



MOLECULAR CYTOGENETIC ANALYSES IN WHEAT AND RELATED SPECIES

Main points of the thesis

Gabriella Linc

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Title of the PhD programme:
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Branch of science:
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Head of programme:
Dr László Heszký
Corresponding Member of the Hungarian Academy of Sciences
Head of the Department of Genetics and Plant Breeding,
Szent István University, Gödöllő

Supervisor:
Dr Márta Molnár-Láng, Ph.D.
Senior Research Associate
Agricultural Research Institute of the Hungarian Academy of Sciences,
Martonvásár

1. Introduction

Wheat production is of decisive importance in Hungarian agriculture. In recent years the sowing area of wheat has averaged 1.2 million hectares. Over the last few decades the genetic variability of common wheat (*Triticum aestivum* L.) has declined substantially and the majority of the wheat varieties currently cultivated stem from similar genetic sources, making them particularly vulnerable to pathogens and other stress factors. It is therefore essential to broaden the gene pool of wheat.

Species related to common wheat have broad genetic variability for most useful agronomic traits (disease resistance, salt, heat and drought tolerance, winter hardiness, quality, etc.). Traditional interspecific and intergeneric crosses within the Triticeae can be used to transfer genes controlling useful agronomic traits from related species into the genome of common wheat. If the incorporation of these genes from alien species into the wheat genome is to be efficient and controllable it is essential to make detailed studies on the degree of relationship between the various plant species and varieties and on their genome structures. In recent decades many results have been achieved in this field. Basic breeding material with more favourable agronomic properties have been developed carrying genes or chromosome segments from the *Aegilops*, *Secale*, *Agropyron* and *Triticum* genera. Translocations, and addition and substitution lines developed using various methods are used as initial populations by plant breeders.

Whether basic genetic stocks are developed through traditional crosses or using various gene manipulation techniques, it is important to elaborate techniques for the control of gene transfer. Certain molecular techniques can be used to obtain detailed information on the physical location in the genome of DNA fragments measuring only a few base pairs in length. Molecular cytogenetic techniques make it possible to study the desired DNA segments within the chromosome itself. Continual improvements in the methods and the combination of various molecular cytogenetic techniques allow the physical location of individual genes or groups of genes to be observed on the chromosomes of the given genome.

2. Research aims

- Species belonging to the *Aegilops* genus possess numerous favourable agronomic traits which can be incorporated into the common wheat

genome by means of traditional interspecific hybridisation. The detailed study of the genomic structure of species related to wheat is a basic criterion for efficient gene transfer. The chromosome karyotype of the diploid *Aegilops* species has been published in the literature, but little information is available on the genomic structure of the tetraploid species. **One aim was thus to investigate the genomic structure of the wild tetraploid wheat species *Aegilops cylindrica* Host. using molecular cytogenetic methods and to record differences and similarities between the genomes of *Ae. cylindrica* and the two donor diploid species at the chromosome level.**

- Addition lines developed in a wheat background from wheat × barley crosses contain a barley chromosome pair in addition to the wheat genome. **A further aim was thus to identify the barley chromosomes in wheat-barley disomic addition lines developed in Martonvásár using molecular cytogenetic methods.**
- If satisfactory techniques are used (hormone treatment on flowers, controlled environmental conditions, embryo culture) the wheat and barley genomes can be hybridised at very low frequency. In the progeny of hybrids regenerated in tissue culture chromosome fractures may occur during cell division, giving rise to translocations involving a reorganisation of the various DNA segments between the two genomes. **The aim of the present experiments was to use molecular cytogenetic methods to identify the wheat and barley chromosome segments in wheat-barley translocations previously developed in Martonvásár.**
- A large proportion of the wheat varieties grown in Hungary contain the 1BL/1RS wheat-rye translocation. The rye chromosome arm incorporated into the wheat genome carries a gene complex responsible for favourable agronomic traits, but may lead to a deterioration in the flour quality. Both geneticists and breeders are interested in the possibility of developing new breeding stocks which retain the useful gene loci while eliminating traits having a negative influence on flour quality, with the final purpose of developing new wheat varieties. **Martonvásár wheat varieties with different breadmaking properties were examined using molecular cytogenetic methods in order to determine the size of the 1BL/1RS translocation and its role in determining the flour quality of the varieties. It was assumed that the chromosome section having a negative effect on quality traits had been eliminated in varieties**

with better breadmaking quality and that the translocated chromosome arm would be shorter in these cases.

