Development of early maturing submergence-tolerant rice varieties for Bangladesh

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ABSTRACT

Flash flooding imparts adverse effect on rice production worldwide. Because of the needs for multiple cropping and to avoid incidences of cold and diseases later in the season, early maturing submergence tolerant varieties have been urgently needed in some rice producing areas. Marker-assisted backcrossing (MABC) was used to introgress the submergence-tolerance *SUB1* QTL from BRRI dhan52 into a short-duration rice variety, BRRI dhan33. In this particular study, a combination of foreground and phenotypic selection was performed during the BC₁F₁-BC₄F₁ stages, while the whole set of foreground, recombinant and background markers were used at the BC₄F₂ stage. At the final stage, the recovery of recipient parent genome ranged from 90.7 to 95.2% in 15 BC₄F₃ promising lines. The introgression sizes of the different Sub1 lines were estimated to be around 2.4 to 5.6 Mb. The submergence tolerance of line BR9157-12-2-37-13-15-40 was found to be the best, having 87.7% survival. The grain yield of the Sub1 lines was also significantly higher compared with that of the original variety, BRRI dhan33, under both flooded and non-flooded conditions in on-station and on-farm trials. The maximum grain yield obtained from a BRRI dhan33-Sub1 line was 4.8 t/ha under on-farm non-flooded conditions and 3.8 t/ha under on-farm flooded conditions. The best selected Sub1 line may be released in the future as a short-duration, submergence-tolerant high-yielding variety for flood-prone rainfed areas in Bangladesh.

Keywords: Foreground and phenotypic selection; Marker-assisted backcrossing; *SUB1* QTL; Submergence tolerance

1. INTRODUCTION

The total production of rice has plateaued over recent years, with the gradual decrease of rice crop-growing area because of expansion of other crops, other enterprises and development of infrastructure. The static production of rice is still attributable to the lack of suitable improved cultivars for different agroclimatic conditions, particularly unfavorable ecosystems. Among the rice-growing ecosystems, the rainfed lowland areas are the most challenging due to the prevalence of many abiotic and biotic stresses. Submergence is the most important abiotic stress in the rainfed lowland rice (RLR) ecosystem in Bangladesh. More than 2.0 M ha of land in Bangladesh is affected by different types of floods (Iftekharuddaula et al. 2011). Submergence can result in yield losses of up to 100% depending on different environmental and floodwater conditions (Neeraja et al. 2007).

The *SUB1* QTL on chromosome 9, which accounts for 70% of the phenotypic variation for survival under submergence, has been fine-mapped, and the cluster of genes underlying the QTL has been cloned (Xu et al. 1996; 2000; 2006). To enable more precise molecular breeding, a number of gene-based and tightly-linked markers in this *SUB1* region have been developed (Neeraja et al. 2007; Septiningsih et al. 2009; 2013). This QTL has successfully been introgressed into a number of different varieties at the International Rice Research Institute (IRRI) (Iftekharuddaula et al. 2011; Neeraja et al. 2007; Septiningsih et al. 2009; 2015). In a relatively short time, some of these *Sub1* varieties have had a profound impact. Bangladesh Rice Research Institute (BRRI) has so far released two submergence-tolerant varieties (BRRI dhan51 and BRRI dhan52) but the duration of these varieties becomes longer after flooding that

flowering becomes affected by cold temperature stress. Hence, short-duration Sub1 varieties are essential.

BRRI dhan33 is an early-maturing rainfed lowland rice variety with a 118-day growth duration. This variety does not possess the submergence-tolerance QTL *SUB1*, hence, it is susceptible to submergence stress. This study was performed to convert this early rainfed lowland rice variety BRRI dhan33 into a submergence-tolerant type by incorporating *SUB1* from BRRI dhan52, using marker-assisted backcrossing (MABC). In order to conserve resources and simultaneously select for other traits, a combination of marker-based and phenotypic selection was performed. It is expected that the improved BRRI dhan33 line with submergence tolerance, BRRI dhan33-Sub1, will be more adaptable in submergence-prone areas of Bangladesh and preferred by farmers, particularly in the flash flood-affected northern parts of the country. This modified MABC approach can be used for rapid trait conversion for programs with limited resources and where low-cost phenotyping is available.

2. MATERIAL AND METHODS

2.1. Planting materials and crossing scheme

The experiments were carried out to introgress the *SUB1* locus into BRRI dhan33, an early rainfed lowland rice variety and lodging tolerance. This variety was derived from the cross BG388/BG367-4, with accession number BG850; it was released by BRRI in 1997. The yield potential of this variety is 4.5 tons/ha. BRRI dhan52, the high-yielding, flash flood-tolerant, rainfed lowland rice variety of Bangladesh, served as the *SUB1* donor parent (Pedigree: BR11*3/IR40931-33-1-3-2). The pedigree number of this variety is IR85260-66-654-Gaz2. For

the MABC scheme, BRRI dhan33 was crossed with BRRI dhan52 to obtain F₁ seeds in T. aman (transplanted aman season, July to December) of 2009 at the experimental farm of BRRI, Gazipur. The F₁ plants (and the following backcross generations) were confirmed through morphological character comparison (i.e. leaf color, panicle emergence, tiller height and pattern, flag leaf length, breadth, and attitude, etc.) with the parents, especially the female parent, BRRI dhan33. The selected F₁ plants were backcrossed with BRRI dhan33 to obtain a large number of BC₁F₁ seeds. Repeated backcrosses were then followed by marker-assisted selection to recover the genetic background of BRRI dhan33 (Fig. 1).

2.2. Molecular marker genotyping and analysis

In MABC, one tightly-linked SSR marker (RM8300) and/or one gene-based insertion-deletion (indel) marker (Sub1C173), two flanking SSR markers (RM296, RM23915) (Neeraja et al. 2007; Septiningsih et al. 2009) and 84 background SSR markers (polymorphic markers identified through a survey of 615 primers) (Table A.1) were used for foreground, recombinant and background selection, respectively. DNA was extracted from young leaves of 14-day-old plants following the mini-scale method (Zheng et al. 1995). SSR marker genotyping was carried out following previously-used methods for the development of Sub1 varieties (Iftekharruddaula et al. 2011; Neeraja et al. 2007; Septiningsih et al. 2009).

