IMPROVEMENT OF EARLY MATURITY IN RICE VARIETY BY MARKER ASSISTED BACKCROSS BREEDING OF $Hd2$ GENE

Perbaikan Umur Masak Varietas Padi melalui Pemuliaan Silang Balik Berbantuan Marka Gen $Hd2$

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ABSTRACT

Early-maturing and high-yielding rice variety is very useful for increasing rice production in Indonesia. The aim of this research was to develop new lines of Indonesian rice containing $Hd2$ gene using Code variety as a recipient parent and Nipponbare variety as a donor parent through targeted MAB approach using RM1362 and RM7601 in chromosom 7 for foreground selection. After two generations of backcrossing, the positive alleles of $Hd2$ gene from Nipponbare had successfully transferred into Code. The plant number CdNp_29 in BC$_2$F$_2$ population had the highest genome recovery of 82.7%. The twelve BC$_2$F$_2$ plants were selected for self-pollination to generate BC$_2$F$_3$. These selected lines that carried the $Hd2$ gene were screened in the greenhouse for the evaluation of heading date and agronomic traits. All improved lines had $Hd2$ gene similar to the donor parent Nipponbare. The heading date of the breeding lines ranged from 73 to 89 days (Code 85 days) or fill the third criterion of rice maturity that is 103-104 days compared to Code of 116-119 days) with agronomic performances similar with that of Code. Application of MABc for improving rice early maturity has accelerated the development and selection in early generation of superior lines having genetic background of Code. It is expected that the newly developed lines of Code will be utilized to increase rice production in Indonesia.

[Keywords: Padi, umur genjah, silang balik berbantuan marka, gen $Hd2$]

INTRODUCTION

Early-maturing and high-yielding rice varieties are very useful for increasing rice production in Indonesia. This is because improved rice varieties have higher yield, 5-9 t ha$^{-1}$ within 110-135 days, while the yields of local varieties are only 3-4 t ha$^{-1}$ in 150-180 days. Early-maturing variety allows farmers to increase cropping intensity from two to three or four crops of rice per year. The grouping criteria of rice maturity based on harvest time are (1) ultra maturity, less than 85 days, (2) super maturity, 85-94 days, (3) early maturity, 95-104 days, (4) mature, 105-124 days, (5) intermediate maturity, 125-164 days and (6) late maturity, >165 days (IAARD 2012).

Code rice variety as a recipient parent has been crossed with Nipponbare as a donor parent for early maturity heading date ($Hd$) gene. In 2002, this line was nationally released as a new lowland rice variety. Because it is derived from an existing popular variety, this variety is well accepted by farmers and consumers (Toenniessen 2003; Jena and Mackill 2008).

Heading date is one of crucial factors determining regional and seasonal adaptation of rice and has been a major target of selection in breeding programs. During the last decade, genetic studies using DNA
markers have facilitated the genetic dissection of heading date, and many quantitative trait loci (QTLs) for heading date have been identified using several mapping populations (Yano et al. 2001; Lin et al. 2002, 2003; Gu and Foley 2007; Nonoue et al. 2008). Rice heading date is controlled by major and minor genes and QTL analysis is a useful method for identifying the rice heading-date-related genes (Shao et al. 2009).

Several rice heading-date-related genes have been identified and isolated throughout 12 chromosomes. $Hd1$ (Heading date 1), a major photoperiod sensitivity gene, is closely related to the Arabidopsis flowering time gene CO (CONSTANS). It encodes a B-box Zinc finger protein with a CCT domain (Yano et al. 2000). Heading date-related genes $Hd2$ (Yamamoto et al. 1998), $Hd3$ (Yamamoto et al. 1998), $Hd3a$ (Kojima et al. 2002), $Hd3b$ (Monna et al. 2002), $Hd4$ (Lin et al. 2003), $Hd5$ (Lin et al. 2003), $Hd6$ (Takahashi et al. 2001) and $Hd9$ (Lin et al. 2002) have also been isolated in rice.

