Recombination analysis on bread wheat chromosome 3B

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ABSTRACT

Recombination plays a major role in determining the structure and evolution of eukaryotes. In wheat, it was shown that recombination increases gradually with the distance from the centromere but the resolution of the analyses remained limited until now. Here, we undertook a more precise characterization of the recombination pattern along chromosome 3B of bread wheat by comparing a very dense genetic map (102 markers) with the cytogenetic map (16 deletion bins). This showed that 90% of the recombination occurs in the distal 40% of the chromosome. Moreover, a clear recombination gradient was observed from the centromere towards the telomere, with a strong decrease at the telomeric end on the short arm. To better understand the mechanisms underlying this gradient, we developed and analyzed lines with a patchwork of homozygous and heterozygous regions in proximal low recombinogenic regions of chromosome 3B and compared the recombination with references maps. We also compared female and male recombination on chromosome 3B. At the whole chromosome level, no differences were observed but significant changes were found in distal regions of the long arm. Finally, a BAC based physical map of chromosome 3B (cv. Chinese Spring) was exploited to study the relationships between genetic and physical distances at the level of megabase-sized sequenced regions. A 3.2 Mb contig located at the telomeric end of the short arm was used for these studies. Recombinants in this region were isolated by screening a large F2 segregating population derived from a cross between Chinese Spring and Renan with SSR markers derived from the ends of the contig sequence. Recombination breakpoints were identified using more than 100 markers distributed within the sequence and the relationship between recombination and the presence of genes, TEs and other sequence features was studied in details.

INTRODUCTION

Meiotic recombination is essential for proper chromosome segregation during the formation of gametes and generates also diversity in the progeny. One way to better understand recombination and its regulation is to study the distribution of crossing over (CO) on chromosomes. This has been done previously in *Arabidopsis*, rice and human [1] and one major feature that has emerged from these studies is the existence of hot and cold spots of recombination. Some cereals with large genomes such as wheat, barley and maize show particular patterns of recombination. In these species, recombination increases gradually from the centromere to the telomere [2]. Understanding this gradient can improve significantly our knowledge on the regulation of recombination. Moreover, in addition to its fundamental aspect, recombination analysis in wheat is very useful for supporting breeding programs in particular to monitor the introgression of genes in elite varieties.

Recombination mapping provides also a mean to assess the order of markers/genes along a chromosome. This is extremely valuable for the construction of physical maps when contigs need to be ordered along a chromosome before sequencing. In wheat, the International Wheat Genome Sequencing Consortium (IWGSC, www.wheatgenome.org) has established a road map for the construction of a physical map of the hexaploid wheat genome. In this framework, our laboratory has constructed a physical map of chromosome 3B using a chromosome specific approach [3]. Several approaches that include deletion mapping and meiotic mapping.were undertaken to produce an integrated physical map of the chromosome. One of the major problems in using meiotic mapping concerned the ordering of contigs in the centromeric regions where no recombination is detected in wheat.

Finally, recombination is also essential in map-based cloning projects where it impacts the genetic to physical distance ratio. Thus, a better understanding of the recombination distribution and the possibility to increase it in target regions would greatly improve the efficiency of gene isolation in wheat by positional cloning.

Here, we have used chromosome 3B as a model to study in details the distribution and impact of recombination at different levels (chromosome and sequences).

MATERIALS AND METHODS

All materials and methods used to study recombination at the whole chromosome level are described in Saintenac et al. [4]. The deleted chromosome 3B population, was developed following the methodology described by Qi et al. [5]. A Chinese Spring deletion line 3BS-4 (FL: 0.55) was crossed with the French cultivar Renan. F2 individuals were screened with molecular markers to identify one individual that carries a deleted chromosome 3BS. This individual was subsequently self pollinated to give a Chinese Spring x Renan deleted chromosome 3B F3 population of 379 individuals. We then used 19 molecular markers to compare the recombination rates in the different regions of chromosome 3B between the deleted population and the original CsRe cross.