The marker data was analyzed using the software Graphical Genotyper (GGT 2.0) (Van Berloo, 2008). The homozygous recipient allele, homozygous dominant allele and heterozygous allele were scored as 'A', 'B' and 'H', respectively. The percentage of homozygous markers for recipient parent (%A) heterozygous markers (%R), were calculated. The introgression sizes in

the six BRRI dhan33-Sub1 lines were determined using 23 SSR and indel primers in *SUB1* region.

2.3. Phenotyping and adaptability test of newly developed Sub1-lines

Three BRRI dhan33-Sub1 introgression lines (bulk BC₄F₃ lines), along with recipient parent BRRI dhan33 and donor parent BRRI dhan52, were evaluated in the submergence tanks of the BRRI farm during the 2013 T. aman season. The population derived from plant no. 82 was discarded due to poor agronomic performance and susceptibility to bacterial blight. The experiment used an RCBD with three replications using standard management practices. Thirty-day-old seedlings were transplanted at 2 seedlings /hill with a spacing of 25 cm x 15 cm. The unit plot size was 5.4 m x 10 rows. Complete submergence stress was imposed at 15 d after transplanting and maintained for 15 d. The average depth of water was 100 cm. The average water temperature was 32°C and water pH was 7.2. Parameters, including grain yield (t/ha), along with growth duration (d), plant height (cm), panicle length (cm), effective tillers per hill, spikelet fertility, thousand-grain weight (g) and survival percentage were recorded.

Furthermore, 15 plants were selected from three BC₃F₄ bulk populations. These 15 Sub1 genotypes together with 3 susceptible checks: BRRI dhan33, BRRI dhan44 and BRRI dhan49; and 5 tolerant checks: BRRI dhan51, BRRI dhan52, IR64-Sub1, Ciherang-Sub1 and FR13A—were tested under controlled submergence in 2014. Fourteen-day-old seedlings were transplanted in submergence tank. The spacing used was 25 cm x 15 cm with 2 rows (25 hills/row). At 14 d after transplanting, the crop was exposed to complete submergence, maintaining a 100-cm water depth for 14 d. During the submergence period, the water in the tank was made turbid twice daily

134 manually using clay soil and the light intensity in upper level (normal), mid-level (30 cm below
135 the water surface) and lower level (75 cm below the water surface) of the tank were measured
136 using light meter (LI-250) (Table A.2). At 14 d after submergence, the water was drained. Data
137 for different parameters were taken following the methodologies described below:

138 **Elongation %** = $100 \times (\text{height 5 d after desubmergence} - \text{height before submergence}) / \text{height}$
139 before submergence.

140 Percent increase in dry matter (DM) during submergence was calculated, taking destructive
141 samples of 5 plants before submergence and then 5 d after desubmergence. The samples were
142 dried in a vacuum oven at 72°C for 72 h. The formula for calculating this parameter was:

143 **% increase in DM** = $100 \times (\text{DM 5 d after desubmergence} - \text{DM at submergence}) / \text{DM at}$
144 submergence.

145 **Recovery ability and tolerance score:** These were recorded at 5 and 30 d after desubmergence
146 on the basis of tillering ability, % survival and vegetative growth following the IRRI (2002)
147 Standard Evaluation System, with slight modification (SES, Table A.3). The survival data was
148 recorded at 5 d after desubmergence using SES, with 1 being highly tolerant and 9 being highly
149 susceptible (Table A.4). Recovery status was also evaluated 30 d after de-submergence.

150 **Survival %** = $100 \times (\text{number of surviving plants 5 d after desubmergence} / \text{total plants before}$
151 submergence)

152 Six submergence-tolerant high-yielding genotypes, along with two check varieties from IRRI
153 that are submergence tolerant and early, IR10F365 (IR 87439-BTN-145-2-1) and IR09F130 (IR
154 87107-47-2-1-1) were evaluated in five locations, including submergence-prone farmers' fields.
155 The experimental design was a RCBD with three replications. Standard management practices
156 for flooded and non-flooded conditions were carried out following Iftekharuddaula et al. (2015).

A detailed evaluation of grain quality parameters was performed. The samples were milled raw and analyzed for physicochemical properties. Milled rice recovery was determined by dehulling 200g rough rice in Satake Rice Mill, followed by 45 second polishing in a Satak Grain testing Mill TM-5. Head rice recovery was determined by separating broken rice from milled rice by hand. Milled rice recovery and head rice recovery were expressed as percentage of rough rice and milled rice respectively. Grain length and breadth were measured by slide calipers. In determining the size and shape, milled rice was first classified into three classes based on length, long (>6 mm in length), medium (5-6 mm in length) and short (<5 mm in length). Then again classified into three classes according to the ratio of length to breadth, slender (ratio>3.0), bold (ratio 2-3) and round (ratio<2.0). Amylose content was determined according to procedure of Little *et al.* (1958). Protein content was determined by micro Kjeldahl method (AOAC, 1970). Elongation ratio was calculated by measuring the length of cooked and uncooked milled rice by slide calipers. Volume of cooked and uncooked milled rice was measured by water displacement method. Data presented in the tables are the mean of three replications.

The brief definition of each grain quality parameters are given as follows:

Milling recovery: Milling recovery is the estimate of the quantity of total milled rice that can be produced from a unit of rough rice. It is generally expressed as a percentage.

Head rice yield: Head rice yield is the estimate of the quantity of whole grains (head rice) that can be produced from a unit of rough rice. It is generally expressed as a percentage.

Appearance: Grain appearance refers to the size and shape of the kernel and translucency and chalkiness of the grain.

Chalkiness: Chalkiness is the white portion of the rice grain which is formed on the dorsal side, ventral side and in the center of the grain caused by temporal reduction in starch biosynthesis

during osmotic adjustment. The starch granules in the chalky areas are less densely packed than translucent areas. Therefore, the chalky areas are not as hard as the translucent areas and the grains with chalkiness are more prone to breakage during milling.

Alkali spreading value: A test for estimating the gelatinization temperature of starch utilizing a seven point scale of the degree of spreading of milled rice grains in potassium hydroxide solution. A low alkali spreading value (2-3) indicates a high gelatinization temperature implying a longer cooking time; a high value (6-7) indicates low gelatinization temperature and shorter cooking time.

