

Development of Contiguous Introgression Lines Covering Entire Genome of the Sequenced *Japonica* Rice

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Abstract

A series of near-isogenic introgression lines were developed from an advanced backcross population derived from a cross between the sequenced *japonica* cv. Nipponbare, and an elite *indica* hybrid parent (Zhenshan 97B). Each line contains only a single defined introgression segment from Nipponbare in the *indica* genetic background. Furthermore, a novel population consisting of 88 lines carrying different but mutual overlapping chromosomal segments provides approximately a complete coverage of the *japonica* rice genome. These contiguous introgression lines (CILs) present an extremely useful base for fine mapping and functional analysis of genomic regions (genes) underlying traits of agronomical importance.

Media summary

A set of contiguous introgression lines developed from the sequenced *japonica* rice provides a valuable stock for fine mapping and functional analysis of important genes.

Key words

Contiguous introgression lines, Marker assisted selection, *Oryza sativa* L.

Introduction

Indica and *Japonica* are two major subspecies of Asian cultivated rice (*O. sativa* L.). There are apparent differences in phenotypic variations, physiological specialty and ecological adaptability between the two subspecies. Molecular dissection of these differentiations will not only increase our understanding of phenotypic adaptations but also facilitate the genetic improvement of cultivated rice. Recently, two draft sequences of genome in *japonica* c.v. Nipponbare and *indica* 93-11 have been reported. Genome-wide comparative analysis of the DNA sequences of the two subspecies revealed intraspecific sequence polymorphisms commonly occurred in both coding and non-coding regions (Han and Xue 2003). These progresses have provided an opportunity to develop new molecular markers for rice molecular breeding and to find allelic variations associated with subspecies differentiations. Near isogenic introgression lines (NILs) has shown extremely useful for mapping important genes or quantitative trait locus (QTLs) because of desirable reduction of the effect of genetic background and gene interaction (Eshed and Zamir 1995). Moreover, Specific near-isogenic lines could be selected to examine the effect of targeted chromosomal regions and used for high-resolution mapping and map-based cloning (Yano and Sasaki 1997). The objectives of the study presented here is to develop a set of contiguous introgression lines (CILs) through marker-assisted backcrossing for identification and functional analysis of QTLs that affect agronomic differentiations between the subspecies, and for systematic evaluation of the *japonica* allelic effects in *indica* genetic background.

Methods

The sequenced variety of *japonica* rice (Nipponbare) was used as female to cross with an *indica* variety (Zhenshan 97B), the maintainer of a widely cultivated hybrid in China. An advanced backcross (BC₄) population consisting of more than 1200 lines was generated through sequential backcrossing using Zhenshan97B as the recurrent parent. Phenotypic variations and agronomic performances of the population were evaluated in experimental field. About five hundred lines exhibiting some *Japonica*-like

characteristics and less genetic segregations were selected to genotype and to further cross with the recurrent parent. Meanwhile, over 180 microsatellite markers evenly distributed on the published framework map (Temnykh et al. 2001) were selected to survey the polymorphism between Zhenshan 97B and Nipponbare. As a result, one hundred and eight polymorphic markers with an average interval of 15 cM on the twelve chromosomes were obtained and used to genotype the targeted lines. A number of near isogenic introgression lines (NILs) containing small introgression chromosomal segments derived from Nipponbare in the same genetic background were determined based on their graphical genotypes. In order to construct a set of contiguous introgression lines with mutual overlapping introgression segments to cover the entire genome of Nipponbare, one more round of marker-assisted selection are made to eliminate some undesirable introgressions in those NILs that carried more than one alien segment.

Results

One hundred and eight polymorphic markers were used to genotype five hundreds BC₄ lines. One hundred and forty putative near-isogenic introgression lines were obtained by graphical genotyping. Regarding the genetic composition of the NILs, the percentage of genome restoration to the recurrent parent ranged from 75.2% to 97.2% with an average of 90.6%, the ratio of heterozygous regions ranged from 23.4% to 0.4% with an average of 8.1%. Generally, most of the NILs carry a single heterozygous introgression chromosomal segment from the *japonica* variety in the genetic background of Zhenshan 97B.

