

Characterization of Donor Genome Segments of BC₂ and BC₄ Way Rarem x *Oryzica Llanos-5* Progenies Detected by SNP Markers

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ABSTRAK

Karakterisasi Segmen Genom Tetua Donor pada Populasi Silang Balik BC₂ dan BC₄ Way Rarem x *Oryzica Llanos-5* dengan Bantuan Marka SNP. Wening Enggarini, Surjono H. Sutjahjo, Trikoesoemaningtyas, Sriani Sujiprihati, Utut Widyastuti, Kurniawan R. Trijatmiko, Sugiono Moeljopawiro, Masdiar Bustamam, dan Casiana V. Cruz. Silang balik diterapkan pemulia tanaman untuk mentransfer karakter yang diinginkan dari tetua donor kepada tetua penerima. Dengan beberapa kali silang balik dapat diperoleh progeni yang memiliki sebagian besar proporsi genom yang telah kembali pada tetua penerima tetapi sebagian kecilnya merupakan introgesi dari tetua donor yang diinginkan. Pendugaan proporsi segmen donor pada populasi silang balik telah banyak dilakukan dengan menggunakan simulasi teori matematika. Pada penelitian ini dilakukan analisis proporsi segmen introgesi donor langsung pada populasi hasil silang balik lanjut, BC₂F₇ dan BC₄F₂. Analisis dilakukan dengan menggunakan satu set marka *Single Nucleotide Polymorphism* (SNP) yang mencakup semua kromosom padi. Dari 384 marka SNP yang digunakan terdapat sebanyak 124 marka yang polimorfis terhadap Way Rarem dan tetua donor, *Oryzica Llanos-5*. Namun hanya 55 marka yang dapat mendeteksi adanya alel *Oryzica Llanos-5* pada individu BC₂F₇ dan BC₄F₂. Hasil analisis menunjukkan bahwa rata-rata jumlah segmen donor sebesar 14,5 pada BC₂F₇ dan 12,3 pada BC₄F₂. Terjadi penurunan jumlah segmen donor sebesar 15% dari BC₂F₇ ke BC₄F₂. Rata-rata panjang segmen donor sebesar 31,2 cM (centiMorgan) pada BC₂F₇ dan 8,79 cM pada BC₄F₂. Terdapat penurunan panjang segmen donor sebesar 72% setelah dua kali proses silang balik. Rata-rata ukuran genom donor sebesar 343,95 cM pada BC₂F₇ dan 71,35 cM pada BC₄F₂. Terdapat penurunan ukuran genom donor sebesar 79% dari BC₂F₇ ke BC₄F₂. Hasil penelitian ini menunjukkan penggunaan metode yang lebih sederhana untuk mengetahui komposisi segmen genom target yang diinginkan dari tetua donor, yang dibutuhkan sebagai salah satu kriteria seleksi dalam program silang balik.

Kata kunci: MABc, introgesi tetua donor, grafik genotype.

ABSTRACT

Characterization of Donor Genome Segments of BC₂ and BC₄ Way Rarem x *Oryzica Llanos-5* Progenies Detected by SNP Markers. Wening Enggarini, Surjono H. Sutjahjo, Trikoesoemaningtyas, Sriani Sujiprihati, Utut Widyastuti, Kurniawan R. Trijatmiko, Sugiono Moeljopawiro, Masdiar Bustamam, and Casiana V. Cruz. Plant breeders make a succession of backcrosses to introgress a character from a donor parent into genomic background of a recurrent parent. In several backcrossing, the proportion of a genome tends to return almost fully to recurrent parent, except the small donor genome segment harboring the character of interest. The estimation of the proportion donor segment through backcross generations has been analyzed theoretically using complex mathematical simulations. In this study, the proportion of donor introgression segments were directly analyzed in advanced backcross populations, BC₂F₇ and BC₄F₂. The analysis was done by using a set of single nucleotide polymorphism (SNP) markers covering the entire rice genome. Of the 384 SNP markers we found 124 markers which provide polymorphism between recurrent parent, Way Rarem and *Oryzica Llanos-5* as donor parent. But only 55 SNP markers could detect *Oryzica Llanos-5* alleles in BC₂F₇ and BC₄F₂ progenies. The result of this analysis demonstrated that the average of donor segment number was 14.5 in BC₂F₇ and 12.3 in BC₄F₂. It was reduced 15% from BC₂F₇ to BC₄F₂. The average of donor segment length was 31.2 cM (centiMorgan) in BC₂F₇ and 8.79 cM in BC₄F₂. It was decreased 72% during twice backcrossing. The average of donor genome size was 343.95 cM in BC₂F₇ and 71.35 cM in BC₄F₂, which means there was 79% decrease from BC₂F₇ to BC₄F₂. These results offered a simple method to describe the proportion of target genome segment from donor parent. It was required as one of the main selection criteria in backcross programs.