Amylose: Amylose is a helical polymer made of α -D-glucose units, bound to each other through $\alpha(1\rightarrow4)$ glycosidic bonds. This polysaccharide is one of the two components of starch, making up approximately 20-30% of the structure. Amylose content correlates negatively with taste panel scores for cohesiveness, tenderness, color, and gloss of boiled rice.

Protein: Large molecules composed of one or more chains of amino acids in a specific order determined by the base sequence of nucleotides in the DNA coding for the protein.

Cooking time: Cooking time is largely determined by the properties of the starch that makes up 90% of milled rice. Gelatinization temperature, amylose content, and gel consistency are the important starch properties that influence cooking duration.

Elongation ratio: Elongation ratio is defined as the ratio of length of 10 sound cooked rice grains to the length of the 10 sound milled rice grains. Length of cooked rice and milled rice is measured by slide calipers.

Imbibition ratio: Imbibition or volume expansion ratio is defined as ratio of the volume of 100 sound cooked rice grains to volume of 100 sound milled rice grains. Volume of cooked and milled rice is measured by water displacement.

3. RESULTS

3.1. BRRI dhan33-Sub1 development

A total of 48 F₁ seeds were produced. The F₁ plants were selected based on morphological characters and the selected F₁s were backcrossed with BRRI dhan33. A total of 137 BC₁F₁ plants were produced from the five best plants (plant numbers 12, 9, 11, 13 and 6), which were selected after foreground and morphological selection. Then 745 BC₂F₁ seeds were produced through subsequent backcrossing of these 5 best plants. In the BC₂F₁ generation, the 3 best plants were selected based on foreground and morphological selection, from which 304 BC₃F₁ seeds were produced.

In the BC₃F₁ generation, foreground selection was performed for 94 phenotypically selected plants using RM8300. Out of these 94 plants, 32 were heterozygous (Score H), 39 showed fixed recipient alleles (susceptible allele; Score A) and 4 showed fixed donor alleles (resistant allele; Score B) for the *SUB1* locus. Furthermore, 11 plants were phenotypically selected based on morphological criteria from 32 plants possessing the submergence tolerance QTL (H allele), and additional backcrossing was conducted to recover the recipient parent genome. A total of 716 BC₄F₁ seeds were produced from 11 BC₃F₁ plants, but only 14 plants survived in the main field due to cold injury. Foreground selection was performed using SUB1C173. Only one plant that was homozygous for *SUB1* allele and which phenotypically resembled BRRI dhan33 was self-pollinated to produce the BC₄F₂ seeds. In the BC₄F₂ generation, foreground selection was performed in 94 plants that had been selected based on morphological criteria out of 304 plants. Out of these 94 plants, 35 were heterozygous, 53 showed fixed recipient alleles and 4 showed

fixed donor alleles for RM8300. All of the plants had fixed donor alleles for Sub1C173. The 4 plants with the 'B' score for both markers were subjected to recombinant and background selection. Recombinant selection was performed in the BC₄F₂ generation. The scores for all flanking markers were 'B', which indicated that the plants had fixed donor alleles containing *SUB1* in the BC₄F₂ generation of this plant.

Background selection was performed in 4 segregants possessing the fixed donor alleles of the *SUB1* QTL and the three flanking markers in the BC₄F₂ generation. A total of 47 background markers were used. All the 4 plants possessed homozygous recipient alleles for 44 markers and homozygous donor alleles for two markers, RM8300 and Sub1C173. Two plants, plant numbers 15 and 17, had homozygous donor alleles for RM211. The percentage of recipient alleles recovered in the two best plants was 91.43 and 93.47%.

Background selection was again carried out for the 15 selected lines in the BC₄F₃ generation, which possessed the fixed donor alleles for *SUB1*. A total of 84 background markers were used. The maximum number of background markers used was 11 each for chromosomes 1 and 3, and the minimum number of background markers used was 4 for chromosomes 5, 7, 8 and 12. This low number of polymorphic markers on these particular chromosomes might be due to the level of genetic similarity between BRRI dhan33 and BRRI dhan 52. Here, 8 lines—BR9157-12-2-37-13-15-25, BR9157-12-2-37-13-15-30, BR9157-12-2-37-13-15-35, BR9157-12-2-37-13-15-40, BR9157-12-2-37-13-15-42, BR9157-12-2-37-13-17-27, BR9157-12-2-37-13-17-39 and BR9157-12-2-37-13-17-41—possessed homozygous recipient alleles for 80 background markers for non-carrier chromosomes; homozygous donor alleles for RM296, Sub1C173,

RM8300 and RM23915 for chromosome 9 (all of these referred to as Type A). Six plants, with the designation BR9157-12-2-37-13-17-32, BR9157-12-2-37-13-17-36, BR9157-12-2-37-13-71-26, BR9157-12-2-37-13-71-32, BR9157-12-2-37-13-71-38 and BR9157-12-2-37-13-71-41 had homozygous recipient alleles for 78 background markers for non-carrier chromosomes; homozygous donor alleles for RM296, Sub1C173, RM8300, RM23915, RM3912 and RM1896 for chromosome 9 (all of these referred to as Type B). The percentage of recipient alleles of lines ranged from 90.7 to 95.2% (Table A.5). In the best selected line, BR9157-12-2-37-13-15-25, all background markers used were identical to the recipient type, thus, the recipient genome percentage is 100% (Fig. 2).

3.2. Phenotyping and adaptability test of newly developed Sub1 lines

The grain yield and survival of all the introgression lines (BC₃F₄ bulk lines) were significantly higher than the recipient parent BRRI dhan33, indicating satisfactory agronomic performance and successful introgression of submergence tolerance in the new lines (Fig. A.1). Under controlled submergence conditions, the grain yields of the introgression lines were 1.24 to 1.71 t/ha higher than that of the recipient parent, which were statistically significant. The newly introgressed BRRI dhan33-Sub1 lines are similar to BRRI dhan33 with respect to the growth duration, effective tillers per plant, filled and unfilled grains per panicle and spikelet fertility. However, the new Sub1 lines have better survival rate, 1000 grain weight, and panicle length (Table 1).

