

Dissecting the hexaploid wheat genome by chromosome sorting

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BACKGROUND

The presence of three homoeologous genomes and large genome size (1C~17,000 Mbp) hamper physical mapping and positional cloning in hexaploid wheat (*Triticum aestivum* L., 2n=6x=42). An attractive approach to reduce the complexity of these accomplishments is to dissect the genome to smaller parts such as chromosomes and chromosome arms. We have shown previously that laser flow cytometry is a suitable method to achieve this goal in a number of species. This approach involves a preparation of aqueous suspensions of intact mitotic chromosomes. The chromosomes in suspension are stained by a DNA-specific fluorochrome and classified according to relative fluorescence intensity. Any chromosome, which differs in relative DNA content from other chromosomes, can be discriminated and sorted at high speed.

FLOW CYTOGENETICS OF WHEAT

Due to small differences in size among the wheat chromosomes, only the largest chromosome 3B can be resolved and sorted from standard wheat lines (Figure 1a). A preliminary analysis of several double ditelosomic (dDt) lines indicated that they could be used to isolate short and long arms of individual chromosomes. In this work we screened a full set of 21 dDt lines to assess their suitability to dissect the wheat genome into individual chromosome arms.

Based on the position of chromosome peaks on flow karyotypes, the lines were classified into four groups. The first and the largest included sixteen dDt lines in which both arms could be easily discriminated and sorted. One of these lines, dDt7D, is interesting in that the 7DS arm is longer than the 7DL - a fact which is reflected by the relative positions of their peaks (Figure 1b). The purity in the sorted fractions ranged from 85 to 95% as determined by FISH.

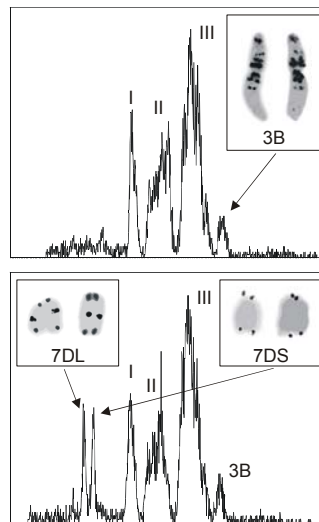
The second category was represented by one dDt line (7A). Both arms of 7A are of similar length and their peaks overlapped so that the telosomes could not be sorted individually. Both arms could be sorted simultaneously at 90% purity.

In the third group of two dDt lines (4A and 7B), the peaks of long arms were close to chromosome peak I. Nevertheless, both arms of 4A and 7B could be sorted with at least 85% purity.

In the last group of two dDt lines (3B and 5B) only the short chromosome arms could be sorted. The peaks of

long arms 3BL and 5BL overlapped the composite peak I and could not be resolved.

Figure 1. (a) Flow karyotype of hexaploid wheat consists of three composite peaks (I - III) representing groups of chromosomes and a peak representing chromosome 3B. (b) Flow karyotype of double ditelosomic line 7D of bread wheat demonstrates the ability to discriminate peaks of both arms of 7D. X axis: relative DAPI fluorescence intensity; Y axis: number of events. The inserts show examples of sorted chromosomes after fluorescent labeling of GAA microsatellites and a telomeric repeat using FISH.



CONCLUSIONS

The use of double ditelosomic and/or ditelosomic lines permits sorting 40 out of the 42 arms of hexaploid wheat. The remaining two arms (3BL, 5BL) can be sorted from lines carrying them as isochromosomes. Thus, the chromosome sorting from cytogenetic stocks wheat offers a powerful tool to dissect the wheat genome to fractions representing only 1 - 3% of the total. This provides an opportunity to divide the complex genome into manageable portions and to structure an international collaboration on wheat genome sequencing.

ACKNOWLEDGEMENTS

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