Korean J. Crop Sci. 43(2) : 128~133(1998)

Variation of Univariate Flow Karyotypes and Chromosomal DNA Contents in Maize (*Zea mays* L.)

Jai-Heon Lee*, Myoung Hoon Lee**†, and Kyung Je Kim**

ABSTRACT

Analyses of flow karyotypes using different maize (*Zea mays* L.) inbred lines have been performed. The accumulation and isolation of high quality and quantity metaphase chromosomes from root tips can be achieved from many kinds of maize lines. The chromosome suspensions were prepared by a simple slicing method from synchronized maize root tips and analyzed with a flow cytometry. The variations of experimental flow karyotypes were detected among inbred lines in terms of the positions and/or the numbers of chromosome peaks. The 2C DNA amount among 8 inbred lines ranged from 5.09 to 5.52 pg. The variability of DNA content in maize chromosome 1 was 9.1 % ranging from 0. 685 to 0.747 pg. The selection of appropriate maize lines is critical for sorting specific single chromosome types. At least five different chromosome types can be discriminated and sorted from five maize lines.

Key words : flow karyotype, flow cytometry, maize, chromosome isolation, DNA content.

Flow cytometric analyses of isolated nuclei or chromosomes have been proven to be a rapid and reliable research tool in the area of molecular cytogenetics area. For example, flow cytometry has been used to determine ploidy levels or to detect aneuploidy in plants (reviewed in Dolezel, 1991; Bashir et al., 1993; Pfosser et al., 1995). Flow cytometry can be used for the classification of chromosomes based on DNA content and the identification of a chromosomal variation within species, or the detection of abnormal chromosomes (Gary et al., 1988; Dolezel & Lucretti, 1995; Lee et al., 1996). In addition, flow-sorted chromosomes have been used for the generation of chromosome-specific DNA libraries, for physical mapping of a specific chromosome, and for cloning of defined regions of a complex genome (Van Dilla & Deaven, 1990; Wang et al., 1992; Macas et al., 1993; McCormick et al., 1993; Arumuganathan et al., 1994).

For the construction of chromosome-specific libraries, it is necessary that chromosome peaks containing only single chromosome-types be separated from the rest of chromosome peaks. Among different maize lines, it is known that there is variation in genome size and the chromosomal knob positions and/or DNA contents. A change in chromosomal knob constitution might account for the variation in flow karyotype among different lines. Therefore, it is necessary to find lines with as many specific chromosome types in the flow karyotype as possible. However, it has been known to be difficult for the preparation of chromosome from plants in good quality as well as quantity for flow cytometric analysis and chromosome sorting. Therefore only a few successful reports for flow karyotyping in plants have been published (De Laat & Blaas, 1984; Conia et al., 1987; Arumuganathan et al., 1991; Lucretti et al., 1993; Gualberti et al., 1996; Lee et al., 1996 & 1997).

The purpose of this research was to compare the flow karyotypes of several inbred lines and the DNA amount of individual chromosomes among these lines, and to determine the number of single chromosome types that can be discriminated from other chromosomes for specific chromosome sorting.

MATERIALS AND METHODS

Plant materials

Eight maize inbred lines were used in this study (Table 1): 'A188', 'A619', 'B73', 'B79', 'KYS', 'N28', 'Oh43', and 'W23'. The pedigree and origin of eight maize inbred lines are shown in Table 1. Seeds of inbred lines were obtained from the Agronomy farm of the University of Nebraska-Lincoln in 1997.

Estimation of 2C DNA content and nucleotide composition

Maize nuclei were isolated from 7-day-old seedlings by the chopping method using nuclear isolation buffer (50 mM KCl, 10 mM $MgSO_4$, 5 mM Hepes, 3 mM dithiothreitol, 0.25 % Triton X-100)as described by Arumuganathan & Earle (1991). After the nuclear suspension was filtered through a 30 μm nylon mesh, the suspension was stained with one of the three fluorochromes: for calculating the amount of total nuclear DNA, propidium iodide (PI) was used at a final concentration of 100 μg/ml (incubation for 30 min); for measuring the percentage of GC composition, mithramycin was used at a final con-

* Division of Natural Resources and Life Science, Dong-A University, Pusan 604-714, Korea.
** Department of Plant Resources, Dongguk University, Seoul 100-715, Korea.
† Corresponding author.
Received 30 Mar. 1998.

Table 1. DNA content and base composition (%GC) of 2C nuclear DNA from 8 different maize inbred lines.

