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 targetted 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_2F_2 population had the highest genome recovery of 82.7%. The twelve BC_2F_3 plants were selected for self-pollination to generate BC_2F_4. 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, whereas their agronomic performances were 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*:** Rice, early maturity, marker assisted backcrossing, *Hd2* gene]

ABSTRAK

Padi umur genjah dengan hasil tinggi sangat bermanfaat untuk meningkatkan produksi padi Indonesia. Penelitian ini bertujuan untuk memasukkan gen umur genjah (gen Hd2*) yang terdapat dalam padi Nipponbare ke dalam varietas unggul Indonesia, yakni Code dengan menggunakan primer RM1362 dan RM7601 pada kromosom 7. Setelah dua generasi silang balik, gen* Hd2 *telah berhasil dimasukkan ke dalam varietas Code. Tanaman BC_2F_2 CdNp_29 memiliki pengembalian genom terbesar, yaitu 82.7%. Sebanyak 12 galur BC_2F_3 telah dipilih untuk membentuk generasi BC_2F_4 dan dievaluasi fenotipenya untuk karakter agronomi dan umur berbunga. Galur-galur dengan gen* Hd2 *memiliki umur berbunga sekitar 73-89 hari (Code 85 hari) atau masuk ke dalam kriteria ketiga umur panen varietas padi, yaitu 103-104 hari (Code 116-119 hari) dengan penampilan agronomi mirip dengan Code. Aplikasi MABc dalam perbaikan padi berumur genjah telah berhasil mempercepat pembentukan dan seleksi pada generasi awal dengan latar belakang genetik varietas Code. Galur-galur turunan Code tersebut diharapkan dapat dimanfaatkan untuk meningkatkan produksi padi 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. *Hd*1 (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*SUB*1 for developing a new submergence tolerant rice variety ASS996-*SUB*1 (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_2F_4 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_1, 200 BC_2F_2, 96 BC_2F_3 and 85 BC_2F_4breeding 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_1F_1 seeds. In the BC_1F_1 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

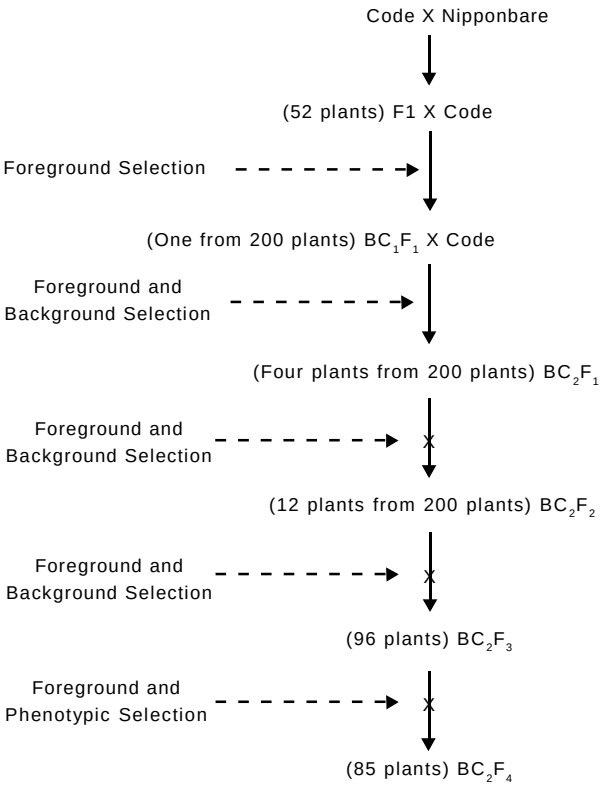


Fig. 1. Scheme for the development of *Hd2* backcross breeding lines of rice using marker-assisted foreground and background selection.