Using conventional breeding methods, it typically takes 6-8 backcrosses to fully recover the recurrent parent genome. Marker assisted backcrossing (MABC) is the process of using markers to select target loci (donor), minimize the length of the donor segment containing a target locus, and/or accelerate the recovery of the recurrent parent genome during backcrossing (Hospital 2001). Foreground selection as the selection of a target locus and background selection as the selection of the recurrent parent genome use markers on non-carrier chromosomes and also on the carrier chromosome (Hospital and Charcosset 1997). Background selection can greatly accelerate a backcrossing program compared to using conventional backcrossing (Frisch et al. 1999).

MABC has previously been used in rice breeding to incorporate the bacterial blight resistance gene $Xa21$ (Chen et al. 2000), waxy gene (Zhou et al. 2003), $Sub1$ gene of mega-variety Swarna to a submergence tolerant variety and IR64$SUB1$ for developing a new submergence tolerant rice variety ASS996-$SUB1$ (Neeraja et al. 2007; Septiningsih et al. 2009; Luu et al. 2012). It is also used to transfer $badh2$ and $Wx$ gene from Basmati into Manawthukha for cooking quality trait (Yi et al. 2009), and $Pup1$ under P-deficient lowland/irrigated conditions into Situ Bagendit and Batur (Chin et al. 2011). Rice salt tolerance on BT7 cultivar, FL478 was used as a donor parent of Saltol QTL (Linh et al. 2012) and three resistance genes ($Xa4 + xa5 + Xa21$) to bacterial leaf blight were transferred from an indica donor (IRBB57) to Korean rice Mangeumbyeo (Suh et al. 2013).

The aim of this research was to develop new lines of Indonesian rice containing early maturity gene $Hd2$ using Code as a recipient parent and Nipponbare is a donor parent through a targetted MABC approach until generation $BC_4F_7$ and background selection for the recurrent parent genome.

MATERIALS AND METHODS

Plant Materials and Breeding Scheme

The study used an Indonesia rice variety Code as a recipient parent which was back-crossed with Nipponbare as a donor parent for early maturity (regulated flowering time) Heading date ($Hd$) gene. Backcross populations consisted of 195 $BC_1F_1$, 146 $BC_2F_2$, 200 $BC_3F_3$, 96 $BC_4F_4$ and 85 $BC_5F_5$ breeding lines.

For the MABC scheme, Code was crossed with Nipponbare to obtain $F_1$ seeds (Fig. 1) then the $F_1$ was back-crossed with Code to obtain a large number of $BC_2F_2$ seeds. In the $BC_2F_2$ generation, individual plants that were heterozygous at the $Hd2$ locus were identified to reduce the population size for further screening (foreground selection). It was carried out

![Fig. 1. Scheme for the development of $Hd2$ backcross breeding lines of rice using marker-assisted foreground and background selection.](image-url)
using 200 individuals in each generation of backcrossing population. From these plants, individuals with the largest number of markers from the recipient genome were selected (background selection). In the second BC generation, the same strategy was applied for selection of individual plants with the desired allele at the target loci and crossed with the recipient parent to develop the next generation. The selected BC₂ plants were self-pollinated for further analysis.

**Molecular Marker Analysis**

Total genomic DNA was extracted after crushing in liquid nitrogen in microfuge tubes using a Tris/SDS extraction buffer (100 mM Tris-HCl pH 8, 50 mM EDTA pH 8, 500 mM NaCl, 1.25% SDS, w/v, and 0.38 g sodium bisulfite per 100 ml of buffer) and chloroform extraction followed by ethanol precipitation. The PCR amplification was generated using MJ research Tetrad Thermal Cycler PCR machine by following PCR conditions: (1) an initial denaturation step of 2 minutes at 94°C, (2) 30 cycles of 45 seconds at 94°C, 45 seconds at 55°C, 1 minute at 72°C and (3) a final extension step for 5 minutes at 72°C. Amplified products were separated by electrophoresis in 8% polyacrylamide gel at 100 v (Dual Triple-Wide Mini-Vertical System, CBS Scientific, CA, USA) then observed by ethidium bromide or silver staining and photographed under ultraviolet light using the gel documentation system (BioRad).