Maize line	Pedigree /origin	2C DNA content (pg) ± SD	Base composition (% GC) ± SD
A188	4-29 (Silver King) /46 (N.W. Dent)	5.09 ± 0.10	45.16 ± 0.24
A619	(A171 /Oh43)Oh43	5.25 ± 0.11	45.22 ± 0.21
B73	Iowa stiff stalk synthetic C5	5.16 ± 0.13	45.49 ± 0.18
B79	BS10 (Iowa 2-ear synthetic)	5.36 ± 0.15	45.74 ± 0.19
KYS	Yellow selection, pride of Saline	5.52 ± 0.13	45.78 ± 0.17
N28	Stiff stalk synthetic	5.20 ± 0.11	45.58 ± 0.15
Oh43	Oh40B /W8	5.29 ± 0.13	45.32 ± 0.16
W23	Golden Glow	5.23 ± 0.18	45.72 ± 0.19

Note: The DNA content of chicken red blood cells (CRBC) was used as internal standards (2C=2.33 pg, 42.7 % GC base composition). Propidium iodide (PI) was used for 2C DNA content, and bisbenzimide Hoechst 33342 and mithramycin for base composition. Four nucleus isolations were performed for each line. Analyses were repeated twice for four nucleus suspensions per line.

centration of 50 μg/ml (incubation for 30 min); and for determining the percentage of AT composition, bisbenzimide Hoechst 33258 was used at a final concentration of 5 μg/ml (incubation for 10 min). The nuclei isolated from chicken red blood cells (CRBC) were used as internal standards (2C = 2.33 pg, 42.7% GC composition; Galbraith et al., 1983) for the determination of 2C nuclear DNA content and nucleotide composition. The base composition was estimated using the equations developed by Godelle et al. (1993). Each line was analyzed four times, and each nuclear-suspension sample was run twice (i.e. total of 8 replicates per each line).

Cell cycle synchronization and metaphase chromosome isolation

Cell cycle synchronization and isolation of metaphase chromosome were performed as described previously (Lee et al., 1996) with minor modifications; briefly, seedlings with about 0.5 cm long radicle were incubated in 10 ml Hoagland's solution (Sigma) containing 5 mM hydroxyurea for 18 hours at 28℃ in the dark and then, washed 3 times with distilled water and incubated for 1 hour in 10 ml Hoagland's solution, and then treated with 1 μM trifluralin (a gift from Dow Elanco, Indianapolis, IN., S.A.) for 4 hours. Metaphase chromosomes were prepared in chromosome isolation buffer [50 mM KCl, 10 mM $MgSO_4$, 5 mM K_2HPO_4, 5 mM Hepes, 2 mM dithiothreitol, 0.2 % Triton X-100, 25 μg/ml propidium iodide (PI)] by chopping.

Flow karyotype analysis

The isolated chromosomes or nuclear suspensions stained with PI were analyzed using FACScan flow cytometer (Becton Dickinson Immunocytometry system, San Jose, CA), and the isolated nuclear suspensions stained with mithramycin or Hoechst were analyzed by FACS vantage flow cytometer and sorter (Becton Dickinson Immunocytometry system, San Jose, CA) equipped with Argon-ion laser. The laser was tuned to UV light (351~365 nm) at 100 mW for the analysis of Hoechst stained samples, and the fluorescence was collected through 424 nm long pass filter. For analysis of mithramycin stained samples, the laser was tuned to 457.9 nm at 100 mW and the fluorescence was collected through 475 nm long pass filter. Approximately 2,000 G1 nuclei per line were analyzed. Analyses were repeated twice for each nuclear suspension. Fluorescence pulse area was measured in all cases.

Number of expected chromosome types in each chromosome peak were determined by the proportion of total events of each chromosome peak in linear flow karyotypes. The DNA content of individual chromosome peaks was calculated based on relative PI fluorescence intensity of individual chromosome peaks and G1 nuclei. Each peak revealed in the flow karyotypes was sorted and the content of the peak was analyzed under the fluorescence microscope for identification of clumps, chromosomes, chromatids, or cell debris as described previously (Lee at al., 1996). The instrument settings were adjusted to place the peak of the largest chromosome (chromosome 1) of B73 inbred line at channel 600. The same instrument settings were used for analyses of chromosomes isolated from other inbred lines.

