Characterization of Donor Genome Segments of BC$_2$ and BC$_4$
Way Rarem x Oryzica Llanos-5 Progenies Detected by SNP Markers

Wening Enggarini$^1$, Suryono H. Sutjajho$^2$, Trikoesoemaningtyas$^3$, Sriani Sujiprihati$^2$, Utut Widyastuti$^4$, Kurniawan R. Trijatmiko$^1$, Sugiono Moeljopawiro$^1$, Masdiar Bustamam$^3$, and Casiana V. Cruz$^4$

$^1$Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Jl. Tentara Pelajar 3A, Bogor 16111 Phone (0251) 8337975; Faxes. (0251) 8338820; *E-mail: whening@yahoo.com
$^2$Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Jl. Meranti, Campus Darmaga, Bogor 16680
$^3$Department of Biology, Faculty of Mathematics and Science, Bogor Agricultural University, Jl. Meranti, Campus Darmaga, Bogor 16680
$^4$International Rice Research Institute, College, Los Banos, Laguna 4031, Philippines

Submitted: 2 November 2011; Accepted: 16 March 2012

ABSTRACT

Characterization of Donor Genome Segments of BC$_2$ and BC$_4$ Way Rarem x Oryzica Llanos-5 Progenies Detected by SNP Markers. Wening Enggarini, Suryono H. Sutjajho, 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, BC$_2$F$_2$ and BC$_4$F$_2$. 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 BC$_2$F$_2$ and BC$_4$F$_2$ progenies. The result of this analysis demonstrated that the average of donor segment number was 14.5 in BC$_2$F$_2$ and 12.3 in BC$_4$F$_2$. It was reduced 7% during twice backcrossing. The average of donor genome size was 343.95 cM in BC$_2$F$_2$ and 71.35 cM in BC$_4$F$_2$, which means there was 79% decrease from BC$_2$F$_2$ to BC$_4$F$_2$. 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.

Karakterisasi Segmen Genom Tetua Donor pada Populasi Silang Balik BC$_2$ dan BC$_4$ Way Rarem x Oryzica Llanos-5 dengan Bantuan Marka SNP. Wening Enggarini, Suryono H. Sutjajho, Trikoesoemaningtyas, Sriani Sujiprihati, Utut Widyastuti, Kurniawan R. Trijatmiko, Sugiono Moeljopawiro, Masdiar Bustamam, dan Casiana V. Cruz. Pada penelitian ini dilakukan analisis proporsi segmen intrograsi donor langsung pada populasi hasil silang balik lanjut, BC$_2$F$_2$ dan BC$_4$F$_2$. 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 polimorfs terhadap Way Rarem dan tetua donor, Oryzica Llanos-5. Namun hanya 55 marka yang dapat mendeteksi adanya alel Oryzica Llanos-5 pada individu BC$_2$F$_2$ dan BC$_4$F$_2$. Analisis menunjukkan bahwa rata-rata jumlah segmen donor sebesar 14.5 pada BC$_2$F$_2$ dan 12.3 pada BC$_4$F$_2$. Terjadi penurunan jumlah segmen donor sebesar 15% dari BC$_2$F$_2$ ke BC$_4$F$_2$. Rata-rata panjang segmen donor sebesar 31.2 cM (centiMorgan) pada BC$_2$F$_2$ dan 8.79 cM pada BC$_4$F$_2$. Terdapat penurunan panjang segmen donor sebesar 72% setelah dua kali proses silang balik. Rata-rata ukuran genom donor sebesar 343.95 cM pada BC$_2$F$_2$ dan 71.35 cM pada BC$_4$F$_2$. Terdapat penurunan ukuran genom donor sebesar 79% dari BC$_2$F$_2$ ke BC$_4$F$_2$. 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, introgressi tetua donor, grafik genotipe.
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, 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 BC3F2 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 F₁ progenies were backcrossed to Way Rarem to develop BC₁F₁ generation. Five seeds for each BC₁F₁ line were backcrossed to Way Rarem, resulting in 400 BC₁F₂ families. One hundred BC₁F₂ lines were selected randomly and self-crossed to produce 200 BC₂F₃ until BC₂F₅ families.

One hundred plants of each BC₂F₅ lines were evaluated by SSR markers which were linked to blast resistance QTL (Lestari et al., 2011). Two plants of BC₂F₅ which carry blast resistance QTL were backcrossed to Way Rarem to obtain BC₃F₁ and then BC₃F₂. One hundred plants of BC₃F₁ were self-crossed to produce BC₃F₂ lines. The BC₃F₂ plants were evaluated their blast resistance using artificial blast inoculation in the green house. After that the plants of BC₃F₂ and BC₄F₂ 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 BC₃F₇ and BC₄F₂ plants. Genotyping using Illumina Beadxpress 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 BC₃F₇ and BC₄F₂ 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 BC₃F₇ to BC₄F₂.

**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$_{2}$F$_{7}$ and BC$_{4}$F$_{2}$ 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$_{2}$F$_{7}$ 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 BC$_2$F$_7$ to BC$_4$F$_2$ 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 BC$_2$F$_7$ and from 10 to 15 in BC$_4$F$_2$. The average of donor segment number was decreased from 14.5 in BC$_2$F$_2$ to 12.3 in BC$_4$F$_2$ with the reduction of 15%.

Xi et al. (2008) calculated that the average of donor segment number was 8.39 in BC$_2$F$_1$ and 4.13 in BC$_3$F$_1$, 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 BC$_2$F$_1$ to BC$_3$F$_1$ was

![Figure 3](image-url)
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 BC₃F₁. 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 BC₃F₂ (Table 2). The average of donor segment length was decreased from 31.2 cM in BC₂F₇ to 8.79 cM in BC₃F₂. The reduction of 72% occurred in twice backcrossing from BC₂F₇ to BC₃F₂.