RESULTS AND DISCUSSION

Recombination along chromosome 3B was assessed by comparing a dense genetic map (102 markers) obtained from the analysis of a cross between the varieties Chinese Spring and Renan (CsRe) and a physical map established by deletion bin mapping (16 deletion bins). With a total genetic length of 179 cM and a physical length of 995 Mb, the average recombination rate on chromosome 3B is 0.18 cM/Mb. This is a very low compared to small genomes such as those of the human (1.1 cM/Mb), *Arabidopsis* (4.6 cM/Mb) and rice (0.4 cM/Mb) but it is very close to the average rate observed in other cereals such as barley (0.2 cM/Mb). Recombination was mainly concentrated on the distal 40% of the chromosome arms. The two distal bins (3BS8-0.78-0.87 and 3BL7-0.63-1.00) that represent 25% of the physical size of the chromosome contain 77% of the recombination. On the contrary, regions around the centromere, representing 27% of the chromosome, showed no crossing-over at all. On the short arm, a clear gradient of recombination was observed from the centromere to the telomere with a strong decrease at the telomeric end in the last deletion bin 3BS3-0.87-1.00 where a ratio of 0.09 cM/Mb was observed compared to 0.85 in the more proximal bin 3BS8-0.78-0.87. Whereas this pattern was never observed in crosses between two wheat varieties before, a similar decrease has been reported in a cross between rye and wheat [6] as well as in a cross between *festuca* and *lollium* at the end of the short arm of chromosome 3 which is orthologous to wheat chromosome group 3 [7]. Experiments are underway to confirm this interesting observation. No such decrease of the recombination gradient was observed at the end of the long arm. However, the large size of the distal deletion bin (3BL7-0.63-1.00, 208 Mb) does not allow to study the recombination gradient with sufficient resolution and new deletion lines would be needed to complete the analysis.

To study the basis of recombination, in particular the impact of chromosome size and/or of the telomeric vs centromeric position of sequences on the recombination pattern observed along chromosome 3B, we developed a F3 population in which the size of chromosome 3B was reduced (see M&Ms) and compared the recombination rate with the reference map of chromosome 3B. The F3 population originated from a self-pollination of an F2 individual that was deleted for 45% of the short arm of the chromosome.

Marker analysis indicated that except for a very short telomeric end, the short arm of chromosome 3B of the deleted individual is heterozygous. Linkage analysis suggested an increased recombination rate in this part of the short arm. However, because recombination could only be evaluated in this heterozygous region, we cannot yet conclude about a direct impact of the deletion on recombination frequency. To answer this question, we are currently analysing the chiasma numbers in the progeny of the deleted population by FISH to determine the whole recombination on this deleted arm.

On the long arm, marker analysis indicated that two homozygous regions were flanked by heterozygous regions which allowed us to study recombination on the whole arm. Comparison of chromosome 3B recombination in the different intervals between the deleted F3 CsRe population and the F2 CsRe population indicated an increase rate of recombination in the homozygous regions thereby suggesting that sequence similarity plays a major role in determining the recombination pattern along the chromosomes. Thus, the gradient of recombination observed along the wheat chromosomes might reflect a gradient of sequence similarity.

We also compared female and male recombination along chromosome 3B. To do this, we used two different populations originating from a cross between Chinese Spring and Courtot produced respectively by andro- (CSCt-A) and gyno-genesis (CSCt-G). A set of 32 common loci was mapped on the two populations. At the whole chromosome level, no differences were observed but three significant changes in the crossing over (CO) ratio (2 female CO>male CO and 1 male CO>female CO) were found in specific regions in the distal part of the long arm. The largest deviation was observed in the proximal part of the deletion bin 3BL7-0.63-1.00 where the number of CO was 17 fold higher in female recombination compared to that of male recombination.

Finally, we have studied the recombination distribution within a 3.2 Mb sequenced contig present in the distal part of the short arm (deletion bin 3BS-8/3BS-3). For this, 1800 F2 Chinese Spring x Renan individuals were screened and more than 70 recombinants were identified with SSR markers derived from the ends of the contig sequence. More than 120 SSRs and 75 ISBPs markers were designed from the sequence and used for linkage analysis. The relationship between the recombination rate and the presence of genes, TEs, other sequence features as well as the level of polymorphism is currently under detailed analysis.

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