RESULTS AND DISCUSSION

The 2C nuclear DNA contents and percent of GC base pair composition of maize inbred lines are shown in Table 1. The variation in genome size was observed to be 8.5 %, ranging from 5.09 to 5.52 pg/2C nucleus. Other researchers also found significant variation of genome size in maize (Laurie & Bennett, 1985; Rayburn & Auger, 1990. These previous studies expected that about 26 % variation in genome size was observed, ranging from 4.7 to 5.9 pg, and the authors suggested that the variation was caused by the number and size of heterochromatic knobs (McClintock et al., 1981; Laurie & Bennett, 1985). Races of maize vary in knob numbers and positions, and so far all of 23 knob locations on the ten maize chromosomes have been identified. Rayburn et al. (1985)

found a positive correlation between 2C nucleus DNA content and C-banded heterochromatic knob number. KYS, W23, and Oh43 inbred lines have five large heterochromatic knobs (references in Rayburn et al., 1989). In this study, these lines have relatively a higher genome size compared to other inbred lines. The GC base pair composition of 8 inbred lines studied did not show significant variability. The variation was 1.3 %, ranging from 45.16 to 45.74 % GC composition.

Ten pairs of maize chromosomes (n = 10) can be classified into 5 groups based on the relative chromosome size data from Bennett & Laurie (1995) as shown in the theoretical flow karyotype constructed with a coefficient of variation (CV) of 3 % (Fig. 1, A). The modeling of a maize theoretical flow karyotype was performed as described in Lee et al. (1996). The theoretical model showed two peaks with single chromosome types (chromosome 1 and 10, respectively) and three composite chromosome peaks (chromosome 2 and 4, chromosome 3 and 5, and chromosome 6, 7, 8 and 9, respectively). The experimental flow karyotype of Seneca 60 (Fig. 1, B) was closely matched to the theoretical flow karyotype (Fig. 1, A). However, the experimental flow karyotype might be different among maize lines since genome size and/or number of heterochromatic knobs vary among lines (McClintock et al., 1981; Laurie & Bennett, 1985; Rayburn et al., 1985).

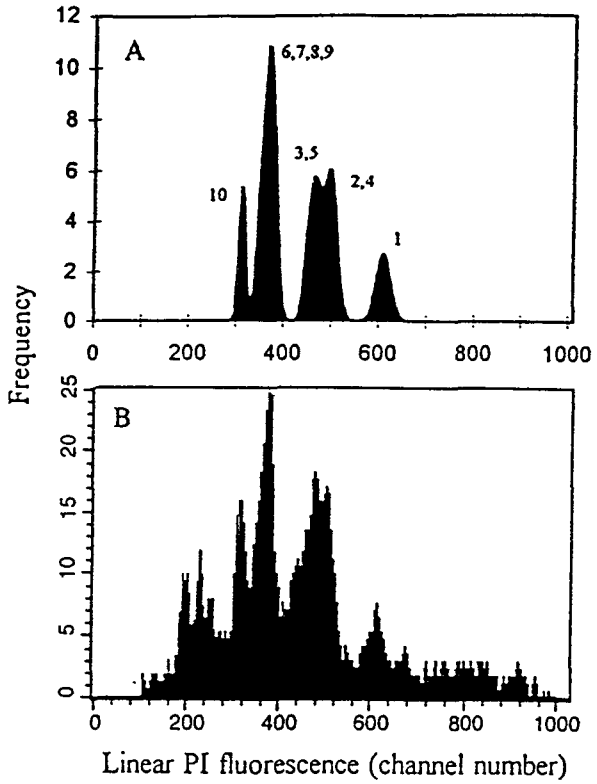

Fig. 1. Theoretical flow karyotype (A) and experimental flow karyotype (B) of maize Seneca 60. The theoretical flow karyotype was calculated on the bases of relative chromosome size in CV = 3% (Bennett & Laurie, 1995); the peaks corresponding to chromosome 1, 10, three composite peaks corresponding to chromosome 2, 4, chromosome 3, 5, and chromosome 6, 7, 8 and 9, respectively, were shown. The experimental flow karyotype (B) closely matched to the theoretical flow karyotype (A).

The flow karyotypes presented in Fig. 2 provide information on 8 maize inbred lines regarding the chromosomal DNA content of each peak, the number of chromosome peaks, and different chromosomal types. The CVs of the individual chromosome peaks analyzed ranged from 1.7 to 3.8 %. Each peak revealed in the flow karyotypes was sorted and the content of the peak was analyzed under the fluorescence microscope for identification of clumps, chromosomes, chromatids, or cell debris. Five to six chromosome peaks resolved from each inbred line ranged from channel 279 for the smallest chromosome to channel 642 for the largest chromosome. The peak of the largest chromosome (chromosome 1) was located between channel 589 and channel 642.