**Foreground Selection of Hd2 Gene**

For selection of BC₁,F₁, BC₂,F₂, BC₃,F₃, BC₄,F₄ and BC₅,F₅ generations, rice microsatellite markers RM1362 (F: TGATCTAAACAGGCCCTTAG and R: CATCATCAA GACCACACATC) and RM7601 (F: GCCTCGCTGTC GCTAATATC and R: CAGCCTCTCCTTGTGTTGTG) were used which were linked with the QTLs for Hd2 locus. These markers were located on chromosome 6 at the genetic distance of 116.1 cM and 116.6 cM (Fujino and Sekiguchi 2008).

**Background Selection**

Among 134 SSR primers surveyed, 43 markers were used for selection of BC₁,F₁ and 66 markers for BC₂,F₂ which at least three markers on each chromosome were used. On BC₃,F₃ generation, additional microsatellite markers were used to check the fixation of the recipient genome. Five hundred SSR primers were surveyed, of which 237 markers showed clear polymorphisms between the two parents and well distributed on all twelve chromosomes.

**Agronomic Performance**

The research was conducted in the greenhouse of ICABIOGRAD in 2009-2012. Traits measured included days to heading, plant height, tiller number, number of effective tillers per plant, number of filled grains per panicle, number of empty grains per panicle, 100 grain weight, total grain weight and grain yield. Days to heading were recorded when 50% of the individual plants in each plot flowered. Plant height, number of effective tillers per plant, number of filled grains per panicle and number of empty grains per panicle were measured at maturity and based on five individual plants selected in each plot. Plant height was measured from the soil surface to the tip of the panicle. Number of filled grains per panicle and number of empty grains per panicle were counted manually. The 100 grain weight and total grain weight measurements were replicated three times. Grain yield of each plot was adjusted to 14% moisture content and extrapolated to tons per hectare.

**Data Analysis**

The marker data were analyzed using the software Graphical Genotyper (GGT 3.2) (Berloo 2008). Polymorphisms in the DNA profiles were scored visually by comparing with two parents and a standard DNA ladder. The homozygous recipient allele, homozygous dominant allele and heterozygous allele were scored as “A”, “B” and “H”. The agronomic data revealed each line were written into Excel (Microsoft 2007) and statistically analyzed by Duncan significant difference and Pearson correlation using SPSS version 17.

**RESULTS AND DISCUSSION**

**Transferring Early Maturity Hd2 Gene**

The validated markers could be used successfully to confirm the early maturity gene in several backcross generations (Fig. 2). The foreground selection result is summarized on Table 1. Of the 195 BC₁,F₁ plants, 90 plants (46.2%) were heterozygous for the marker
RM7601 and 44.6% for RM1362. Of the 146 BC\textsubscript{2}F\textsubscript{1} plants, 31 plants (21.2%) were heterozygous for the marker RM7601 and RM1362. Of the 200 BC\textsubscript{2}F\textsubscript{2} plants, 37 plants (18.5%) were homozygous to Nipponbare for the marker RM7601 and RM1362. Of the 96 BC\textsubscript{2}F\textsubscript{3} plants, 72 plants (75%) were homozygous to Nipponbare for the marker RM7601 and 66% for RM1362. Of the 84 BC\textsubscript{2}F\textsubscript{4} plants, 56 plants (66.7%) were homozygous to Nipponbare for the marker RM7601 and 65.6% for RM1362.