Keywords: Marker-assisted backcrossing, donor introgression, graphical genotype.

INTRODUCTION

Backcross breeding is a long established procedure for the introgression of a character from a donor parent into genomic background of a recurrent parent. The character can be a trait, a gene or even an anonymous locus or chromosome segment of interest. The objective of backcrossing is to reduce the donor genome content of the progenies (Hospital, 2005). Repeated backcrossing increases recombination events between the remaining donor genome and recurrent genome, which lead to smaller donor fragments and enhances chances of separating linked genes. It is generally assumed that, after 5-6 backcrosses under selection for the desired phenotype of the recurrent parent, the genotype of the backcross product is nearly identical to that recurrent parent, except for the desirable introgressed gene (Sleper and Poehlman, 2006).

In several recent studies it has been shown that molecular markers can be used to identify the introgression genes in a backcross population. The introgression locus is kept heterozygous by selection for the donor type allele at each generation (Hospital *et al.*, 1992; Van Berloo *et al.*, 2001). The presence or absence of a favorable allele cannot be determined by only screening the plant phenotype. An approach of using molecular marker information is needed to verify, in each generation, the presence of the favorable allele (Visscher *et al.*, 1996; Hospital, 2005).

Screening backcross population with molecular markers with known position on genetic map provides valuable information. Not only can the origin of the donor allele be determined, but also the remainder of the genome can be monitored. Molecular markers give a direct estimate of the proportion of the genome which is from recurrent parent. It can accelerate recovery of the recurrent parent genome and reduce number of backcross generations required for gene introgression (Hospital, 2009).

In each generation of the backcross program, the progeny that carry the gene introgression are chosen, and then among these, those carrying the lowest proportion of donor genes at other loci are selected. The evolution of introgression donor segments through backcross generations has been analyzed theoretically using simulations. The probability distribution of the length for heterozygosity at the target loci and homozygosity for the recurrent allele at flanking markers for a diploid species was investigated by Frisch and Melchinger (2001). Hospital (2001) also constructed simulation of the efficiency of marker-assisted selection based on its ability to reduce the

length of the donor chromosome segment retained around a locus held heterozygous by backcrossing. However, most of the theoretical papers related to marker-assisted selection presented complex mathematical models. Only little started to use DNA marker to analyze the evolution of introgressed segments across backcross generations.

Xi *et al.* (2008) studied the reduction of the proportion of donor genome contents represented by donor segment number and donor segment length using SSR markers in rice backcross generations. They also compared their results to the results which calculated using theoretical formula of Naveira and Barbadilla (1992). It is clear that these two groups of data were approachable. The objective of this paper is to describe the proportion of target genome segment from donor parent using directly analyzed of SNP markers in backcross progenies which has already been selected for blast resistance. The proportion of target donor segment was required as one of the main selection criteria in backcross programs.

MATERIALS AND METHODS

Rice Plant Material

The recurrent parent is Way Rarem, an upland Indica rice cultivar from Indonesia. It is high yielding and resistant to blast when it was first released in 1994. Recently, however, it was damaged by blast disease. Therefore, there is a need to increase its blast resistance (Suwarno *et al.*, 2001).