The DNA content of individual chromosome peaks and the number of chromosome types in the chromosome peak are shown in Table 2. The DNA content of each chromosome ranged from 0.324 to 0.747 pg. In each inbred line, the difference in DNA content between the largest and the smallest chromosome peak was nearly double. The variation of DNA content in chromosome 1 was 9.1 % among the 8 inbred lines. At least five single chromosome types (chromosome 1, 2, 10, and two others not identified) could be discriminated on flow karyotypes from 4 inbred lines. The total genomic DNA content was calculated by adding up the values of each chromosome peak ranging from 4.735 to 5.095 pg. These values were on average 7.5 % lower than those of the 2C DNA content calculated from interphase nuclei. This discrepancy may result from differences of fluorochrome binding and/or chromatin coiling between interphase and metaphase chromosomes.

On the bases of these results, the selection of appropriate lines is critical for the sorting of different chromosome types. For example, all of maize lines are suitable sources for sorting chromosome 1; B79 and KYS inbred lines are good for sorting at least 3 single chromosome types; Oh43 inbred line is a better choice for sorting chromosome 1 and 10.

In conclusion, the accumulation and isolation of high quality and quantity of metaphase chromosomes from maize root tips can be performed from different maize lines. The knowledge of the experimental flow karyotype of maize lines is useful for sorting specific chromosomes. The variability of DNA amounts in maize chromosome 1 may be useful information for maize genome analysis.