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 BC_2 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 BC_1F_1, BC_2F_1, BC_2F_2, BC_2F_3 and BC_2F_4 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 BC_1F_1 and 66 markers for BC_2F_1 which at least three markers on each chromosome were used. On BC_2F_2 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 BC_1F_1 plants, 90 plants (46.2%) were heterozygous for the marker

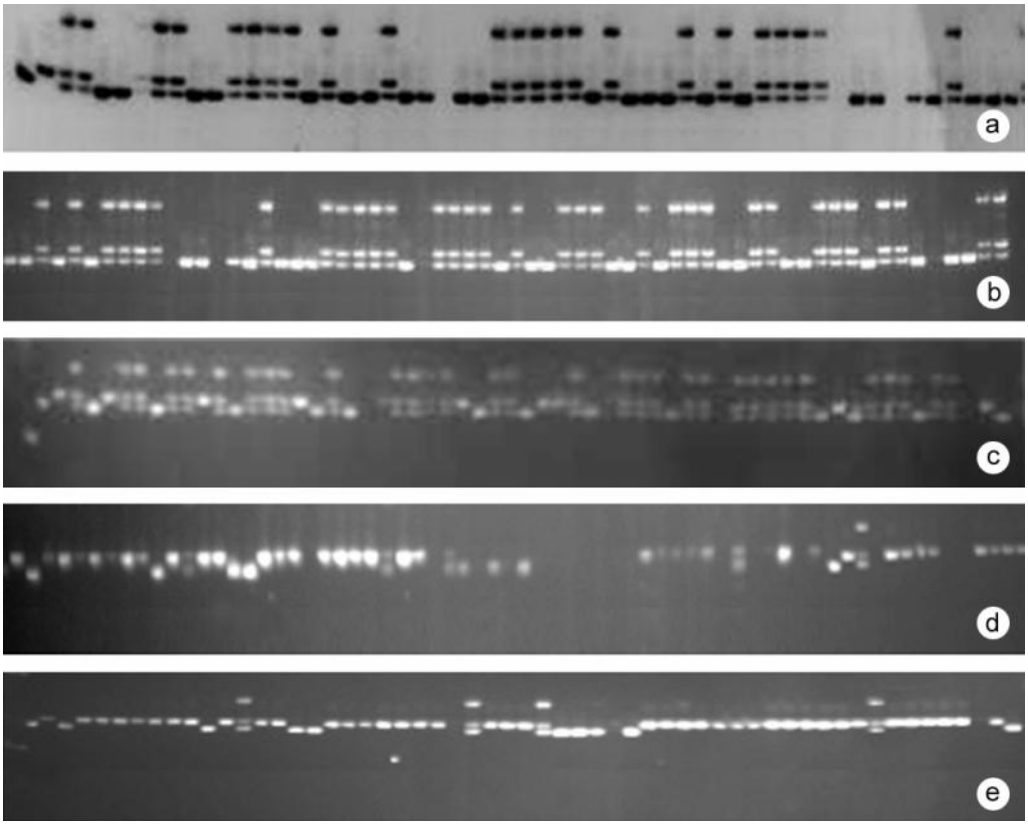


Fig. 2. Screening of backcross generation of Code and Nipponbare rice varieties using *Hd2* linked primer RM7601; a = BC_1F_1, b = BC_2F_1, c = BC_2F_2, d = BC_2F_3 and e = BC_2F_4 individuals. Lane 1 = 100 bp marker, lane 2 = Code, lane 3 = Nipponbare, lane 4-56 = individuals on 8% polyacrylamid gel electrophoresis.

