J. Plant Biol. 40 (3) : 149~155 (1997)

This paper was presented at the 11th Symposium on Plant Biotechnology entitled "Plant Genes and Genetic Resources" organized by Gynheung An, held July 4-5, 1997, by the Botanical Society of Korea

Molecular Cytogenetics of Plant Genome

Bang, Jae Wook*, Jeong Soon Kim and Mi Kyung Lee

Department of Biology, College of Natural Sciences, Chungnam National University, Daejon 305-764, Korea

Cytogenetics-the F1 of cytology and genetics-was born in the 1920s and the 1930s were the golden period of cytogenetic research. Cytogenetics is challenged by two basic questions (Lacadena, 1995): (1) When will we have a complete structural model of the eukaryotic chromosome?; (2) When will we be able to draw a chart presenting diagramatically the intricate network of genetic signals?

Chromosomes have three function: to conserve, transmit and express genetic information that they carry. In some plant species, it is not easy to distinguish individual chromosomes by using conventional staining methods. To identify individual chromosomes, fluorochromes and Giemsa staining techniques have been employed by Vosa and Marchi (1972). Since then, chromosome banding techniques have proved to be useful tools for detailed cytogenetic analyses. Giemsa C-banding technique has enabled the complete identification of individual chromosomes and chromosome arms in many plant species: wheat (Natarajan and Sarma, 1974), rye (Bennett *et al.*, 1977), barley (Noda and Kasha, 1978), *Triticale* (Darvey and Gustafson, 1975), oats (Yen and Filion, 1977) and maize (Ward, 1980; Chow and Larter, 1981). By establishment of a series of methods from identification and quantification to manipulation of plant chromosomes, understanding and utility of chromosome information in both basic and applied fields would be promoted (Fukui, 1992).

Computer-aided image analysis offers another approach to plant karyotypes. Imaging methods have been used for localization of genes precisely on plant chromosomes. Recent rapid development of micro electronics has made it possible to analyse the plant chromosomes digitally by an image analysis method (Fukui, 1985, 1986, 1988, 1989). Fukui (1985) developed a chromosome image analyzing system (CHIAS) based on the universal image analyzer, IBAS-I and II.

While traditional cytogenetics has contributed much to our understanding of evolutionary processes generating karyotypic diversity, molecular cytogenetics such as *in situ* hybridization (ISH) have been successfully applied to reveal new insight into the origin and evolution of DNA sequences, chromosomes and whole genomes in a diverse array of plant species (Leitch *et al.*, 1995).

Knowledge on the relationships and diversification of different species is useful for the selection of suitable material for crossing, maximizing compatability, and understanding species phylogenies. Once hybrid is produced, it is important to know its karyotype, the chromosomal stability, and the ancestral origin of its chromosomes. At the moment the techniques of molecular cytogenetics using total genomic DNA as a probe (Schwarzacher *et al.*, 1989; Heslop-Harrison, 1992, 1993; Mukai *et al.*, 1993) are useful for discrimination of chromosomes in hybrids and for detecting chromosomal rearrangement (Schwarzacher *et al.*, 1993).

By all means, the present and the preceding decade have brought much excitement in cytogenetics field as well and we are reliving the glory of its renaissance (Gill and Friebe, 1997).

The wide array of currently available FISH (fluorescence *in situ* hybridization) techniques extends the resolution of visual mapping from some megabases to only a few kilobases. Ordering of clones on metaphase chromosomes requires that differentially labelled clones are separated by at least 1 Mb.

We will concentrate on recent advances in the plant molecular cytogenetics, with special emphasis on FISH, GISH, flow cytogenetics and chromosome microdisssection for genome analysis, and prospective applications are also being discussed.

IN SITU HYBRIDIZATION

In situ hybridization (ISH) was first reported in late 1960s as a cytological method to visually detect specific DNA or RNA sequences located on the chromosome, nucleus, cell or tissue samples (Gall and

*Corresponding author: Fax +82-42-822-9690

Pardue, 1969).