3. Materials and methods

Plant material

In order to investigate the genome structure of the tetraploid wild wheat species *Aegilops cylindrica* Host., lines originating from five different locations were examined:

TA2201	France
TA2202	Romania
TA2203	Iran
TA2204	Armenia
TA2205	Of unknown origin

The barley chromosomes were identified in the following wheat-barley disomic addition lines:

1. Disomic addition lines selected from the progeny of a Chinese Spring-Betzes wheat-barley hybrid (previously developed in Martonvásár) multiplied in tissue culture, backcrossed twice with a Japanese winter wheat variety (Asakaze komugi) and self-pollinated for several generations.
2. Disomic addition lines selected from the progeny of a Chinese Spring-Betzes wheat-barley hybrid (previously developed in Martonvásár) multiplied in tissue culture, backcrossed twice with the Mv9kr₁ wheat line, pollinated with maize (cv. Seneca), treated with colchicine and self-pollinated for two generations.

Plants carrying wheat-barley translocations, selected from the progeny of the Chinese Spring-Betzes wheat-barley hybrid previously developed in Martonvásár after regeneration in tissue culture and backcrossing with the winter wheat line Mv9kr₁ were multiplied, after which mitotic chromosome preparations were made from root tips undergoing cell division in order to identify the translocations.

Fifteen Martonvásár wheat genotypes with different breadmaking quality were examined, namely: Martonvásári (Mv) 16, Mv 17, Mv 19, Mv 21,

Mv 23, Mv 25, Optima, Fatima, Magdaléna, Emma, Pálma, Irma, Vilma, Madrigál, Mv-1485.

DNA clones

The DNA clone **pTa71** is 9.05 kb in length and carries segments from wheat ribosomal genes (18S, 5.8S and 25S rDNA) cloned to the pUC19 vector (Gerlach and Bedbrook 1979).

The DNA clone **pTa794** is 410 bp in length and carries a segment from the wheat ribosomal genes (5S rDNA) incorporated in the pBR322 plasmid (Gerlach and Dyer 1980).

The DNA clone **pAs1** is a 1 kb fragment isolated from *Ae. tauschii* Coss. and inserted into the pUC8 plasmid, carrying a repetitive, non-coding DNA sequence (Rayburn and Gill 1986a).

The DNA probe **pSc119**, isolated from rye, is 120 bp in length and carries a DNA segment with repetitive sequences, incorporated into the pBR322 plasmid (Bedbrook et al. 1980).

Methods

Feulgen staining

Giemsa C-banding

Sequential Giemsa N-banding and genomic *in situ* hybridisation (GISH)

Genomic *in situ* hybridisation (GISH)

Fluorescent *in situ* hybridisation (FISH)

3. Results

Molecular cytogenetic analysis of the genome structure of *Aegilops cylindrica* Host.

Five lines of *Aegilops cylindrica* Host. originating from different geographical locations were examined by means of genomic *in situ* hybridisation and by mapping various DNA probes on the individual chromosomes (FISH). Conclusions were generally drawn after analysing 10–12 cells from each line.

The GISH analysis revealed that three of the lines carried intergenomic translocations between the C^C and D^C genomes, while no reorganisation of the chromosomes was observed between the two genomes in the case of lines TA2204 and TA2205.

In line TA2203, which was of Iranian origin, a homozygous reciprocal translocation was identified between one of the long acrocentric chromosomes of the C^C genome and a submetacentric chromosome of the D^C genome. The translocational breakpoint was at around 0.85 on the long arm of the C^C genome chromosome (85 % of the chromosome arm was labelled after genomic *in situ* hybridisation), indicating that the distal end of the arm (15 %) was of D^C genome origin. The translocational breakpoint on the submetacentric D^C genome chromosome involved in the intergenomic exchange could be seen at 0.70 on the short arm (70 % of the arm was unlabelled), indicating that the distal end of the arm (30 %) originated from the C^C genome.

Judging by the size of the translocated chromosomes and the ratio of the chromosome arms it is highly probable that the exchange of genetic material occurred between the B or D chromosomes of the C^C genome and the 3D or 5D chromosomes of the D^C genome.