All of the 15 (plant selections from bulk population) genotypes were compared with the tolerant genotypes BRRI dhan51, BRRI dhan52, IR64-Sub1, Ciherang-Sub1 and FR13A, and the

susceptible genotypes of BRRI dhan33, BRRI dhan44 and BRRI dhan49. Among the tested genotypes, 3 lines (BR9157-12-2-37-13-71-41, BR9157-12-2-37-13-15-30 and BR9157-12-2-37-13-15-40; [Table 2](#)), had good survival and excellent recovery; and were non-elongating (11.4-11.5% elongation). The elongation ranged from 0.8 to 99.4, and % survival from 48.6 to 94.7 in the tested genotypes. The increase in dry matter in the selected three genotypes ranged from 6.7-114.2, and SES score was 3. The rest of the Sub1 introgression lines were as tolerant as the tolerant checks but superior than the susceptible checks after 14 d of complete submergence. The rest of the Sub1 lines were also non-elongating types with survival scores ranging from 4 to 5.

Out of the 23 markers in the Sub1 region, 12 markers were polymorphic between BRRI dhan33 and BRRI dhan52. Based on the alleles of six gene-based markers and ten additional polymorphic markers within the Sub1 QTL region, the size of donor introgression among BRRI dhan33-Sub1 lines was ~ 5.6 Mb, with the exception for BR 9157-12-2-37-13-71-4, which was ~2.4 Mb. The presence of *SUB1* genes among those individuals was also confirmed ([Table A.6](#)). The different alleles of SUB1BC2 and RM23902 among BRRI dhan33-Sub1 lines are shown as an example ([Fig. A.2](#)).

The milling recovery and head rice yield of BRRI dhan33-Sub1 lines ranged from 70.7 to 71.9% and 51.2 to 65.6%, respectively, whereas these were 70.5 and 49.0%, respectively, in BRRI dhan33. Chalkiness was categorized as Wb₅ (10-20% chalkiness) in all the BRRI dhan33-Sub1 lines, but it was Wb₉ (>20% chalkiness) in BRRI dhan33. Length to breadth ratio and alkali spreading value ranged from 2.1 to 2.4 and 3.5 to 5.0, respectively, in BRRI dhan33-Sub1 lines, and the values were 2.1 and 4.5, respectively, in BRRI dhan33. Amylose content of BRRI

dhan33-Sub1 lines and BRRI dhan33 was >26%, hence, these values can be categorized as high amylose content. Protein was slightly higher in BRRI dhan33-Sub1 lines, ranging from 7.4 to 8.5%, whereas it was 6.6% in BRRI dhan33. The cooking time was more or less similar among BRRI dhan33-Sub1 (18.3-19.3 min) lines compared with BRRI dhan33 (20.0 min). The elongation ratio was a bit higher in BRRI dhan33-Sub1 lines (1.4-1.8) compared with that in BRRI dhan33 (1.3) and the imbibition ratio was similar in BRRI dhan33-Sub1 lines (2.4-3.1) compared with that in BRRI dhan33 (2.8) (Table 3).

There were no flash floods in three trials conducted at Gazipur, Nilphamari and Kawnia. There were no significant differences between BRRI dhan33-Sub1 lines and BRRI dhan33 with respect to growth duration (d), plant height (cm), panicles/plant, filled grains/panicle and sterility (%). Moreover, all BRRI dhan33-Sub1 lines produced significantly higher grain yield than BRRI dhan33. Importantly, two breeding lines, BR9157-12-2-37-13-71-32 and BR9157-12-2-37-13-15-30, produced 4.8 t/ha of grain yield, which was significant higher (0.8 t/ha) than the grain yield of BRRI dhan33 (Table 4) under control conditions.

At Lalmonirhat, there were two flash flood events for 15 and 9 d during T. aman 2014 (Table A.7). There was a period of 23 d between the two flash floods, and the average depth of flood water was 110-120 cm. Sixteen d of controlled submergence with a water depth of 80 cm was imposed at BRRI, Rangpur. The average performance of the breeding lines under flooded conditions reflected that growth durations ranged from 142 to 143 d among BRRI dhan33-Sub1 lines, whereas it was 143 d in BRRI dhan33. Four BRRI dhan33-Sub1 lines showed similar values compared with BRRI dhan33, with respect to plant height, panicles/plant and sterility.

The same breeding lines showed significantly higher values than BRRI dhan33 for filled grains/panicle and % survival. Moreover, BRRI dhan33-Sub1 lines produced 2.2-2.3 t/ha more grain yield than the original recipient variety, BRRI dhan33, under flooded conditions, with the highest grain yield (3.8 t/ha) obtained from 2 lines, BR9157-12-2-37-13-17-27 and BR9157-12-2-37-13-15-25 (Table 5).

4. DISCUSSION

In this study, *SUB1* was introgressed into BRRI dhan33 to develop a short-duration, submergence-tolerant rice variety using MABC. We did not perform background selection from the very beginning as was previously done (Iftekharruddaula *et al.*, 2011; Neeraja *et al.* 2007; Septiningsih *et al.* 2009), but used a combination of foreground marker and phenotypic selection and several rounds of additional backcrossing instead, in order to reduce resources and more importantly to allow generating more recombinations to break any negative linkage drags which enables selecting the best plants that closely resembled the recipient parent, yet with more beneficial attributes such as higher yield or other desirable traits. Although recombinant selection was practiced using three flanking markers in the advanced backcross generation, the introgression size in the vicinity of *SUB1* in BRRI dhan33-Sub1 lines was around 2.4 to 5.6 Mb. The previous Sub1 line introgression sizes ranged from ~0.8 to 8 Mb (Iftekharruddaula 2011; Neeraja *et al.* 2007; Septiningsih *et al.*, 2009; 2015). Nonetheless, and as for the previous Sub1 lines, the ultimate phenotype of BRRI dhan33-Sub1 lines was not affected with respect to agronomic performance. In addition, the donor of this ‘second generation’ of Sub1 line was BRRI dhan52, a variety that possesses high yield and desirable agronomic traits. In conventional backcrossing, large donor segments are likely to be introgressed in the carrier chromosome

(Young and Tanksley 1989) and, in this approach, a large donor segment has been inherited from the carrier chromosome due to the lack of recombinant selection done in early backcross generations. Young and Tanksley (1989) also 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 has returned to approximately zero if no selection using markers was applied. Since the donor parent was an improved genotype, this large introgression did not cause any negative effect on the phenotype of BRRI dhan33-Sub1 lines.