Several painting tools have been developed for the differentially staining of whole genomes, chromosomes, tandem repeats and single copy sequences. FISH technique has had a great impact on the practice of molecular cytogenetics (Lawrence, 1990; Trask, 1991). Estimation of the copy number of the genes at a locus and localization of single-copy genes are also being achieved (Ohmido and Fukui, 1995).

Three improvements in FISH procedure to obtain reproducibly clear signals have been made (Fukui *et al.*, 1994): (1) a combination of post-treatment to avoid the thin fluorescent layer often observed to cover all the field after FISH treatment; (2) using a rearranged thermal cycler throughout the denaturation process of the chromosomal and the probe DNA to facilitate hybridization of the chromosomal DNA with the probes; (3) Using image method to analyze the FISH signals. True chromosome painting protocols based on FISH with chromosome specific DNA libraries and widely applied to human and mammalian karyotypes have been challenging plant cytogenetics for direct visualizing chromosomal rearrangements in kayotype evolutionary processes. The feasibility, applicability, and utility of FISH to plant genetics will contribute significantly to long-standing questions, such as genome evolution and behaviour, chromosomal position effects on gene silencing, and effects of linkage drag in mapping and breeding programs.

Most of differences in composition and complexity of higher plant genomes can be defined by repetitive DNA. Two main types of repetitive DNA sequences are distinguishable by their genomic organization (Charlesworth *et al.*, 1994; Lapitan *et al.*, 1991): (1) families of repetitive DNA sequences found as tandem arrays along chromosomes; (2) the repeats dispersed throughout the whole genome (Mukai, 1996). The class of repetitive sequences, known as satellite DNA, is organized in tandem arrays, after amplified up to 105 to 106 copies/1C (Singer, 1982). Dispersed repetitive DNA is a heterogeneous class sequences with repeats interspersed throughout the genome and scattered over many chromosomes of the complement (Bennetzen *et al.*, 1994; Ganal *et al.*, 1988; Hueros *et al.*, 1993; McIntyre *et al.*, 1990; Rogowsky *et al.*, 1991). However, it has been shown that the interspersion pattern of some repeats is often not random and that mobile sequences like retrotransposons occur clustered in distinct regions of the genome (Schmidt *et al.*, 1995).

The ribosomal sequences at the nucleolar organizing regions (NORs) are particularly useful because of their universality and rapid evolution sites (Leitch and Heslop-Harrison, 1992).

By using repeated DNA probes for chromosome identification, the location of a target gene labeled by different reporter molecule was allocated to a specific chromosome from a single metaphase. FISH technique is an effective method for physical mapping of specific RNA genes and/or DNA sequences on chromosomes. The information obtained with FISH mapping provides the differences between genetic and cytological maps. The precise localization and molecular size estimation of the DNA sequences using FISH are powerful tools for genetics and breeding. Two approaches have been applied to physical mapping of plant genes as follows; (1) chromosome *in situ* suppression and (2) extended DNA fiber FISH. High-resolution mapping in low copy gene was performed by FISH to extended fibers from interphase nuclei.

Knowledge about the higher order structural organization of eukaryotic chromosome ends is restricted. The telomeric regions of chromosomes, including the telomeric repeat and subtelomeric sequences, are important to study the genome organization because they are responsible for the stability of chromosomes (Richard *et al.*, 1993). Some aspects of the complex organization of the telomeric DNA sequences are being found in many plant species. An important step toward understanding the organization of the various telomeric and subtelomeric DNA sequences can be made by the physical mapping approaches of FISH and PFGE (pulse field gel electrophoresis), which together bridge the gap between the level of a few kilobases in a clone and C-bands along metaphase chromosomes that are undefined at the molecular level (Vershinin *et al.*, 1995).

