Phyton (Horn, Austria) Special issue: "Bioindication ..."	Vol. 36	Fasc. 3	(121)-(128)	15.09.96

Molecular and Cytogenetic Mapping of Plant Genomes

By

R.G. HERRMANN[1)], W. BUSCH[1)], U. HOHMANN[1)], G. WANNER[1)] & R. MARTIN[1)]

Key words: Genome analysis, molecular markers, high-resolution mapping, chromosome microsurgery, synteny, in situ gene mapping, chromosome ultrastructure.

Summary

HERRMANN R.G., BUSCH W., HOHMANN U., WANNER G. & MARTIN R. 1996. Molecular and cytogenetic mapping of plant genomes. - Phyton (Horn, Austria) 36 (3): (121) - (128).

Three principal approaches are used in our laboratory to analyze plant genomes: (i) The construction of high density molecular maps using a refined microdissection procedure to construct chromosomal and subchromosomal libraries. (ii) Synteny analysis: The Aegilops-based deletion mapping system in wheat that has led to the construction of a high-density physical consensus map of wheat. The integration of wheat, barley and oat RFLP markers has proven the colinearity between the wheat A-, B- and D-genomes, the H- genome of barley, and the E-genome of Agropyron. (iii) Gene mapping in situ, and chromosomes at high resolution: Sensitivity enhancement of fluorescence in situ hybridization (FISH) and an efficient preparation of plant chromosomes has been developed. A tandemly amplified repetitive sequence element from microdissected barley chromosomes has enabled the karyotyping of individual Gramineae genomes in a single step. These sequences are also useful for double or triple hybridization experiments. The low copy genes for the storage proteins Sec-1 and Hor-B have been topographically mapped on the satellite of chromosome 1R of rye and the syntenic locus on barley chromosome 1H.

Introduction

The analysis of complex eukaryotic genomes with their huge amounts of DNA and compartmentalized genomes has made substantial progress during the past two decades (e. g., BERNATZKY & TANKSLEY 1989, TANKSLEY & al. 1989, JORDAN 1988). One of the central elements in modern analysis of genomes has been the development of a molecular marker system based on restriction fragment length polymorphisms (RFLPs), mini- or microsatellites, polymerase chain reaction

[1)] Botanisches Institut der Ludwig-Maximilians-Universität München, Menzinger Strasse 67, D-80638 München, Germany

(PCR), random amplified polymorphic DNAs (RAPDs), and arbritrary fragment length polymorphisms (AFLPs) as well as of megabase technologies (JORDAN 1988, SAIKI & al. 1985). Molecular markers possess a highly superior and significantly wider potential for both, basic and applied research than customary phenotypic and isozyme markers used in the analysis of complex genomes, due to their codominant expression (environmental stability) and applicability in practically unlimited number per experiment (high resolution). Molecular markers not only represent a useful tool in genetic fingerprinting, evolutionary and pedigree studies but are valuable also for the characterization of germ plasm stocks. They can further serve for the isolation of developmentally crucial or economically important genes, for instance, genes conferring pathogen or drought resistance. In addition, they offer the opportunity to study quantitative, polygenic traits (QTLs, OSBORN & al. 1987, PATERSON & al. 1988), e.g. crop quality or yield.

In contrast to advances in our understanding of the "DNA thread", the dimensions between the macromolecule on the one hand and the cytological range on the other, and their consequences for the processes of maintenance, evolution and expression of genetic information have largely escaped analysis. Their importance is illustrated by the enormous differences seen in the expression of a discrete transgene which are generally attributed to (ill-defined) "position effects". The recent development of the field emission scanning electron microscope (SEM) in combination with non-radioactive probe hybridization (LICHTER & al. 1990) offer unique perspectives and promises to bridge this second gap in gene and genome research (WANNER & al. 1990, MARTIN & al. 1994, 1995).

Material and Methods

The materials and methods used are described in JUNG & al. 1992 and SCHONDELMAIER & al. 1993 for section I, in HOHMANN & al. 1994, 1995 for section II, and in LEHFER & al. 1993, BUSCH & al. 1994 and MARTIN & al. 1994, 1995 for section III.