Two different types of chromosome composition were observed in plants of the *Ae. cylindrica* Host. genotype from Romania, line TA2202.

Three of the plants carried a homozygous translocation between the long arm of a C^C genome chromosome with a satellite and the long arm of a submetacentric D^C genome chromosome. The translocation breakpoint was at around 0.85 on the long arms of both the C^C and the D^C genome chromosomes, so around 15 % of the long arms of these chromosomes originated from the other genome. The C^C genome chromosome involved in the translocation was chromosome A, while it could be assumed from

the size of the chromosome and the ratio of the chromosome arms that the translocated chromosome from the D^C genome was either 3D or 5D.

Five further plants of line TA2202 exhibited no intergenomic translocation between the chromosomes of the C^C and D^C genomes in the course of genomic *in situ* hybridisation.

Of the eight plants examined from the French *Ae. cylindrica* Host. line TA2201, six carried a double homozygous reciprocal translocation. The genomic reorganisation involved both arms of the 1C^C (A) and 5D^C chromosomes, both of which have satellites.

The satellite and the distal end of the short arm of the 1C^C chromosome pair remained unlabelled after GISH, indicating that these chromosome segments originated from the D^C genome. The whole of the satellite on the short arm of the chromosome was of D^C genome origin, and the translocation breakpoint could be seen at 0.70 on the long arm. By contrast the distal end of the arms of the 5D^C chromosome pair were distinctly labelled after genomic *in situ* hybridisation, showing that these chromosome segments originated from the C^C genome. The translocation breakpoints were at 0.85 on the short arm and 0.70 on the long arm.

The segments of the wheat ribosomal genes which exhibited deviations were mapped by means of fluorescent *in situ* hybridisation using a combination of DNA probes pTA71 and pTa794.

The DNA probe pTA71, labelled with tetramethyl-rhodamine, exhibited a total of six hybridisation signals of various strength (3 gene loci) on the chromosomes of line TA2204. The strongest fluorescent signal was found in the NOR region of the short arm of the 1C^C chromosome pair (loci 18S–5.8S–25S). Two smaller, less strongly stained hybridisation signals were observed at the terminal end of the short arm of the 5D^C chromosome pair and at the distal end of the short arm of the 5C^C chromosome pair.

Hybridisation with the DNA probe pTa794, labelled with fluorescein-11-dUTP, mapped a total of eight 5S rDNA hybridisation signals (4 gene loci). Three of these loci were located terminally compared with the NOR region of the 1C^C, 5D^C and 5C^C chromosome pairs. The fourth locus was observed at the distal end of the short arm of the 1D^C chromosome pair.

Probes pSc119 and pAs1, which contain repetitive DNA sequences, were used with the sequential FISH technique to identify the individual chromosomes.

The pSc119 DNA probe was labelled with tetramethyl-rhodamine. After FISH carried out with the pAs1 clone, which was labelled with

fluorescein-11-dUTP and which carried a repetitive DNA sequence from *Ae. tauschii* Coss., the chromosomes of the D^C genome exhibited specific labelling on the basis of which each chromosome could be identified. FISH analysis of the *Ae. cylindrica* Host. lines TA2201, TA2202 and TA2203 resulted in hybridisation signals of similar strength and location to those described above.

Demonstration and identification of new wheat-barley disomic addition lines in the winter wheat genome

In the experiments two wheat-barley disomic addition lines developed in a winter wheat background were identified by means of Giemsa C-banding. The chromosome numbers of the addition lines were checked by Feulgen staining in metaphase I of meiosis and in mitosis. The barley chromosomes could be clearly distinguished from the wheat chromosomes after genomic *in situ* hybridisation.

The C-band karyotype of barley was prepared for the comparative analysis of the patterns of the individual barley and wheat chromosomes. The banding of the chromosomes on the karyotype of the Betzes barley variety was similar to that of the karyotypes reported after C-banding for other barley genotypes.

On the basis of their specific patterns, the seven chromosome pairs of the barley variety Betzes could be clearly distinguished from the wheat chromosomes after C-banding. Each of the chromosomes exhibited various centromeric, close to centromeric and numerous interstitial bands. The individual chromosomes of the two new wheat-barley disomic addition lines were identified on the basis of karyotypes reported by Gill et al. (1991) for wheat and Kakeda et al. (1991) for barley, as well as the barley karyotype developed in Martonvásár.