Arabidopsis thaliana is the model plant for molecular cytogenetic studies. *A. thaliana* has five pairs of chromosomes; three metacentric and two telocentric, which are not easily distinguishable by common staining. Recently two repetitive sequence families (18S-5.8S-25S rRNA genes and 180 bp tandem repeat family) have successfully been localised by FISH to the short arms of two telocentric chromosome pairs and the centromeric regions of all chromosomes, respectively (Maluszynska and Heslop-Harrison, 1991; Murata *et al.*, 1994). The 5S rDNA is also shown to be a good probe to identify the chromosomes of *A. thaliana*. More recently, low copy DNA sequences could be visualized efficiently on mitotic metaphase chromosomes. Mapping of low-copy DNA sequences and genes on *Arabidopsis* by FISH is almost established.

Single copy sequences on human chromosomes have

been routinely detected by standard FISH techniques. However, no signals have been detected clearly so far in less than 4 kb low copy DNA sequences. Recently a new technique to detect DNA sequences on extended DNA fibers prepared from interphase nuclei by FISH was developed (Fransz *et al.*, 1996).

Recently many probe-labeling methods and fluorescent reagents are being available for simultaneous detection of target DNA sequences in different colors. Multi-colour FISH (McFISH) using directly cloned and labeled DNA probes has been successfully employed in plants. This opens the way, not only for more effective mapping of different DNA sequences, but also for determining the order of different clones within a chromosome (Montogomery *et al.*, 1993). McFISH also allows the development of more detailed maps of specific chromosome regions. The strategy and applications of McFISH to visualize simultaneously with different colors are shown in Table 1.

Methods for discrimination of two different genomes in *Scilla scilloides* Complex are as follows. The A genome probe was labeled by biotin and detected with FITC. The B genome probe was labeled by digoxigenin and detected with rhodamine (Fig. 1).

In FISH experiments, the technique using total genomic DNA as a probe is called Genomic *in situ* hybridization (GISH). GISH technique has provided new insights into genomic evolution following the formation of both naturally occurring polyploids and man-made dihaploid and asymmetric somatic hybrids. GISH has been recently developed as an important technique for identification and analysis of alien and wheat chromosomes in interspecific wheat hybrids (Schwarzacher *et al.*, 1989, 1992; Jiang and Gill, 1994: Chen *et al.*, 1995). Repeated sequences, which comprise most of the genomic DNA in higher plants, reanneal more rapidly than more highly conserved unique sequences in the genome. Therefore, total genomic DNA can be species-specific labeling probe. Genomic interaction may be expressed in two different ways: (1) the preferential elimination of chromosomes from one of the parental genomes in a hybrid; (2) the preferential inactivation of genes from one plant (Neves *et al.*, 1996). GISH is able to give three types of information about chromosomes and genomes: (1) the identification of the parental origin of chromosomes in hybrid plants (Schwarzacher *et al.*, 1989); (2) understanding the intergenomic chromosome rearrangements (Schwarzacher *et al.*, 1992); (3) data about genome relationships (Heslop-Harrison and Schwarzacher, 1996). With tremendous impact for plant breeding and plant taxonomy, genome painting enables

Table 1. Labeling-detection combinations in multicolor FISH using total genomic DNA probes (From Mukai, 1996)

	Labeling of probe DNA	Detection		Application
		Reagent	Fluorescence	
Tetraploid Method 1 1st genome 2nd genome	Biotin-dUTP Digoxigenin-dUTP	Avidin-FITC* Antin-dig.-rhodammine	Yellow Orange	Aegilops(CCDD, CCUU, DDNN) Synthetic amphidiploid (AASS)
Method 2 1st genome 2nd genome	Biotin-dUTP Cold(blocking)	Avidin-FITC* Propidium iodide	Yellow Red	Tetraploid wheat (AABB, AAGG) Aegilops (DDMM, UUMM, UUSS)
Method 3 1st genome 2nd genome	Biotin-dUTP None	Avidin-FITC* Propidium iodide	Yellow red	Milium montianum Tobacco
Hexaploid 1st genome 2nd genome 3rd gonome	Biotin-dUTP Digoxigenin-dUTP Cold(blocking)	Avidin-FITC* Anti-dig.-rhodamine	Yellow Orange Brown	Common wheat Synthetic amphidiploid (AABBNN, AAGGUU)
Octoploid 1st genome 2nd genome 3rd genome 4th genome	Biotin-dUTP Digoxigenin-dUTP Cold(blocking) 2/3bio.+1/3dig	Avidin-FITC* Anti-dig.-rhodamine Avidin-FITC* + Anti-dig.-rhodamine	Yellow Orange Brown Yellow	Synthetic amphidiploid (AABBDDEE, AABBDDRR)

