and BR11 had 24.8% and 30.6% survival, respectively. The survival (%) of the backcross recombinant lines were also found more (max. 15%) than that of precision introgression line (PIL) of BR11-Sub1. The plant heights of recombinant lines of BR11-Sub1 (e.g. IR85260-66 lines) were around 5 cm more than the PIL of BR11-Sub1 (Table 4).

Table 5. Performances of Sub1-lines under natural flash flood condition, T. Aman in 2010, Borobari, Lalmonirhat.

Designation	Plant height (cm)	Survival rate (%)	Days to maturity (d)	Yield (t/hm^2)
BRRI dhan52	100.7	94.9	159	4.9
IR64-Sub1	84.9	96.8	138	3.0
Samba Mahsuri-Sub1	89.2	93.2	153	3.9
IR85260-66-1192	95.7	93.2	155	4.1
BRRI dhan51	82.1	95.6	161	4.6
IR85260-391-148	95.8	94.6	163	4.3
BRRI dhan33 (CK)	95.2	74.2	140	2.5
BINA dhan7 (CK)	91.7	88.4	141	2.8
LSD (0.01)	2.27	1.35	1.05	0.27

LSD (0.01), Least significant difference at 0.01 level of probability; Date seeded on 25 June, 2010; Date transplanted on 30 July, 2010.

Adaptability tests of BRRI dhan52

RLR season 2010

At Lalmonirhat (Borobari), the experimental plots were submerged for 12 d at 20 d after transplanting (submergence on 19 August 2010 and desubmergence on 31 August 2010). The average depth of water was around 80 cm. IR64-Sub1 gave around 0.5 t/hm^2 higher yield than corresponding check varieties BRRI dhan33 and BINA dhan7 which was around three weeks earlier than BRRI dhan51 and BRRI dhan52 and also showed highest survival. However, BRRI dhan51 and BRRI dhan52 were the highest yielder genotypes. The range of plant height was 82.1–100.7 cm and growth duration ranged from 138–163 d (Table 5).

RLR season 2011

There was 12 d of flash flooding at Godadhardangi,

Table 6. Performance of entries of phenotypic variations trial, T. Aman in 2011, Gadadhardangi, Faridpur.

Designation	Plant height (cm)	Growth duration (d)	Yield (t/hm^2)
BRRI dhan52	115	145	5.2
IR64-Sub1	95	119	2.5
BRRI dhan51	89	150	2.1
BRRI dhan33 (CK)	104	117	2.6
LSD (0.01)	2.52	0.94	0.46

LSD (0.01), Least significant difference at 0.01 level of probability. Date transplanted on 3 August 2011; Date submerged on 10 August, 2011; Date de-submerged on 25 August, 2011.

Table 7. Performance of entries of phenotypic variations trial in submergence prone farmers' field, T. Aman in 2012.

Designation	Plant height (cm)			Growth duration (d)			Yield (t/hm^2)		
	L1	L2	Ave	L1	L2	Ave	L1	L2	Ave
IR09F147	123	121	122	137	134	136	3.0	3.1	3.1
IR09F177	133	129	131	143	139	141	2.6	2.9	2.7
IR09F189	124	105	114	139	135	137	2.8	3.1	3.0
IR09F226	137	132	135	143	138	141	3.0	3.2	3.1
IR09F236	136	121	128	140	137	138	2.7	2.9	2.8
IR09F253	123	114	118	142	139	140	2.8	3.0	2.9
BRRI dhan51	96	86	91	148	143	146	4.5	4.8	4.6
BRRI dhan52	131	136	134	138	133	135	4.2	4.2	4.2
LSD (0.01)	6.05	12.2		2.05	2.87		0.32	0.3	

LSD (0.01), Least significant difference at 0.01 level of probability; Ave, Average; L1, Mojiterpar, Hilmari, Kurigram; Date seeded on 28 June, 2012; Date transplanted on 04 August, 2012; Date submerged on 13 September, 2012; Date de-submerged on 23 September, 2012; L2, Sariferhat, Chilmari, Kurigram; Date seeded on 26 June, 2012; Data transplanted on 26 July, 2012; Date submerged on 14 September, 2012; Date de-submerged on 24 September, 2012; Average water height: 60cm.

Faridpur Sadar. The crops at Godadhardangi recovered well from submergence pressure, but due to slow receding of flood water, BRRI dhan51 cannot perform well (2.1 t/hm^2 grain yield). At Gadadhardangi, Faridpur Sadar, flooding was occurred 7 d after transplanting, and the duration was 12 d. Flooding water depth was 1–2.5 feet. Grain **y**ield of BRRI dhan52 was the highest (5.2 t/hm^2) followed by BRRI dhan33 (2.6 t/hm^2) and IR64-Sub1 (2.5 t/hm^2) (Table 6).