Oryzica Llanos-5, an Indica rice cultivar which was released from the International Center for Tropical Agriculture (CIAT) Rice Breeding Program in 1989 with durable blast resistance (Correa-Victoria and Zeigler, 1995) was used as the donor parent. It has been cultivated for many years on large areas without high losses yield caused by blast incidence. This cultivar have one or more complete resistance genes in addition to a high level of partial resistance controlled by polygenic based on the study of Fanny/Oryzica Llanos-5 population (Lopez-Gerena, 2006).

A previous study using BC₂F₂ population derived from a cross between Way Rarem x Oryzica Llanos-5 identified a total of 16 QTLs located at chromosome 1, 3, 5, 6, 7, 8, 9, and 11. The range of R square value of these QTLs were 11.31-45.11%. The Oryzica Llanos-5 allele was favorable for 19% of the QTLs (Lestari *et al.*, 2011).

Monitoring of the Introgression of Chromosome Segments from Donor Parent

The backcross process is illustrated in Figure 1. The Way Rarem (as female parent) was crossed with *Oryzica Llanos-5*, the donor parent, and the F_1 progenies were backcrossed to Way Rarem to develop BC_1F_1 generation. Five seeds for each BC_1F_1 line were backcrossed to Way Rarem, resulting in 400 BC_2F_1 seeds, from which 200 BC_2F_1 plants were chosen randomly and self-crossed to produce 200 BC_2F_2 families. One hundred BC_2F_2 lines were selected randomly and self-crossed to produce BC_2F_3 until BC_2F_7 families.

One hundred plants of each BC_2F_3 lines were evaluated by SSR markers which were linked to blast resistance QTL (Lestari *et al.*, 2011). Two plants of BC_2F_3 which carry blast resistance QTL were backcrossed to Way Rarem to obtain BC_3F_1 and then BC_4F_1 . One hundred plants of BC_4F_1 were self-crossed to produce BC_4F_2 lines. The BC_4F_2 plants were evaluated their blast resistance using artificial blast inoculation in the green house. After that the plants of BC_2F_7 and BC_4F_2 were analyzed by SNP markers.

DNA Molecular Markers

Mini-scale DNA extraction was carried out using Mini Kit DNA Extraction (Biorad). Three hundred and eighty-four SNP primers distributed evenly over 12 chromosomes (Figure 2) were used in genome-wide scan to identify the introgression of donor segments and the coverage of Way Rarem segments on the genome of BC_2F_7 and BC_4F_2 plants. Genotyping using

Illumina Beadexpress 384-plex SNP set for *indica-indica* (650011861) was conducted at Molecular Marker Applications Laboratory, International Rice Research Institute (IRRI), Philippines.

Estimation of the Length of Donor Chromosome Segments

The length of chromosome segments from the donor was estimated based on graphical genotypes (Young and Tanksley, 1989). GGT is a computer program for the display of graphical genotypes (Van Berloo, 2008) that used to analyze the SNP data. The marker data were arranged in a format commonly used for genetic mapping. Marker loci carrying the Way Rarem alleles in homozygous condition were labeled as 'A', heterozygous loci were labeled as 'H'. GGT allowed a quick estimation of the length of donor chromosome segments in BC_2F_7 and BC_4F_2 progenies. The total number of donor segments in a genome was defined as the donor segment number and the total length of all donor segments in a genome was used as the donor genome size. By analyzing the donor segment length and the donor genome size, we could assume the proportion of donor chromosome segments in each progenies and the reduction of donor proportion from BC_2F_7 to BC_4F_2 .

