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Marker assisted foreground selection for identification of aromatic rice genotype to develop a modern aromatic line

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ABSTRACT

Marker-assisted backcrossing is one of the feasible methods to develop a new katariboghtype rice cultivar with semi dwarf and modern type plant character to cope with the challenge. The study was focused on the introgression of Badh2 QTL from katariboghinto some elite promising line-an early, agronomically suitable and susceptible variety. Backcrossing was done during T. Aman season; where G52, GOR509, G27, G49 and IR 93358:4-B-21-4-3-1RGA-2RGA-1-B were the recurrent parent and katariboghwas the donor parent. At 1st season 160 true F1 and then in the next season 134 BC1F1 Plant were developed, which were subjected to foreground selection; the first level of selection of marker assisted backcrossing program. The aim of foreground selection was to identify the introgressed lines. 141 BC1F1 populations were evaluated with tightly linked BADEX7-5 marker. A total of 134 heterozygous BC1F1 Plant were selected finally, which have alleles of both of the parents. Those introgressed lines could be efficiently used in further development of a stable modern type aromatic rice variety.

Keywords: *The aim of foreground selection was to identify the introgressed lines.*

1. INTRODUCTION

Fragrant rice (*Oryza sativa*) is gaining widespread popularity among consumers worldwide (Bhattacharjee et al., 2002); thus, its market price is much higher than that of nonfragrant rice (Qiu and Zhang, 2003). Growing rice is an inevitable part in half of the world population who depends on it as their staple food. Rice consumers all over the world have strong preferences for the aromatic rice, the aroma A mixture of 114 different volatile compounds was detected in the flavor of cooked rice (Yajima et al., 1978). One of them, 2-acetyl-1-pyrroline (2AP), is a potent flavor component and this trait is monogenic recessive. 2AP is found in all parts of plants of fragrant rice varieties except for the roots (Buttery et al., 1983b). The 2AP level is relatively higher in the aerial parts of plants than in milled rice grains (Yoshihashi et al., 1999).

Genetic analysis shows that a single recessive gene (badh2) on chromosome 8 is associated with rice fragrance and that the dominant badh2 allele is associated with lack of fragrance (Sood and Siddiq, 1978; Huang et al., 1994; Jin et al., 2003). A number of markers were identified that are closely linked to fgr (Ahn et al., 1992; Causse et al., 1994; Chen et al., 1997; Cho et al., 1998; Jin et al., 2003).

Kataribhog rice is a famous aromatic traditional variety of Bangladesh having a problem of lodging due to its height and photoperiod sensitivity resulting in its seasonal productivity and seasonal restricted cultivation. Improvement of kataribhog with modern plant high yielding type using molecular technique by foreground selection for aroma is the one of the strategy to improve kataribhog to modern type. The use of DNA markers in backcrossing greatly increases the efficiency of selection which is known as Marker-assisted backcrossing(MABC). The basis of MABC strategy is to incorporate one or a few genes into an adapted or elite variety or to transfer a gene/QTL from a donor line to a recipient line by repeated backcrossing. This approach develops an ideal genotype within a very short time than conventional breeding approaches. MABC approach is very advantageous with the following steps, (1) recombination and identification of target locus, known as 'foreground selection'; (2) minimizing linkage drag as recombinant selection; (3) harvesting maximum recurrent parent genome as background selection (Collard et. al. 2008). Although, the extent of effectiveness of this program is delimited by some factors, molecular breeding technologies are upgrading day by day, which has already been proven as the most effective technology for the development of HYV type aromatic varieties. Hence, the attempt of this study was to introgression Badh2 QTL into some promising line by MABC method and to identify the introgressed lines through foreground selection using SSRs markers at early generation.