Foreground selection confirmed from previous study by Moeljopawiro et al. (2010) showed that of 45 primers related to QTL of \textit{Hd} genes, only \textit{Hd2}, \textit{Hd3}, \textit{Hd7} and \textit{Hd14} gave a high polymorphism pattern between Code and Nipponbare. In this study, the \textit{Hd2} gene on chromosome 7 used primer RM1362 (116.1 cM) and RM7601 (116.6 cM) in all the breeding lines. The use of two precise primers located in the \textit{Hd2} region around 0.5 cM of LOD value of 7.5 which corresponds to infinitely dense of 1 cM between markers calculated a difference in LODs of about 7% (Lander and Kruglyak 1995; van Ooijen 1999) resulted in the minimized size of the \textit{Hd2} in Code variety. The closely linked DNA markers can be used in accelerating the allele fixation and increasing the efficiency of plant breeding with the maximum

**Table 1. Foreground selection of backcross generation of Code and Nipponbare rice varieties using \textit{Hd2} linked primer.**

| Primer  | BC\textsubscript{2}F\textsubscript{1} Progeny | Heterozygous (%) | BC\textsubscript{2}F\textsubscript{2} Progeny | Heterozygous (%) | BC\textsubscript{2}F\textsubscript{3} Progeny | Homozygous (%) | BC\textsubscript{2}F\textsubscript{4} Progeny | Homozygous (%) |
|---------|--------------------------------|-----------------|--------------------------------|-----------------|--------------------------------|---------------|--------------------------------|---------------|
| RM1362  | 195                             | 87 (44.6)       | 146                             | 31 (21.2)       | 200                             | 37 (18.5)     | 96                             | 64 (66)       |
| RM7601  | 195                             | 90 (46.2)       | 146                             | 31 (21.2)       | 200                             | 37 (18.5)     | 96                             | 72 (75)       |

**Fig. 2.** Screening of backcross generation of Code and Nipponbare rice varieties using \textit{Hd2} linked primer RM7601: a = BC\textsubscript{1}F\textsubscript{1}, b = BC\textsubscript{2}F\textsubscript{1}, c = BC\textsubscript{2}F\textsubscript{2}, d = BC\textsubscript{2}F\textsubscript{3} and e = BC\textsubscript{2}F\textsubscript{4} individuals. Lane 1 = 100 bp marker, lane 2 = Code, lane 3 = Nipponbare, lane 4-56 = individuals on 8% polyacrylamid gel electrophoresis.
percentage of recurrent parent genome (Babu et al. 2004). Suh et al. (2013) reported that selection of the target genes through foreground selection and flanking marker analysis aimed to reduce the persistent linkage drag.

**Genetic Background Profiling**

Microsatellite markers covering all the 12 chromosomes were used for the background selection. These polymorphic markers were used for assessing BC$_{1}$F$_{1}$, BC$_{2}$F$_{1}$ and BC$_{2}$F$_{2}$ generations and resulted the average polymorphic markers of 25%, 49.3% and 47.4%, respectively (Table 2).

Among 134 SSR primers surveyed, 43 markers were used for initial selection on BC$_{1}$F$_{1}$. The maximum number of background markers used was five for chromosome 11. The microsatellite markers with homozygous alleles on non-target loci in one generation were not screened in the next backcross generation and the segregants with homozygous donor alleles were discarded from the selection. The highest recipient allele was CdNP_37 and continuing to develop BC$_{2}$F$_{1}$ generation (Fig. 3a).

On BC$_{1}$F$_{1}$ plants, the maximum number of background markers used was 10 for chromosome 2. BC$_{2}$F$_{1}$ plants no. CdNp_01, CdNp_03, CdNp_07, and CdNp_73 were used to develop BC$_{2}$F$_{2}$ generation. Among 500 SSR primers surveyed, 237 markers were used on BC$_{2}$F$_{2}$ generation. The maximum number of background markers used was 23 for chromosome 6. The best plant was CdNp_29 of which the recipient allele was 82.7% (Fig. 3b). The data of 12 selected individuals of BC$_{2}$F$_{2}$ showing the donor segment of Hd2 gene located in distal end of chromosome 7 are presented in Figure 4.