A barley chromosome pair in the disomic addition line originating from the combination (*Triticum aestivum* cv. Chinese Spring × *Hordeum vulgare* cv. Betzes)*T. aestivum* cv. Asakaze komugi²)×⁵ was identified as 2H after Giemsa C-banding. The 2H barley chromosome does not possess any centromeric bands, while a band close to the centromere and an interstitial band could be observed on each arm. The band on the long arm was in the distal position, while a pale terminal band could be seen on the short arm.

In the case of the disomic addition line developed from the (*T. aestivum* cv. CS × *H. vulgare* cv. Betzes(*T. aestivum* cv. Mv9kr₁²)Seneca)×²

combination C-banding indicated that the plants carried the 6H barley chromosome pair in addition to the 42 wheat chromosomes. A pronounced centromeric band and other bands in the NOR region were observed after C-banding on the 6H barley chromosome. Less strongly stained bands were found in a proximal position in the region close to the centromere on both arms. Terminal bands were identified on both the short and long arms of the 6H barley chromosome.

Demonstration and identification of wheat-barley translocations by means of sequential N-banding and GISH

Sequential N-banding and GISH were used in an attempt to identify the five different translocations demonstrated between the progeny of the wheat×barley hybrid previously developed in Martonvásár after backcrossing with wheat.

One of the genomic translocations (Type I) involved a metacentric wheat chromosome: approx. half (FL 0.50) of the short arm of the chromosome exhibited bright staining after *in situ* hybridisation, indicating that it originated from the barley genome. The second type of translocation (Type II) was located on the long arm of a barley chromosome with a satellite (5H or 6H). The translocational breakpoint was observed on the long arm of the barley chromosome; 20 % of the distal end of this arm was of wheat origin (FL 0.20). Plants carrying this translocation on the satellited barley chromosome did not survive.

A centric fusion was demonstrated in the progeny of two plants: the short arm of the chromosome originated from barley and the long arm from the wheat genome (CF). Plants carrying this Robertson translocation were viable and fertile.

The Type III translocation involved a barley chromosome with a satellite. The translocational breakpoint was on the long arm, some 50 % of which derived from the barley genome. The short arm was of wheat origin, but much of the arm was missing. Two of the three plants carrying this translocation proved to be fertile.

The Type IV translocation involved a metacentric wheat chromosome and the translocational breakpoint was observed on the short arm (FL 0.80). Around 20 % of the distal end of the chromosome arm originated from the barley genome and exhibited bright staining after hybridisation.

The cells examined from each of the plants carrying these five types of translocations also contained further telocentric or complete barley chromosomes.

The chromosomes of the plants carrying the translocations were analysed after sequential N-banding and genomic *in situ* hybridisation. The translocated chromosomes involved in the Type I, II, III and IV translocations could not be identified as they did not exhibit characteristic N bands, but the chromosomes involved in the Robertson translocation gave chromosome- and species-specific patterns in the course of N-banding. The short arm of the chromosome exhibited intense heterochromatic staining at the centromere. On the long arm bands were identified at the centromere and at interstitial locations after staining. On the basis of the patterns and specific location of the individual bands it was established that the chromosome carrying the translocation was formed from the long arm of the 2B wheat chromosome and the short arm of the 4H barley chromosome. This was later confirmed by Giemsa C-banding.

GISH analysis of Martonvásár wheat varieties carrying the 1BL/1RS wheat-rye translocation

Fifteen wheat varieties bred in Martonvásár and possessing different breadmaking quality were analysed by means of genomic *in situ* hybridisation in order to determine whether there was any difference between the varieties in the size of the 1BL/1RS wheat-rye translocation. For each of the 15 Martonvásár wheat genotypes tested, the presence of the 1BL/1RS translocation was examined in 6–12 cells from each of 3–5 plants. The length and ratio of the chromosome arms were also recorded. Pairing between the chromosome carrying the 1BL/1RS wheat-rye translocation and the wheat chromosomes was studied in fifty pollen mother cells (PMCs) in anther preparations.

Under normal conditions the wheat chromosomes form 21 ring bivalent configurations when pairing in Metaphase I of meiosis, though occasionally open rod bivalents are also observed.

