High-resolution radiation hybrid mapping in wheat: an essential tool for the construction of the wheat physical maps

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ABSTRACT

Methods for physical mapping of chromosomes, which do not rely on meiotic recombination, are necessary for species with large genomes like wheat where uneven distribution of recombination and significant variation in genetic to physical distance ratios dramatically affect the capacity to order physical contigs in large portions of the chromosomes. In this context, physical mapping using a radiation hybrid (RH) mapping approach has proved valuable in a number of non-plant and plant systems. RH maps of chromosome 1D, utilizing 87 lines (irradiated with 35 Krad), and chromosome 3B, utilizing 184 lines (94 from 25 Krad and 90 from 35 Krad), were generated by mapping different marker classes without the need for polymorphism. Analysis of 1D RH panel with 378 marker loci identified a total of 2,312 obligate breaks for an average resolution of ~201 kb (size of chromosome/total breaks = 464 Mb/2,312 breaks). Remarkably, analysis of several large sequenced segments (3 Mb average size) of chromosome 3B with the 3B RH panels similarly indicated an average map resolution of ~263 Kb/break. Since these mapping resolutions are within the range of BAC contig alignment, these panels have been used to align BAC contigs to regions of chromosomes 1D and 3B and to further refine the location of s*pecies cytoplasm specific* (*scs*^{ae}) locus on chromosome 1D. The *scs*^{ae} locus could not be conventionally mapped in the durum alloplasmic background indicating an added benefit of RH panels.

INTRODUCTION

The wheat genome is characterized by uneven distribution of gene-rich and gene-poor regions on a given chromosome[1-4]. Gene density in wheat tends to increase with relative distance from the centromere, with some of the highest densities observed in several distal regions of chromosomes[1,2]. As in many plant species, recombination along chromosome arms in wheat is not evenly distributed, being absent in proximal regions of all arms[1-4]. Recombination rate increases with approximately the square of the relative distance of a given segment from the centromere[1]. Due to this uneven distribution of recombination, significant variation in correlations of genetic to physical distances along the length of a given chromosome is evident ranging from ~17 Mb/cM in the most proximal regions to ~1 Mb/cM in the most distal intervals[1]. However, no correlation between recombination rate and local gene density has been observed[1-4]. By some estimates one-fourth to one-third of the wheat genome, present around the centromeres, accounts for less than 1% of total recombination[1-3]. Thus development of high-resolution genetic maps through the use of traditional recombination methods for at least one-fourth of the wheat genome will not be possible. By some recent estimates nearly 30% of wheat genes are in recombination-poor regions that are inaccessible to map-based cloning[1-4]. Studies in wheat and other organisms indicate that essential genes are located in regions of low recombination[1,5,6]. Therefore, cloning and analysis of essential genes located in the proximal low recombination regions of the wheat genome may not be possible by the traditional map-based cloning approaches.

In order to develop a complete map and sequence of the human genome, a sizeable effort was directed towards the task[7]. In this process, BAC libraries were developed and assembled into physically separate contigs, representing the majority of the human genome that served as a substrate for large-scale DNA sequencing[8]. These BAC contigs were separated by physical gaps of unknown size, and the sequences derived from BAC contigs were unordered and unoriented with respect to each other. Thus, the resulting assembled sequence was referred to as the "working draft" to distinguish it from the completed genomic sequence in which size of all gaps, and the order and orientation of all sequences are known. Ninety (90) whole-genome radiation hybrids (RHs) were used in conjunction with 40,322 unique STSs to identify segments of the human genome that were absent from the working draft[9]. This provided an independent estimate of the size and location of missing sequences in relation to the existing ones, and information about the linear order for more than 15,000 of the clones was used to create the human genome map[7-9].

Somatic cell hybrids with human sub-chromosome fragments (radiation hybrids) were used to develop a high-density gene map of the human genome using the

RH mapping approach[8]. Given that a single sub-chromosome fragment stock may contain multiple non-contiguous pieces of a chromosome, it is not possible to use any single stock as a mapping reagent. However, the frequency of radiation induced breakage between two markers or loci can be used as a measure of the distance between these markers or loci. Physically close markers will show little breakage and more distant markers will show greater breakage. Thus, a population of sub-chromosome fragment stocks can be used to calculate the frequency of breakage between all pair-wise combinations of markers or loci to construct a map analogous to a meiotic linkage map[7,8]. The success of RH mapping in humans has fueled its use in the construction of complete physical maps of a large group of animal genomes prior to complete sequencing[10-17].

Although radiation has been used in cereals for the mutation and introgression of genes from related species[18-20], this approach has not been widely used for mapping or cloning experiments. The isolation of self-fertile partial oat × maize hybrids and the production of oat lines with maize-chromosome additions created the opportunity to evaluate radiation-induced chromosome breakage for mapping in plants[21]. Utilizing oat lines carrying maize chromosome 9, the first RH map for a plant chromosome was produced[22]. This work has been expanded to produce low-resolution RH maps of other maize chromosomes[23,24]. Recently, other RH mapping studies in diverse plant species were reported, indicating the broad applicability of this method[25-28]. These plant RH maps are reportedly of low-resolution and only valuable in placing markers on a chromosomal segment. Here we report the results of two studies demonstrating the development of high-resolution radiation hybrid map of plant chromosomes that provide the starting point for BAC contig alignment. Consequently, our RH approach permits mapping at a significantly higher resolution than any current mapping resource for wheat.