FITC*, fluorecein isothiocyanate

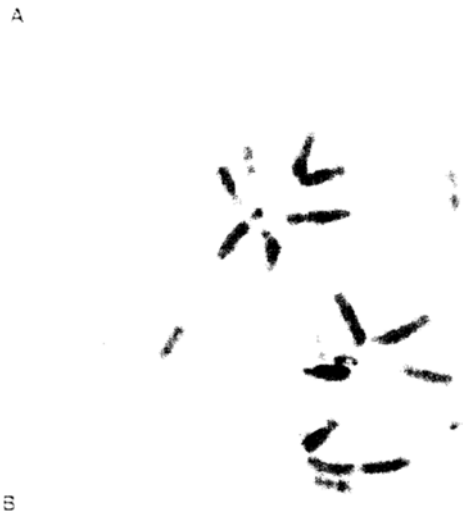

Fig. 1. Multicolor *in situ* hybridization in *Scilla scilloides* Complex with total genomic DNA probes from allotetraploid AABB genome plant. (A) A complete metaphase cell counter stained with DAPI. (B) Metaphase chromosomes showing discrimination of A and B genome by fluorescent *in situ* hybridization using genomic DNAs. The A genome probe was labeled by biotin and detected with FITC (green colored chromosomes). The B genome probe was labeled by digoxigenin and detected with rhodamine (red colored chromosomes).

the distinction of parental genomes and chromosomes in intergeneric and interspecific hybrids (Schwarzacher *et al.*, 1989; Anamthawat-Jonsson *et al.*, 1990; Leitch *et al.*, 1990).

T-DNA which is inserted on mitotic chromosomes in the *Agrobacterium*-mediated transformants of *A. thaliana* was detected (Murata and Motoyoshi, 1997). Copies of the T-DNA inserted at one locus were one to six, and the detection efficiency by FISH was correlated with the copy number. Since even a single T-DNA insertion could be detected by this technique, it will be useful to estimate how many T-DNAs are involved in the T-DNA-tagged mutants.

BAC-FISH in many species revealed that BACs are not only the best contemporary big-DNA cloning tool, but they provide an almost unlimited number of molecular cytogenetic markers for mitotic and meiotic FISH (Hanson *et al.*, 1995). Cytogenetic mapping of most BAC-cloned sequences is simple and facilitates expeditious development of integrated physical maps by contig assembly. Meiotic chromatin as amenable to FISH as mitotic chromatin. Meiotic applications are underway to help address numerous practical and philosophical questions, such as cytostock definition, pairing, recombination, disjunction, viability and transmissibility.

FLOW CYTOGENETICS

Flow cytogenetics is defined as the sorting and analysis of mitotic chromosomes by flow cytometry. Plant flow cytogenetics was established by De Laat and Blass (1984) and is to be expected that it will play a significant role in gene isolation and mapping in plants. Recent results obtained in several plant species confirmed the usefulness of flow cytogenetics for plant genome mapping (Dolezel *et al.*, 1995). Although chromosome flow sorting is the prime interest to plant geneticists and breeders, methods will have to be developed for preparation of chromosome suspensions and discrimination of individual chromosome types.