2. MATERIAL AND METHODS

Experiment approach on field

The experiment was conducted on T.Aman season 2018 to Boro 2019 in Central Research Station at Mawna, Gazipur. Five semi-dwarf modern types, small grain varieties (G52, GOR509, G27, G49 and IR 93358:4-B-21-4-3-1RGA-2RGA-1-B) were selected as recurrent recipient parent and one local popular aromatic variety was selected as donor parent for aroma. The plants were labeled with stick labeler on the field.Total 169 F1 population were grown for F1 confirmation and then true F1 has been selected through MAS for crossing with respective recurrent parent. Seeds from one cross has been grown in one line.For confirming true BC1F1 through marker assisted selection BC1F1s were planted in field. Seeds from one cross were grown in one line for the identification of aromatic rice genotype with Marker Assisted Backcrossing (MABC) technique.

Parent selection and sample collection

Kataribhog is a very popular local aromatic variety which has specific aroma has been selected as donor parent and five non-aromatic lines were selected as the recurrent parent. This low yielding traditional variety contains specific aroma and grain quality, which is very popular and has great demand in the market. This aromatic variety was crossed with 5 semi modern type varieties whose are semi dwarf and photoperiod insensitive. Seeds of selected F1 lines were grown in the pot. Leaf samples were collected for foreground selection. Then selected heretogeneous F1 plants were crossed with the 5 non aromatic recurrent parents. 170 BC1F1 seeds from 5 crosses were grown for backcrossing with their recurrent parents. Among these seeds, 141 seeds were germinated in the experimental field. For foreground selection, leaf sample were collected from these 141 BC1F1 plants.

F1 plant production

Hybridization was carried out between 1 aromatic variety and 5 non aromatic varieties. 5 non aromatics were used as female parents while 1 aromatic was used as male parent in the hybridization scheme. Five sets parental lines were seeded at an interval of 7 days starting from. Twenty- five days old seedlings were transplanted in the hybridization block

Production of BC1F1

For producing BC1F1 seeds, F1 seeds were seeded. Four sets of non aromatic recurrent parents were seeded for synchronization of flowering with F1 plants for producing BC1F1 seeds. They were sown at an interval of 7 days. The total number of F1 seeds produced was 169 and after marker assisted selection we selected 160 heterozygous plants as true F1 plants. In order to handle all the selection approaches and also for introgression of (badh2) gene by backcrossing all the true F1 plants were used.

Raising of F1 and BC1F1 plants

The mature F1 and BC1F1seeds were seeded on the pot with proper care and tagging. So 5 pots were prepared for 5 crosses. Seeds from one cross were seeded in the same pot. Before seeding in the plot F1 seeds were dried in the oven at 40*C temperature for 72 hours for breaking dormancy. Seeds were seeded and then 25 days old seedlings were transferred on the field.

Collection of leaf sample for DNA extraction

DNA extraction following marker assisted selection was done using young vigorously growing fresh leaf samples. Leaf sample were collected from 30 day old seedlings to extract genomic DNA from the parent plants along with the F1 plants. Initially, healthy portion of the youngest leaves of the plants were cut apart with sterilized scissors and for avoiding contamination, the scissor was washed in distilled water and ethanol (70%) and dried on fresh tissue paper to remove spore of microorganisms and any other source of foreign DNA. The collected leaf samples from the parent plants were then kept in zipper bags, and leaf sample from hybridized plants were kept in the deep well PCR box. avoiding any damage of the leaf tissues the bags and plate were placed in an ice box to carry to the lab. Finally, the samples were stored in -20*C freezer.

Genomic DNA extraction

Two types of DNA extraction methods Cetyl Trimethyl Ammonium Bromide CTAB and IRRI methods of DNA extraction only for confirmation of heterogeneous plants were used for extracting the genomic DNA from the parent plants. As parents sample's DNA were preserved for the next generation, we used CTAB method and for F1 sample we used simple IRRI methods for DNA extraction. For extracting DNA from F1 only 2 reagents (NaOH and Tris) were used. The simplified mini scale procedure for DNA isolation in PCR analysis developed at IRRI was followed. The quality of the isolated DNA in the protocol was sufficient for PCR analysis (Zheng et al., 1995). The following steps were followed in PCR-based DNA marker analysis.