Results and Discussion

I. Chromosome-specific DNA libraries; high-resolution RFLP maps

A refined microdissection procedure was applied to chromosomes of telotrisomic or ditelotetrasomic barley lines and telosomic wheat/barley addition lines or sugar beet material (JUNG & al. 1992, SCHONDELMAIER & al. 1993) which are all well discernible by their morphology in mitotic metaphase spreads (Fig. 1). Microdissection and construction of chromosomal or subchromosomal libraries have been significantly improved by the use of (i) mitotic metaphase spreads which is time-saving compared to that of meiotic preparations since the plant material is available within one week, (ii) synchronized meristematic root tip tissue from which metaphase spreads of appropriate quality can be readily obtained, (iii)

fixation times of less than three minutes that are possible with a novel drop-spread technique, (iv) a specific vector (LÜDECKE & al. 1989) in combination with nl-scale PCR that allows to clone the DNA of 10 or less chromosomes or chromosome segments, and (v) an instrumental set-up which allows to select suitable metaphases, to store the coordinates of their positions, and to address sequentially individual metaphases or the collection drop automatically (SCHONDELMAIER & al. 1993). The outlined approach is clearly superior to conventional microcloning techniques. It may serve to establish subchromosomal libraries for entire genomes and to construct high-resolution maps from discrete genome regions which can greatly facilitate gene isolation and QTL studies.

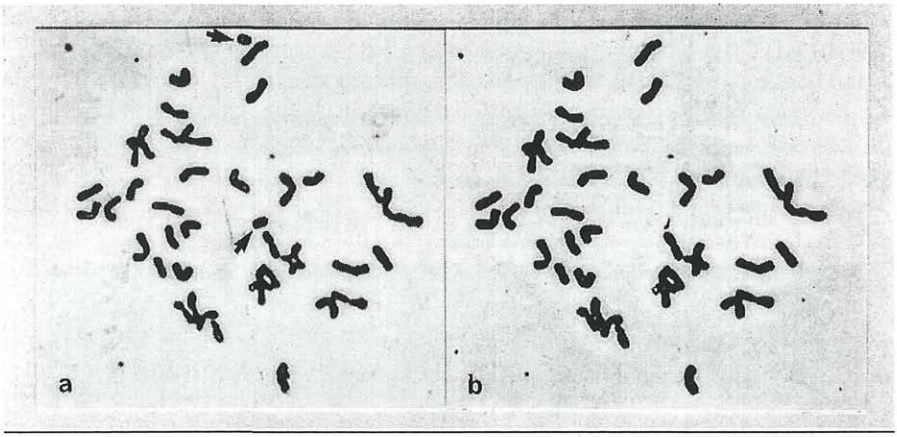

Fig. 1. Metaphase of the disomic barley/wheat addition line 3HL (42+2t) (a) before and (b) after the excision of the two telosomes (arrows in A).

II. Synteny analysis

One of the most intriguing findings in the genome analysis is the detection of a conserved linear order of genes along a chromosome. This phenomenon, designated synteny, is found among related species, for example the *Triticeae*. The identification of a region with synteny in one genome can thus facilitate the isolation of genes, for instance resistance genes from another organism in a time- and money-saving way. We have used the potential of this approach to clarify the synteny between wheat and barley by analyzing *Aegilops*-induced terminal deletion lines in wheat with RFLP markers from different Triticeae species (HOHMANN & al. 1994, 1995). The high-density physical map for homoeologous group 7 chromosomes based on 54 deletion lines and over 100 RFLP markers has proven the synteny and microsynteny of homoeologous chromosomes from the A-, B-, and D-genome of wheat as well as of the genome of barley (Fig. 2.)

The construction of a physical map and the comparison of physical and genetic maps of wheat and barley has uncovered significant differences between genetic and physical distances. The genetic distance in proximal chromosome

regions is larger than in terminal regions. Therefore, the recombination in Gramineae genomes is mainly restricted to the telomeric region, whereas recombination in the proximal region appears to be suppressed (HOHMANN & al. 1994, 1995).