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

Primer	BC_1F_1		BC_2F_1		BC_2F_2		BC_2F_3		BC_2F_4	
	Progeny number	Heterozygous (%)	Progeny number	Heterozygous (%)	Progeny number	Homozygous (%)	Progeny number	Homozygous (%)	Progeny number	Homozygous (%)
RM1362	195	87 (44.6)	146	31(21.2)	200	37 (18.5)	96	64 (66)	84	63 (65.6)
RM7601	195	90 (46.2)	146	31 (21.2)	200	37 (18.5)	96	72 (75)	84	56 (66.7)

RM7601 and 44.6% for RM1362. Of the 146 BC_2F_1 plants, 31 plants (21.2%) were heterozygous for the marker RM7601 and RM1362. Of the 200 BC_2F_2 plants, 37 plants (18.5%) were homozygous to Nipponbare for the marker RM7601 and RM1362. Of the 96 BC_2F_3 plants, 72 plants (75%) were homozygous to Nipponbare for the marker RM7601 and 66% for RM1362. Of the 84 BC_2F_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 *Hd* genes, only *Hd2*, *Hd3*, *Hd7* and *Hd14* gave a high polymorphism pattern between Code and Nipponbare. In this study, the *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 *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 *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

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_1F_1, BC_2F_1 and BC_2F_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_1F_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_2F_1 generation (Fig. 3a).

On BC_2F_1 plants, the maximum number of background markers used was 10 for chromosome 2. BC_2F_1 plants no. CdNp_01, CdNp_03, CdNp_07, and CdNp_73 were used to develop BC_2F_2 generation. Among 500 SSR primers surveyed, 237 markers were used on BC_2F_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_2F_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_2F_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.

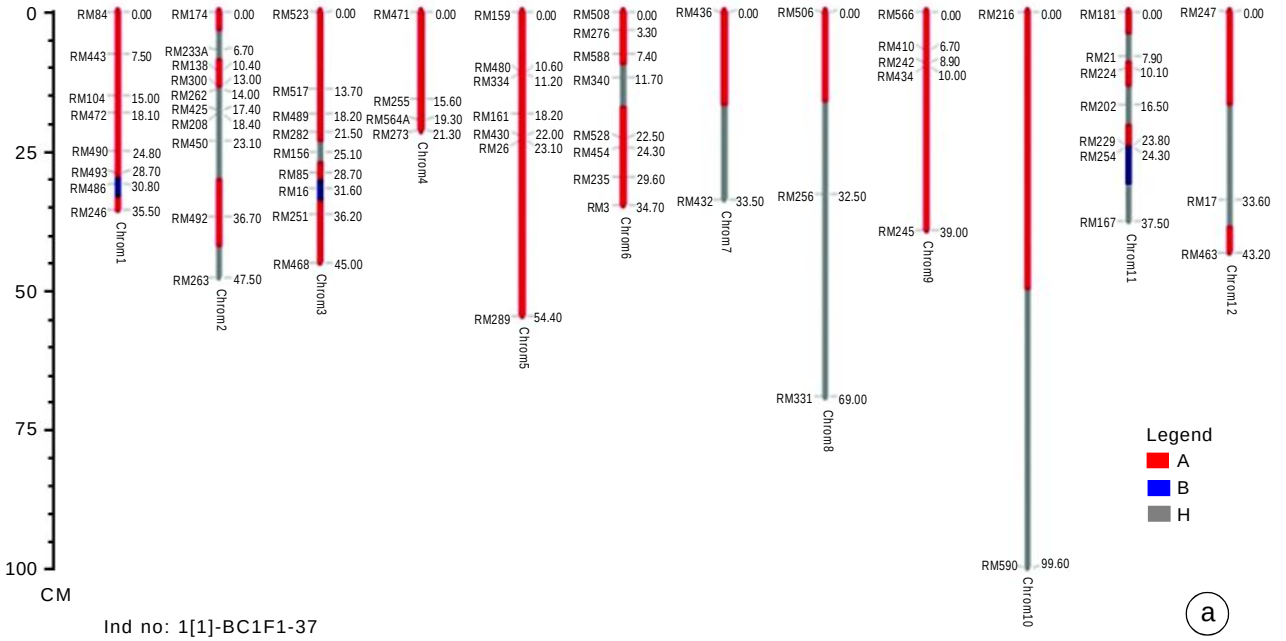
Most of the remaining donor genome occurred on the chromosomes where the target genes were located. This may be caused by the introduction of additional chromosome segments from the donor or from linkage drag in the target chromosomes. Yano *et al*. (1997) reported that five QTLs (*Hd1–Hd5*) caused variation 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.