In *Vicia faba* sorted chromosomes were used for PCR with sequence-specific primers to localize the seed-specific protein genes (Macas *et al.*, 1993). In this work, the use of reconstructed karyotypes not only solved the problem with dicrimination of different chromosome types, but enabled sub-chromosomal localization. A modified method called PRINSES (primed *in situ* DNA labeling en suspension), labels repetitive sequences specifically on *P. sativum* and *V. faba* chromosomes in suspension (Macas *et al.*, 1995).

MICRODISSECTION AND MICROMANIPULATION OF CHROMOSOME

By establishment of a series of methods from identification and quantification to manipulation of plant chromosomes, understanding and utility of chromosome information in both basic and applied fields would be promoted (Fukui, 1992). It is possible to isolate the DNA sequences from specific chromoso-

mes or chromosome-fragments by microdissection. Fine glass needles and a micromanipulator are often used for the mechanical dissection of chromosomes. Alternatively, microdissection by a laser microbeam may be used. The laser dissection method enables one to obtain genetic information directly from specific chromosomal regions. The dissected chromosomes are amplified by degenerate oligonucleotide-primed PCR in a single tube reaction. The amplified DNA is used as a complex probe mixture for FISH. Chromosome dissection may provide an alternative method for the analysis of the genome of various organisms, since chromosomal samples are readily available (Fukui *et al.*, 1992).

Microisolated DNA can be used for the preparation of chromosome specific DNA libraries. Furthermore, microisolated DNA can be used as a target for PCR with gene specific primers or as a source of chromosome specific painting probes for *in situ* hybridization.

CONCLUSIONS

Using high resolution FISH techniques it is possible to obtain simultaneous information rapidly in a convenient visual form about the order, transcriptional orientation and physical distances separating clones. Modern FISH techniques facilitate physical mapping over distances that range from megabases down to a few kilobases. This wide range of resolution power and applicability to multiple areas of genome research is currently not obtained with any other mapping techniques.

Multicolor FISH (McFISH) techniques are an immensely powerful tool. Visual mapping by FISH represents the most direct approach for the ordering and orientation of genomic clones. The simultaneous detection of hybridization signals of several probes using fluorescent labels with different colours has made FISH a practical tool at multiple stages of various mapping projects.

McFISH techniques allow the following research applications: (1) simultaneous discrimination of each genome and identification of diploid progenitors in alloploids; (2) simultaneous mapping of different DNA sequences; (3) physical ordering of multiple probes in a single chromosome; (4) genome allocation of genes of interest; (5) detection of chromosomal aberration; (6) examining chromosome organization in interphase nuclei.

Distribution pattern of individual genes and repetitive DNA sequences should contribute to the analysis of the physical organization and genome dynamics of chromosomes. The data obtained by using newly developed FISH techniques will enable to further promote research on plant genetics and breeding program. GISH technique allows a detailed description of the genomic composition of the hybrids. GISH technique is powerful for information on genomic relationships of the parental species involved and the study of morphology and behaviour of alien chromosomes in backcross derivatives at mitotic and meiotic stages.

The genome mapping for sorted chromosomes in plants was confirmd. Flow cytogenetics will play a significant role in gene isolation and mapping in plants. Further improvement of the sensitivity of PRINSES could permit sorting of all chromosome types within a given karyotype and finally revolutionize plant flow cytogenetics.

The microdissection and micromanipulation methods enable one to obtain genetic information directly from specific chromosome regions. Direct cloning and direct labeling of certain DNA sequences from microdissected chromosomes and nuclei become a standard chromosome technique.

Chromosome engineering allows plant breeders to produce chromosome or chromosome arm addition, substitution or translocation lines. Once a new chromatin is introduced into cultivated crops it is hoped that this new information is stably transmitted in every cell cycle and from generation to another. Furthermore, the regular expression of the inserted genes is also expected.

LITERATURE CITED

Anamthawat-Jonsson, K., T. Schwarzacher, A.R. Leitch, M.D. Bennett and J.S. Heslop-Harrison. 1990. Discrimination between closely related Triticeae species using genomic DNA as a probe. *Theor. Appl. Genet.* **79**: 721-728.