PCR analysis for microsatellite (SSR) markers for badh2 gene

Polymorphism survey for primer selection

Polymorphism survey of parent plants was carried out using 7 microsatellite markers. Out of these SSR markers, one primer (BADEX7-5) showed clear polymorphisms between aromatic(Kataribhog) and non aromatic (G-27, G-49) which were used in genotyping for the foreground selection of the 169 F1 plants and 141 BC1F1 plants. The details of the primer are given in Table 1.

Polymerase chain reaction (PCR)

The PCR cocktail including DNA had total volume of 10 µl/reaction based on rice protocol, here we used master mix for preparing the PCR cocktail which was placed in the PCR tubes and run in the DNA thermal cycler. The PCR amplification conditions for SSR analysis were applied as initial extended step of denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 second, primer annealing at 55 °C for 30 second, elongation at 72 °C for 30 second followed by extension step at 72 °C for 5 minutes. As we used master mixed for preparing the PCR cocktail, no loading dye was mixed with PCR product. The amplification products were electrophoresed on Polyacrylamide gel at 80 Volt in 1x TAE buffer for 80-100 minutes. Genomic DNA was quantified by UV absorbance at 260 nm using UV syngene gel documentation system.

Components of PCR cocktail for foreground selection on F1 and BCIFI generation

The following components were used to prepare PCR cocktail (Table 2). The total volume of PCR cocktail for this study was 8.5 µl per sample.

Marker Assisted Foreground Selection

The use of DNA markers in backcrossing greatly increases the efficiency of selection. Three general levels of marker-assisted backcrossing (MABC) were described (Holland 2004). In the first level, markers can be used in combination with or to replace screening for the target gene or QTL. This is referred to as 'foreground selection' (Hospital and Charcosset, 1997). This may be particularly useful for traits that have laborious or time-consuming phenotypic screening procedures. It can also be used to select for reproductive-stage traits in the seedling stage, allowing the best plants to be identified for backcrossing. Furthermore, recessive alleles can be selected, which is difficult to do using conventional methods (Fig. 1).

Allele scoring for foreground selection

In F1 and BC1F1 generation, foreground selection was initially carried out by the robust tightly-linked marker BADEX7-5 and the individual plants that were heterozygous at the badh2 locus

were identified reducing the population size for further selection, then were marked with sticks in the field. DNA samples were collected from F1 and BC1F1 plants and PCR was carried out using one SSR marker BADEX7-5. Aromatic parent was denoted as "A" and non aromatic parent was denoted as "B. Score "H" represented heterozygous alleles for donor and recipient parent.So, true heterozygous F1s and BC1F1s were scored as "H" (Fig. 3).

Confirmation of F1 and BC1F1 plants

In the F1s and BC1F1 generation, foreground selection was performed on 169 and 141 plants respectively using BADEX 7-5, a marker tightly linked to the aroma containing QTL (badh2). Out of 169 plants, 160 plants showed heterozygous alleles in F1 generation and selected as true F1. These 160 true F1 were selected for backcrossing with recipient parent. out of 141 BC1F1 plants, 134 plants showed heterozygous alleles (score 'H') in BC1F1 generation. 7 plants showed fixed recipient allele (non aromatic allele, score 'B'). The seven plants with 'B' score were produced due to accidental failure of backcrossing. The 134 plants with the 'H' score were subjected for foreground selection. Fig. 2 shows the partial view of the most intensely amplified band for each microsatellite marker. It was determined based on its migration relative to a molecular weight size marker (1kb+ DNA ladder) The band having same level of Kataribhog was scored as 'A' which indicated the homozygous allele of the donor parent for the particular SSR marker. Again, the band having same level of non aromatic was scored as 'B' which indicated the homozygous allele of the recipient parent for the particular SSR marker. However, heterozygous alleles were scored as 'H' having both the bands of two parents. Score 'N' indicated the absence of band.