Heading Date Selection

Heading date selection on each backcross and selfing generation was conducted to eliminate plants with linkage drag traits such as late flowering, high sterility and tall plant type. The population size for MAS could be reduced by eliminating the plants with

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

Chromosomes	BC_1F_1			BC_2F_1			BC_2F_2		
	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



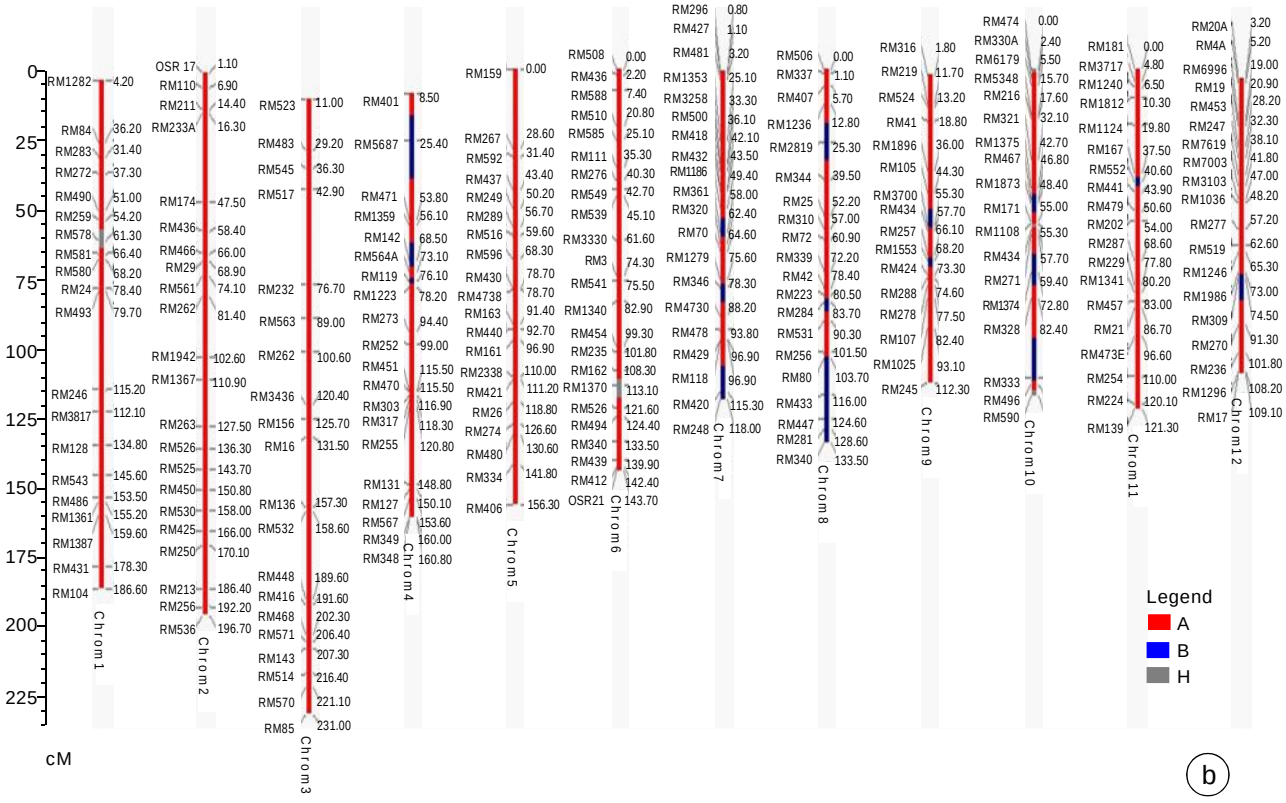


Fig. 3. Background recovery across the genome in two backcross generations. (a) BC_1F_1 plant CdNp_37 as the best plant with 77.1% recipient alleles from Code and (b) BC_2F_2 plant CdNP_29 as the best plant with 82.7% recipient alleles from Code.

