Marker Assisted Backcross for the Development of Submergence Tolerant Rice (Oryza sativa L.) By

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Received: 26/10/2013 Revised: 30/12/2013 Accepted: 04/01/2014 Marker Assisted Backcross for the Development of Submergence Tolerant Rice *(Oryza sativa L.)* B.P. Ray, K.M. Iftekharuddaula and S. Ghosal

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ABSTRACT

The present study was undertaken to study the genetic variability among different submergence tolerant genotypes. Submergence stress regularly affects 15 million hectares or more of rainfed lowland rice areas in South and Southeast Asia. Flash floods can result in yield loss up to 100% depending on different climatic & agronomic factors. In Bangladesh, ~2.0 mha of rice lands are unfavorably affected by excess water and periodically suffer from flash-floods and reduces 5% average yield in Bangladesh. In this circumstance, improvement of germplasm is likely the best option to withstand submergence and stabilize productivity in these environments. Progress in germplasm improvement has been slow but can substantially be enhanced if the physiological and genetic bases of submergence tolerance are well understood .This review focuses on current physiological understanding of tolerance to submergence in rice with greater emphasis on flood water environments new genetic resources. This approach demonstrates the effective use of marker assisted selection for a major QTL in a molecular breeding program. Key words: Marker, Submergence, Backcross and QTL.

INTRODUCTION

"Rice is life" was the theme of International Year of Rice 2004 signifies its devastating importance on global food system (FAO, 2004). It is the most important cereal crop providing energy, protein and vitamins for half of the world's population (Nguyen, 2010; Tiwari et al., 2011). Rice is the only cereal crop that is well adapted to the conditions of water logging or partial flooding. In Bangladesh,~2.0 mha of rice lands are unfavorably affected by excess water and periodically suffer from flash-floods and reduces 5% average yield in Bangladesh.

Performance of high-yielding varieties can further be augmented if their submergence tolerance is enhanced, which is now becoming more feasible. Selection for submergence tolerance by rainfed lowland rice breeders is very important in Bangladesh .Current understanding of the physiological and biochemical bases of submergence tolerance has progressed well in recent years, making it possible to design efficient phenotypic protocols and has laid the infrastructure for further genetic and molecular studies to discover genes underlying component traits associated with tolerance. This will subsequently speed up the breeding process. Therefore, the present study was undertaken to study the genetic variability among different submergence tolerant genotypes.

The basis of a marker-assisted backcrossing (MAB) strategy is to transfer a specific allele at the target locus from a donor line to a recipient line while selecting against donor introgressions across the rest of the genome. The use of molecular markers which permit the genetic dissection of the progeny at each generation increases the speed of the selection process, thus increasing genetic gain per unit time (Tanksley et al. 1989; Hospital 2003). The main advantages of MAB are: (1) efficient foreground selection for the target locus, (2) efficient background selection for the recurrent parent genome, (3) minimization of linkage drag surrounding the locus being introgressed, and (4) rapid breeding of new genotypes with favorable traits. The effectiveness of MAB depends on the availability of closely linked markers and/or flanking markers for the target locus the size of the population, the number of backcrosses and the position and number of markers for background selection (Frisch et al. 1999a; Frisch and Melchinger 2005). MAB has previously been used in rice breeding to incorporate the bacterial blight resistance gene Xa21 (Chen et al. 2000, 2001) and waxy gene (Zhou et al. 2003) into elite varieties. The availability of the large-effect QTL Sub1 for submergence tolerance, a theoretical frame-work for MAB and the existence of intolerant varieties that are widely accepted by farmers provided an opportunity to develop cultivars that would be suitable for larger areas of submergence prone rice (Mackill 2006). The objectives of our project were (1) To introgress gSUB1 into RLR varieties viz. BR22 and BRRI dhan39 through Marker Assisted Breeding and (2) To develop efficient genotype giving more yield under submerged and water stagnant condition but will give similar yield under rainfed condition compared to the standard check varieties.

MATERIAL AND METHODS

Two parents' viz. BR22 and BRRI dhan39 were transplanted four times at an interval of seven days starting from 01st of July 2012 to synchronize flowering times for achieving desired backcross combinations. $F_{1}s$ [BR22/BRRI dhan51 (170 plants), BRRI dhan39/IR64-Sub1 (65 plants)] were transplanted along with third and fourth set of parents. Thirty days old seedlings were transplanted with a spacing of 25 x 15 cm. Single seedling was used for transplanting. Fertilizers were applied 80-60-40-100-10 kg N, P_2O_5 , K_2O , Gypsum and Zinc sulphate/ha. Nitrogen was applied in three splits at 15, 30 and 45 days after transplanting.