In background selection, 84 markers spaced at an average distance of 21 cM were used in the advanced backcross generations in order to recover the homozygous recipient genetic background of BRRI dhan33. Background selection was postponed in the early backcross generations in order to make the marker genotyping less labour-intensive. This approach is applicable for resource-poor countries using SSR markers for genotyping.

Submergence tolerance of the newly developed BRRI dhan33-Sub1 lines was found to be superior to that of the recipient parent, including some agronomic parameters. In term of survival rate and recovery score, BR9157-12-2-37-13-15-40, BR9157-12-2-37-13-15-30, and BR9157-12-2-37-13-71-41 are among the three best lines (Table 2). Grain yields of BRRI dhan33-Sub1 lines were significantly higher compared to that of the original recipient variety BRRI dhan33 under both flooded and non-flooded conditions (Table 4 and 5). The significantly higher grain yield of BRRI dhan52, in comparison with that of the recurrent parent variety BR11 under controlled submergence conditions, was reported by Iftekharuddaula et al. (2015). The original variety, BRRI dhan33 produced 4.0 t/ha grain yield under on-farm non-flooded condition while

under on-farm flooded condition the variety produced 1.5 t/ha grain yield. The maximum grain yield obtained from a BRRI dhan33-Sub1 line was 4.8 t/ha under on-farm non-flooded conditions and 3.8 t/ha under on-farm flooded conditions. However, since the results of the screening were not available yet by the time of the on-farm trials were conducted, not all the best lines can be included in these trials due to limited resources. To some extent, the lower grain yield of BRRI dhan33-Sub1 lines under both flooded and non-flooded conditions was associated with the occurrence of bacterial blight and sheath rot diseases (data not shown). However, the amount of grain yield advantages over the original recurrent parent (0.8-2.3 t/ha) was encouraging. Additional trials will be conducted in the future to further test these lines. The selected BRRI dhan33-Sub1 lines may be used in participatory variety selection trials for release as a variety or as a parent to further develop high-yielding short-duration, submergence-tolerant and disease resistant varieties through conventional and molecular breeding. The physicochemical properties of BRRI dhan33-Sub1 lines were more or less similar to the recurrent parent BRRI dhan33. Iftekharuddaula et al. (2011) also reported similar types of grain physicochemical properties in BR11-Sub1 and in the original recurrent parent, BR11.

The growth duration of BRRI dhan33-Sub1 lines was around 140-143 d after two weeks of controlled submergence stress, as well as under two natural occurrences of flash floods of 24 d. Although the growth duration was delayed by around 20 d compared with the growth duration (119 d) of BRRI dhan33 under non-flooded conditions, it could still be possible to cultivate *rabi* (winter) crops after harvesting BRRI dhan33-Sub1 lines even after multiple flash floods. Again, the growth duration of BRRI dhan52, a submergence-tolerant high-yielding variety developed earlier (Iftekharuddaula et al. 2015), was found to be around 157 d, which was 17 d later than

BRRI dhan33-Sub1 lines. The delayed growth duration of BRRI dhan52 creates a problem in fitting this variety to some cropping sequences. The scenarios become worse when there are multiple flash floods, which often occur in Bangladesh. After multiple flash flood events, the duration of BRRI dhan52 is further delayed that it is affected by cold stress at the reproductive phase (unpublished data, K. M. Iftekharuddaula). These problems are now partially solved by introgressing *SUB1* into the early, high-yielding rainfed lowland variety, BRRI dhan33. However, BRRI dhan52 produced around 2.0 ton more grain yield than BRRI dhan33-Sub1 lines implicating necessity of this variety in the land type where multiple flash flood does not occur and where farmers do not need early *rabi* crops.

In summary, by combining foreground selection and morphological phenotyping from the very beginning of the MABC process, while delaying the background selection until the last round, we successfully developed early-maturing submergence tolerant rice lines, BRRI dhan33-Sub1, with significantly higher yield compared to the original recurrent parent, BRRI dhan 33, under both flooded and non-flooded conditions of on-station and on-farm trials. It is hoped that one or two of the best BRRI dhan33-Sub1 lines could soon be released in the flood-prone areas, especially those that in need of short duration varieties due to the onset of other abiotic and biotic stresses late in the planting season. These improved lines could help improve the livelihood of farmers in submergence-prone areas of Bangladesh.

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Figure legends

Figure 1. Marker assisted backcrossing scheme used indicating “Foreground and Phenotypic” selection steps. The number of plants to develop BRRI dhan33-Sub1 lines at each step is shown.

Figure 2. Graphical genotype of the selected best line. BR9157-12-2-37-13-17-25 was selected as the best genotype of the BC₄F₃ generation. The black coloured regions on the chromosomes indicated a homozygous region for the recipient genome, whereas the grey coloured regions indicated the fixed donor allele regions. The distances are represented in cM based on the published map of Temnykh et al. (2001).

Appendices

Table A.1. List of polymorphic markers of all 12 chromosomes used for background selection in the development of BR dhan33-Sub1 lines.

Table A.2. Range of light intensity, pH and temperature of water during submergence period to test the performance of submergence-tolerant lines along with selected references and checks under non-flooded conditions, BRRI, Gazipur, T. aman 2014.

Table A.3. Criteria for recovery status after submergence stress. Recovery data was taken 30 d after submergence.

Table A.4. Modified Standard evaluation system (SES) for survival after submergence. The survival data was taken 5 d after submergence.

Table A.5. Results of background selection, Observational Trial (BC₄F₃ generation), T. Aman 2013. Type A refers to the eight lines possessing homozygous recipient alleles for 80 background markers for non-carrier chromosomes; homozygous donor alleles for RM296, Sub1C173, RM8300 and RM23915 for chromosome 9. Type B refers to the six lines had homozygous recipient alleles for 78 background markers for non-carrier chromosomes; homozygous donor alleles for RM296, Sub1C173, RM8300, RM23915, RM3912 and RM1896 for chromosome 9.