The background recovery of selected BC$_{2}$F$_{2}$ progenies was lower than the expected value (85%). Further continued MAB among progenies in subsequent selfing generations would not only lead to higher background recovery, but also need homozygosity for the target traits for stability. However, Singh et al. (2012) reported that background analysis of the advanced lines using 60 polymorphic STMS markers across the genome revealed up to 89.50% of the faster recovery of the recurrent parent genome that had been recovered in only two backcross generations.

Most of the remaining donor genome occurred on the chromosomes where the target genes were located. This may be caused by the introduction of additional chromosome segments from the donor or from linkage drag in the target chromosomes. Yano et al. (1997) reported that five QTLs (Hd1–Hd5) caused variation in rice heading date in crosses between Nipponbare and Kasalath. Yamamoto et al. (2000) reported that three photoperiod-sensitive QTLs (Hd1, Hd2 and Hd3) were interacted each other.

**Table 2. Distribution of SSR markers in 12 chromosomes of three backcross rice line of code and Nipponbare.**

| Chromosomes | No. of markers tested | No. of polymorphic markers | %  | No. of markers tested | No. of polymorphic markers | %  | No. of markers tested | No. of polymorphic markers | %  |
|-------------|-----------------------|-----------------------------|----|-----------------------|-----------------------------|----|-----------------------|-----------------------------|----|
| 1           | 16                    | 4                           | 25.0 | 16                    | 7                           | 43.8 | 61                    | 20                           | 32.8 |
| 2           | 16                    | 4                           | 25.0 | 16                    | 10                          | 62.5 | 55                    | 22                           | 40.0 |
| 3           | 13                    | 4                           | 30.8 | 13                    | 8                           | 61.5 | 46                    | 20                           | 43.5 |
| 4           | 9                     | 4                           | 44.4 | 9                     | 3                           | 33.3 | 43                    | 20                           | 46.5 |
| 5           | 12                    | 3                           | 25.0 | 12                    | 7                           | 58.3 | 33                    | 20                           | 60.6 |
| 6           | 12                    | 4                           | 33.3 | 12                    | 8                           | 66.7 | 53                    | 23                           | 43.4 |
| 7           | 11                    | 3                           | 27.3 | 11                    | 3                           | 27.3 | 45                    | 20                           | 44.4 |
| 8           | 12                    | 3                           | 25.0 | 12                    | 3                           | 25.0 | 38                    | 20                           | 52.6 |
| 9           | 6                     | 4                           | 66.7 | 6                     | 5                           | 83.3 | 29                    | 16                           | 55.2 |
| 10          | 10                    | 2                           | 20.0 | 10                    | 3                           | 30.0 | 35                    | 18                           | 51.4 |
| 11          | 10                    | 5                           | 50.0 | 10                    | 6                           | 60.0 | 23                    | 19                           | 82.6 |
| 12          | 7                     | 3                           | 42.9 | 7                     | 3                           | 42.9 | 39                    | 19                           | 48.7 |
| Total       | 134                   | 43                          | 25.0 | 134                   | 66                          | 49.3 | 500                   | 237                          | 47.4 |
Fig. 3. Background recovery across the genome in two backcross generations. (a) BC$_1$F$_1$ plant CdNP$_{37}$ as the best plant with 77.1% recipient alleles from Code and (b) BC$_2$F$_1$ plant CdNP$_{29}$ as the best plant with 82.7% recipient alleles from Code.
an undesirable phenotype. The selected $BC_2F_1$ and $BC_2F_2$ lines showed heading date earlier than Code; only three plants had a longer heading date than Code. The differences were found when comparing among the breeding lines and Code. Most of the breeding lines flowered earlier than Code, ranged from 74 to 86 days (Fig. 5). The selected $BC_2F_1$ and $BC_2F_2$ lines also had a heading date earlier than Code, ranged from 73 to 89 days (Fig. 5). The average of Code is 86 days in $BC_2F_1$, Code and Nipponbare, respectively, were 82 and 57 days in $BC_2F_2$ while 77 and 56 in $BC_3F_1$, and 85 and 66 in $BC_3F_2$. Significant differences were found when comparing among the breeding lines and between the breeding lines and Code. These breeding lines fill the third criteria of rice maturity that is 103-104 days compared to Code that matures at 116-119 days.