Bennett, M.D., J.P. Gustafson and J.B. Smith. 1977. Variation in nuclear DNA in the genus *Secale*. *Chromosoma* **61**: 149-176.

Bennetzen, J.L., K. Schrick, P.S. Spriger, W.E. Brown and P. Miguel. 1994. Active maize genes are modified and flanked by diverse classes of modified, highly repetitive DNA. *Genome* **37**: 565-576.

Charlesworth, B., P. Sniegowski and W. Stephan. 1994. The evolutionary dynamics of repetitive DNA in eukaryuotes. *Nature* **370**: 215-220.

Chen, Q., R.L. Conner and A. Laroche. 1995. Identification of the parental chromosomes of the wheat-alien amphiploid Agrotana by genomic *in situ* hybridization. *Genome* **38**: 1163-1169.

Chow, C. and E. N. Larter. 1981. Centromeric banding

in maize. Can. *J. Genet. Cytol.* **23**: 255-258.

Darvey, N.L. and J.P. Gustafson. 1975. Identification of rye chromosomes in wheat-rye addition lines and triticale by heterochromatin bands. *Crop Sci.* **15**: 239-243.

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

Dolezel, J., S. Lucretti and J. Macas. 1995. Flow cytometric analysis and sorting of plant chromosomes. *In*: Brandham P. E. and M. D. Bennett eds. *Kew Chromosome Conferrence IV*, pp 185-200. Royal Botanic Garden, Kew.

Fransz, P.F., C. Alonso-Blancd, T.B. Riharska, A.J.M. Peerters, P. Zabel and J. Hans de Jong. 1996. High-resolution physical mapping in *Arabidopsis thaliana* and tomato by fluorescence *in situ* hybridization to extended DNA fibers. *Plant J.* **9**: 421-430.

Fukui, K. 1985. Identification of plant chromosomes by image analysis method. *Cell* (Tokyo) **17**: 145-149.

Fukui, K. 1986. Standardization of karyotyping plant chromosomes by a newly developed chromosome image analyzing system (CHIAS). *Theor. Appl. Genet.* **72**: 27-32.

Fukui, K. 1988. Analysis and utility of chromosome information by using the chromosome image analyzing system, CHIAS. *Bull. Natl. Inst. Agrobiol. Resour.* **4**: 153-176.

Fukui, K. 1989. Application of image analysis methods in plant chromosome research. *In*: Deyuan H. ed. pp 195-200. Academia Sinica, Beijing.

Fukui, K. 1992. Chromosome engineering: Historical perspective and prospect. *Proc. Sec. Jpn. Symposium Pl. Chromos. Plant Chromosome Research* pp. 61-72.

Fukui, K., M. Minezawa, Y. Kamisugi, M. Ishikawa, N. Ohimido, T. Yanagisawa, M. Fujishima and F. Sakai. 1992. Microdissection of plant chromosomes by argon-ion laser beam. *Theor. Appl. Genet.* **84**: 787-791.

Fukui, K., N. Ohimido and G.S. Khush. 1994. Variability in rDNA loci in the genus *Oryza* detected through fluorescence *in situ* hybridization. *Theor. Appl. Genet.* **87**: 893-899.

Gall, J.G. and M.L. Pardue. 1969. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. *Proc. Natl. Acad. Sci. USA* **63**: 378-383.

Ganal, M.W., N.L.V. Lapitan and S.D. Tanksley. 1988. A molecular and cytogenetic survey of major repeated DNA sequences in tomato (*Lycoperisicon esculentum*). *Mol. Gen. Genet.* **213**: 262-268.

Gill, B.S. and B. Friebe. 1997. Unique contributions of plant molecular cytology. A historical perspective and some recent results. Inter. Worshop on Analysis and Utility of Plant Chromosomes Information, p 4.

Hanson, R.E., M.S. Zwick, S. Choi, M.N. Islam-Faridi, T.D. McNight, R.A. Wing, H.J. Price and D. M. Stelly. 1995. Fluorescent *in situ* hybridization of a bacterial artificial chromosome. *Genome* **38**: 646-651.