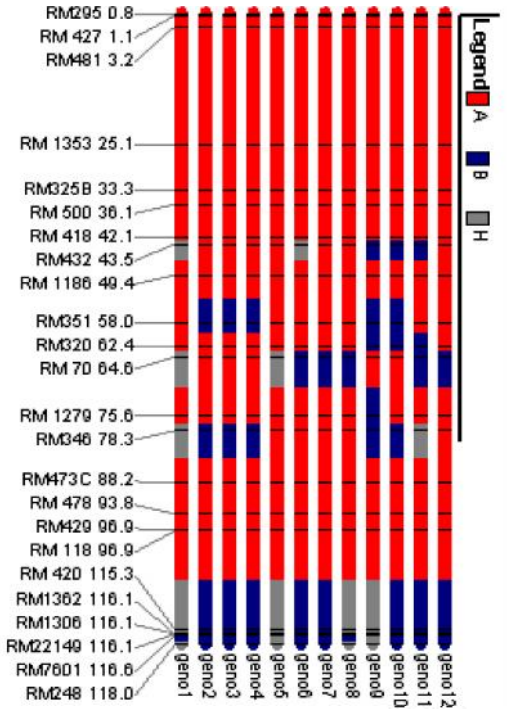


Fig. 4. The donor segment of *Hd2* gene in selected twelve BC_2F_2 plants of Code and Nipponbare crosses located in distal end of chromosome 7.

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 $BC_2F_3$and BC_2F_4 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_2F_3 and 85 and 66 in BC_2F_4. 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*