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 BC₃F₁. The average of donor segment length in BC₂F₇ was similar compared to the average of donor segment length in BC₃F₁. But the average of donor segment length in BC₂F₂ was different compared to the prediction of donor segment length in BC₂F₁. 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 BC₂F₇ were already evaluated by phenotypic selection. The phenotypic selection was performed to obtain the BC₂F₇ progenies which have almost same phenotype with recurrent parent. The high level of phenotypic selection could make the donor segment length of BC₂F₇ 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 BC₂F₇ progenies were shorter compared to BC₃F₁ 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 BC₃F₂. The average of donor genome size was 343.95 cM in BC₂F₇ and 71.35 cM in BC₃F₂. 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 BC₂F₁ to 6.65% on BC₃F₂ (Table 2). This tendency held true from BC₂F₁ to BC₃F₂ with reduction of 79%. Sleeper 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 BC₂F₇ 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 BC₃F₂. It was reduced 15% from BC₂F₂ to BC₃F₂. The average of donor segment length was 31.2 cM in BC₂F₇ and 8.79 cM in BC₃F₂. It was decreased 72% during twice backcrossing. The average of donor segment length in BC₂F₇ 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 BC₂F₇ were already evaluated by phenotypic selection. The average of donor genome size was 343.95 cM in BC₂F₇ and 71.35 cM in BC₃F₂, which means there was 79% decrease from BC₂F₇ to BC₃F₂. 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 |
| BC₂F₇ | 70.88 | 31.2 | 850.58 | 343.95 | 71.21 | 28.79 |
| BC₃F₂ | 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.

ACKNOWLEDGEMENTS

This work was supported by Grants from the Generation Challenge Program (GCP) Capacity Building (G4007.13.06) and SNP analysis at IRRI was supported by Grants from Japan Rice Breeding Project. We would like to thank Dr. Michael Thompson for SNP genotyping at MMAL, IRRI.

REFERENCES

Correa-Victoria, F.J. and R.S. Zeigler. 1995. Stability of partial and complete resistance in rice to Pyricularia grisea under rainfed upland conditions in Eastern Colombia. Phytopathology 85(9):977-982.

Frisch, M. and A.E. Melchinger. 2001. The length of the intact donor chromosome segment around a target gene in marker-assisted backcrossing. Genetics 157:1343-1356.

Hospital, F. 2001. Size of donor chromosome segments around introgressed loci and reduction of linkage drag in marker-assisted backcross program. Genetics 158:1363-1379.

Hospital, F. 2005. Selection in backcross program. Phil. Trans. R. Soc. B 360:1503-1511.

Hospital, F. 2009. Challenges for effective marker-assisted selection in plants. Genetica 136:303-310.

Hospital, F., C. Chevalet, and P. Mulsant. 1992. Using markers in gene introgression breeding program. Genetics 132:1199-1210.

Lestari, P., K.R. Trijatmiko, Relfinur, A. Warsun, Tasliah, I. Ona, and C. Vera Cruz. 2011. Mapping quantitative trait loci conferring blast resistance in upland indica rice (Oryza sativa L.). J. Crop Sci. Biotech. 14(1):57-63.

Lopez-Gerena, J. 2006. Mapping QTL controlling durable resistance to rice blast in variety Oryzica Llanos-5. PhD Thesis. Kansas State University. USA.

Nasu, S., J. Suzuki, R. Ohta, K. Hasegawa, R. Yui, N. Kitazawa, L. Monna, and Y. Minobe. 2002. Search for and analysis of single nucleotide polymorphisms (SNPs) in rice (Oryza sativa, Oryza rufipogon) and establishment of SNP markers. DNA Research 9:163-171.

Naveira, H. and A. Barbadilla. 1992. The theoretical distribution of lengths of intact chromosome segments around a locus held heterozygous with backcrossing in a diploid species. Genetics 130:205-209.

Ribaut, J.M., C. Jiang, and D. Hoisington. 2002. Simulation experiments on efficiencies of gene introgression by backcrossing. Crop Sci. 42:557-565.

Sleper, D.A. and J.M. Poehlman. 2006. Breeding Field Crops. 5th edition. Blackwell publishing. USA.

Suwarno, E. Lubis, and E. Soenarjo. 2001. Breeding of upland rice for resistance to blast in Indonesia. In M.K. Kardin, I. Prasadja, and M. Syam (eds.) Upland Rice Research in Indonesia. Current Status and Future Directions. Central Research Institute for Food Crops, Agency for Agricultural Research and Development. Bogor. Indonesia. p. 7-14.

Van Berloo, R., H. Aalbers, A. Werkman, and R.E. Niks. 2001. Resistance QTL confirmed through development of QTL-NILs for barley leaf rust resistance. Molecular Breeding 8:187-195.

Van Berloo, R. 2008. GGT 2.0: Versatile software for visualization and analysis of genetic data. J. Heredity 99(2):232-236.

Visscher, P.M., C.S. Haley, and R. Thompson. 1996. Marker-assisted introgression in backcross breeding program. Genetics 144:1923-1932.

Xi, Z.Y., F.H. He, R.Z. Zeng, Z.M. Zhang, X.H. Ding, W.T. Li, and G.Q. Zang. 2008. Characterization of donor genome contents of backcross progenies detected by SSR markers in rice. Euphytica 160:369-377.

Young, N.D. and S.D. Tanksley. 1989. Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor. Appl. Genet. 77:95-101.