RESULTS AND DISCUSSION

Screening of *Oryzica Llanos-5* Using DNA Markers

Recurrent backcrossing with selection for presence of a target gene is a well-established

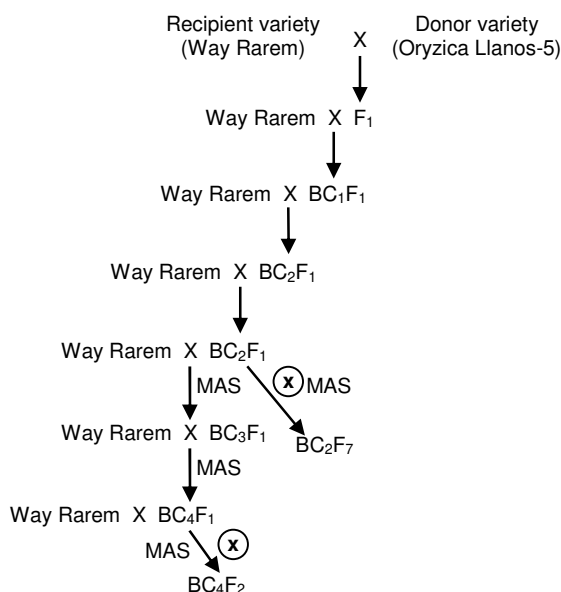


Figure 1. Schematic diagram of backcross breeding. MAS = marker-assisted selection.

breeding method for introgression desirable genes from a donor into the genetic background of a recurrent parent. With the development of high-density linkage maps in most crop species, it becomes possible to monitor the parental origin of alleles at DNA markers throughout the entire genome. Selection of individuals, which not only carry the target gene but also have an allele homozygous for the recurrent parent alleles at a large portion of DNA markers, can accelerate recovery of the recurrent parent genome and reduce the number of backcross generations required for gene introgression (Frisch and Melchinger, 2001).

Three hundred and eighty four SNP markers were tested for finding out the donor introgression of *Oryzica Llanos-5*. Of the total SNP markers, 125 markers showed polymorphism. They were selected and used in this study for monitoring the introgression of segments from donor parent (Figure 2). The polymorphism level of the markers was 32.5% (Table 1).

In early generations, few recombination events have occurred, so that donor genes should be represented by a few long segments on each

chromosome. As recombination events accumulate over time, the number of donor segments spreading over the genome increases, and their length decreases, so that more and more markers are needed to monitor all of them. In our study, all polymorphic SNP markers were recognized to be evenly distributed with an interval size of 11.38 cM (in average 10 markers per chromosome). Using the polymorphic SNP markers, we have constructed the graphical genotype of BC_2F_7 and BC_4F_2 progenies. Based on the estimation of accurate graphical genotype (Young and Tanksley, 1989), it could be assumed that with SNP markers spaced every 11.38 cM, an inferred graphical genotype will be accurate in describing the genomic constitution for over 98% of the genome. Visscher *et al.* (1996) proposed that a marker density of 10-20 cM seems appropriate to determine donor segment accurately. The higher density of markers was useful in later generations to eliminate these donor segments more rapidly (Hospital, 2009).

There was the *Oryzica Llanos-5* segment in all chromosomes of BC_2F_7 progenies except in chromosome 5, 8, and 9. These three chromosomes