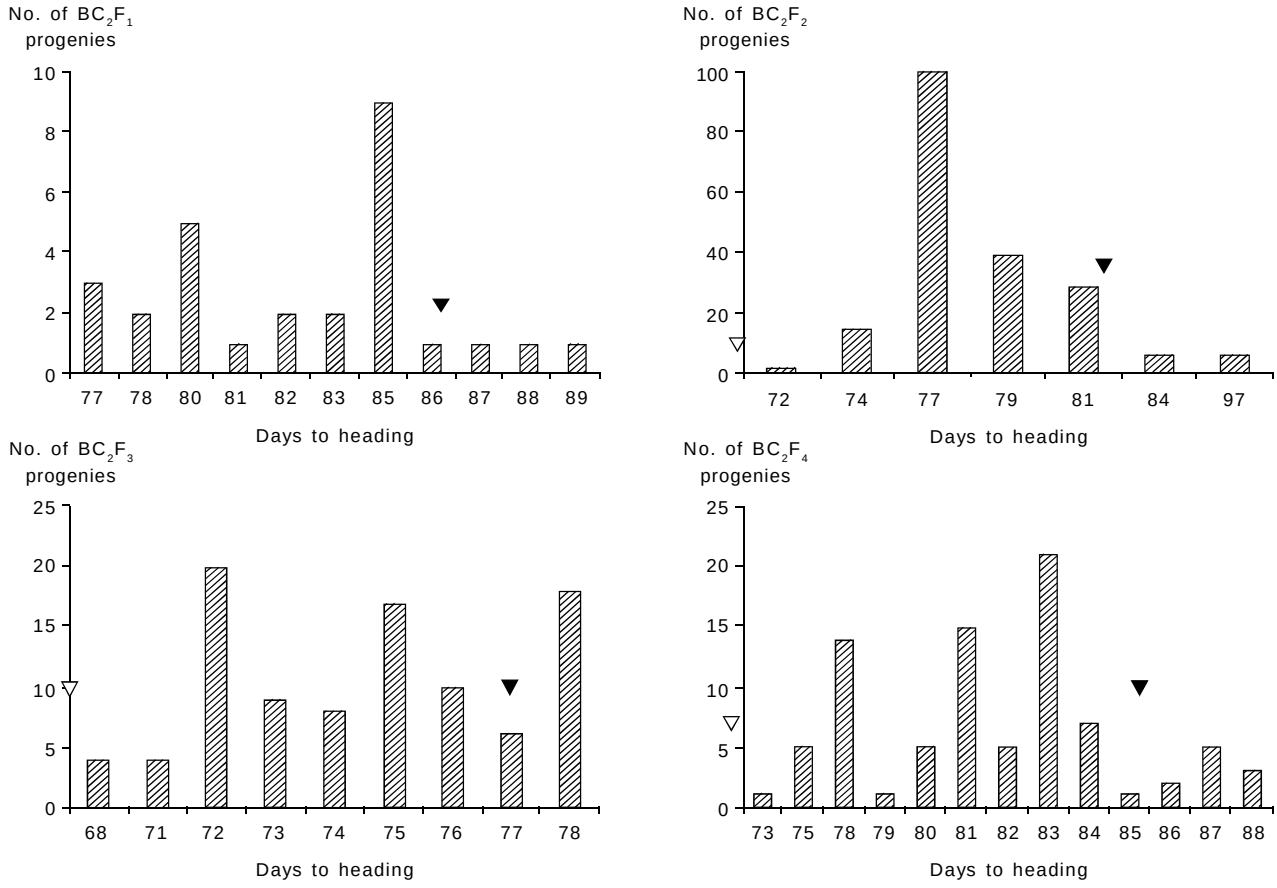


Fig. 5. Distribution of breeding population of backcross generation of Code and Nipponbare rice using *Hd2* linked primer for days to heading.

Table 4. Comparison of agronomic performance of twelve selected breeding rice lines of BC_2F_4 and their parents.

Breeding lines	Days to heading	Plant height (cm)	No. of tilllers	No. of maximum tillers	No. of effective tillers	Panicle lenght (cm)	No. of filled grains	No. of empty grains	100 grain weight (g)	Total grain weight (g)
BC_2F_4 Code x Nip-03	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_2F_4 Code x Nip-05	82.7 efgh	90.0 ab	10.0 ab	10.0 ab	8.7 ab	24.0 def	223.4 bcd	51.4 bcd	2.5 g	36.1 c
BC_2F_4 Code 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 abc	2.1 cde	35.6 c
BC_2F_4 Code 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_2F_4 Code 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 abcde	35.7 c
BC_2F_4 Code 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_2F_4 Code 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_2F_4 Code x Nip-95	83.3 efgh	85.2 ab	11.2 b	11.2 b	9.9 bc	22.3 bcd	218.b cd	59.7 cd	1.9 a	32.1 c
BC_2F_4 Code x Nip-121	84.3 fgh	116.3 cd	9.1 ab	9.1 ab	7.1 a	26.0 f	281.7 de	80.5 d	2.0 abcd	34.2 c
BC_2F_4 Code x Nip-131	80.4 cde	116.4 cd	7.9 a	7.9 a	7.4 ab	24.7 efg	282.8 de	46.4 abc	2.1 bcde	33.7 c
BC_2F_4 Code x Nip-144	81.3 cdef	123.3 d	8.4 ab	8.4 ab	8.1 ab	25.3 fg	319.8 e	31.5 abc	2.3 f	42.3 c
BC_2F_4 Code x Nip-180	82.8 efgh	97.6 abc	9.8 ab	9.8 ab	9.6 ab	22.6 cde	232.6 cd	39.2 abc	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	32.3 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_2F_1 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 lenght and 100 grain weight between the breeding lines and Code. In BC_2F_2 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_2F_3 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_2F_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_2F_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_2F_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 targetted MABc approach for the *Hd2* gene using RM1362 and RM7601 in chromosom 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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