Field Crops Research 190 (2016) 44–53

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Development of early maturing submergence-tolerant rice varieties for Bangladesh

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ARTICLE INFO

Article history:
Received 3 May 2015
Received in revised form 8 November 2015
Accepted 2 December 2015
Available online 16 March 2016

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

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 BC_1F_1-BC_4F_1 stages, while the whole set of foreground, recombinant and background markers were used at the BC_4F_2 stage. At the final stage, the recovery of recipient parent genome ranged from 90.7 to 95.2% in 15 BC_4F_3 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.

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 and Mackill, 1996; Xu et al., 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

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http://dx.doi.org/10.1016/j.fcr.2015.12.001
0378-4290/

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 F_1 seeds in T. aman (transplanted aman season, July to December) of 2009 at the experimental farm of BRRI, Gazipur. The F_1 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, BRR1 dhan33. The selected F_1 plants were backcrossed with BRRI dhan33 to obtain a large number of BC_1F_1 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 A1) 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 (Iftekharuddaula 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.

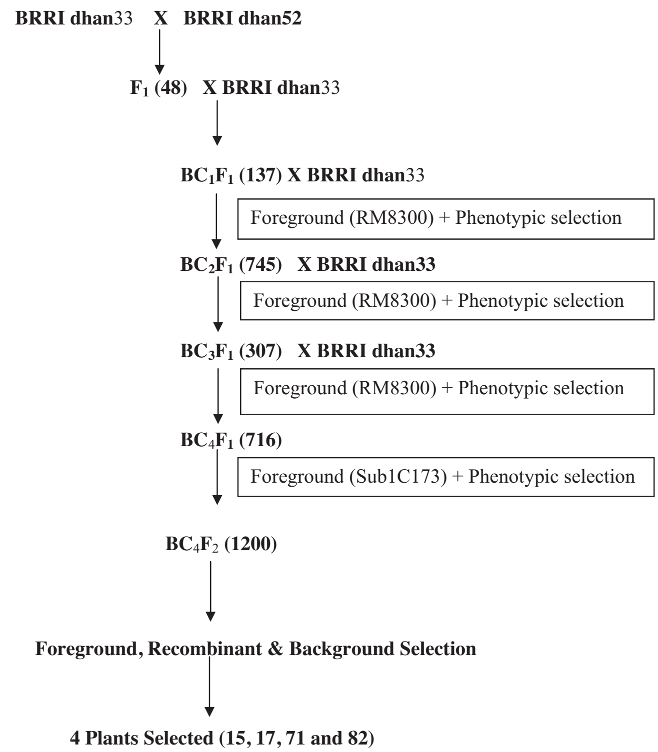

Fig. 1. Marker assisted backcrossing scheme used indicating "Foreground and Phenotypic" selection steps. The number of plants to develop BRRI dhan33-Sub1 lines at each step is shown.

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

Three BRRI dhan33-Sub1 introgression lines (bulked BC_4F_3 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 × 15 cm. The unit plot size was 5.4 m × 10 rows. Complete submergence stress was imposed at 15 days 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 BC_3F_4 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 × 15 cm with 2 rows (25 hills/row). At 14 days after transplanting, the crop was exposed to complete submergence, maintaining a 100-cm water depth for 14 days. During the submergence period, the water in the tank was made turbid twice daily manually using clay soil and the light intensity in upper level (normal), mid-level (30 cm below the water surface) and lower level (75 cm below the water surface) of the tank were measured using light meter (LI-250) (Table A2). At 14 days after submergence, the water was drained. Data for differ-