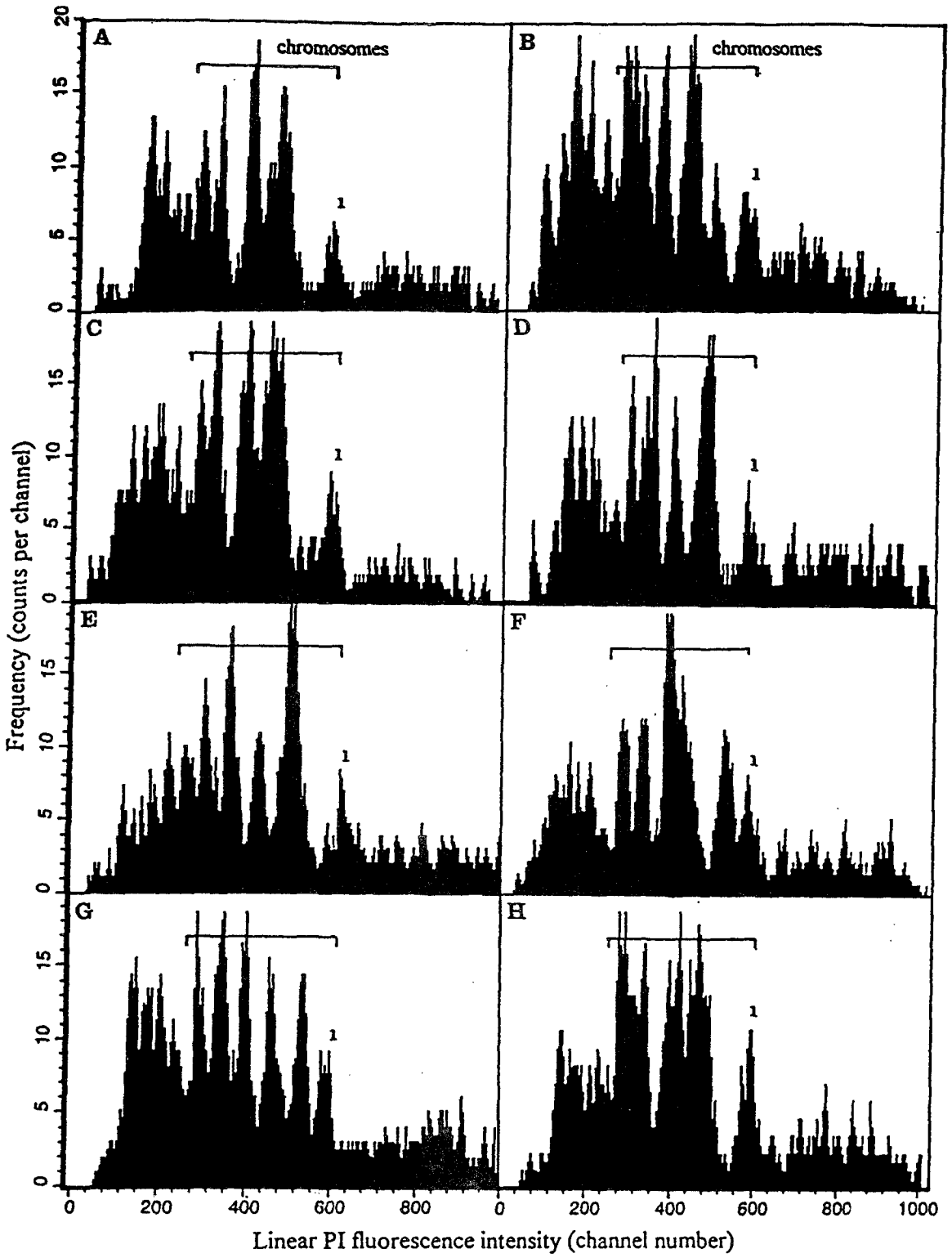

Fig. 2. Variability of flow karyotypes in maize inbred lines. A. A188; B. A619; C. B73; D. B79; E. KYS; F. N28; G. Oh43; H. W23. The instrument settings were adjusted to channel 600 for the largest chromosome (chromosome 1) of B73 inbred line. The same instrument settings were used for analyses of chromosomes isolated from other inbred lines. The chromosome 1 indicated as 1 in all flow karyotypes.

Table 2. DNA content of chromosome 1 and individual chromosome peaks based on relative PI fluorescence intensity from different maize lines.

FL chr	A188		A619		B73		B79		KYS		N28		Oh43		W23	
peak†	DNA (pg)‡	Chr no§	DNA (pg)	Chr no	DNA (pg)	Chr no	DNA (pg)	Chr no	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	0.688 ±0.013	1	0.687 ±0.012	1	0.696 ±0.015	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	0.616 ±0.015	2	0.626 ±0.012	2	0.556 ±0.016	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	0.480 ±0.025	3	0.542 ±0.016	2	0.488 ±0.011	2
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	0.388 ±0.011	2	0.467 ±0.011	2	0.397 ±0.010	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	0.337 ±0.009	2	0.400 ±0.012	2	0.346 ±0.008	
6									0.324 ±0.010	1			0.338 ±0.007	1		
Total	4.763		4.778		4.735		4.866		5.053		4.810		5.095		4.826	

† Chromosome peaks of flow karyotypes were described by numerical designation. Chromosome peak 1 in all of flow karyotypes is expected as a chromosome 1.

‡ 2C DNA content of each chromosome peak.

§ Numbers 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 relative PI fluorescence intensity in metaphase chromosomes may be less than that of interphase nuclei due to chromatin coiling and fluorochrome binding difference.

ACKNOWLEDGMENTS

This research was supported by the Dong-A University Interdisciplinary grant program number 98-2.

REFERENCES

Arumuganathan, K., J. P. Slattery, S. D. Tanksley, and E. D. Earle. 1991. Preparation and flow cytometric analysis of metaphase chromosomes of tomato. Theor. Appl. Genet., 82: 101-111.

_____ , and E. D. Earle. 1991. Estimation of nuclear DNA content of plants by flow cytometry. Plant Mol. Biol. Reporter 9: 229-233.

Arumuganathan K., G. B. Martin, H. Telenius, S. D. Tanksley, and E. D. Earle. 1994. Chromosome 2-specific DNA clones from flow-sorted chromosomes of tomato. Mol. Gen. Genet., 242: 551-558.

Bashir, A., D. G. Bullock, and A. L. Rayburn. 1994. Nuclear DNA amount, growth, and yield parameters in maize. Theor. Appl. Genet. 88: 557-560.

_____ , J. A. Auger, and A. L. Rayburn. 1993. Flow cytometric DNA analysis of wheat-rye addition lines. Cytometry 14: 843-847.

Bennett, M. D., and D. A. Laurie. 1995. Chromosome size in maize and sorghum using EM serial section reconstructed nuclei. Maydica 40: 199-204.

Coe, E. H. 1994. A-A translocations: breakpoints and stocks. *In* The Maize Handbook. Edited by M. Freeling and V. Walbot. Springer-Verlag, New York, Inc. USA. pp. 365-376.

Conia, J., C. Bergounioux, C. Perennes, P. Muller, S. Brown, and P. Gadal. 1987. Flow cytometric analysis and sorting of plant chromosomes from *Petunia hybrida* protoplasts. Cytometry 8: 500-508.

De Laat and A. M. M., J. Blaas. 1984. Flow-cytometric characterization and sorting of plant chromosomes. Theor. Appl. Genet. 67: 463-467.

