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Plant genome analysis using flow cytometry

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Abstract

The goal of this research was (1) to describe the conditions and parameters required for the cell cycle synchronization and the accumulation of large number of metaphase cells in maize and other cereal root tips, (2) to isolate intact metaphase chromosomes from root tips suitable for characterization by flow cytometry, and (3) to construct chromosome-specific libraries from maize. Plant metaphase chromosomes have been successfully synchronized and isolated from many cereal root-tips. DNA synthesis inhibitor (hydroxyurea) was used to synchronize cell cycle, followed by treatment with trifluralin to accumulate metaphase chromosomes. Maize flow karyotypes show substantial variation among inbred lines. This variation should be useful in isolating individual chromosome types. In addition, flow cytometry is a useful method to measure DNA content of individual chromosomes in a genotype, and to detect chromosomal variations. Individual chromosome peaks have been sorted from the maize hybrid B73/Mo17. Libraries were generated from the DOP-PCR amplification product from each peak. To date, we have analyzed clones from a library constructed from the maize chromosome 1 peak. Hybridization of labeled genomic DNA to clone inserts indicated that 24%, 18%, and 58% of the clones were highly repetitive, medium repetitive, and low copy, respectively. Fifty percent of putative low copy clones showed single bands on inbred screening blots, and the remaining 50% were low copy repeats. Single copy clones showing polymorphism will be mapped using recombinant inbred mapping populations. Repetitive clones are being characterized by Southern blot analysis, and will be screened by *in situ* hybridization for their potential utility as chromosome specific markers.

Materials and Methods

1. *Cell synchronization and metaphase accumulation* : Optimal parameters for accumulation of maize root tip cells at metaphase were determined to be the treatment of a 0.5 cm long radicle with 5 mM hydroxyurea for 18 hours, incubation for 1 hour after removal of hydroxyurea, followed by treatment with 1 μM trifluralin for 4 hours.

2. *Flow cytometric analysis and chromosome sorting* : Suspensions of metaphase chromosomes were prepared by chopping synchronized root tips in chromosome isolation buffer, stained with propidium iodide, and analyzed by flow cytometry. Peaks corresponding to nuclei, chromosome clumps, chromosomes, and cellular debris were identified by examination of sorted particles under a fluorescent microscope.

3. *Cloning and analysis of DNA sequences from maize sorted chromosomes* : DNA sequences specific to maize sorted chromosomes were amplified using the DOP-PCR method. After generating plasmid libraries from amplified fractions, inserts isolated from individual bacterial colonies were analyzed by hybridization and mapping analysis . To date, we have analyzed 104 clones from a library constructed from maize chromosome 1. Hybridization analysis with labeled genomic DNA to cloned inserts indicated that 27%, 48%, and 25% of the clones were highly repetitive, medium repetitive, and low copy, respectively. Fifty percent of putative low copy clones showed single bands on inbred screening blots, and the remaining 50% were low copy repeats. Two single copy clones showing polymorphism were mapped using a B73/Mo17 recombinant inbred population. Both clones were mapped to chromosome 1. Repetitive clones were characterized by Southern blot analysis, and will be screened by *in situ* hybridization for their possible utility as chromosome specific markers.

Table 1. DNA content of chromosome 1 and individual chromosome peaks based on fluorescence intensity from different maize lines.

FL chr	A188		A619		B73		B79		KYS	
peak[1]	DNA (pg)[2]	Chr no[3]	DNA (pg)	Chr no	DNA (pg)	Chr no	DNA (pg)	Chr no	DNA (pg)	Chr no
1	0.701 ±0.016	1	0.685 ±0.018	1	0.698 ±0.013	1	0.687 ±0.019	1	0.747 ±0.012	1
2	0.552 ±0.021	3	0.594 ±0.011	1	0.543 ±0.015	3	0.550 ±0.017	4	0.607 ±0.019	3
3	0.475 ±0.010	2	0.519 ±0.017	3	0.470 ±0.012	2	0.463 ±0.011	1	0.521 ±0.013	1
4	0.388 ±0.010	2	0.440 ±0.011	2	0.388 ±0.007	2	0.392 ±0.014	3	0.443 ±0.013	2
5	0.340 ±0.011	2	0.354 ±0.008	3	0.346 ±0.007	2	0.340 ±0.008	1	0.377 ±0.010	2
6									0.324 ±0.010	1
Total	4.763		4.778		4.735		4.866		5.053	

[1]Chromosome peaks of flow karyotypes were described by numerical designation. Chromosome peak 1 in all of flow karyotypes is expected as a chromosome 1. [2]2C DNA content of each chromosome peak. [3]Number of expected chromosomes were calculated by the proportion of total events of each chromosome peak in linear flow karyotypes. Note: Total DNA content based on PI fluorescence intensity in metaphase chromosomes may be less than that of interphase nuclei due to chromatin coiling and fluorochrome binding difference.

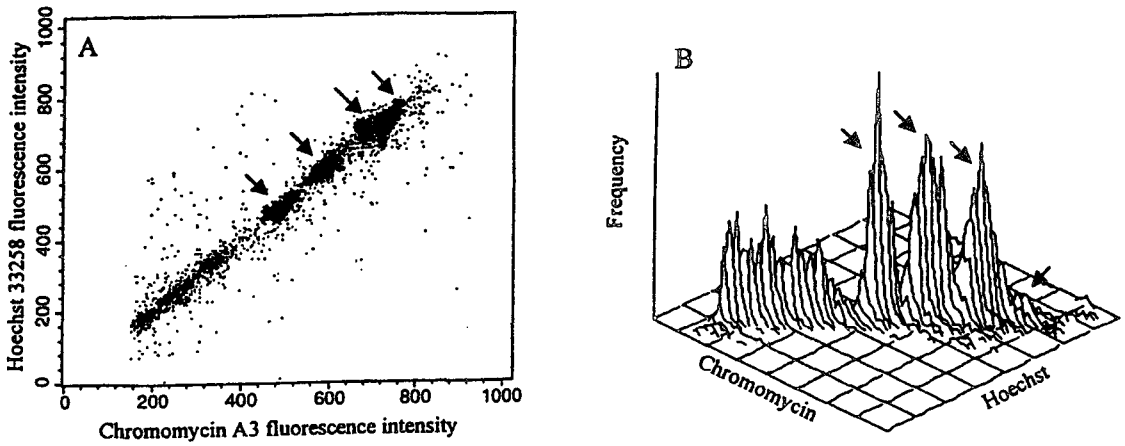

Figure 1. Wheat bivariate flow karyotypes. Chromosome suspension was stained with Hoechst 33258 and Chromomycin A3, and analyzed with a dual-beam flow cytometer. The arrows indicate four peaks of wheat chromosomes on a dot-plot (A) and the 3D-plot (B) derived from analysis of about 20,000 objects in the preparation.