In 78 % of the cells examined the 1RS rye chromosome formed a closed ring with the wheat chromosome, while in 22 % rod bivalents involving the rye arm were observed. In one of the fifty cells a trivalent chromosome configuration was observed involving the 1RS rye arm.

In preparations made from dividing root tips genomic *in situ* hybridisation (GISH) showed that the 1BL/1RS wheat-rye translocation was of a similar size in the all the varieties tested. The NOR region, and thus the satellite, could be clearly distinguished on the 1RS rye chromosome arm. The translocational breakpoint was found to be at the centromere in all cases. Genetic rearrangements may have occurred within the 1RS rye arm involved in the translocation, but this could not be demonstrated by means of GISH. The results are in line with those published in the literature; many authors have recorded similar observations in the course of *in situ* hybridisation studies on different wheat lines.

5. New scientific results

1. The genome structure of the tetraploid wild wheat species *Aegilops cylindrica* Host., a species related to common wheat, was analysed using molecular cytogenetic methods. In the course of studies on *Ae. cylindrica* Host. genotypes of different origins three types of intergenomic translocation were identified.
2. The pSc119, pAs1, pTa71 and pTa794 DNA clones were mapped on the chromosomes of the species *Ae. cylindrica* Host.
3. C-banding was used to identify the 2H and 6H barley chromosomes in wheat-barley disomic addition lines developed in Martonvásár in a winter wheat background.
4. Among the five different wheat-barley translocations developed from wheat-barley hybrids in Martonvásár, the wheat and barley chromosome arms involved in the centric fusion (CF) were identified by means of sequential N-banding and genomic *in situ* hybridisation. The CF was found to have taken place between the long arm of the 2B wheat chromosome and the short arm of the 4H barley chromosome.
5. The size of the 1BL/1RS wheat-rye translocation in fifteen wheat varieties bred in Martonvásár was determined by means of genomic *in situ* hybridisation. The translocation was found to be stable; the whole of the 1RS rye arm was found in the genome of all the varieties examined.

Conclusions and recommendations

Intergenomic translocations were identified by means of genomic *in situ* hybridisation in three of five *Aegilops cylindrica* Host. lines of different geographical origin. The three translocations involved different chromosomes in the genomes of the various lines and could probably be attributed to the different geographical and microclimatic conditions. Further detailed molecular cytogenetic analysis of the genome structure of the tetraploid and hexaploid *Aegilops* species is definitely justified, since wild wheat species form an important gene reserve for wheat breeding. They can only be used efficiently if the precise structure of the genomes is known.

Hybridisation between the wheat and barley genomes makes it possible to incorporate certain favourable characteristics from barley into wheat. The development of addition lines allows genes responsible for agronomically useful traits to be transferred directly into the genome of common wheat. In later stages these addition lines could be suitable for the development of planned translocations. The new disomic addition lines identified in the present work contain barley chromosomes in a winter wheat background. It is intended to apply molecular cytogenetic methods to select further addition lines carrying barley chromosome pairs. The expression of the genes located on the added barley chromosomes in the wheat genome will be analysed in the near future to determine their effect on the morphological and agronomic properties of wheat.

The formation of translocations was observed in the progeny of wheat-barley hybrids developed in Martonvásár after regeneration in tissue culture. Compensating translocations make it possible to study the homoeology between the wheat and barley chromosomes. The translocations which have not yet been identified will be analysed in detail with the use of specific DNA markers. Work has begun on the use of microsatellite markers and the mapping of specific DNA probes originating from the barley genome on the chromosomes carrying translocations. It is hoped to determine the effect of the translocated barley chromosome segments on the agronomic properties of wheat. The translocations can also be used in the physical mapping of barley. It is planned to develop new translocations in order to incorporate useful traits (e.g. earliness) from the barley genome into wheat.

In the course of genomic *in situ* hybridisation on Martonvásár wheat varieties containing the 1BL/1RS wheat-rye translocation it was found

that all the varieties carried the whole 1RS rye arm. The ω -secalin allele which has an unfavourable effect on the breadmaking quality of flour is probably still present in the chromosome pool of the genotypes examined. The differing breadmaking quality of the varieties must have evolved due to the variability of the genetic background, or it could be that intragenomic translocations have taken place within the 1RS rye chromosome arm. Using molecular cytogenetic methods (FISH) species- and chromosome-specific DNA clones can be mapped on the translocated chromosome. Based on the specific location of the individual DNA probes, rearrangements within the chromosome can be demonstrated and traced.