Heslop-Harrison, J.S. 1992. Molecular cytogenetics, cytology and genomic comparisons in the Triticeae. *Hereditas* **116**: 93-99.

Heslop-Harrison, J.S. 1993. Applications of molecular cytogenetics in the Triticeae. *In*: Damania A. ed. *Biodiversity and Wheat Improvement*, Chichester; Wiley, pp 31-38.

Heslop-Harrison, J.S. and T. Schwarzacher. 1996. Genomic Southern and *in situ* hybridization for plant genome analysis. *In*: Jauhar, P.P. ed. *Methods of genome analysis in plants.* Boca Raton: CRC Press, pp. 163-179.

Hueros, G., Y. Loarce and E. Ferrer. 1993. A structural and evolutionary analysis of a dispersed repetitive sequence. *Plant Mol. Biol.* **22**: 635-643.

Jiang, J. and B.S. Gill. 1994. Nonisotopic *in situ* hybridization and plant genome mapping: the first 10 years. *Genome* **37**: 717-725.

Lapitan, N.L.B., M.W. Ganal and S.D. Tanksley. 1991. Organization of the 5S ribosomal RNA genes in the genome of tomato. *Genome* **34**: 509-514.

Lacadena, J.R. 1995. Cytogenetics yesterday, today and forever: a conceptual and historical review. *Chromosome Research* 3, Suppl. 1: 9

Lawrence, J.B. 1990. A fluorescence *in situ* hybridization approach for gene mapping and the study of nuclear organization. *In*: Davies, K.E. and Tilghman, S.M. eds, *Genome Analysis* Vol. 1: Genetic and Physical Mapping. Cold Spring Harbor Laboratory Press, New York, pp. 1-38.

Leitch, I.J., A.S. Parokonny and M.D. Bennett. 1995. New insights into chromosome evolution in plants from molecular cytogenetics. *Chromosome Research* 3, **Suppl. 1**: 10

Leitch, I.J. and J.S. Heslop-Harrison. 1992. Physical mapping of the 18S-5.8S-26S rRNA genes in barley by *in situ* hybridization. *Genome* **35**: 1013-1018.

Leitch, A.R., W. Mosgoller, T. Schwarzacher, M.D. Bennett and J.S. Heslop-Harrison. 1990. Genomic *in situ* hybridization to sectional nuclei shows chromosome domains in grass hybrids. *J. Cell. Sci.* **95**: 335-341.

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

Macas, J., J. Dolezel, G. Gualberti, U. Pich, I. Schubert and S. Lucretti. 1995. Primer-induced labelling of pea and field bean chromosomes *in situ* and in suspension. *Bio Techniques* **19**: 402-408.

Maluszynska, J. and J.S. Heslop-Harrison. 1991. Localization of tandemly repeated DNA sequences in *Arabidopsis thaliana*. *Plant J.* **1**: 159-166.

McIntyre, C.L., S. Pereira, L.B. Moran and R. Appels. 1990. New *Secale cereale* (rye) DNA derivatives for the detection of rye chromosome segments in wheat. *Genome* **33**: 635-640.

Montogomery, K.T., J.M. Leblanc, P. Tsai, J.S. Mcninch, D.C. Ward, P.J. Jong, R. Kucherlapati and K.S. Krauter. 1993. Characterization of two chromosome-12 cosmid libraries and development of ITSs from cosmid mapped by FISH. *Genomics* **17**: 682-693

Mukai, Y. 1996. Multicolor fluorescence *in situ* hybridization: A new tool for genome analysis. *In*: Jauhar, P. P. ed, *Methods of Genome Analysis in Plants,* CRC Press, pp. 181-194.

Mukai, Y., Y. Nakahara and M. Yamamoto. 1993. Simultaneous discrimination of three genomes in hexaploid wheat by multicolour fluorescence *in situ* hybridization using total genomic and highly repeated DNA probes. *Genome* **36**: 489-494.