Yamamoto et al. (1998) reported that large variation in days to heading was observed in the population of crosses between Nipponbare and Kasalath. This variation attributed to the segregation of $Hd2$. Progeny testing found heading-late-fixed (homozygous for Nipponbare at $Hd2$), segregated (heterozygous) and early-fixed (homozygous for Kasalath). Ebana et

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**Fig. 4.** The donor segment of $Hd2$ gene in selected twelve $BC_2F_2$ plants of Code and Nipponbare crosses located in distal end of chromosome 7.

**Fig. 5.** Distribution of breeding population of backcross generation of Code and Nipponbare rice using $Hd2$ linked primer for days to heading.
Table 4. Comparison of agronomic performance of twelve selected breeding rice lines of BC$_2$F$_4$ and their parents.

| Breeding lines       | Days to heading | Plant height (cm) | No. of tillers | No. of maximum tillers | Panicle length (cm) | No. of filled grains | No. of empty grains | 100 grain weight (g) | Total grain weight (g) |
|----------------------|-----------------|------------------|----------------|------------------------|---------------------|----------------------|----------------------|----------------------|-----------------------|
| BC$_2$F$_4$ x Code    | 85.4 h          | 79.9 a           | 10.1 ab        | 10.1 ab                | 9.9 bc              | 22.9 cde             | 226.8 bcd            | 31.0 abc             | 2.0 abcd 33.1 c        |
| BC$_2$F$_4$ x Nip-03  | 82.7 efgf       | 90.0 ab          | 10.0 ab        | 10.0 ab                | 8.7 ab              | 24.0 def             | 233.4 bcd            | 51.4 bcd             | 2.5 g 36.1 c           |
| BC$_2$F$_4$ x Nip-27  | 82.0 defg        | 88.5 ab          | 9.3 ab         | 9.3 ab                 | 9.1 ab              | 22.9 cde             | 235.5 cd             | 27.4 ab              | 2.1 cde 35.6 c          |
| BC$_2$F$_4$ x Nip-29  | 82.0 defg        | 73.4 a           | 9.7 ab         | 9.7 ab                 | 8.6 ab              | 20.7 abc             | 162.7 ab             | 45.4 abc             | 1.9 ab 20.3 ab         |
| BC$_2$F$_4$ x Nip-75  | 78.9 c           | 96.7 abc         | 9.4 ab         | 9.4 ab                 | 9.0 ab              | 23.4 def             | 240.9 cd             | 14.6 a               | 2.0 abde 35.7 c         |
| BC$_2$F$_4$ x Nip-78  | 79.6 cd          | 107.1 bcd        | 8.4 ab         | 8.4 ab                 | 7.9 ab              | 26.2 f               | 246.9 cd             | 41.9 abc             | 2.1 def 33.3 c          |
| BC$_2$F$_4$ x Nip-92  | 76.1 b           | 82.8 ab          | 15.1 c         | 15.1 c                 | 12.3 c              | 20.3 ab              | 190.1 abc            | 50.8 bcd             | 1.9 abc 30.4 bc         |
| BC$_2$F$_4$ x Nip-95  | 83.3 efgf        | 85.2 ab          | 11.2 b         | 11.2 b                 | 9.9 bc              | 22.3 bcd             | 218.3 cd             | 59.7 cd              | 1.9 a 32.1 c           |
| BC$_2$F$_4$ x Nip-121 | 84.3 fgh         | 116.3 cd         | 9.1 ab         | 9.1 ab                 | 7.1 a               | 26.0 f               | 281.7 d             | 80.5 d               | 2.0 abcd 34.2 c         |
| BC$_2$F$_4$ x Nip-131 | 80.4 cde         | 116.4 cd         | 7.9 a          | 7.9 a                  | 7.4 ab              | 24.7 efgf            | 282.8 de             | 46.4 abc             | 2.1 bde 33.7 c          |
| BC$_2$F$_4$ x Nip-144 | 81.3 cdef        | 123.5 d          | 8.4 ab         | 8.4 ab                 | 8.1 ab              | 25.3 fg              | 319.8 e             | 31.5 ab              | 2.3 f 42.5 c           |
| BC$_2$F$_4$ x Nip-180 | 82.8 efgf        | 97.6 abc         | 9.8 ab         | 9.8 ab                 | 9.6 ab              | 22.6 cde             | 232.6 cd             | 39.2 ab              | 2.2 ef 35.0 c          |
| Code                 | 85.0 gh          | 92.6 abc         | 10.4 ab        | 10.4 ab                | 8.8 ab              | 23.2 def             | 246.4 cd             | 24.4 ab              | 2.1 cdef 32.5 c         |
| Nipponbare           | 66.5 a           | 81.0 a           | 8.6 ab         | 8.6 ab                 | 8.9 ab              | 19.5 a               | 138.25 a             | 20.8 ab              | 2.1 def 18.3 a          |