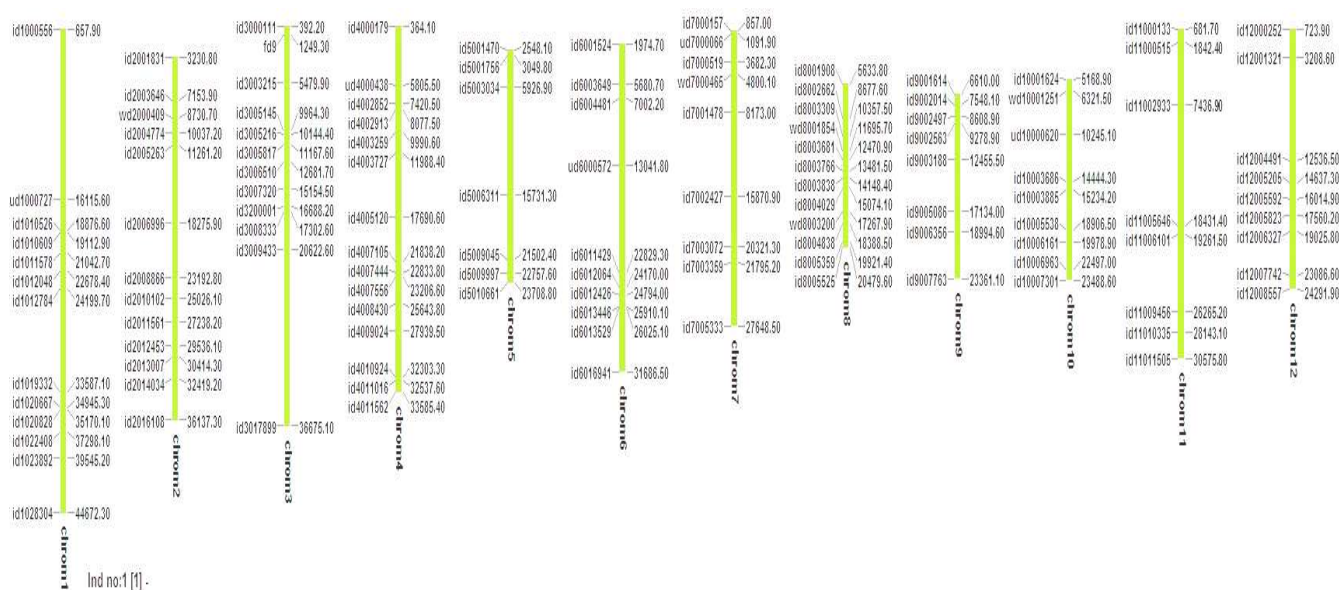


Figure 2. The SNP markers map of backcross population.

Table 1. SNP markers with polymorphism between *Oryzica Llanos-5* as donor parent and Way Rarem.

Characteristic of SNP markers	
Number of SNP markers tested	384
Number of polymorphic markers	125
Polymorphisms level of markers (%)	32.5
Average size of interval between polymorphic markers (cM)	11.38

had completely Way Rarem segments. Only donor segments in chromosome 2, 4, and 7 were almost inherited fully from BC₂F₇ to BC₄F₂ progenies. The length of donor segments in chromosome 3, 10, and 11 were decreased in great number. The graphical genotype could show accurately that the reduction of donor segments were occurred after several backcrossing (Figure 3).

Decrease of Donor Segment Number by Backcrossing

The donor segment number ranged from 13 to 16 in BC₂F₇ and from 10 to 15 in BC₄F₂. The average of donor segment number was decreased from 14.5 in BC₂F₇ to 12.3 in BC₄F₂ with the reduction of 15%.

Xi *et al.* (2008) calculated that the average of donor segment number was 8.39 in BC₂F₁ and 4.13 in

BC₃F₁ using SSR markers, which was lower than our result. It might be due to the type of molecular marker which was used. In our research, we used SNP markers to identify the donor segment. The SNP markers have much abundance in rice genome compare to SSR markers. So the number of polymorphic SNP markers in rice genome could be higher than polymorphic SSR markers. More dense markers can capture recombination event more accurately than less dense markers. Also there were other advantages if we used SNP markers: (1) a large number of samples can be processed systematically in 96-well plates for SNP-typing, and (2) time and labor can be saved, as no electrophoresis is needed (Nasu *et al.*, 2002).