Dolezel, J. 1991. Flow cytometric analysis of nuclear DNA content in higher plant. Phytochem Anal. 2: 143-154.

Dolezel, J. and S. Lucretti. 1995. High-resolution flow karyotyping and chromosome sorting in *Vicia faba* lines with standard and reconstructed karyotypes. Theor. Appl. Genet. 90: 797-802.

Galbraith, D. W., K. R. Harkins, J. M. Maddox, N. M. Ayres, D. P. Sharma, and E. Firoozabady. 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science 220: 1049-1051.

Gary, J. W., B. Trask, G. Van den Engh, A. Silva, C. Lozes, S. Grell, S. Schonberg, L. Yu, and M. S. Golbus. 1988. Application of flow karyotyping in prenatal detection of chromosome aberrations. Am. J. Hum. Genet. 42: 49-59.

Godelle, B., D. Cartier, D. Marie, S. C. Brown, and S. Siljak-Yakovlev. 1993. Heterochromatin study demonstrating the non-linearity of fluorometry useful for calculation genomic base- composition. Cytometry 14: 618-626.

Gualberti, G., J. Dolezel, J. Macas, and S. Lucretti. 1996. Preparation of pea (*Pisum sativum* L.) chromosome and nucleus suspensions from single root tips. Theor. Appl. Genet. 92: 744-751.

Laurie, D. A. and M. D. Bennett. 1985. Nuclear DNA content in the genera *Zea and Sorghum*. Intergeneric, interspecific, and intraspecific variation. Heredity 55:

307-313.

Lee, J. -H., K. Arumuganathan, S. M. Kaeppler, H. F. Kaeppler, and C. M. Papa. 1996. Cell synchronization and isolation of metaphase chromosomes from maize (*Zea mays* L.) root tips for flow-cytometric analysis and sorting. Genome 39: 697-703.

_____ , Y. Yen, S. M. Kaeppler, P. S. Baenziger, and K. Arumuganathan. 1997. Synchronization of cell cycle in root-tips and flow karyotype of metaphase chromosomes of common wheat (*Triticum aestivum* L.). Genome 40: 633-638.

_____ , _____, K. Arumuganathan, and P. S. Baenziger. 1998. The absolute DNA content of wheat chromosomes at interphase measured by laser-based flow cytometry. Theor. Appl. Genet. (in press).

Lucretti, S., J. Dorezel, I. Schubert, and J. Fuchs. 1993. Flow karyotyping and sorting of *Vicia faba* chromosomes. Theor. Appl. Genet. 85: 665-672.

Macas, J., J. Dolezel, I. Lucretti, U. Pich, A. Meister, J. Fuchs, and I. Schubert. 1993. Localization of seed storage protein genes on flow-sorted field bean chromosomes. Chrom. Res. 1: 107-115.

McClintock, B., T. A. Kato-Y, and A. Blumenschein. 1981. Chromosome constitution of races of maize. Colegio de postgraduados, Chapingo, Mexico.

McCormick, M., E. Campbell, L. Deaven, and R. Moyzis. 1993. Low frequency chimeric yeast artificial chromosome libraries from flow-sorted chromosomes 16 and 21. Proc. Natl.

Pfosser, M., A. Amon, T. Lelley, and E. Heberle-Bors. 1995. Evaluation of sensitivity of flow cytometry in detecting aneuploidy in wheat using disomic and ditelosomic wheat-rye addition lines. Cytometry 21: 387-393.

Rayburn, A. L. and J. A. Auger. 1990. Genome size variation in *Zea mays* ssp. *mays* adapted to different altitudes. Theor. Appl. Genet. 79: 470-474.

_____ , _____, E. A. Benzinger, and A. G. Hepburn. 1989. Detection of intraspecific DNA content variation in *Zea mays* L. by flow cytometry. J. Exp. Bot. 40: 1179-1184.

_____, H. J. Price, J. D. Smith, and J. R. Gold. 1985. C-band heterochromatin and DNA content in *Zea mays*. Am. J. Bot. 72: 1610-1617.

Van Dilla, M. A., and L. L. Deaven. 1990. Construction of gene libraries for each chromosome. Cytometry 11: 208-218.

Wang, M. L., A. R. Leitch, T. Schwarzacher, J. S. Heslop-Harrison, and G. Moore. 1992. Construction of a chromosome-enriched HpaII library from flow-sorted wheat chromosomes. Nucleic Acids Res. 20: 1897-1901.