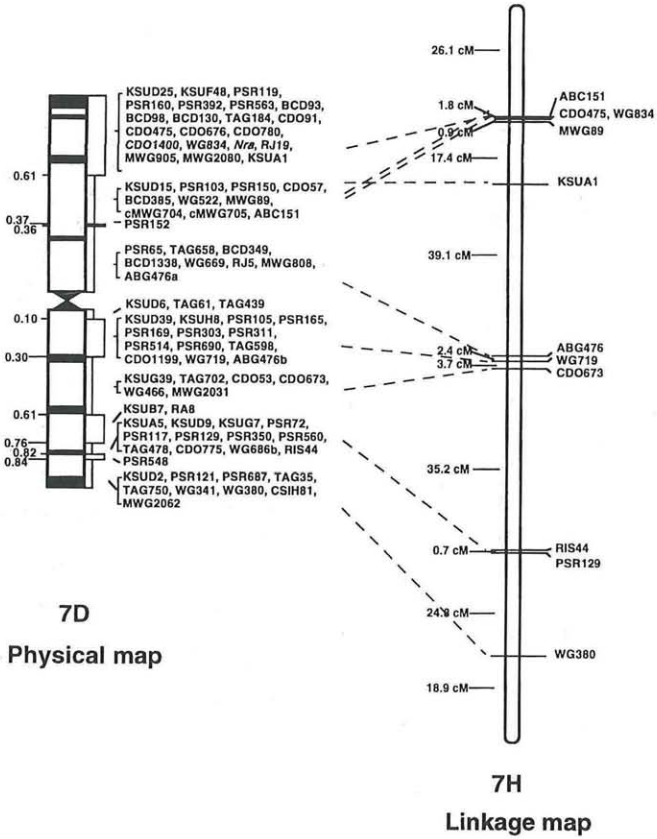

Fig. 2. Comparison of the physical map of chromosome 7D of wheat (left) and linkage map of barley chromosomes 7H (right). The linkage map includes only markers that were localized on the physical map.

III. Chromosomes at high resolution; gene mapping in situ

The relationships between topographic, genetic and physical distances and their impact on genetic events (e.g., recombination frequency), gene expression, and perhaps evolution are largely unknown. It is therefore highly desireable to

unravel details of chromosome structure and gene arrangement at the (ultra)structural level.

Using a B-hordein-specific (i.e., low-copy) probe and FITC as fluorescence dye, we have recently mapped the Hor2 locus near the telomer of chromosome 1HS of barley (Fig. 3; LEHFER & al. 1991, 1993). An improved in situ hybridization procedure combining synchronization of root meristems, enzymatic removal of cell walls and the use of the advantageous fluorescent dye Cy3 has resulted in a significant increase of well spread metaphases, and to an enhanced frequency and intensity of hybridization signals (BUSCH & al. 1994). When compared to FITC, Cy3 exhibits higher resistance to fading and a stronger signal intensity. Under appropriate conditions, practically all metaphases display signals, more than 60% of them on both chromatides of the gene-carrying chromosomes. This fulfils a crucial criterion for the construction of topographic maps (LICHTER & al. 1990), preferably with mapped, chromosome-specific (RFLP) probes, and opens the perspective to tackle the relationship between the topographic position of a gene and its impact on genetic events or expression. The technique is not restricted to metaphase chromosomes, but should also allow the detection of single-copy sequences.