References

- Bedbrook, J.R., Jones, J., O'Dell, M., Thompson, R.J., Flavell, R.B. 1980.** A molecular description of telomeric heterochromatin in *Secale* species. *Cell* 19:545-560.
- Gerlach, W.L., Bedbrook, J.R. 1979.** Cloning and characterization of ribosomal RNA genes from wheat and barley. *Nucleic. Acids. Res.* 7:1869-1885.
- Gerlach, W.L., Dyer, T.A. 1980.** Sequence organization of the repeat units in the nucleus of wheat which contains 5S rRNA genes. *Nucleic. Acids. Res.* 8:4851-4865.
- Gill, B.S., Friebe, B., Endo, T.R. 1991.** Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum* L.). *Genome* 34:830-839.
- Kakeda, K., Fukui, K., Yamagata, H. 1991.** Heterochromatic differentiation in barley chromosomes revealed by C- and N-banding techniques. *Theor. Appl. Genet.* 81:144-150.
- Rayburn, A.L., Gill, B.S. 1986a.** Isolation of a D genome specific repeated DNA sequence from *Aegilops squarrosa*. *Plant.Mol.Biol. Rep.* 4:102-109.

Publications by Gabriella Linc on the subject of the thesis

Reviewed journals

- Molnár-Láng, M., **Linc, G.**, Sutka, J. 1996. Transfer of the recessive crossability allele *kr1* from Chinese Spring into the winter wheat variety Martonvásári 9. *Euphytica* 90: 301-305.
- Linc, G.**, Karsai, I., Molnár-Láng, M., Bedő, Z. 1996. Comparison of RFLP probes and RAPD primers for studying genetic diversity in barley. *Cereal Research Communications* 24: 283-290.

Molnár-Láng, M., Kőszegi, B., **Linc, G.**, Galiba, G., Sutka, J. 1996. Chromosome instability of wheat/barley ditelosomic addition lines in tissue culture. *Cereal Research Communications* 24: 275-281.

Molnár-Láng, M., Kőszegi, B., **Linc, G.**, Sutka, J. 1996. Detection of wheat (*Triticum aestivum* L.)/ *Triticum timopheevii* Zhuk. addition, substitution and wheat/rye translocation using Giemsa C-banding and *in situ* hybridisation. *Növénytermelés* 45: 237-245.

Molnár-Láng, M., **Linc, G.**, Sutka, J. 1999: Production and molecular cytogenetic identification of wheat-barley hybrids and translocations. *Journal of Plant Biotechnology* 1: 8-12.

Linc, G., Friebe, B., Kynast, R., Molnár-Láng, M., Kőszegi, B., Sutka, J., Gill, B.S. 1999. Molecular cytogenetic analysis of *Aegilops cylindrica* Host. *Genome* 42: 497-503.

Molnár-Láng, M., **Linc, G.**, Friebe, B., Sutka, J. 2000. Detection of wheat-barley translocations by genomic *in situ* hybridization in derivatives of hybrids multiplied *in vitro*. *Euphytica* 112: 117-123.

Molnár-Láng, M., **Linc, G.**, Logojan, A., Sutka, J. 2000. Production and meiotic pairing behaviour of new hybrids of winter wheat (*Triticum aestivum*)×winter barley (*Hordeum vulgare*). *Genome* 43: 1045-1054

Kőszegi, B., **Linc, G.**, Juhász, A., Láng, L., Molnár-Láng, M. 2000. Occurrence of the 1RS/1BL wheat-rye translocation in Hungarian wheat varieties. *Acta Agr. Hung.* 48: 227-236.

Oral presentations, posters, other journals

Molnár-Láng, M., **Linc, G.**, Sutka, J. 1994. Transfer of the *kr1* crossability gene from the wheat variety Chinese Spring into Martonvásári 9.

I. Magyar Növénytermesztési Tudományos Napok. Budapest, 1994. január 11-12. pp 61.

Linc, G., Karsai, I., Molnár-Láng, M. 1995. Use of the RFLP method for the analysis of wheat-barley hybrids. II Magyar Növénytermesztési Tudományos Napok. Budapest, 1995. január 16-17. pp 52.