3. RESULTS AND DISCUSSION

Parental Survey

A comprehensive primer survey is required for successful foreground, recombinant and background selection so a primer survey is an important prerequisite before starting marker-assisted selection technique. Polymorphic markers are essential for a MABC scheme. A marker which is monomorphic bears no value in selection work because this type of marker cannot distinguish the two parental genotypes viz. aromatic, the recurrent or recipient parents and non aromatic, the donor parent of the MABC program. A total of 7 SSR primers were surveyed for finding out polymorphic markers and among them one primer was found as polymorphic as this marker shows different length band is gel electrophoresis.

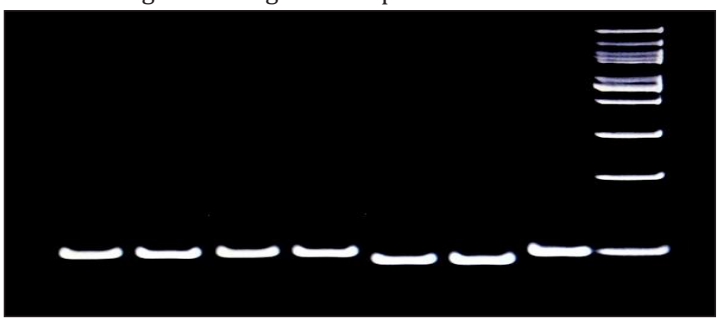

Fig 1: Gel showing the polymorphism among the varieties

Introgression of the badh2 gene through hybridization

The main aim of this study was to introgress the badh2 gene into 5 semi dwarf modern type varieties using a marker assisted backcrossing approach in order to maximize the recovery recurrent parent genome and all of the desirable characteristics of the recurrent parent. During this process, further selection strategies associated with marker-assisted backcross breeding were done. The results of these selection strategies were as follows: Confirmation of F1 plants DNA samples were collected from 169 F1 plants and PCR was carried out using one SSR marker BAD EX7-5. PCR bands from all the 160 F1 plants were scored as "H". Score "H" represented heterozygous alleles for donor and recipient parent. Fig. 1 shows the gel picture of F1 confirmation using a SSR marker BADEX7-5. All the F1s were confirmed as true F1 (Fig. 3). Then true F1 plants were backcrossed with the respective recurrent parents. Again MAS was conducted on 141 BC1F1 plants and 143 were selected as true BC1F1.