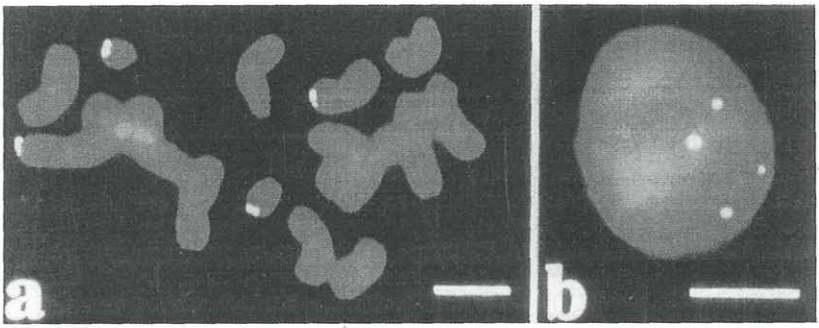

Fig. 3. Metaphase plate of a ditelosomic addition line of barley (14 +2t) after hybridization with the B-hordein probe pBSC21. (a)The signals are located near the telomeres on both chromosomes 1H as well as on both telosome chromosomes 1Hs and visible on both chromatids. (b) Interphase nuclei of this line displayed also four signals in G1.

In spite of satisfactory progress to visualize and map individual or even several plant genes with the "intermediate" approach of the light microscopic hybridization in situ using fluorescent dyes, attempts were made to enter high resolution in the study of these questions. Modern high resolution field emission scanning electron microscopy in combination with various detection systems (PETERS 1986) has now reached the "nanometer scale" of resolution. We have outlined relevant technical aspects of these approaches in four recent publications (WANNER & al. 1990, LEHFER & al. 1991, MARTIN & al. 1994). These approaches should provide access to yet largely unexplored topics such as (i) the arrangement of chromosomes or chromatin within the nuclear matrix, (ii) chromosomal fine

structure at all stages of the cell cycle, (iii) the localization of individual genes including transgenes and, perhaps, (iv) to expression structures of genes. Satisfactory progress has been made in the preparation of plant specimen (Fig. 4, WANNER & al. 1990, MARTIN & al. 1994). The various structures seen or deduced include supercoiling of 12-nm fibres (nucleosomes as "beads on a string") into a 35-nm fibre (solenoid), and other stages of packing in condensed, condensing or decondensing chromatin or long fibrillar arrangement with little or no drilling. A newly developed technique of in situ hybridization for gene localization at the ultrastructural level using colloidal gold for the signal detection (MARTIN & al. 1995) may help to answer the outlined aspects.

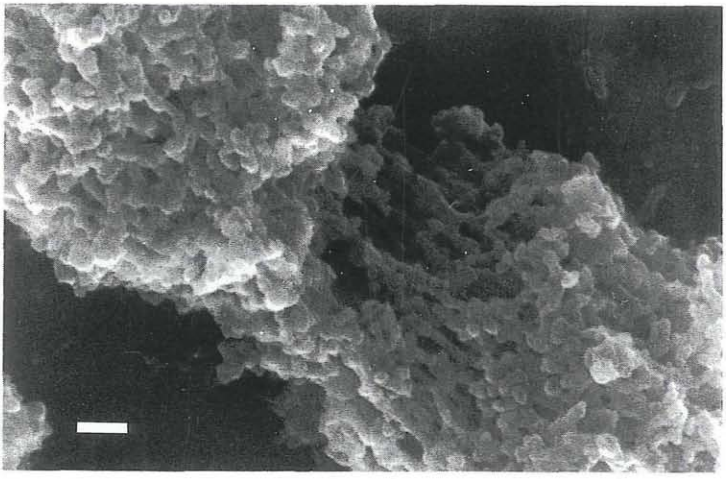

Fig. 4. Scanning electron micrograph of a centromeric region of a wheat chromosome. Bar: 100 nm.

Acknowledgements

This work was supported by grants from the Bundesministerium für Forschung und Technologie (0318990F), the Deutsche Forschungsgemeinschaft (SFB 184 and Ma 1673/1-1) and the Fonds der Chemischen Industrie.

References

BERNATZKY R. & TANKSLEY S. D. 1989. Restriction fragments as molecular markers for germplasm evaluation and utilization. In: BROWN A. D. H., FRANKEL O. H., MARSHALL D. R., WILLIAMS J. T. (eds.), The use of plant genetic resources (pp. 353-362). - Cambridge University Press, Cambridge

BUSCH W., MARTIN R. & HERRMANN R. G. 1994. Sensitivity enhancement of fluorescence in situ hybridization on plant chromosomes. - Chromosome Res. 2: 15-20.