RLR season 2012

The trials were submerged for 11 d with around 60 cm water depth at the two flash flood prone locations of Kurigram, Bangladesh. Under these situations, BRRI dhan52 produced the second highest yield 4.2 t/hm^2 (average) in both the locations. The average plant height and growth duration obtained were 134 cm and 135 cm, respectively in two locations (Table 7).

DISCUSSION

The present study established the utilization of marker-assisted backcrossing technique for developing transgressive backcross recombinant line (BRL) having different phenotypes other than the recurrent parent mega variety. This type of variety was developed so that the new stress tolerant variety can be easily identified by the farmers. If the newly developed stress tolerant variety is exactly similar to the original recipient mega variety, apprehension arises that the dishonest seed dealers might cheat the farmers. That's why, phenotypically different backcross recombinant lines were developed which possessed some recombination in their genetic background. The BRLs were produced through self-pollination of BC_2F_1 plants which had five heterozygous loci in addition to the heterozygous target gene locus. The BC_2F_1 plants had around 15 Mb heterozygous chromosomal segments on the carrier chromosome 9. This large donor segment on the carrier chromosome was obtained due to not practising recombinant selection. In this study, we did not perform recombinant selection as used previously (Iftekharuddaula et al, 2011), but we used phenotypic selection instead in order to select plants that closely resembled the recipient parent, and obtained transgressive segregants for yield or other desirable traits.

Precision introgression lines as proposed by Collard et al (2008) possess very small donor segments on the carrier chromosome like BR11-Sub1 (Iftekharuddaula et al, 2009), which had only 800 kb donor introgression. Takeuchi et al (2006) developed three isogenic lines for the heading date QTLs, where introgresion size ranged from 170–625 kb which was possible due to performing recombinant selection. The selected BC_3F_2 individual had a fragment of less than 3.8 cM from the donor line in the *Xa21* region on chromosome 11 (Chen et al, 2000). Since the selected BC_2F_2 individuals in this study had a 15 Mb fixed donor chromosomal segment and the lines were produced by two backcrosses utilizing marker-assisted background selection, the newly developed lines were designated as BRLs. The BRLs were produced from the cross combinations BR11/IR40931-33-1-3-2 which was good × poor combination for grain yield. Iftekharuddaula et al (2005) reported high specific combining ability effect for grain yield from good × poor cross combination.

The submergence tolerance of the newly developed BRLs was again found superior compared to the PILs. The submergence tolerance of the BRL IR85260-66-

654-Gaz2 (BRRI dhan52) had the highest survival rate and the highest grain yield under controlled submergence trial during T. Aman in 2009 at BRRI, Gazipur. In comparison, BR11-Sub1 had the lowest survival rate and significantly lower grain yield. The submergence tolerant lines were also tested in the flash flood affected farmers' field. The BRL IR85260-66-654-Gaz2 (BRRI dhan52) produced the highest grain yield being half more ton per herctare and significantly higher survival rate compared to the PIL BR11-Sub1. The higher submergence tolerance of the BRL IR85260-66-654-Gaz2 (BRRI dhan52) might be associated with some minor genes remaining in the 15 Mb donor segment on the carrier chromosome which again can be inherited from FR13A in the donor parent IR40931-33-1-3-2 or might be due to some positive interaction of *SUB1* QTL with the genes remaining in that region. Submergence tolerance is a polygenic trait, and *SUB1* QTL does not completely represent the trait alone (Grover et al, 2000). The donor parent of the present study is already a moderately improved breeding line (Mackill et al, 1993) which possesses very strong submergence tolerance.

The introgression size in the three BRLs *viz*. IR85260-66-217, IR85260-66-258, IR85260-66-654 was 15 Mb on chromosome 9 including *SUB1* QTL from IR40931-33-1-3-2. In conventional backcrossing, large donor segments are likely on the carrier chromosome (Young and Tanksley, 1989).