Leaf samples were collected from the F₁ plants, recurrent and donor parents. Genotyping was done following standard protocol of SSR markers using polymorphic marker Sub1C173.

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Confirmed and selected F_1 plants were used to produce BC_1F_1 seeds. Leaf samples were collected from young leaves from the plants at 10-12 DAT. About 2 cm long leaf tips were collected from the plants and kept inside 1.5 ml eppendorf tubes. DNA was extracted modified Miniscale method (Zheng *et al.*, 1995).

PCR was performed in 10 μ l reactions containing around 25 ng of DNA template (3 μ l DNA with 10-20X dilution factor), 1 μ l 10X TB buffer (containing 200 mM Tris–HCl pH 8.3, 500 mM KCl, 15 mM MgCl₂), 1 μ l of 1 mM dNTP, 0.5 μ l each of 5 μ M forward and reverse primers and 0.25 μ l of *Taq* DNA polymerase (4 U/ μ l) using MJ Research single or dual 96-well thermal cycler or G Storm thermal cycler (Chen *et al.*, 1997). Twelve-channel electronic pipette was used for transferring DNA from dilution plate to PCR plate. Ten micro liter of mineral oil was added in each well to prevent evaporation and the PCR plate was wrapped with adhesive film. After initial denaturation for 5 min at 94°C, each cycle comprised 1 min denaturation at 94°C, 1 min annealing at 55°C (for example), and 2 min extension at 72°C with a final extension for 7 min at 72°C at the end of 35 cycles. The PCR products were mixed with bromophenol blue gel loading dye and were analyzed by electrophoresis on 8% polyacrylamide gel using mini vertical polyacrylamide gels for high throughput manual genotyping (CBS Scientific Co. Inc., CA, USA). The gels were stained in 0.5 mg/ml ethidium bromide and were documented using Alpha Imager 1220 (Alpha Innotech, CA, USA). Microsatellite or simple sequence repeats (SSRs) markers were used for selection (IRGSP 2005).

RESULTS

 F_1 plants having heterozygous bands for Sub1C173 were confirmed as true F_1s . Gel pictures (Figure 1 and 2) are showing the heterozygous bands having bands from both the parents representing confirmed F_1s . In case of BR22/BRRI dhan51 F_1 population, 74, 78 and 62 BC₁ F_1 seeds were produced from selected and confirmed plant numbers 2, 3 and 14. Again, in case of BRRI dhan39/IR64-Sub1 F_1 population, 75 and 35 BC₁ F_1 seeds were produced from selected and confirmed F_1 plant numbers 2 and 8, respectively. This approach was not adopted because the main objective of the project was to reduce the timeframe of the backcrossing scheme.



Figure 1. Gel picture confirming F_1 s for BR22/BRRI dhan51 cross combination.

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Figure 2. Gel picture confirming F₁s for BRRI dhan39/IR64-Sub1 cross combination. DISCUSSION

The present study clearly demonstrated that the conversion of the variety BRRI dhan51, which is grown on around Bangladesh to submergence tolerant within a two year time span for the BC2 and 2.5-year-time span for the BC3. To the best of our knowledge, this is the first report of the introgression of a QTL with the specific aim of reducing the size of a donor segment using tightly linked flanking markers (i.e. recombinant selection). However, the extent of the size of the donor chromosomal segment was not monitored. In addition, this study demonstrates the practical use of molecular markers for the introgression of the *Sub1* QTL into important varieties grown in areas prone to submergence stress. The MAB strategy has thus been shown to be an effective means of utilizing QTLs with large effects in rice breeding programs

The development of submergence-tolerant versions of popular varieties with high grain guality and wide adaptability offers a model for an alternative to the time-consuming, labor-intensive task of developing new varieties in a conventional crossing program. Adoption of a completely new variety could take considerable time, whereas the chances of acceptability of converted popular varieties are relatively higher (Mackill 2006). In summary, we have developed an enhanced mega variety material of rice by using a marker assisted backcrossing approach to incorporate submergence tolerance, which was controlled by a major QTL. The recovery of the recipient parent genome was greatly accelerated emphasizing the increased efficiency of using markers to assist selection of backcross lines. More importantly given the agronomic characteristics of the donor parent, the size of the donor chromosomal segment containing the target locus was reduced to ensure that there were minimal changes to the genetic composition of the recipient variety. This practical example of marker-assisted selection clearly illustrates the superiority of using MAB compared to conventional backcrossing because obtaining such a small donor region within only a few backcross generations would be impossible using conventional methods. This approach is currently being used to enhance several other rice mega varieties for submergence tolerance in rice breeding program.

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