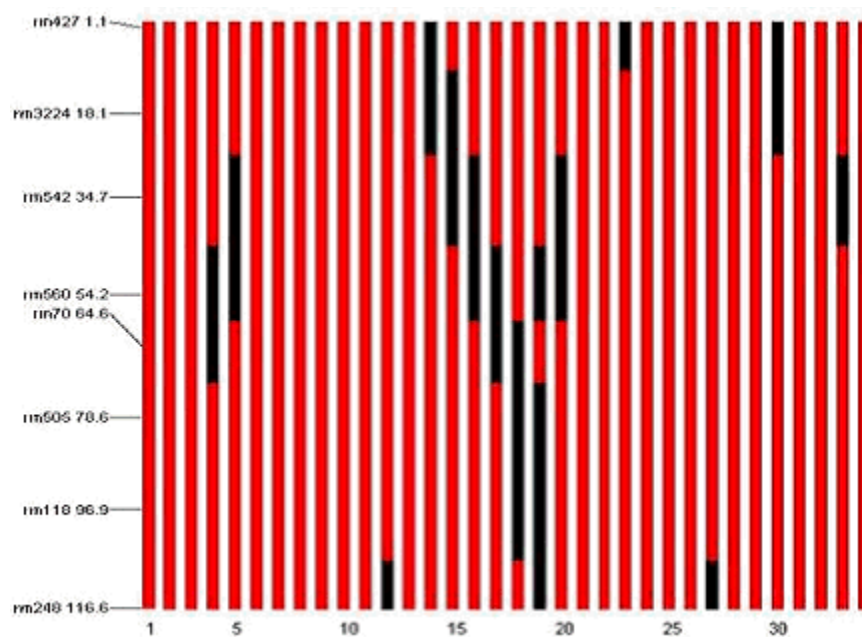


Figure 1. Graphical genotypes of some near-isogenic introgression lines on chromosome 7 show each line carrying a defined introgression chromosomal segment. The lines numbered 14-19 collectively containing mutual overlapping introgression segments that cover the whole chromosome were selected for development of contiguous introgression lines. The red and black bars represent the genomic regions of *indica* and introgression segments from the *japonica* variety, respectively.

A graphical genotyping scheme is present as Figure 1 that show thirteen out of thirty four near-isogenic introgression lines on chromosome 7 each contain only one defined segment coming from the variety of *japonica*. For the whole genome of rice, eighty eight introgression lines were consequently selected to construct a contiguous introgression line population. In the population, each CIL contains a small defined introgression chromosomal segment, and all lines together cover the entire genome of the *japonica* variety. However, two genomic regions respectively on chromosome 2 and 6 were not covered by the

overlapping introgression segments (Fig. 2). The lack of introgression segments in the CILs may be due to random selection during the introgression process. We are now conducting selection of more putative NILs for the two uncovered regions and other potential introgression segments in the large advanced backcross population.