Table A.6. Different alleles of the Sub1-region markers among the BRRI dhan33-Sub1 lines. ‘A’ represents the recipient’s allele, BRRI dhan 33; ‘B’ represents the donor’s allele, BRRI dhan52.

Table A.7. Status of flash flood at different locations of Bangladesh in T. aman 2014.

Figure A.1. Survival of BRRI dhan33-Sub1 line (BR9157-12-2-37-13-17) and BRRI dhan33 after 16 d of controlled submergence at BRRI, Gazipur, T. aman 2013. The survival of BRRI dhan33 (recurrent parent check) was 22.1% whereas that of BRRI dhan33-Sub1 line (BR9157-12-2-37-13-17) was 88.9%.

Figure A.2. Examples of different alleles of SUB1BC2 and RM23902 among the BRRI dhan33-Sub1 lines. ‘A’ refers to BRRI dhan33, the recurrent parent’s alleles, while ‘B’ refers to BRRI dhan52, the donor parent’s allele. It showed that all the BRRI dhan33-Sub1 lines already have fixed donor alleles.

Table 1. Performance of BRRI dhan33-Sub1 under submergence in T. aman 2013 season at BRRI, Gazipur. Data were means of three replicates. Duration of flooding was 14 d. The survival data was taken at 5 d after de-submergence and recovery status was evaluated at 30 d of de-submergence.

Sl #	Designation	PH ^a (cm)	GD ^b (days)	PL ^c (cm)	ET ^d	SFT ^e	TGW ^f (g)	Survival %	GY ^g (t/ha)
1	BR9157-12-2-37-13-15 ^h	107.3	140	26.3	14.9	78.8	27.6	84.6	3.01
2	BR9157-12-2-37-13-17	108.1	141	27.3	13.9	74.8	28.4	88.9	3.44
3	BR9157-12-2-37-13-71	106.3	139	26.7	13.5	72.7	28.2	83.0	2.97
4	BRRI dhan33 (Ck)	103.7	138	23.2	15.1	73.2	25.5	22.1	1.73
5	BRRI dhan52 (Ck)	110.7	157	24.7	15.6	85.2	27.6	97.5	4.95
LSD (0.05)		3.58	1.46	2.09	ns	6.59	1.63	4.34	0.76

^aPlant height (taken from 10 random plants in each replication)

^bGrowth duration (taken from whole population of each replication)

^cPanicle length (taken from 10 random panicles of each replication)

^dEffective tillers per plant (taken from 10 random hills of each replication)

^eSpikelet fertility (taken from 10 random panicles of each replication)

^fThousand-grain weight (taken from random 1000 filled grains of each replication)

^gGrain yield (taken from 10 m² area of each replication)

^hBC₄F₂

Table 2. Recovery parameters of the tested Sub1 lines after de-submergence in T. Aman 2014 season at the submergence tank of the Plant Breeding Division, BRRI, Gazipur-1701. The duration of flooding was 18 d. Data were mean of three replicates. The survival data was taken at 5 d after de-submergence and recovery status was evaluated 30 d of de-submergence. Percentage elongation and percentage increase in dry matter were taken from 5 plants of each replication. Percentage survival was calculated from all the individuals of each replication.

Sl#	Designation	% elongation	% increase in dry matter	% survival	SES ^a Score	Recovery Status
1.	BR157-12-2-37-13-71-26 ^b	6.6	22.2	71.6	4	Very good
2.	BR157-12-2-37-13-71-32	12.0	39.8	66.6	5	Very good
3.	BR157-12-2-37-13-71-38	12.0	73.8	66.5	5	Very good
4.	BR157-12-2-37-13-71-41^c	11.5	50.0	86.8	3	Very good
5.	BR157-12-2-37-13-71-46	14.3	16.6	62.3	5	Very good
6.	BR157-12-2-37-13-17-27	11.7	55.2	73.2	4	Very good
7.	BR157-12-2-37-13-17-32	3.0	6.7	65.4	5	Very good
8.	BR157-12-2-37-13-17-36	9.1	33.9	70.5	4	Very good
9.	BR157-12-2-37-13-17-39	17.7	53.1	66.5	5	Very good
10.	BR157-12-2-37-13-17-41	13.1	43.5	64.9	5	Very good
11.	BR157-12-2-37-13-15-25	13.6	30.2	72.7	4	Very good
12.	BR157-12-2-37-13-15-30	11.4	108.4	83.0	3	Very good
13.	BR157-12-2-37-13-15-35	10.0	54.1	68.3	5	Very good
14.	BR157-12-2-37-13-15-40	11.4	46.3	87.7	3	Very good
15.	BR157-12-2-37-13-15-42	0.8	12.1	77.7	4	Very good
16.	BRRI dhan 33 (Ck)	21.0	71.9	75.2	5	Fair
17.	BRRI dhan 44(Ck)	91.8	89.1	48.6	6	Fair
18.	BRRI dhan 49 (Ck)	99.4	85.4	85.1	5	Fair
19.	BRRI dhan 51 (Ck)	5.1	26.3	79.9	4	Very good
20.	BRRI dhan52 (Ck)	13.5	50.2	82.6	3	Very good
21.	IR64-Sub1	17.5	84.2	73.3	4	Very good
22.	Ciherang-Sub1	16.3	114.0	94.7	2	Very good
23.	FR13(A)	23.6	114.2	94.7	2	Very good
LSD at 0.05		17.04	27.08	16.28		
Correlation with % survival		-0.150 ns	-0.150 ns			

^aStandard evaluation system

^bBC₄F₃

^cTested lines in bold font had good survival and excellent recovery

Table 3**Table 3.** Physical and chemical properties of rice grains and kernels of BRRI dhan33-Sub1 lines along with standard check varieties.

Data were means of three replicates.