Due to not practising recombinant selection, the size of the donor introgression on the chromosome 9 was 15 Mb in the BRLs. The best plant of BC_1F_1 (plant number 396) and the best plant of BC_2F_1 (plant number 396-66) had heterozygous chromosomal segment with a size 15 Mb on the carrier chromosome 9. The selected BC_2F_2 plants *viz*. 396-66-258, 396-66-217, 396-66-654 also had 15 Mb linkage drag ultimately, which was a fixed chromosomal segment of donor parent. With this context, Young and Tanksley (1989) pointed out that the donor genes on the carrier chromosome were the most difficult to eliminate, and could persist long after the donor genome content of non-carrier chromosomes had returned to approximately zero if no selection on markers was applied. According to the report of IRGSP (2005), one gene has been predicted per 9.9 kb of rice genome. Therefore, the 15 Mb portion of donor genome can contain approximately 1 500 genes. If the donor parent possesses many genes conferring undesirable agronomic traits in this region, this can reflect negatively on the agronomic performance of the selected plants. In general, this large introgression indicated considerable weakness of this approach with respect to minimizing linkage drag. However, in the selected best plant where recombinant selection was practised (Iftekharuddaula et al, 2009), the introgression size was 0.8 Mb in BR11-Sub1. The findings demonstrated the superiority of recombinant selection with respect to minimizing linkage drag. The results were in agreement with Frisch et al (1999) and Hospital (2001), who minimized the linkage drag using markers that were flanked a target gene (e.g. < 5 cM on either side) in simulated populations. Interestingly, as the donor parent was already a moderately improved genotype, this huge introgression did not reflect any negative effect on the phenotype of the BRL. In fact, there appeared a positive effect on yield in this study which was unexpected. Therefore, a major recommendation for MABC programs based on this study is for a range of advanced backcross lines should be evaluated together including the lines with the highest proportion of recipient parent genome and other lines with larger introgression segments or unlinked donor segments.

The national seed board of Bangladesh recommended the proposal of national technical committee to release the backcross recombinant line IR85260-66-654-Gaz2 as BRRI dhan52 on 6 April, 2010. The newly released variety is the first high yielding submergence tolerance variety with plant type closed to mega variety BR11 in Bangladesh.

The average grain yield performance of BRRI dhan52 under different flash flood prone areas of Bangladesh under consecutive three seasons ranged from 4.2 to 5.2 t/hm^2 which was satisfactory. However, the duration of BRRI dhan52 was around 15 d delayed where the severity of flash flooding was probably more intensified at Lalmonirhat in 2010 compared to the duration of the original non-Sub1 mega varieties. This delay in growth duration due to BRRI dhan52 is weakly photosensitive. The delaying of growth duration along with longer growth duration of the Sub1-varieties might create problem in fitting them in some cropping sequences. The scenarios become worse because there are multiple flash floods particularly in Bangladesh. Under multiple flash flooding situations of Bangladesh, the duration of Sub1-varieties like BRRI dhan52 is so delayed that it is affected by cold shock at the reproductive phase (data not shown). But the problems can be solved by introgressing *SUB1* QTL into early or strongly photo-sensitive but high

yielding RLR varieties like BRRI dhan33 and BR 22. Recently, *SUB1* QTL has been introgressed into early RLR varieties of Bangladesh *viz*. BRRI dhan33 and BRRI dhan49, which will be reported elsewhere soon. However, it is expected that the released submergence tolerant variety BRRI dhan52 will increase the rice production in the submergence prone areas of Bangladesh where arises single flash flood but the suitability studies of different submergence tolerant varieties under different cropping patterns in the flash flood prone areas of Bangladesh should be undertaken.

CONCLUSIONS

In conclusion, BRRI dhan52 was released as a high yielding and submergence tolerant variety using marker-assisted backcrossing. Submergence tolerance and adaptability tests of this variety under multi-location trials in the farmers' field showed satisfactory performance with respect to grain yield and some yield contributing parameters. Hopefully, this variety will be a mega variety for the submergence prone areas of Bangladesh in T. Aman season. The efficiency of recombinant selection was established with respect to minimizing the linkage drag. In absence of recombinant selection, the size of the donor introgression was around 15 Mb. It is expected that this variety will contribute to the national GDP and also alleviate poverty from submergence prone northern region of Bangladesh. But the variety can be affected by cold shock due to delayed growth duration under the multiple flash flood conditions of T. Aman season in Bangladesh. That's why; short duration submergence tolerant high yielding varieties will be required particularly for the multiple flash flood prone areas.

ACKNOWLEDGEMENTS

Technical assistance from Scientists of Plant Breeding, Genetics and Biotechnology division, IRRI is gratefully acknowledged. The author is thankful to BRRI and IRRI authorities for providing supports in this research work. The work was also supported in part by a grant from the German Federal Ministry for Economic Cooperation and Development and Bill and Melinda Gates Foundation.

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