Molnár-Láng, M., **Linc, G.** 1996. Demonstration of species-alien chromosomes and chromosome segments in wheat by means of *in situ* hybridisation. *Martonvásári* 2: 14.

Molnár-Láng, M., Kőszegi, B., **Linc, G.**, Sutka, J. 1996. Use of chromosome identification methods for the characterisation of interspecific hybrids and their derivatives. III. Növénytermesztési Tudományos Napok. Budapest, 1996. pp 67.

Molnár-Láng, M., **Linc, G.**, Sutka, J. 1997. GISH analysis of the derivatives of wheat×barley hybrids. In Proc. Int. Symp. "Current Topics in Plant Cytogenetics Related to Plant Improvement" February 21-22, 1997. Tulln, Austria

Linc, G., Molnár-Láng, M. 1997. Molecular cytogenetic analysis of wild wheat species. *Martonvásár* 2:16.

Molnár-Láng, M., **Linc, G.** 1997. Identification of the chromosomes of different cereal genomes by genomic *in situ* hybridization. European Wheat Aneuploid Co-operative Meeting, June 16-19, 1997. Viterbo, Italy, EWAC Newsletter

Linc, G., Friebe, B., Kynast, R., Molnár-Láng, M., Kőszegi, B., Sutka, J., Gill, B.S. 1998. Genome differentiation in *Aegilops cylindrica* Host. In Proc. Plant and Animal Genome VI. January 18-22, 1998. San Diego, CA, USA.

Molnár-Láng, M., **Linc, G.** 1998. Demonstration of barley chromosomes in wheat×barley hybrids and their derivatives using *in situ* hybridisation. IV. Növénynevelési Tudományos Napok. Budapest, 1998. január 28-29. pp 23.

Molnár-Láng, M, **Linc, G.** 1998. Production and molecular cytogenetic identification of wheat-barley hybrids and translocations. II International Symposium of Plant Biotechnology Kiev, Ukraine, 4-8 October, 1998. International Institute of Cell Biology, pp 89.

Molnár-Láng, M., **Linc, G.**, Sutka, J. 1998. Detection of barley chromosomes in wheat-barley hybrids and their derivatives by genomic *in situ* hybridization. In Proc. 9th International Wheat Genetics Symposium, Vol. 3. 28-31, Saskatoon, Kanada

Molnár-Láng, M., **Linc, G.**, Sutka, J. 1998. GISH analysis of the derivatives of wheat×barley hybrids. In Lelley T (ed) Proc. Int. Symp. "Current Topics in Plant Cytogenetics Related to Plant Improvement" WUV Universitätsverlag, Wien, Austria, pp 191-197.

Molnár-Láng, M., **Linc, G.**, Sutka, J. 1999. Development and identification of wheat-barley translocations by means of genomic *in situ* hybridisation. IV. Magyar Genetikai Kongresszus, 1999. április 11-14, Siófok, pp 30-31.

Molnár-Láng, M., **Linc, G.**, Kőszegi, B., D. Nagy, E., Sutka, J. 1999. Incorporation of alien chromosomes and chromosome segments into wheat and their demonstration using molecular cytogenetic methods. 50 éves az MTA Mezőgazdasági Kutatóintézete. Tudományos Tanácskozás, Martonvásár, 1999. június 2-3. pp 102-108.

Molnár-Láng, M, **Linc, G.**, Sutka, J. 1999. Production and molecular cytogenetic identification of wheat-barley hybrids and translocations. In: Wedzony, M., Skrudlik, G. (eds) Proc. COST-824 Gametic embryogenesis "Wide crosses in cereals – problems and application" Krakow, Poland, 18-20 February, 1999. Department of Plant Physiology, Polish Academy of Sciences, pp 23-25.

Molnár-Láng, M., **Linc, G.** 2000. Production and molecular cytogenetic analysis of wheat-barley hybrids. 6th International Wheat Conference, 5-9 June 2000, Budapest, Hungary, Abstracts. pp 111.

Linc, G., Friebe, B., Molnár-Láng, M. 2001. Molecular cytogenetic analysis of *Aegilops cylindrica* Host. VII. Növénynevelési Tudományos Napok. Budapest, 2001. január 23-24. pp 30.