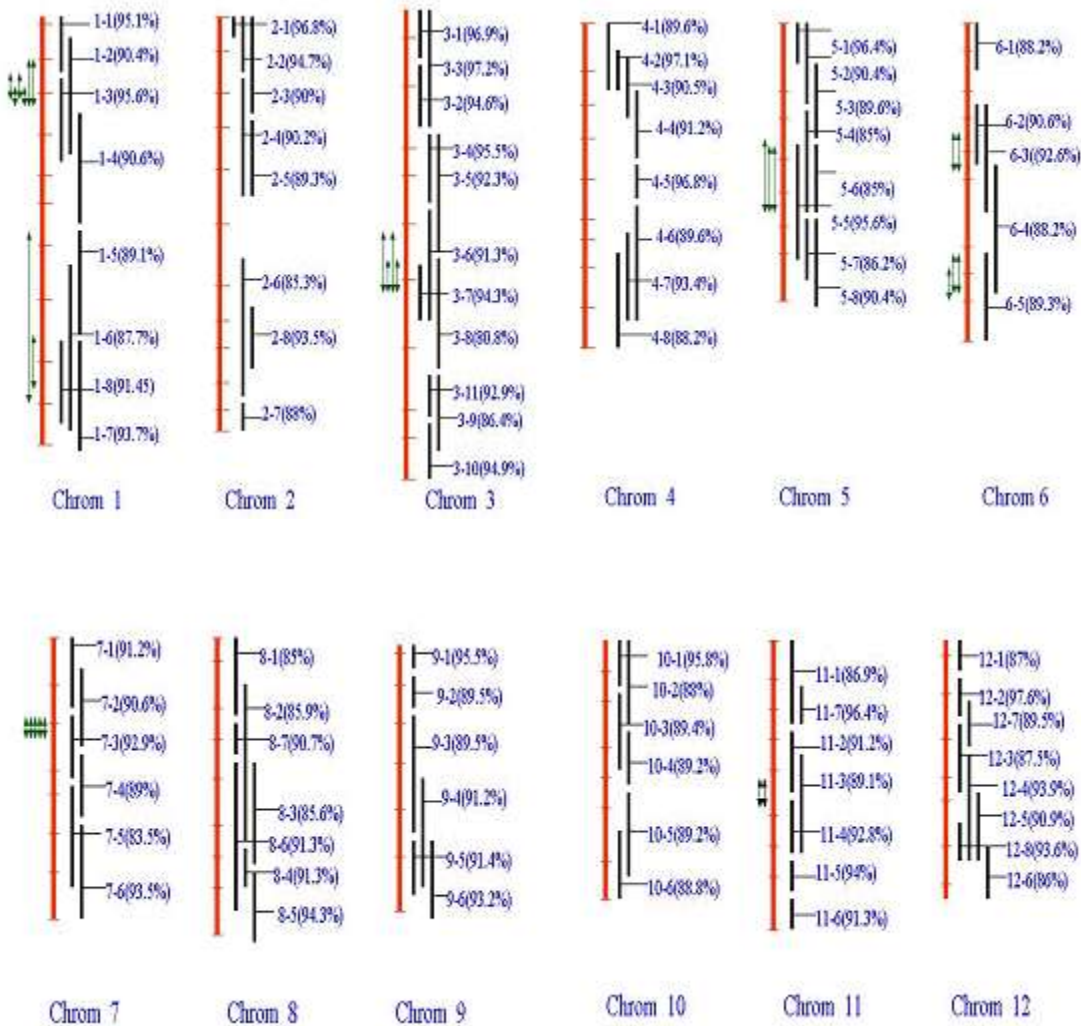


Figure 2. A population consisting of eighty-eight contiguous introgression lines (CILs) almost covers the entire genome of the *japonica* rice. The red bars represent the 12 chromosomes. Each black bar represents the introgression segment in each line (e.g., 1-1, 1-2 etc.), respectively. The percentages in parentheses represent the genome restorations of each CIL. The arrows on the left of each chromosome indicate approximate locations of the common QTLs for grain numbers per panicle detected at least in two mapping populations or two environments.

Comparison and evaluation of agronomic performances and yield components traits among these CILs and the recurrent parent were conducted in experimental field. A remarkable result from a preliminary trial on yield indicates some genomic regions affecting grains per panicle match well with those common QTL regions previously reported in the website of gramene (Fig. 2).

Conclusion

A population consisting of eighty eight contiguous introgression lines (CILs) was developed. Each CIL contains only a single defined introgression segment from the sequenced *japonica* variety in the genetic background of *indica*, and all CILs together can provide a complete coverage of the *japonica* rice genome. One noteworthy feature using these CILs is that it enables the study of specific genomic regions affecting agronomic important traits and also precise estimation of the QTL effects which are often difficult to obtain because of the influence of environments and genetic backgrounds. Secondly, the CILs population provides an attractive tool to select candidate genes in the annotated data of the sequenced genome corresponding to the QTLs. Finally, the CILs enable systematic assessment of *japonica* allelic effects in the *indica* genetic background, and thus facilitate transferring genes from one subspecies to the other in molecular rice breeding.

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