Xi *et al.* (2008) also presented that the decrease of donor segment number from BC₂F₁ to BC₃F₁ was

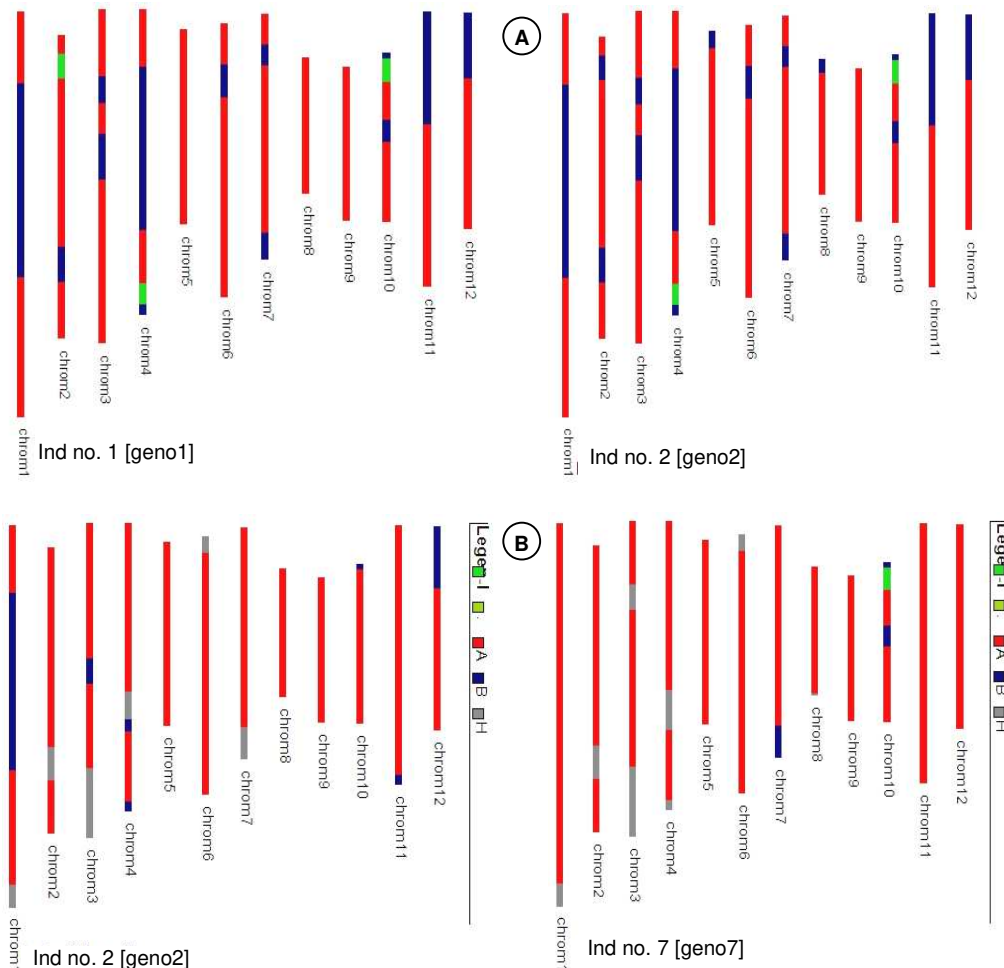


Figure 3. Graphical genotypes of backcrossing progenies. A = BC₂F₇ population, B = BC₄F₂ population. *Intervals filled with red* indicate that the segment was derived exclusively from the Way Rarem parent. *Blue intervals* indicate that the segment was derived from the Oryzica Llanos-5 parent, while *gray intervals* indicate the presence of a crossover event. *Green intervals* indicate that the segment could not be recognized by the SNP markers.

50%, which was higher than our result. It might be because they did not apply any phenotypic or genotypic selection to their plants. In our study, we applied phenotypic selection to BC₂F₇ and BC₄F₁. The phenotypic selection would maintain the interest character of donor in each backcross population so the decrease of donor segment number in the next backcross population was lower than 50%.

Decrease of Donor Segment Length by Backcrossing

To know the length of donor chromosome segments after a series of backcrosses is very important for plant breeders who are interested in the development of stocks with desired genetic traits. During backcrossing, all donor chromosome segments would be heterozygous and any recombination in these segments would result in reduction of the donor segment length. In our investigation, the donor segment length ranged from 5.64 cM to 71.37 cM in BC₂F₇ and from 1.36 cM to 22.87 cM in BC₄F₂ (Table 2). The average of donor segment length was decreased from 31.2 cM in BC₂F₇ to 8.79 cM in BC₄F₂. The reduction of 72% occurred in twice backcrossing from BC₂F₇ to BC₄F₂.