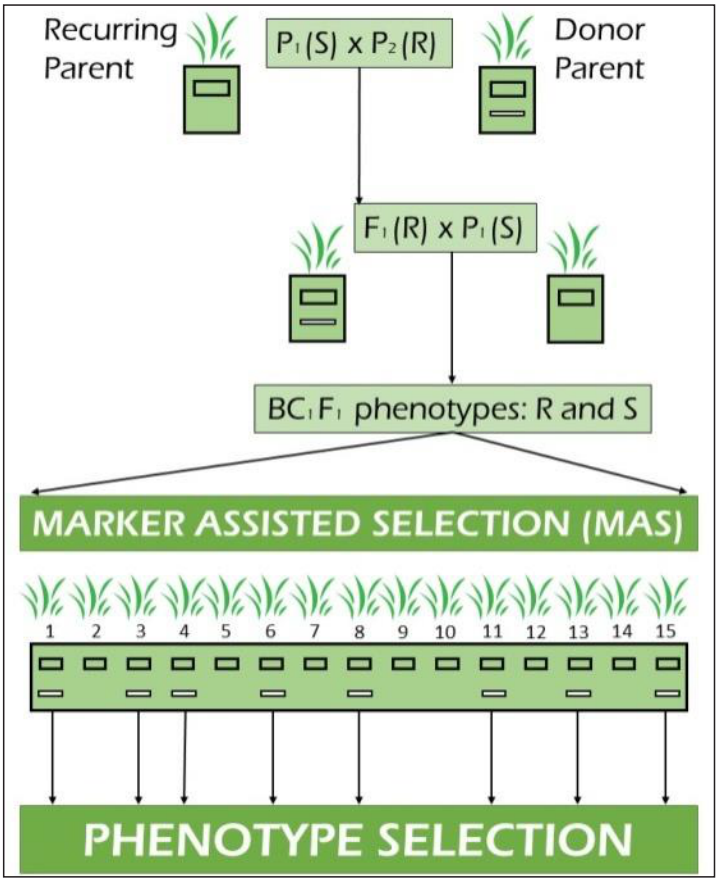

Fig 2: Marker assisted selection scheme Fig 2: Hybridization

Fig 3: F1 and BC1F1 seeds seeded in the pots

Table 1: Summery of microsatellite (SSR) marker used for foreground selection

Primer name	Expected PCR Product size	Chromosome Position	Primer Sequence	Annealing temperature
BADEX 7-5	**95 (Aromatic) 103(Non Aromatic)**	**8**	5' TGTTTTCTGTTAGGTTGCATT 3' 3' ATCCACAGAAATTTGGAAAC 5'	**55*C**

3.2. Confirmation of F1 plants

DNA samples were collected from F1 plants and PCR was carried out using one SSR marker BADEX5-7. Score "H" represented heterozygous alleles for donor and recipient parent, all the heterozygous F1 plants showing double bands in gel electrophoresis were scored "H". PCR bands from all the 5 F1 plants were scored as "H". Fig. 1 shows the gel picture of F1 confirmation using a SSR marker BADEX5-7. From total 169 F1 plants, 160 showed heterogeneous allele for badh2 QTL and 9 showed allele same as their recurrent parents (Fig. 3).

Table 1: List of F1 seeds produced

Sl no.	Cross	Recurrent Parents	F1 Seeds
1.	Kataribhog / G52	G52	42
2.	Kataribhog / GOR509	GOR509	20
3.	Kataribhog / G27	G27	35
4.	Kataribhog / G49	G49	48
5.	Kataribhog / IR 93358:4-B-21-4-3-1RGA-2RGA-1-B	IR 93358:4-B-21-4-3-1RGA-2RGA-1-B	24
	Total		169

Table: Components of PCR mixture for foreground selection for 94 samples

SL	Components	Quantity (for single sample).....	For 94 samples
1	Master mix	05	470
2	Forward primer	0.5	47
3	Reverse primer	0.5	47
4	ddH2O	04	376
5	Total		940

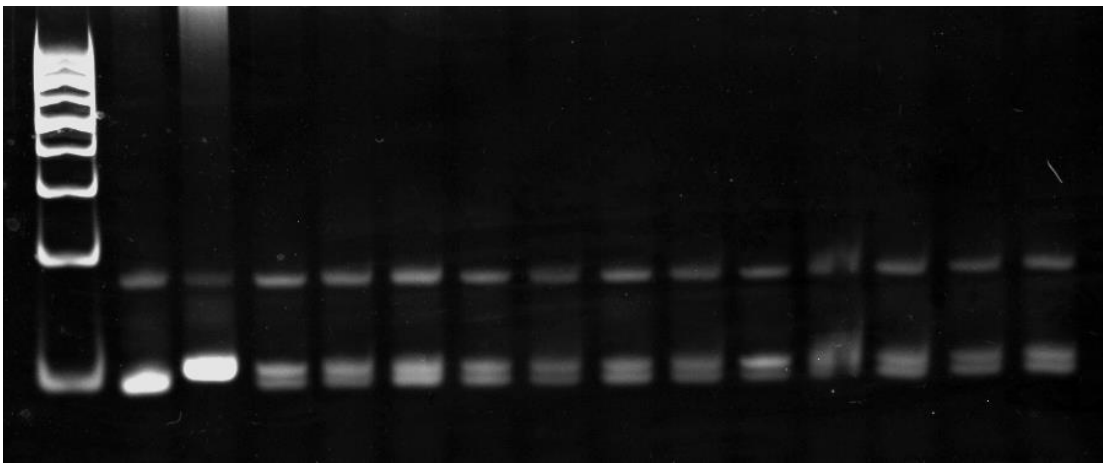

Fig 4: Complete view of gel showing confirmation of F1s of aromatic X non-aromatic using SSR marker BAD Ex7