Sl #	Variety/Line	Milling recovery (%)	Head rice yield (%)	Appearance	Chalkiness ^a	Length (L) (mm)	Breadth (B) (mm)	L/B ratio	Size and shape ^b	Alkali spreading Value	Amylose (%)	Protein (%)	Cooking time (min.)	ER ^c	IR ^d
1.	BR 9157-12-2-37-13-15-25 ^e	71.6	51.2	Good	Wb ₅	5.4	2.5	2.4	SM	3.5	26.4	8.0	19.3	1.4	2.5
2.	BR 9157-12-2-37-13-15-30	71.0	65.0	Good	Wb ₅	5.4	2.5	2.2	SM	4.8	26.8	7.6	18.3	1.8	2.4
3.	BR 9157-12-2-37-13-17-27	71.9	58.0	Good	Wb ₅	5.5	2.6	2.1	SM	4.3	26.9	7.4	18.3	1.6	2.4
4.	BR 9157-12-2-37-13-17-32	71.5	65.6	Good	Wb ₅	5.4	2.5	2.1	SM	5.0	26.6	8.3	18.3	1.4	2.4
5.	BR 9157-12-2-37-13-71-32	70.7	51.9	Good	Wb ₅	5.3	2.5	2.1	SM	4.4	26.2	7.5	19.0	1.4	2.4
6.	BR 9157-12-2-37-13-71-41	71.4	65.1	Good	Wb ₅	5.4	2.5	2.1	SM	5.0	25.4	8.5	18.3	1.7	3.1
7.	BRRI dhan33	70.5	49.0	Good	Wb ₉	5.4	2.5	2.1	SM	4.5	27.8	6.6	20.0	1.3	3.1
8.	BRRI dhan52	74.0	56.0	Good	Tr	5.7	2.7	2.1	IM	6.0	26.8	7.3	19.3	1.5	2.8
LSD at 16 df		0.56	2.35			0.17	0.08	ns ^f		0.39	0.48	0.21	0.41	0.11	0.43

^aTr=Translucent, Wb=White belly, Wb₅=10-20% endosperm chalkiness, Wb₉≥20% endosperm chalkiness

^bSM=Short Medium, IM=Intermediate Medium,

^cElongation ratio

^dImbibition ratio

^eBC₄F₃

^fnon-significant

Table 4. Performance of submergence-tolerant lines and selected references and checks under non-flooded conditions. Data were averages of three locations (Gazipur, Rangpur, and Nilphamari) in Bangladesh in T. aman 2014. Plant height was taken from 10 random plants of each replication. Panicles/plant, filled grains/panicle and sterility% were taken from 10 random plants of each replications. Grain yield (t/ha) was recorded from 10m² area and the data was adjusted at 14% moisture content.

Sl#	Designation	Growth Duration (days)	Plant Height (cm)	Panicles/ plant	Filled Grains/ panicle	Sterility%	Grain Yield (t/ha)
1.	IR10F365	125	114.9	10	99	25.8	4.4
2.	IR09F130	124	117.4	9	118	18.7	4.5
3.	BR9157-12-2-37-13-71-32^a	119	108.7	7	114	24.1	4.8
4.	BR9157-12-2-37-13-17-27 ^b	119	105.7	7	128	21.1	4.6
5.	BR9157-12-2-37-13-15-30	118	105.6	7	111	23.7	4.8
6.	BR9157-12-2-37-13-15-25	119	108.6	7	121	22.8	4.5
7.	BINA dhan11 (Ck)	118	105.8	8	81	29.9	4.0
8.	BRRI dhan33 (Ck)	119	105.9	8	116	21.8	4.0
	LSD (0.05)	1.17	4.36	0.93	16.2	4.37	0.31

^aTested lines in bold font had very good recovery status and SES score of 3.

^bBC₄F₃

Table 5. Performance of tested lines under flooded conditions. Data were averages of two locations in Bangladesh, Lalmonirhat and Rangpur, in T. aman 2014. There were 3 replications in each location. At Lalmonirhat, there were two flash flood events for 15 and 9 d (Table A.7). There was a period of 23 d between the two events, and the average depth of flood water was 110-120 cm. Sixteen d of controlled submergence with a water depth of 80 cm was imposed at BRRI, Rangpur.

Sl#	Designation	Growth duration (days)	Plant height (cm)	Panicle/ plant	Filled grains/ panicle	% sterility	Grain yield (t/ha)	% survival
1.	IR10F365	152	86.6	8	91	23.0	3.6	86.4
2.	IR09F130	149	102.9	8	141	14.0	3.8	93.1
3.	BR9157-12-2-37-13-71-32 ^a	143	91.6	7	135	11.2	3.7	90.0
4.	BR9157-12-2-37-13-17-27	143	93.6	7	126	10.2	3.8	93.1
5.	BR9157-12-2-37-13-15-30	142	92.0	7	123	16.5	3.7	95.2
6.	BR9157-12-2-37-13-15-25	142	91.5	7	115	16.1	3.8	93.9
7.	BINA dhan11 (Ck)	140	77.1	7	83	23.4	3.3	96.3
8.	BRRI dhan33 (Ck)	143	89.0	9	48	15.2	1.5	46.6
LSD (0.05)		28.78	23.95	ns	26.66	5.75	0.83	20.39

^aBC4F3

Entry number 1 to 7 have *SUB1*, only BRRI dhan33 does not have *SUB1*

Figure 1

Figure 1. Marker assisted backcrossing scheme used indicating “Foreground and Phenotypic” selection steps. The number of plants at each step to develop BRR I dhan33-Sub1 lines is shown.

Figure 2

Ind no:1 [BR33-Sub1] - BR9157-12-2-37-13-17-25

Table A.1. List of polymorphic markers of all 12 chromosomes used in background selection in the development of BR dhan33-Sub1 lines.

Chr 1	Chr 2	Chr 3	Chr 4	Chr 5	Chr 6
RM495	RM110	RM231	RM5953	RM6300	RM589
RM3521	RM211	RM517	RM16313	RM405	RM8258
RM283	RM555	RM546	RM5414	RM289	RM527
RM272	RM327	RM6914	RM518	RM6545	RM6202
RM490	RM561	RM7097	RM2416		RM400
RM583	RM341	RM8209	RM7279		
RM581	RM475	RM6970	RM16734		
RM493	RM5916	RM293	RM3643		
RM212	RM497	RM565	RM3332		
RM472	RM208	RM570	RM2431		
RM104		RM85			
Chr 7	Chr 8	Chr 9	Chr 10	Chr 11	Chr 12
S07024	RM1270	RM296	RM222	RM1761	RM3671
R7M7	RM310	Sub1C173	RM8207	RM5704	RM179
R7M30	RM126	RM8300	RM5758	RM26482	RM270
S07101	RM4997	RM23915	RM228	RM202	RM12
		RM3912	RM484	RM287	
		RM1896	RM590	RM229	
		RM245		RM254	
				RM224	

Table A.2. Range of light intensity, pH and temperature of water during submergence period to check the performance of submergence-tolerant lines along with selected references and checks under non-flooded conditions, BRRI, Gazipur, T. aman 2014.