Applying the formula of Naveira and Barbadilla (1992) we predicted the average of donor segment length of the backcross rice progeny. Using an average of 127 cM per chromosome, the prediction was 35.96 cM in BC₂F₁ and 23.03 cM in BC₄F₁. The average of donor segment length in BC₂F₇ was similar compared to the average of donor segment length in BC₂F₁. But the average of donor segment length in BC₄F₂ was different compared to the prediction of donor segment length in BC₄F₁. This might be due to the formula of Naveira and Barbadilla (1992) that did not incorporate the phenotypic selection as parameter to predict the donor segment length. In our study, the progenies of BC₄F₂ were already evaluated by phenotypic selection. The phenotypic selection was performed to obtain the BC₄F₂ progenies which have almost same phenotype with recurrent parent. The high level of phenotypic selection could make the donor segment length of BC₄F₂ progenies shorter. Ribaut *et al.* (2002) stated that in each generation of the backcross program, the progeny which carry the introgression gene are

chosen, and then among these, those carrying the lowest proportion of donor genes at other loci are selected. The lowest proportion of donor genes also means that the progenies genome mostly returned to recurrent parent.

Decrease of Donor Genome Size by Backcrossing

In our result, the graphical genotype showed that the length of Oryzica Llanos-5 segments in chromosome 4, 10, and 11 of BC₄F₂ progenies were shorter compared to BC₂F₇ progenies. The size of donor genome ranged from 329.13 cM to 358.76 cM in BC₂F₇ and from 13.3 cM to 140.21 cM in BC₄F₂. The average of donor genome size was 343.95 cM in BC₂F₇ and 71.35 cM in BC₄F₂. This clearly showed that the donor segment size reduced with increased backcrossing (Table 2).

The proportion of donor segment was also decreased from 28.79% on BC₂F₇ to 6.65% on BC₄F₂ (Table 2). This tendency held true from BC₂F₇ to BC₄F₂ with reduction of 79%. Sleper and Poehlman (2006) revealed that the proportion of donor genome, when no selection is applied, is 25% in BC₂, 12.5% in BC₃ and 6.25% in BC₄. It is decreased 75% in twice backcrossing. In our study, the reduction of donor segment proportion was higher might be because the BC₄F₂ progenies were evaluated by the phenotypic selection.

CONCLUSION

The analysis of estimation donor segment proportion in backcross population revealed that the average of donor segment number was 14.5 in BC₂F₇ and 12.3 in BC₄F₂. It was reduced 15% from BC₂F₇ to BC₄F₂. The average of donor segment length was 31.2 cM in BC₂F₇ and 8.79 cM in BC₄F₂. It was decreased 72% during twice backcrossing. The average of donor segment length in BC₄F₂ was differed comparing to the prediction of Naveira and Barbadilla (1992) might be because the prediction did not incorporate the phenotypic selection as parameter to predict the donor segment length. The progenies of BC₄F₂ were already evaluated by phenotypic selection. The average of donor genome size was 343.95 cM in BC₂F₇ and 71.35 cM in BC₄F₂, which means there was 79% decrease from BC₂F₇ to BC₄F₂. This revealed that the

Table 2. The length, size and proportion of recurrent and donor genome segment in backcross progenies.

	Genome segment length (cM)		Genome segment size (cM)		Genome proportion (%)	
	Recurrent	Donor	Recurrent	Donor	Recurrent	Donor
BC ₂ F ₇	70.88	31.2	850.58	343.95	71.21	28.79
BC ₄ F ₂	84.45	8.79	1025.42	71.35	93.35	6.65

reduction in the proportion of donor segment was approximately 75% with twice backcrossing.

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