HOHMANN U., ENDO T. R., GILL K. S. & GILL B. S. 1994. Comparison of genetic and physical maps of group 7 chromosomes from *Triticum aestivum* L. Mol. Gen. Genet. 245: 644-653

--, GRANER A., ENDO T. R., GILL B. S. & HERRMANN R. G. 1995. Comparison of wheat physical maps with barley linkage maps for group 7 chromosomes. - Theor. Appl. Genet., in press

JORDAN B. R. 1988. Megabase methods: a quantum jump in recombinant DNA techniques. - Bio Essays 8: 140-145

JUNG C., CLAUSSEN V., HORSTHEMKE B., FISCHER F. & HERRMANN R. G. 1992. A DNA library from an individual *Beta patellaris* chromosome conferring nematode resistance obtained by microdissection of meiotic metaphase chromosomes. - Plant Mol. Biol. 20: 503-511

LEHFER H., WANNER G. & HERRMANN R. G. 1991. Physical mapping of DNA sequences on plant chromosomes by light microscopy and high resolution scanning electron microscopy. - In: HERRMANN R. G., LARKINS B. (eds.), Plant molecular biology 2 (pp. 277-284). - Plenum Publ. Corp, New York

--, BUSCH W., MARTIN R. & HERRMANN R.G. 1993. Localization of the B-hordein locus on barley chromosomes using fluorescense in situ hybridization. - Chromosoma 102:428-432

LICHTER P., CHANG TANG C. & CALL K. 1990. High-resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. - Science 24: 64-69

LÜDECKE H. J., SENGER G., CLAUSSEN U. & HORSTHEMKE B. 1989. Cloning defined regions of the human genome by microdissection of banded chromosomes and enzymatic amplification. - Nature 338: 348-350

MARTIN R. & HERRMANN R. G. 1991. High-resolution scanning electron microscopy of plant chromosomes. - Chromosoma 100: 103-109

--, BUSCH W.& HERRMANN R.G. 1994. Efficient preparation of plant chromosomes for high resolution scanning electron microscopy. - Chromosome Res. 2: 411-415.

--,-- ,-- & WANNER G. 1995. In situ hybridization and signal detection by high resolution scanning electron microscopy. - Kew Chromosome Conf. 1995, in press

OSBORN T. C., ALEXANDER D. C. & FOBES J. F. 1987. Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit. - Theor. Appl. Genet 73: 350-356

PATERSON A. H., LANDER E. S., HEWITT J. D., PETERSON S., LINCOLN S. E. & TANKSLEY S. D. 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment polymorphisms. - Nature 335: 721-726

PETERS K. R. 1986. Metal deposition by high-energy sputtering for high magnification electron microscopy. - In: KOEHLER J. K. (ed.), Advanced techniques in biological electron microscopy, vol. III (pp 101-166). Springer, Berlin

SAIKI R. K., SCHARF S., FALCONA F., MULLIS K. B., HORN G. T., ERLICH H. A. & ARNHEIM N. 1985. Enzymatic amplification of ß-globulin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. - Science 230: 1350-1354

SCHONDELMAIER J., MARTIN R., JAHOOR A., HOUBEN A., GRANER A., KOOP H. U., HERRMANN R. G. & JUNG C. 1993. Microdisscetion and microcloning of the barley (*Hordeum vulgare* L.) chromosome 1HS. - Theor. Appl. Genet .86: 629-636

TANKSLEY S. D., YOUNG N. D., PATERSON A. H. & BONIERBALE M. W. 1989. RFLP mapping in plant breeding: new tools for an old science. - Biotechnology 7: 257-264

WANNER G., FORMANEK H. & HERRMANN R. G. 1990. Ultrastructure of plant chromosomes by high-resolution scanning electron microscopy. - Plant Mol Biol Rep 8: 224-236