Water level	Light intensity (w/m ²)		Water pH	Water temperature (°C)
	Before turbidity	After turbidity		
Upper level (above water surface)	100-130		7.1-7.5	30-31.5
Mid-level (30 cm below water surface)	20-27	1-1.9		
Lower level (75 cm below water surface)	2.5-6.6	0-0.023		

Table A.3. Criteria for recovery status after submergence stress. Recovery data was taken 30 days after submergence.

Recovery status	Criteria
Excellent	Highly satisfactory growth and tillering, erect dark green leaves
Very good	Satisfactory growth and tillering, erect green leaves
Good	Normal growth and tillering, erect green leaves
Fair	Low growth and tillering, droopy, pale green leaves
Poor	Very low growth and tillering, long pale green leaves

Table A.4. Standard evaluation system (SES) for survival after submergence. Survival data was taken 5 days after submergence.

Scores	% Survival
1	100
2	90-99
3	80-89
4	70-79
5	60-69
6	50-59
7	40-49
8	30-39
9	<30

Table A.5. Results of background selection, Observational Trial (BC₄F₃ generation), T. Aman 2013. Type A refers to the eight lines possessing homozygous recipient alleles for 80 background markers for non-carrier chromosomes; homozygous donor alleles for RM296, Sub1C173, RM8300 and RM23915 for chromosome 9. Type B refers to the six lines had homozygous recipient alleles for 78 background markers for non-carrier chromosomes; homozygous donor alleles for RM296, Sub1C173, RM8300, RM23915, RM3912 and RM1896 for chromosome 9.

Allele	Type A lines ^a	Type B lines ^b
A (homozygous recipient alleles)	80	78
H (heterozygous alleles)	0	0
B (donor alleles)	4	6
% A	95.24	90.7
%Recipient allele	95.24	90.7

^aBR9157-12-2-37-13-15-25, BR9157-12-2-37-13-15-30, BR9157-12-2-37-13-15-35, BR9157-12-2-37-13-15-40, BR9157-12-2-37-13-15-42, BR9157-12-2-37-13-17-27, BR9157-12-2-37-13-17-39 and BR9157-12-2-37-13-17-41

^bBR9157-12-2-37-13-17-32, BR9157-12-2-37-13-17-36, BR9157-12-2-37-13-71-26, BR9157-12-2-37-13-71-32, BR9157-12-2-37-13-71-38 and BR9157-12-2-37-13-71-41

Table A.6. Different alleles of the Sub1-region markers among the BRRI dhan33-Sub1 lines. ‘A’ represents the recipient’s allele, BRRI dhan 33; ‘B’ represents the donor’s allele, BRRI dhan52.

Marker	Position (Mb)	G1 ^a	G2	G3	G4	G5	G6	BRRI dhan33	BRRI dhan52
RM8300	5.17	B	B	B	B	B	B	A	B
RM23843	6.21	B	B	B	B	B	B	A	B
SC41	6.95	B	B	B	B	B	B	A	B
RM23901	6.99	B	B	B	B	B	B	A	B
ART5	Sub1C promoter	B	B	B	B	B	B	A	B
Sub1BC1	Between Sub1B and C	B	B	B	B	B	B	A	B
Sub1BC2	Between Sub1B and C	B	B	B	B	B	B	A	B
Sub1BC3	Between Sub1B and C	B	B	B	B	B	B	A	B
Sub1C173	Exon of Sub1C	B	B	B	B	B	B	A	B
GnS2	Exon of Sub1A	B	B	B	B	B	B	A	B
SC23	7.22	B	B	B	B	B	B	A	B
RM23917	7.31	B	B	B	B	B	B	A	B
RM23915	7.31	B	B	B	B	B	B	A	B
RM23902	7.58	B	B	B	B	B	B	A	B
RM296	10.79	B	B	B	B	B	A	A	B
RM3912	10.83	A	A	A	A	A	A	A	B
Introgression size (Mb)		5.62	5.62	5.62	5.62	5.62	2.41		

^aG1=BR 9157-12-2-37-13-15-25, G2=BR 9157-12-2-37-13-15-30 (the same as BR 9157-12-2-37-13-15-40), G3=BR 9157-12-2-37-13-17-27, G4=BR 9157-12-2-37-13-17-32, G5=BR 9157-12-2-37-13-71-32, G6=BR 9157-12-2-37-13-71-41

Table A.7. Status of flash flood at different locations of Bangladesh in T. aman 2014.

Locations	Flood status	Date of Submergence	Date of desubmergence	Duration of submergence	Average flood water height (cm)
Lalmonirhat	First Flood (natural)	15 August 2014	29 August 2014	15 days	110
	Second Flood (natural)	22 September 2014	30 September 2014	9 days	120
Rangpur	Controlled submergence	14 August 2014	30 August 2014	16 days	80

Figure A1

Figure A.1. Survival of BRRI dhan33-Sub1 line (BR9157-12-2-37-13-17) and BRRI dhan33 after 16 d of controlled submergence at BRRI, Gazipur, T. aman 2013. The survival of BRRI dhan33 (recurrent parent check) was 22.1% whereas that of BRRI dhan33-Sub1 line (BR9157-12-2-37-13-17) was 88.9%.

Figure A2

Figure A.2. Examples of different alleles of SUB1BC2 and RM23902 markers among the BRRI dhan33-Sub1 lines. ‘A’ refers to BRRI dhan33, the recurrent parent’s alleles, while ‘B’ refers to BRRI dhan52, the donor parent’s allele. It showed that all the BRRI dhan33-Sub1 lines already have fixed donor alleles.