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Genomics goes chromosomal to explore the wheat genome

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BACKGROUND

Tetraploid durum wheat (*Triticum turgidum* Desf. var. *durum*, 2n=4x=28, 1C~12000 Mbp) and hexaploid bread wheat (*Triticum aestivum* L., 2n=6x=42, 1C~17000 Mbp) posses one of the largest genomes among cultivated crops. Their genomes expanded during the evolution and speciation due to propagation of DNA repeats and polyploidy. The resulting genome structures make polyploid wheats suitable models to study genome expansion and the role of polyploidy in plant evolution and speciation. The same features, however, make genome sequencing and dissection of traits of interest a daunting task.

DISSECTING THE COMPLEX GENOMES

The progress in sequencing technologies makes it possible to sequence even the massive genomes. However, the challenge is to order the short reads and reconstruct the complete genomic sequence. A range of methods has been used to avoid sequencing repetitive DNA, including the generation of Expressed Sequence Tags (ESTs), Cot fractionation and methyl filtration. As an alternative to the reduced representation sequencing, wheat genomics profited from using the tetraploid and diploid progenitors and other closely related species. However, the tetraploid and hexaploid wheat genomes diverged from their wild diploid progenitor species, suggesting that they constitute poor surrogates for genomic studies.

CHROMOSOME GENOMICS

The above-mentioned approaches deal with the whole genomes. However, there is a powerful option to dissect large genomes to small and manageable parts and work on them separately. Chromosomes and chromosome arms are the obvious choices as they are well defined functional genome units. Not only they represent small genome fractions, but their use avoids problems due to homoeology in polyploids. In hexaploid wheat, single chromosome arms represent only 1 – 3% of the whole genome. While greatly reducing the complexity, this approach allows working on polyploid genomes without a need to use surrogate species. Flow cytometry is currently the only method that can isolate chromosomes in sufficient quantities. However, as the cytometry discriminates chromosomes according to relative DNA content, only chromosome 3B can be sorted from lines with wild-type karyotypes. This problem can be resolved by using cytogenetic stocks, which carry telocentric chromosomes and/or isochromosomes, and from which particular chromosome arms can be sorted.

DNA of sorted wheat chromosomes is intact and the chromosomes are suitable for a range of applications, including the construction of subgenomic BAC libraries and molecular cytogenetic mapping. Other important uses of sorted chromosomes include physical mapping using PCR, high-throughput mapping on DNA arrays, and targeted isolation of molecular markers (Table 1).

Table 1. A portfolio of applications of flow-sorted chromosomes and their DNA*

Number of sorted chromosomes	Amplification of DNA	Applications
10^2	No	Physical mapping using PCR
10^2 - 10^3	DOP-PCR	Short-insert DNA libraries Generation of markers
10^3	No	Cytogenetic mapping (FISH, PRINS)
10^4	No	DArT markers
10^4	MDA	DNA arrays Generation of markers
10^{6-7}	No	BAC libraries

*Key to acronyms: DArT = Diversity Array Technology; DOP-PCR = degenerate oligonucleotide primed PCR; MDA = Multiple displacement amplification.

CONCLUSIONS

The chromosome-based approach provides important advantages over the whole-genome approach, including the avoidance of problems due to the presence of homoeologs, cost efficiency, reduction of work to manageable portions, and an opportunity to structure collaborative projects where individual laboratories work on particular chromosomes.

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