MATERIALS AND METHODS

Development of radiation hybrid mapping panels (chromosome 1D=DWRH-1D panel of 87 lines and chromosome 3B=DWRH-3B panel of 184 lines) were described previously[26,29]. Genomic DNA extraction and marker assays were performed following Hossain et al.[26]. Large numbers of ISBP (Insertion Site-Based Polymorphism) markers were used in mapping of chromosome 3B panels. Development and application of these markers have been described by Paux et al.[30].

RESULTS AND DISCUSSION

Analysis of the DWRH-1D panel with 378 AFLP, EST, and RFLP markers indicated that 77% of the 87 individual lines had lost at least one marker. Analysis of DWRH-3B panel with 106 ISBP markers indicated that 70% of the 184 individuals had lost at least one marker. The number of markers missing per individual ranged from 18 to 134 (~5% to 35%) in the DWRH-1D and 1 to 97 (~1% to 92%) in the DWRH-3B with an average marker retention frequency per individual RH line of 72%. Of a total of 378 markers in DWRH-1D panel ~96% were lost at least once and of 106 markers in DWRH-3B panel all were lost at least once with an average marker retention frequency per locus of 71%. It was expected that DWRH-1D would retain a number of loci linked to *scs*^{ae} completely as it was generated for positional mapping and cloning of the region surrounding this gene[26,29]. This is the likely explanation for the difference in marker retention for the two different wheat RH panels.

Assuming random distribution, 368 of the 378 markers used in DWRH-1D panel identified at least one break along chromosome 1D, and therefore, the resolution of the RH map can be directly assayed by estimating the average distance between breaks. A total of 2,312 obligate breaks were identified which would indicate that average distance between breaks is ~201 kb/break (size of chromosome/total breaks = 464 Mb/2,312 breaks). Similar approach for the DWRH-3B panel using the 106 marker data available to date indicates 550 obligate breaks or a resolution of ~1.8 Mb/break (1 Gb/550 breaks). However, this value can rapidly change, as was observed with the initial analysis of DWRH-1D by Hossain et al.[26] with 39 markers identifying only 88 breaks, and as more marker data distributed throughout the chromosome is added. To better estimate the map resolution of DWRH-3B, several Mb sized segments identified on this chromosome were analyzed. The results indicate that average size of chromosome fragments generated by irradiation on this panel is ~263 kb. Both panels provide the resolution needed to align BAC clones and contigs to chromosomes 1D and 3B. Previously only 11 chromosome 1D and 17 chromosome 3B deletion stocks had been identified using the *Gc* system[33]. The values and resolutions reported for the two RH panels are much greater than that afforded by the deletion bin mapping.

Over 16,000 EST loci were successfully placed onto various chromosomes by deletion bin mapping[31,32]. Of these, 2,212 EST loci have been placed on wheat homoeologous group 1 chromosomes[32]. Although these ESTs were placed onto seven bins for chromosome 1D, the relative order of the ESTs within the respective bins is not known. Some of these ESTs were mapped on the DWRH-1D panel and ordered in a linkage group demonstrating the value of this approach in physically ordering loci on a chromosome[29]. Similarly 74 ISBP markers derived from BAC clones and contigs assigned to 3BL7 (the largest bin on this chromosome estimated at ~208 Mb) were used to derive physical order for these DNA segments. Only 22% of total individuals in the DWRH-3B showed breaks in this segment of chromosome 3B, identifying 138 obligate breaks. This number of critical lines allowed localization of 36 contigs anchored by ISBP markers (~49%) accurately. More critical lines are needed now to allow localization and ordering of all 180 BAC clones/contigs identified in this segment of chromosome.

Results of two studies reported here indicate that high-resolution physical mapping in wheat based on the radiation hybrid approach is clearly possible. The RH panels do not need to contain a huge number of individuals, as are clearly needed for meiotic based linkage mapping, and their resolution can be adjusted by varying the radiation dosage. RH mapping approach in conjunction with global fingerprinting (FingerPrinted Contigs or FPC) of BAC clones and quick assignment of contigs utilizing ISBP as well as other markers (RH mapping does not rely on polymorphism) can produce a sequence ready physical map of the ENTIRE wheat genome anchored to a high-resolution map. Additionally, as has been demonstrated often with animal genome projects, high-resolution RH maps not only are the pre-requisite for complete genome sequencing, but also provide an excellent resource for detailed comparative analysis of genomes.

In durum wheat, an alloplasmic durum line has been identified with chromosome 1D of *Triticum aestivum* carrying the species cytoplasm specific (scs^{ae}) gene. The chromosome 1D of this line segregates as a whole without recombination precluding the use of conventional genome mapping. DWRH-1D panel has allowed the localization of scs^{ae} to within a small segment on the long arm of chromosome 1D. This approach can easily be expanded to other chromosomes as well as alien introgressions. Majority of alien chromosome transfers in wheat have been terminal in nature carrying a large segment limiting their use. Reducing the size of linkage drag can be a difficult and tedious process while fine mapping of useful aliens genes almost impossible. The RH mapping approach presented here can be used to help reduce the size of alien introgressions as well as map based cloning of valuable genes. Thus, the potential value of this approach goes beyond physical mapping and comparative genomics to include genome engineering.

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