Murata, M., Y. Ogira and F. Motoyoshi. 1994. Centromeric repetative sequences in *Arabidopsis thaliana. Jpn. J. Genet.* **69**: 361-370.

Murata, M. and F. Motoyoshi. 1997. Visualization and mapping of low-copy DNA sequences and genes of *Arabidopsis thaliana* chromosomes by FISH. Inter. Worshop on Analysis and Utility of Plant Chromosomes Information, p 8.

Natarajan, A.T. and N.P. Sarma. 1974. Chromosome banding patterns and the origin of the B genome in wheat. *Genet. Res.* **24**: 103-108.

Neves, N., A. Castilho, M. Silva, J.S. Heslop-Harrison and W. Viegas. 1996. Genomic interactions: gene expression, DNA methylation and nuclear architecture. *Chromosomes Today* **12**: 182-200.

Noda, K. and K.J. Kasha. 1978. A modified Giemsa C-banding technique for *Hordeum* species. *Stain Tech.* **53**: 155-162.

Ohmido, N. and K. Fukui. 1995. Cytological studies of African cultivated rice, *Oryza glaberrima. Theor. Appl. Genet.* **91**: 212-217.

Richard, E. J., A. Vongs, M. Walsh and S. Chao. 1993. Substructure of telomere repeat arrays. *In*; Heslop-Harrison J. S. and R. Flavell, eds, *The Chromosome*, Oxford: BIOS Scientific Publisher, pp 103-114.

Rogowsky, P.M., S. Manning, S.Y. Liu and P. Landridge. 1991. The R173 family of rye-specific DNA sequences: a structural analysis. *Genome* **34**: 88-95.

Schmidt, T., A. Kubis and J.S. Heslop-Harrison. 1995. Analysis and chromosomal location of retrotransposons in sugar beet (*Beta vulgaris* L.): LINEs and *Ty1-copia*-like elements as major components of the genome. *Chromosome Research* **3**: 335-345.

Schwarzacher, T., K. Anamthawat, G.E. Harrison, A. K.M.R. Islam, J.Z. Jia, I.P. King, A.R. Leitch, T.E. Miller, S.M. Reader, W.J. Rogers, M. Shi and J.S. Heslop-Harrison. 1992. Genomic *in situ* hybridization to identify alien chromosomes and chromosome segments in wheat. *Theor. Appl. Genet.* **84**: 778-786.

Schwarzacher, T., A.R. Leitch, M.D. Bennett and J.S. Heslop-Harrison. 1989. *In situ* localization of parental genomes in a wide hybrid. *Annals of Botany* **64**: 315-324.

Schwarzacher, T., A.R. Leitch and J.S. Heslop-Harrison. 1993. DNA: DNA *in situ* hybridization-methods for light microscopy. *In*: Harris, N. and K. J. Oparka, eds. *Plant cell biology: a practical approach*. Oxford: Oxford University Press.

Singer, M.F. 1982. Highly repeated sequences in mammalian genomes. *Int. Rev. Cytol.* **76**: 67-112.

Trask, B.J. 1991. Fluorescence *in situ* hybridization: applications in cytogenetics and gene mapping. *Trends Genet.* **7**: 149-154.

Vershinin, A.V., T. Schwarzacher and J.S. Heslop-Harrison. 1995. The large-scale genome organization of repetitive DNA families at the telomeres of rye chromosomes. *Plant Cell* **7**: 1823-1833.

Vosa, C.G. and P. Marchi. 1972. Quinacrine fluorescence and Giemsa staining in plants. Nature (London). *New Biol.* **237**: 191-192.

Ward, E.J. 1980. Banding patterns in maize mitotic chromosomes. *Can. J. Genet. Cytol.* **22**: 61-67.

Yen, S.T. and W.G. Filion. 1977. Differential Giemsa staining in plants. V. Two types of constitutive heterochromatin in species of *Avena. Can. J. Genet. Cytol.* **19**: 739-743.