Foreground selection at BC1F1 generation

A total of 157 F1 seeds were produced from 5 crosses where one crossing plant was aromatic and another was non aromatic plants (Table 3) and 141 plants were survived in the main fields. Foreground selection was performed on 141 plants. Foreground selection was carried out using a tightly linked aromatic marker BADEX 7-5. The marker produced very conspicuous bands (Fig. 4, 5 & 6) and it was possible to identify the genetic constitution of the badh2 QTLs very easily using polyacrylamide gel electrophoresis.

Table 2: List of F1 seeds produced

Sl no.	BC1F1	Recurrent Parents	F1 Seeds
1.	Kataribhog / G52// G52	G52	5
2.	Kataribhog / GOR509// GOR509	GOR509	10
3.	Kataribhog / G27// G27	G27	8
4.	Kataribhog / G49//G49	G49	7
5.	Kataribhog / IR 93358:4-B-21-4-3-1RGA-2RGA-1-B// IR 93358:4-B-21-4-3-1RGA-2RGA-1-B	IR 93358:4-B-21-4-3-1RGA-2RGA-1-B	9
	Total		39

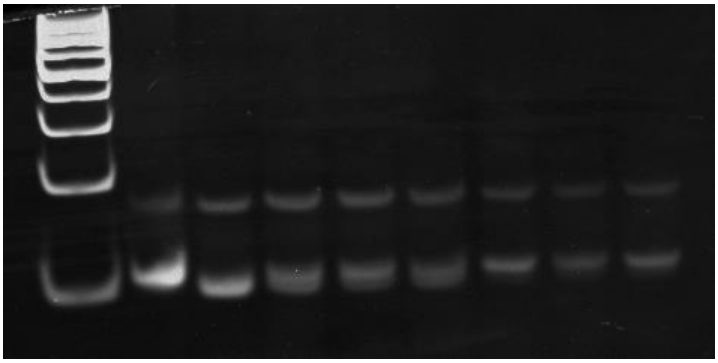

Fig 5: Complete view of gel showing confirmation of BC1F1s of aromatic x non-aromatic using SSR marker BAD EX7-5

3.5. Foreground selection at BC1F1 generation with tightly linked marker BADEX 5-7

Out of 141 plants, 39 plants were found showing the locus for tightly linked marker as heterozygous state (score H), 39 plants were found with the locus fixed for recipient allele (susceptible allele), 2 plants were found with the locus fixed for donor allele (resistant allele). The 39 plants with the 'H' score for the tightly linked marker BADEX 5-7 were subjected for recombinant selection. It was expected that those 39 individuals possessed the fragrance allele. So, these segregants were selected and promoted for further selection. The results were similar to the study of Neeraja et al. (2007) where foreground selection was performed with marker assisted backcrossing of rice for introgression of promising gene. Iftekharuddaula (2009) found similar results in his study on foreground selection in BC1F1 populations with marker assisted backcrossing to popular rice variety BR11.

4. CONCLUSIONS

In the present study we introgress the fragrance gene into five modern semi-dwarf lines for improvement of Kataribhog with the help of Marker Assisted backcross Breeding (MABC). The improve line were kataribhog type aroma with modern semi-dwarf high yielding than kataribhog. In the present investigation, based on the foreground selection with a tightly linked aromatic marker BAD EX 7-5, we selected 39 BC1F1 plants showing 'H' scores were selected in whish 75% recovery of recurrent parent genome with aroma gene. The selected segregates was further foreground, recombinant and background selections will be done with appropriate markers up to BC3 or more for the development of high yielding aromatic rice lines. The improved lines will also be valuable as donor for aroma gene for introgression in aromatic rice breeding.

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