Means followed by the same letter are not significantly different at 5% level of Duncan significant difference.

al. (2011) reported that $Hd2$ has additive effects of the Koshihikari alleles in both directions, either increasing or decreasing days to heading. The range of additive effects reflected the functional status of gene(s) located within the QTLs.

**Agronomic Performance**

In BC$_2$F$_4$ lines, genotype variances were found for plant height, tiller number, number of grains per panicle and total grain weight, however, no differences were observed for panicle length and 100 grain weight between the breeding lines and Code. In BC$_2$F$_4$ lines, the plant height of the breeding lines was higher than that of Code and the total grain weight of the breeding lines was lighter than that of Code. Tiller number and number of effective tillers per plant were not different. In BC$_2$F$_4$ lines, the plant height of the breeding lines was higher than that of Code, and grain number and total grain weight of the breeding lines were less than those of Code. However, the 100 grain weight was not different. In BC$_2$F$_4$, genotype variances were found for plant height, number of empty grains per panicle, and total grain weight, however no significant differences were observed on tiller number, number of effective tillers per plant, number of filled grains per panicle and 100 grain weight when comparisons were made among the breeding lines and Code. Therefore plant no CdNp-29 of BC$_2$F$_4$ had low total weight. The plant also had better agronomic characters and $Hd2$ gene, and were early flowering than Code. The twelve selected BC$_2$F$_4$ lines (Table 4) were also resistant to bacterial leaf blight (data not shown). The results showed that the breeding line had agronomic characters similar to Code.

**CONCLUSION**

After two generations of backcrossing, a targeted MABC approach for the $Hd2$ gene using RM1362 and RM7601 in chromosome 7 for foreground selection has successfully transferred positive allele of $Hd2$ gene from Nipponbare into Code with the highest genome recovery of 82.7%. The heading date of the breeding lines ranged from 73 to 89 days (Code 85 days). These breeding lines fill the third criteria of rice maturity that is 103-104 days (Code 116-119 days). Twelve selected MABC lines were completed using marker selection and their heading date traits confirmed under greenhouse condition before amplifying seed for large-scale testing and validation in farmers’ fields. There is a need for combining $Hd$ gene, not only $Hd2$ gene but also $Hd3$, $Hd7$ and $Hd14$ into Code variety to improve the early maturity trait and develop the pyramiding lines.

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