Lehr- und Forschungszentrum für Landwirtschaft
Raumberg-Gumpenstein

64. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs 2013, 21 – 22
ISBN: 978-3-902849-00-7


# New resources and approaches for gene cloning in cereals

Miroslav Valárik[1*], Barbora Klocová[1], Michael Abrouk[1], Zeev Frenkel[2], Ajay Kumar[3], Shahryar F. Kianian[4], Hana Šimková[1], Jan Šafář[1], Yuqin Hu[5], Mingcheng Luo[5], Abraham Korol[2] and Jaroslav Doležel[1]

## Abstract

Wheat (*Triticum aestivum* L.) is one of the most important crops, but due to its size (17 Gb) and complexity (allohexaploid and over 80% repetitive elements), its genome represents a challenge for mapping, sequencing, gene cloning and marker assisted breeding. To facilitate wheat genomics, the genome of 'Chinese Spring' has been dissected to particular chromosomes and chromosomal arms by flow cytometric sorting. Flow sorted chromosomes found a large portfolio of applications, including physical mapping using PCR, cytogenetic mapping, protein immunolocalization, chromosome ultrastructure, development of DArT markers, linear DNA amplification suitable for DNA markers development, mapping on DNA arrays, and next generation sequencing. Out of variety of applications, the most important and most demanding application has been the construction of chromosome-specific BAC libraries. In the effort to sequence wheat genome, coordinated by the International Wheat genome Sequencing Consortium (IWGSC, www.wheatgenome.org), this allowed sharing the job between laboratories around the world. Physical maps constructed from the chromosomal specific libraries already facilitate marker development and gene cloning. One example is the cloning of powdery resistance gene *QPm-tut-4A* introgressed to hexaploid wheat from tetraploid wheat *T. militinae* Zhuk & Migush. The gene was mapped to 4AL chromosomal arm. However, recombination suppression in the gene region was observed and marker order in the gene locus could not be resolved by traditional approaches. To overcome this difficulty, a combination of traditional approaches and recent advances in wheat genomics were used. For marker ordering, 4AL-specific radiation hybrid panel and three additional recombination mapping populations were used. To facilitate marker development, 4AL-chromosome specific BAC library was constructed, fingerprinted and ordered to contigs. The assembly of 4AL shotgun sequence was used to construct GenomeZipper (virtual gene order along the chromosome) and all genes from the collinear regions were mapped to our mapping population. Marker development was facilitated using DNA amplified from flow-sorted chromosome arm 4AL of 'Chinese Spring' and the same arm carrying the *T. militinae* translocation. Using these resources, the *QPm-tut-4A* gene was delimited to 0.25 cM region flanked with markers *gpw356* and *Mag974*.

The flanking markers were used to anchor the region with contigs of the 4AL physical map. The anchoring was facilitated by sequencing 3D pools of MTP from whole 4A physical map. Any marker sequence can be anchored in this way to the 4AL physical map. Using sequences of the *QPm-tut-4A* flanking markers, three large contigs spanning the *QPm-tut-4A* gene region and the flanking regions were identified and the sequence of their MTP will be used to identify candidate *QPm-tut-4A* gene/genes. The physical map of 4AL and sequences of the MTP 3D pools are becoming very important genomic resource not only for the *QPm-tut-4A* gene cloning, but also for other agronomically important genes. We have provided complete physical map of gene loci for three additional genes affecting pre-harvest sprouting, gene affecting yield and resistance locus *Yr51*.

*Keywords*

Chromosome translocation, DArT marker, physical map, *Triticum aestivum*, wheat genome

## Acknowledgments

This work has been supported by the Ministry of Education, Youth and Sports of the Czech Republic and the European Regional Development Fund (Operational Programme Research and Development for Innovations No. CZ.1.05/2.1.00/01.0007), Internal Grant Agency PrF-2012-003 and by Estonian Ministry of Agriculture.

[1] Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Slechtitelu 31, 78 371 OLOMOUC, Czech Republic
[2] Institute of Evolution, University of Haifa, HAIFA 31905, Israel
[3] Department of Plant Sciences, North Dakota State University, FARGO, Loftsgard Hall 470G, ND 58108, USA
[4] USDA-ARS Cereal Disease Laboratory, University of Minnesota, ST. PAUL, MN 55108, USA
[5] Department of Plant Sciences, University of California, DAVIS, CA 95616, USA
[*] Corresponding author: Miroslav VALÁRIK, valarik@ueb.cas.cz

## References

DOLEŽEL J, VRÁNA J, CÁPAL P, KUBALÁKOVÁ K, BUREŠOVÁ V, ŠIMKOVÁ H, 2014: Advances in plant chromosome genomics. Biotechnol Adv 32, 122-136.

HERNANDEZ P, MARTIS M, DORADO G, PFEIFER M, GÁLVEZ S, SCHAAF S, JOUVE N, ŠIMKOVÁ H, VALÁRIK M, DOLEŽEL J, MAYER KFX, 2012: Next-generation sequencing and syntenic integration of flow-sorted arms of wheat chromosome 4A exposes the chromosome structure and gene content. Plant J 69, 377-386.

JAKOBSON I, REIS D, TIIDEMA A, PEUSHA H, TIMOFEJEVA L, VALÁRIK M, KLADIVOVÁ M, ŠIMKOVÁ H, DOLEŽEL J, JÄRVE K, 2012: Fine mapping, phenotypic characterization and validation of non-race-specific resistance to powdery mildew in a wheat-*Triticum militinae* introgression line. Theor Appl Genet 125, 609-623.

RANDHAWA M, BANSAL U, VALÁRIK M, KLOCOVÁ K, DOLEŽEL J, BARIANA H, 2014: Molecular mapping of stripe rust resistance gene Yr51 in chromosome 4AL of wheat. Theor Appl Genet 127, 317-324.